UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Desarrollo del proceso de obtención de polvos funcionales de uso alimentario a partir de residuos de las líneas de confección de hortalizas, caracterización funcional y evaluación de su respuesta a la digestión simulada *in vitro*

PROGRAMA DE DOCTORADO EN CIENCIA, TECNOLOGÍA Y GESTIÓN ALIMENTARIA

TESIS DOCTORAL

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Dra. Lucía Seguí Gil, Profesora Titular de Universidad, perteneciente al Departamento de Tecnología de los Alimentos de la Universitat Politècnica de València,

Dra. Cristina Barrera Puigdollers, Profesora Titular de Universidad, perteneciente al Departamento de Tecnología de los Alimentos de la Universitat Politècnica de València,

CONSIDERAN que la memoria titulada "**Desarrollo del proceso de obtención de polvos funcionales de uso alimentario a partir de residuos de las líneas de confección de hortalizas, caracterización funcional y evaluación de su respuesta a la digestión simulada *in vitro***" que presenta **Dª Claudia Isabel Bas Bellver** para aspirar al grado de Doctora de la Universitat Politècnica de València, y que ha sido realizada bajo su dirección en el Instituto Universitario de Ingeniería de Alimentos para el Desarrollo de la Universitat Politècnica de València, reúne las condiciones adecuadas para constituir su tesis doctoral, por lo que AUTORIZAN a la interesada para su presentación.

Valencia, marzo de 2023

Fdo.: Lucía Seguí Gil

Fdo.: Cristina Barrera Puigdollers

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RESUMEN

La industrialización de frutas y hortalizas genera una gran cantidad de residuos orgánicos que perjudican el medio ambiente, que generalmente están infravalorados o no se utilizan. La eliminación de estos residuos mediante incineración o su almacenamiento en vertederos no es una opción sostenible, por lo que la industria debe dirigir sus esfuerzos a reducir su producción, así como a reintroducirlos en la cadena agroalimentaria. Estos residuos hortofrutícolas son ricos en compuestos bioactivos beneficiosos para la salud humana, por lo que presentan un gran potencial para su valorización. La reintroducción de estos productos de deshecho en la cadena agroalimentaria, como ingredientes en la formulación de alimentos más nutritivos, por ejemplo, permitiría generar un sistema de economía circular, además de contribuir al consumo de dietas saludables y sostenibles, en línea con los Objetivos de Desarrollo Sostenible (ODS) definidos por la FAO.

En los últimos años, la fabricación de frutas y hortalizas deshidratadas en polvo ha suscitado un creciente interés al tratarse de productos estables, versátiles y concentrados. En línea con esta tendencia, el presente proyecto de tesis doctoral plantea la obtención de este tipo de productos en polvo a partir de los residuos de confección de hortalizas, en colaboración con la cooperativa Agrícola Villena Coop.V. Concretamente, el proyecto se centra en la valorización integral de residuos de zanahoria, col, apio, puerro y brócoli, mediante su transformación en ingredientes funcionales en polvo.

Los resultados de esta tesis se presentan por compendio de artículos organizados en cuatro capítulos. En el primer capítulo se presentan los resultados de una revisión bibliográfica sobre las tecnologías disponibles para la transformación de estos residuos en productos deshidratados en polvo, centrándose en el impacto de las operaciones unitarias sobre las propiedades fisicoquímicas, tecnológicas y funcionales del producto final, incluida la biodisponibilidad de los compuestos bioactivos contenidos en los polvos. La revisión bibliográfica permitió esclarecer que las condiciones de proceso y las técnicas utilizadas definen las características y la funcionalidad de los productos en polvo. Además, fue posible identificar las etapas

principales del proceso y, de acuerdo a criterios de viabilidad técnica y económica, seleccionar las operaciones unitarias más adecuadas para tal fin.

En el segundo capítulo se recogen los trabajos realizados de cara a evaluar el impacto del procesado (pretratamientos, secado y molienda) sobre las propiedades de los productos en polvo obtenidos a partir de residuos generados en las líneas de confección de hortalizas (zanahoria, col, apio, puerro y brócoli). Concretamente, se evaluó la influencia del procesado sobre las propiedades fisicoquímicas, tecnológicas y funcionales de los productos en polvo obtenidos, así como los cambios experimentados por éstos durante el almacenamiento en condiciones controladas por un periodo de 4 meses. Como tratamientos previos a la deshidratación o pretratamientos, se consideraron la congelación (fresco *vs.* congelado), la intensidad de disrupción del material vegetal (triturado *vs.* troceado) y la fermentación con *Lactobacillus plantarum* (en tallos de brócoli); y como técnicas de deshidratación, el secado por aire caliente (a 50, 60 y 70 °C) y la liofilización. Los resultados obtenidos mostraron que los métodos de deshidratación aplicados permitieron disminuir la actividad del agua del producto inicial hasta valores inferiores a 0,3, condición necesaria para garantizar su estabilidad. Asimismo, se demostró que las variables del proceso influyeron de forma diferente según el tipo de material vegetal deshidratado, definiendo de este modo las características de los productos en polvo obtenidos. Se confirmó una interdependencia de las operaciones de disrupción y secado, los cuales tuvieron un impacto significativo en el tamaño de partícula y las propiedades antioxidantes de los productos en polvo. Respecto a sus propiedades tecnológicas, los polvos mostraron resultados positivos en cuanto a sus propiedades de interacción con el agua, aunque una pobre capacidad de interacción con el aceite. A lo largo del almacenamiento se constató una reducción en la calidad de los productos en polvo. Esta parte del estudio reveló la importancia de estudiar, no solo las condiciones de proceso, sino también las de almacenamiento con tal de conseguir las características y calidad adecuadas. Con la fermentación se consiguió mejorar las propiedades antioxidantes de los residuos vegetales. La liofilización permitió además obtener polvos con potencial efecto

probiótico, mientras que el secado por aire caliente afectó negativamente la viabilidad microbiana.

Además de definir las propiedades fisicoquímicas y tecnológicas, la bioaccesibilidad de los compuestos bioactivos presentes en el polvo también queda determinada por el proceso y las características de los polvos obtenidos. En este sentido, se realizaron investigaciones dirigidas a evaluar el impacto del procesado sobre determinados compuestos bioactivos específicos, y sobre la respuesta de los productos en polvo a la digestión simulada *in vitro*. Se analizó por un lado el contenido en carotenoides en polvos de residuos de zanahoria y, por otro, el contenido en glucosinolatos e isotiocianatos en polvos de residuos de col blanca y brócoli. Del mismo modo, se evaluó la liberación de antioxidantes y carotenoides a lo largo de la digestión simulada *in vitro.* Todo ello queda recogido en el tercer capítulo de resultados de la presente tesis doctoral. En general, la liofilización dio lugar a productos en polvo más finos y con mayor contenido en compuestos bioactivos específicos (carotenoides y sulforafano), mientras que el secado por aire caliente mejoró las propiedades antioxidantes. Por su parte, el estudio de la digestión simulada *in vitro* reveló que la digestión favorece la extracción y liberación de compuestos antioxidantes y, en el caso de los polvos de zanahoria, de carotenoides. En los polvos de zanahoria también se evaluó el efecto de dispersar estos polvos en diferentes matrices (agua, aceite o emulsión de aceite en agua) sobre la liberación de carotenoides durante la digestión *in vitro*. La respuesta al proceso de digestión quedó definida tanto por las condiciones de procesado como por la co-ingesta de los polvos con aceite.

En el cuarto capítulo de resultados de esta tesis se describe una primera aproximación a la aplicación de los productos en polvo obtenidos como ingrediente alimentario. En particular, se adicionaron productos en polvo obtenidos a partir de residuos de zanahoria y col a la formulación de magdalenas y rosquilletas, respectivamente, en diferentes porcentajes de sustitución de harina de arroz (5, 10, 20 y 30%). La aplicación de los polvos como ingrediente funcional en la formulación de productos de panadería sin gluten confirmó que la adición de harinas obtenidas a partir de residuos vegetales es una buena estrategia para fortificar y enriquecer

nutricionalmente estos productos horneados. El nivel de sustitución tuvo una influencia significativa sobre las propiedades fisicoquímicas, reológicas y antioxidantes de las masas y los productos obtenidos, obteniéndose mejores propiedades antioxidantes a medida que se incrementó la proporción de harina vegetal en la mezcla.

Los resultados obtenidos en la presente tesis doctoral han demostrado el potencial de los residuos generados en la confección de bandejas y productos de IV gama de zanahoria, col, apio, puerro y brócoli para su valorización integral, habiéndose desarrollado un proceso de transformación de los mismos en productos deshidratados en polvo. Se prevé que esta aproximación pueda contribuir de manera efectiva al concepto de dietas saludables y sostenibles, y al desarrollo de un sistema alimentario más sostenible.

ABSTRACT

Fruit and vegetables industrialisation generates a large amount of organic waste affecting environment negatively, which is undervalued or not used. Disposal of these residues by incineration or storage in landfills is not a sustainable option, so the industry must focus on reducing its production as well as reintroducing them into the agri-food chain. These fruit and vegetable by-products are generally rich in bioactive compounds, which are responsible of beneficial health effects, presenting a great potential for their valorisation. The reintroduction of these waste products into the agri-food chain, as ingredients in the formulation of more nutritious foods, for example, would generate a circular economy system, as well as contribute to the consumption of healthy and sustainable diets, all within the framework of the Sustainable Development Goals (SDG) defined by the FAO.

In recent years, interest in fruit and vegetable dehydrated powders manufacturing has been increased, as they are stable, versatile and concentrated products. In line with this trend, this doctoral thesis project proposes obtaining this type of powdered products from vegetable waste, in collaboration with the cooperative Agrícola Villena Coop.V. Specifically, the project focuses on the integral valorisation of carrot, cabbage, celery, leek and broccoli waste, through its transformation into powdered functional ingredients.

The results of this thesis are presented as a compendium of articles organised in four chapters. The first chapter presents the results of a literature review on the available technologies for the transformation of these wastes into dehydrated powdered products, focusing on the impact of the unitary operations on the physicochemical, technological and functional properties of the final product, including the bioavailability of the bioactive compounds. This literature review stated that the processing conditions and techniques used determine the characteristics and functionality of the powdered products. Furthermore, it was possible to identify the main stages of the process and, according to technical and economic feasibility criteria, to select the most suitable unit operations for this purpose.

In the second chapter, the work performed to evaluate the impact of processing (pretreatment, drying and milling) on the properties of the powdered products obtained from waste generated in the vegetable packaging lines (carrot, cabbage, celery, leek and broccoli) is presented. Specifically, the influence of processing on the physicochemical, technological and functional properties of the powdered products obtained was evaluated, as well as changes experienced by these products during storage under controlled conditions for a period of 4 months. Freezing (fresh vs. frozen), disruption of the plant material (ground vs. chopped) and fermentation with *L. plantarum* (in broccoli stems) were considered as pre-dehydration treatments or pretreatments; hot-air drying (at 50, 60 and 70 °C) and freeze-drying were used as dehydration techniques. Results obtained showed that the dehydration methods applied allowed to reduce water activity of the initial product to values below 0.3, a necessary condition to guarantee its stability. It was also shown that the process variables had a different influence depending on the type of plant material dehydrated, thus determining the characteristics of the powdered products obtained. An interdependence of the disruption and drying operations was confirmed, which had a significant impact on particle size and antioxidant properties of the powders. Regarding their technological properties, the powders showed positive results in terms of water-interaction properties, but poor oil-interaction capacity. During storage, a reduction in the quality of the powdered products was observed. This part of the study revealed the importance of studying not only the process conditions, but also the storage conditions in order to achieve suitable characteristics and quality. Fermentation improved antioxidant properties of the plant residues. Freeze-drying also made it possible to obtain powders with potential probiotic effect, whereas hot-air drying negatively affected microbial viability.

Besides defining the physicochemical and technological properties, bioaccessibility of the powder bioactive compounds is also determined by the process and the characteristics of the powders obtained. In this regard, research was carried out to assess the impact of processing on specific bioactive compounds and on the response of the powdered products to simulated *in vitro* digestion. Thus, the carotenoid content of carrot waste

powders and the glucosinolate and isothiocyanate content of white cabbage and broccoli waste powders were analysed. Similarly, the release of antioxidants and carotenoids during simulated *in vitro* digestion was evaluated. All this is reported in the third chapter of the results of this doctoral thesis. In general, freeze-drying resulted in finer powdered products with a higher content of specific bioactive compounds (carotenoids and sulforaphane), while hot-air drying improved antioxidant properties. The *in vitro* digestion study revealed that digestion favours the extraction and release of antioxidant compounds and, in the case of carrot powders, of specific bioactive compounds such as carotenoids. The effect of dispersing carrot powders in different matrices (water, oil or oil-in-water emulsion) on the release of carotenoids during *in vitro* digestion was also evaluated in carrot powders. The response to the digestion process was defined by both the processing conditions and the co-ingestion of the powders with oil.

The fourth chapter of results of this thesis describes a first approach to the application of the obtained powdered products as a food ingredient. In particular, powdered products obtained from carrot and cabbage waste were added to the formulation of muffins and breadsticks, respectively, at different percentages of rice flour substitution (5, 10, 20 and 30%). The application of the powders as a functional ingredient in the formulation of gluten-free bakery products confirmed that the addition of flours obtained from vegetable waste is a good strategy to fortify and nutritionally enrich these baked goods. The level of substitution had a significant influence on the physicochemical, rheological and antioxidant properties of the doughs and the products obtained, with better antioxidant properties being obtained as the proportion of vegetable flour in the blend increased.

Results obtained in this doctoral thesis have demonstrated the potential of the waste generated in the preparation of trays and pre-prepared convenience food products of carrot, cabbage, celery, leek and broccoli for their integral valorisation, developing a process to transform them into dehydrated powdered products. This approach is expected to contribute effectively to the concept of healthy and sustainable diets and to the development of a more sustainable food system.

RESUM

La industrialització de fruites i hortalisses genera una gran quantitat de residus orgànics en detriment del medi ambient, els quals estan infravalorats o no s'utilitzen. L'eliminació d'aquests residus mitjançant incineració o el seu emmagatzematge en abocadors no és una opció sostenible, per la qual cosa la indústria ha de dirigir els seus esforços a reduir la seua producció, així com a reintroduir-los en la cadena agroalimentària. Aquests residus hortofructícoles generalment són rics en compostos bioactius que poden exercir efectes beneficiosos per a la salut, presentant un gran potencial per a la seua valorització. La reintroducció d'aquests productes en la cadena agroalimentària, com a ingredients en la formulació d'aliments més nutritius, per exemple, permetria generar un sistema d'economia circular, a més de contribuir al consum de dietes saludables i sostenibles, tot això emmarcat en els Objectius de Desenvolupament Sostenible (ODS) definits per la FAO.

En els últims anys, la fabricació de fruites i hortalisses deshidratades en pols ha suscitat un creixent interés perquè es tracta de productes estables, versàtils i concentrats. En línia amb aquesta tendència, el present projecte de tesi doctoral planteja l'obtenció d'aquest tipus de productes en pols a partir dels residus de confecció d'hortalisses, en col·laboració amb la cooperativa Agrícola Villena Coop.V. Concretament, el projecte es centra en la valorització integral de residus de carlota, col, api, porro i bròcoli, mitjançant la seua transformació en ingredients funcionals en pols.

Els resultats d'aquesta tesi es presenten per compendi d'articles organitzats en quatre capítols. En el primer capítol es presenten els resultats d'una revisió bibliogràfica sobre les tecnologies disponibles per a la transformació d'aquests residus en productes deshidratats en pols, centrant-se en l'impacte de les operacions unitàries sobre les propietats fisicoquímiques, tecnològiques i funcionals del producte final, inclosa la biodisponibilitat dels compostos bioactius continguts en les pols. Aquesta revisió bibliogràfica va permetre esclarir que les condicions de processament i les tècniques utilitzades defineixen les característiques i la funcionalitat dels productes en pols. A més, va ser possible identificar les etapes principals del

procés i, d'acord amb criteris de viabilitat tècnica i econòmica, seleccionar les operacions unitàries més adequades per a tal fi.

En el segon capítol es recullen els treballs realitzats de cara a avaluar l'impacte del processament (pretractaments, assecat i molta) sobre les propietats dels productes en pols obtinguts a partir de residus generats en les línies de confecció d'hortalisses (carlota, col, api, porro i bròcoli). Concretament, es va avaluar la influència del processament sobre les propietats fisicoquímiques, tecnològiques i funcionals dels productes en pols obtinguts, així com els canvis experimentats per aquests durant l'emmagatzematge en condicions controlades per un període de 4 mesos. Com a tractaments previs a la deshidratació o pretractaments es van considerar la congelació (fresc vs. congelat), la intensitat de disrupció del material vegetal (triturat vs. trossejat) i la fermentació amb *L. plantarum* (en tiges de bròcoli); com a tècniques de deshidratació, el ssecat per aire calent (a 50, 60 i 70 °C) i la liofilització. Els resultats obtinguts van mostrar que els mètodes de deshidratació aplicats van permetre disminuir l'activitat de l'aigua del producte inicial fins a valors inferiors a 0,3, condició necessària per a garantir la seua estabilitat. Així mateix, es va demostrar que les variables del procés van influir de manera diferent segons el tipus de material vegetal deshidratat, determinant d'aquesta manera les característiques dels productes en pols obtinguts. Es va confirmar una interdependència de les operacions de disrupció i secat, els quals van tindre un impacte significatiu en el tamany de partícula i les propietats antioxidants dels productes en pols. Respecte a les seues propietats tecnològiques, les pols van mostrar resultats positius en quant a les seues propietats d'interacció amb l'aigua, encara que una pobra capacitat d'interacció amb l'oli. Al llarg de l'emmagatzematge es va constatar una reducció en la qualitat dels productes en pols. Aquesta part de l'estudi va revelar la importància d'estudiar, no sols les condicions de procés, sinó també les d'emmagatzematge per tal d'aconseguir les característiques i qualitat adequades. Amb la fermentació es va aconseguir millorar les propietats antioxidants dels residus vegetals. La liofilització va permetre a més obtindre pols amb potencial efecte probiòtic, mentre que el secat per aire calent va afectar negativament la viabilitat microbiana.

A més de definir les propietats fisicoquímiques i tecnològiques, la bioaccesibilitat dels compostos bioactius presents en la pols també queda determinada pel procés i les característiques de les pols obtingudes. En aquest sentit, es van realitzar investigacions dirigides a avaluar l'impacte del processament sobre determinats compostos bioactius específics, i sobre la resposta dels productes en pols a la digestió simulada *in vitro*. Per una banda, es va analitzar el contingut en carotenoides en pols de residus de carlota i, per altra, el contingut en glucosinolatos i isotiocianatos en pols de residus de col blanca i bròcoli. De la mateixa manera, es va avaluar l'alliberament d'antioxidants i carotenoides al llarg de la digestió simulada *in vitro*. Tot això queda recollit en el tercer capítol de resultats de la present tesi doctoral. En general, la liofilització va donar lloc a productes en pols més fins i amb major contingut en compostos bioactius específics (carotenoides i sulforafano), mentre que el secat per aire calent va millorar les propietats antioxidants. Per part seua, l'estudi de la digestió simulada *in vitro* va revelar que la digestió afavoreix l'extracció i alliberament de compostos antioxidants i, en el cas de les pólvores de carlota, de compostos bioactius específics com són els carotenoides. En les pols de carlota també es va avaluar l'efecte de dispersar aquestes pols en diferents matrius (aigua, oli o emulsió d'oli en aigua) sobre l'alliberament de carotenoides durant la digestió *in vitro*. La resposta al procés de digestió va quedar definida tant per les condicions de processament com per la co-ingesta de les pols amb oli.

En el quart capítol de resultats d'aquesta tesi es descriu una primera aproximació a l'aplicació dels productes en pols obtinguts com a ingredient alimentari. En particular, es van addicionar productes en pols obtinguts a partir de residus de carlota i col a la formulació de magdalenes i rosquilletes, respectivament, en diferents percentatges de substitució de farina d'arròs (5, 10, 20 i 30%). L'aplicació de les pols com a ingredient funcional en la formulació de productes de panaderia sense gluten va confirmar que l'addició de farines obtingudes a partir de residus vegetals és una bona estratègia per a fortificar i enriquir nutricionalment aquests productes enfornats. El nivell de substitució va tindre una influència significativa sobre les propietats fisicoquímiques, reològiques i antioxidants de les masses i els

productes obtinguts, obtenint-se millors propietats antioxidants a mesura que es va incrementar la proporció de farina vegetal en la mescla.

Els resultats obtinguts en la present tesi doctoral han demostrat el potencial dels residus generats en la confecció d'hortalisses i productes de IV gamma de carlota, col, api, porro i bròcoli per a la seua valorització integral, havent-se desenvolupat un procés de transformació dels mateixos en productes deshidratats en pols. Es preveu que aquesta aproximació puga contribuir de manera efectiva al concepte de dietes saludables i sostenibles, i al desenvolupament d'un sistema alimentari més sostenible.

ÍNDICE

1. CONTEXTUALIZACIÓN Y JUSTIFICACIÓN DEL ESTUDIO

La pérdida y el desperdicio de alimentos tienen un impacto negativo en la sociedad, el medio ambiente y la economía (FAO, 2020). La generación de residuos alimentarios implica pérdidas económicas y su gestión supone un coste importante para los agentes productivos. Los aspectos relacionados con el impacto medioambiental de la actividad humana son cada vez más preocupantes, ya que la producción de alimentos requiere de un uso intensivo de los recursos, lo cual contribuye a la huella de carbono y al agotamiento de recursos tales como la tierra y el agua. Por otro lado, si no se produce cambio alguno en los hábitos alimentarios, se espera que en el año 2050 la demanda de productos agrícolas se vea incrementada en un 50% como consecuencia del crecimiento demográfico (Food2030 Pathways for Action). Todo ello enmarcado en un sistema alimentario en el que la inseguridad alimentaria y la malnutrición están cada vez más presentes, al tiempo que una cantidad considerable de alimentos que podrían haberse consumido, se pierde a lo largo de la cadena productiva o termina como residuo. Cada año, en la UE se generan cerca de 129 millones de toneladas en pérdidas alimentarias (Caldeira et al., 2019; Sánchez-López et al., 2020) y se estima que un tercio de los alimentos producidos a nivel mundial se desperdicia a lo largo de toda la cadena alimentaria (Comunian et al., 2021), desde la producción primaria hasta su consumo (figura 1). Reducir el desperdicio alimentario en la próxima década es un objetivo prioritario que pretende contribuir a los cuatro pilares sobre los que se sustenta el programa Food2030: nutrición y salud, clima y sostenibilidad, circularidad y uso de los recursos e innovación y comunidades (FAO, 2020).

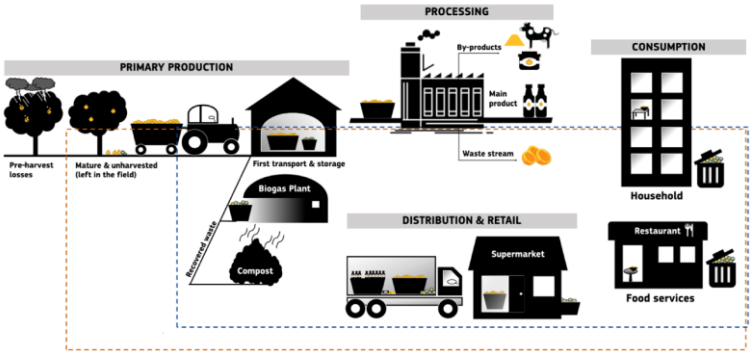

Figura 1. Generación de residuos a lo largo de la cadena alimentaria. FUENTE: Sánchez-López et al. (2020).

Como se muestra en la Figura 2, la mayor cantidad de residuos se genera durante la etapa de consumo (46%), aunque la producción primaria (25%) y el procesado de alimentos (24%) suponen alrededor del 50% de estas pérdidas, siendo las frutas y vegetales los productos que más participan de estos porcentajes (Sánchez-López, et al., 2020). Concretamente, en los canales de distribución de hortalizas, aproximadamente un 30% del total se convierte en desperdicio o subproducto (Gustavsson et al., 2011; Sepúlveda et al., 2021).

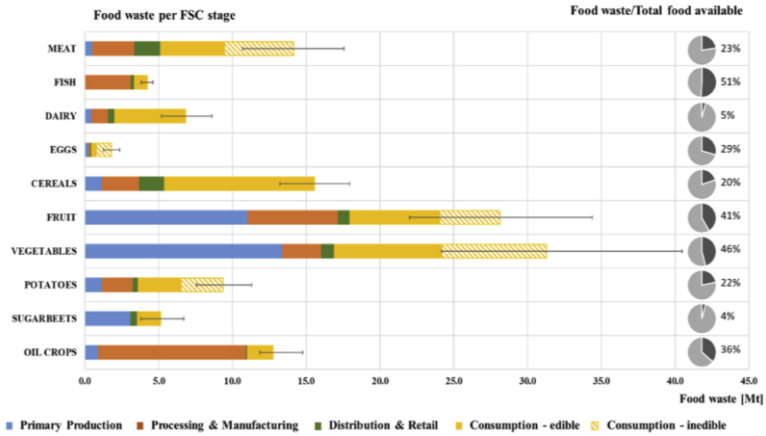

Figura 2. Izquierda: las barras representan la cantidad total de residuos alimentarios (componentes comestibles y no comestibles) a lo largo de toda la cadena alimentaria para cada grupo de alimentos. Derecha: porcentaje de residuos alimentarios (gris oscuro) sobre el total de alimentos disponibles. FUENTE: Caldeira et al. (2019).

En las primeras etapas de procesado, estas pérdidas se deben principalmente a los destríos derivados de los altos estándares de comercialización, así como a partes comestibles que se eliminan por pelado o corte en las líneas de procesado en fresco (hojas exteriores, tallos, partes dañadas). Los excedentes y el deterioro también pueden contribuir a estas cifras. La práctica habitual frente a las grandes cantidades de residuos generadas es su desestimación o su uso en alimentación animal, ya que suelen ser infrautilizados y considerados como material de bajo valor. No obstante, este material vegetal presenta un gran potencial para su valorización y posee un alto valor nutricional y biológico, ya que es rico en nutrientes y compuestos bioactivos, tales como polisacáridos, proteínas,

vitaminas, minerales, polifenoles, carotenoides, etc. (Comunian et al., 2021). La reintroducción de estos productos de deshecho en la cadena alimentaria permitiría generar un sistema de economía circular, contribuyendo al consumo de dietas más saludables y al desarrollo de un sistema alimentario rentable y sostenible. Es necesario por tanto enfocar los sistemas de producción hacia la minimización de residuos y a su reintroducción en la cadena de procesado. Conceptos como "economía circular", "ecología industrial" y "economía de residuo cero", entre otros, han surgido para orientar la ecoinnovación hacia el uso de los residuos como materia prima en el desarrollo de nuevos productos y aplicaciones, y así disminuir el impacto ambiental a la vez que se obtiene un beneficio económico (Mirabella et al., 2014; Stenmarck et al., 2016).

Según la definición de la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), un sistema alimentario sostenible debe ser capaz de garantizar patrones de consumo y producción sostenibles, así como de ofrecer dietas seguras, saludables y nutritivas. En consecuencia, la industria alimentaria debe incorporar procesos y productos que proporcionen, por un lado, un menor impacto medioambiental y, por otro, un mayor impacto positivo en las dietas y la salud. La reformulación de los alimentos procesados ofrece una oportunidad para mejorar la salud de las personas modificando las características nutricionales de los alimentos procesados que se consumen habitualmente, contribuyendo así al objetivo de desarrollo sostenible de la FAO "Garantizar una vida sana y promover el bienestar para todos en todas las edades". Esto está en consonancia con la definición de una dieta saludable sostenible, que establece que un sistema alimentario sostenible debe garantizar patrones de consumo y producción sostenibles, así como ofrecer dietas seguras, saludables y nutritivas (FAO y WHO, 2019).

En línea con esto, el grupo de investigación en alimentos funcionales del Instituto Universitario de Ingeniería de Alimentos para el Desarrollo de la Universitat Politècnica de València que lleva a cabo esta investigación, ha liderado diversos proyectos financiados en convocatorias públicas en los que se ha desarrollado el proceso de obtención de ingredientes funcionales en polvo a partir de residuos procedentes de la industrialización de frutas y

hortalizas: AICO/2017/049 “Desarrollo tecnológico del proceso de obtención de polvos para uso alimentario y con propiedades funcionales a partir de subproductos de mandarina, caqui y arándano”; AGCOOP_D/2018/025 “Obtención de polvos de uso alimentario con propiedades funcionales a partir de residuos de las líneas de confección de hortalizas (RESHORTPOLS)” y AGCOOP_A/2021/020 “Obtención de productos en polvo a partir de las líneas de confección de col blanca para su uso como ingrediente funcional sostenible y para la gestión integrada de plantas arvenses (FUNBIOPEST)”. Los dos últimos se han llevado a cabo en colaboración con la cooperativa agrícola Agrícola Villena Coop. V. Estas colaboraciones demuestran el interés del sistema productivo por valorizar los residuos generados durante el procesado en fresco de frutas y hortalizas, en este caso mediante su transformación en ingredientes funcionales en polvo. De esta forma se contribuye al concepto de dietas sostenibles definido por la FAO, que combina el reto de desarrollar dietas saludables para una población creciente, reduciendo al mismo tiempo su impacto ambiental (FAO y WHO, 2019).

2. INTRODUCCIÓN

2.1. La obtención de ingredientes en polvo como estrategia de valorización de residuos hortofrutícolas

Tradicionalmente, los residuos de frutas, hortalizas y cereales suelen eliminarse en vertederos o incinerarse con la consiguiente contaminación del aire, del agua y del suelo. Para disminuir estos problemas, la Unión Europea (UE) está promoviendo su reducción y su valorización (Socas-Rodríguez et al., 2021). Las estrategias habituales de valorización de estos residuos se han centrado en su utilización como compostaje (Gebremikael et al., 2020), su transformación en alimento para ganado (Kasapidou et al., 2015; Esparza et al., 2020), la extracción de componentes bioactivos en forma de antioxidantes, enzimas, proteínas, fibras, ésteres, etc. para su utilización en productos farmacéuticos, nutracéuticos, suplementos dietéticos o como aditivos en matrices alimentarias (Pleissner et al., 2016; Routray & Orsat, 2017; Esparza et al., 2020), o bien en la producción de biocombustibles y productos químicos (Pleissner et al., 2016) (figura 3). No obstante, la valorización sigue representando un reto debido, entre otros factores, a la diferente estabilidad de los compuestos bioactivos durante el procesado, la dificultad tecnológica, los altos costes del proceso y el uso de disolventes no alimentarios durante los procesos de extracción de los componentes con valor añadido (Socas-Rodríguez et al., 2021).

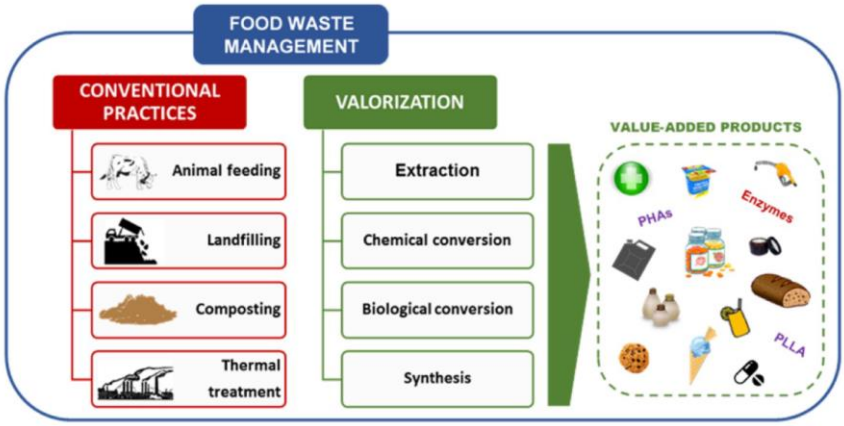

Figura 3. Estrategias habituales de gestión de residuos alimentarios. FUENTE: Esparza et al. (2020).

La utilización de subproductos del procesado de frutas y hortalizas para el desarrollo de nuevos ingredientes y productos alimentarios ha demostrado su importancia comercial (Ferreira et al., 2015; Plazzota et al., 2018).

Por otro lado, la creciente concienciación sobre la asociación entre una dieta inadecuada y problemas de salud ha llevado al desarrollo de una industria alimentaria más saludable. Investigaciones clínicas y epidemiológicas asocian las dietas ricas en vegetales con una reducción del riesgo de padecer enfermedades cardiovasculares, coronarias, metabólicas, enfermedades crónicas y enfermedades degenerativas como el cáncer (Hung et al., 2004; Sagar et al., 2018). Son responsables de estos efectos beneficiosos para la salud sus nutrientes y compuestos bioactivos (vitaminas, fibra, minerales, compuestos antioxidantes, etc.), la mayoría de los cuales también se encuentran en los subproductos y deshechos generados (Ferreira et al., 2015), presentándose como una fuente interesante de compuestos potencialmente funcionales. Sin embargo, la valorización de residuos hortofrutícolas mediante la extracción de fitoquímicos a través de procesos físicos o químicos conlleva un aumento de los costes y otros inconvenientes como la necesidad de etapas adicionales, que generalmente incluyen la eliminación de disolventes (Ramachandraiah & Chin, 2018). Por el contrario, el empleo de técnicas establecidas tales como la combinación de las operaciones de secado y molienda, constituye una alternativa más económica y respetuosa con el medio ambiente para la valorización integral de estos residuos (Lucas-González et al., 2017; Seguí et al., 2022).

La industria alimentaria actual muestra un creciente interés en la aplicación de frutas y verduras deshidratadas en polvo (Karam et al., 2016). Existen ejemplos como la utilización de polvos de cebolla, apio, brócoli, tomate, pimiento, entre otros, como conservantes alimentarios o también como ingredientes en la reformulación de alimentos más saludables (Neacsu et al., 2015). También se ha reportado la utilización de polvos de frutas como colorante, como el caso de polvos de arándano (Camire et al., 2007) o de grosella negra (Toscano Martínez et al., 2021). Además, se han realizado estudios sobre aplicabilidad de estos ingredientes en polvo en diferentes formulaciones alimentarias, especialmente en productos de panadería o

snacks con el fin de mejorar sus propiedades nutricionales, tal es el caso de polvos de zanahoria (Sharma & Kumar, 2017) o polvos de naranja, sandía, lechuga, espinacas, entre otros (Ferreira et al., 2015); en la formulación de sopas instantáneas, utilizando por ejemplo polvos obtenidos a partir de guisantes (Hanan et al., 2020) o calabaza (Dhiman et al., 2017); o bien como ingredientes para aumentar el valor nutricional, modificar la textura y/o como antioxidantes en productos cárnicos, utilizándose, por ejemplo, polvos de granada (Qin et al., 2013), polvos de calabaza (Unal et al., 2022) o harina de caqui (Lucas-González et al., 2019).

Los productos en polvo o harinas obtenidos a partir de frutas o vegetales son productos estables, con una concentración elevada en los nutrientes propios de la materia prima y que resultan versátiles, pudiendo ser utilizados directamente o como ingrediente en la formulación de alimentos. En este contexto y siguiendo esta tendencia, la fabricación de ingredientes en polvo a partir de residuos hortofrutícolas se plantea como un proceso de aprovechamiento integral de los mismos, que permitiría transformar un material de desecho en un producto funcional estable con un alto valor añadido. No obstante, la obtención de estos ingredientes en polvo también supone un reto debido a su heterogeneidad y a las diferentes características macroestructurales, microestructurales y de composición que puedan existir entre productos, y entre las partes comestibles y no comestibles de un mismo producto.

2.2. Efecto del procesado sobre las propiedades de frutas y hortalizas en polvo

El proceso de fabricación de polvos a partir de frutas y hortalizas suele contar con tres etapas principalmente: desestructuración, deshidratación y molienda (Santos et al., 2022), tratamientos con los que se consigue un producto final fisicoquímica y microbiológicamente más estable (Hernández y Blanco, 2015). Otras etapas pueden ser incluidas, fundamentalmente como pretratamientos, en función de la materia prima y de la posterior aplicación del producto obtenido. Los antecedentes o estado del arte del impacto de las etapas de proceso sobre las características de los productos en polvo

supusieron el primer objetivo del presente proyecto de tesis doctoral, de modo que el resultado de dicha revisión se presentará como primer capítulo del apartado de resultados.

Para el desarrollo de polvos con unas propiedades finales adecuadas es necesario estudiar el impacto de las etapas de proceso y sus variables, incluidos los pretratamientos, sobre las propiedades fisicoquímicas y funcionales de los mismos. Las características de los productos en polvo también dependen de la composición y de las propiedades fisicoquímicas de las materias primas, las cuales deben tenerse en cuenta a la hora de seleccionar los niveles de las variables de proceso más adecuadas. Para ello, es importante evaluar las condiciones de proceso a las que se somete el alimento con tal de obtener un producto con las propiedades deseadas.

Debido a su elevado contenido en agua, los residuos vegetales son propensos al deterioro durante el almacenamiento. La **deshidratación o secado** es una técnica útil para reducir la actividad del agua de los residuos y prolongar su vida útil, contribuyendo además a concentrar y preservar los compuestos fitoquímicos que contienen (Sagar et al., 2018; Majerska et al., 2019; Sepúlveda et al., 2021). La deshidratación es una operación ampliamente estudiada y de gran aplicación en la industria alimentaria, además de ser una etapa fundamental para la producción de ingredientes en polvo. Implica la transferencia de calor y materia en régimen transitorio acompañada de transformaciones físicas, químicas y cambios de fase. Estas transformaciones afectan a la calidad del producto y determinan sus propiedades tecnológicas, como las propiedades de interacción con el agua y el aceite (Ramírez-Pulido et al., 2021). Existe una gran variedad de técnicas de deshidratación para la obtención de productos en polvo, como son el secado por aire caliente, la liofilización, el secado por atomización o el secado a vacío, los cuales pueden aplicarse solos o ser asistidos por otras tecnologías, como la tecnología de microondas o ultrasonidos, por ejemplo (Sagar & Suresh Kumar, 2010; Bhatkar et al., 2021). En cualquier caso, debe tenerse en cuenta que el aprovechamiento integral de los residuos requiere de la aplicación de técnicas que permitan la deshidratación de productos sólidos, tales como la liofilización o el secado por aire caliente.

El secado por aire caliente (SAC) se aplica habitualmente en la industria alimentaria debido a su sencillez y a sus menores costes de inversión inicial y operación. Durante el SAC, el alimento se expone a una corriente de aire con bajo contenido en humedad, generalmente porque ha sido calentado, de modo que al incidir sobre la superficie del producto promueve mecanismos de transferencia de materia y calor, que conllevan a la evaporación del agua de la muestra. En primer lugar, la energía se transfiere desde el aire caliente hasta la muestra y, debido al gradiente entre la actividad del agua del producto y la humedad relativa del aire, se produce la evaporación del agua desde la superficie del producto. Inicialmente se evaporará el agua libre o no ligada y, a medida que el secado progresa, parte del agua atrapada en el interior de la muestra migra hacia la superficie para terminar transfiriéndose a la corriente de aire que rodea el producto. Generalmente, la evaporación del agua no ligada se produce durante el conocido como periodo de velocidad de secado constante, mientras que el agua ligada se elimina a lo largo del periodo de velocidad de secado decreciente (Calín-Sánchez et al., 2020). Las condiciones del proceso (largos tiempos de exposición y elevada temperatura) pueden resultar en pérdidas de calidad del producto deshidratado debido a la incidencia de reacciones de oxidación, fenómenos de encostramiento en la superficie del producto y a la modificación de las propiedades organolépticas (Sagar & Suresh Kumar, 2010; Calín-Sánchez et al., 2020). No obstante, existen estudios que demuestran que determinadas condiciones de secado, tales como una elevada temperatura del aire de secado, puede conllevar una mejora de las propiedades antioxidantes (Que et al., 2008; Chen et al., 2011) o bien un empeoramiento de las mismas en el producto seco (Korus, 2011).

Por su parte, la liofilización (LIO) es una técnica que se realiza en condiciones de baja temperatura y vacío, durante la cual el alimento se somete en primer lugar a una etapa de congelación y posteriormente tiene lugar la sublimación de los cristales de hielo formados. Finalmente, la muestra atraviesa una etapa de desorción durante la cual se elimina parte del agua no congelable del producto, que no habría podido eliminarse por sublimación. La liofilización también tendría aplicación para la valorización integral de los residuos con el fin de obtener ingredientes en polvo. Por las

características de esta técnica, los productos obtenidos suelen ser de alta calidad, y conservan en mayor medida las propiedades del producto fresco. Las condiciones en las que se lleva a cabo la liofilización previenen los daños causados por oxidación durante el secado, permiten conservar gran parte de compuestos volátiles y sólidos solubles, minimizan los cambios en la composición química del alimento, y generan una estructura final muy porosa con bajo contenido en humedad (Calín-Sánchez et al., 2020). Sin embargo, esta tecnología tiene aplicaciones industriales más limitadas debido a un mayor consumo de energía y elevados costes de operación en comparación con el SAC (Oyinloye & Yoon, 2020; Stamenković et al., 2020).

El efecto de la deshidratación sobre la materia prima va a depender del tipo de producto, así como de las etapas involucradas en el proceso de transformación y las condiciones en las que se realiza, pudiendo favorecer o disminuir el valor nutricional del material vegetal (Mrkìc et al., 2006). Generalmente, el producto fresco es **pretratado** física o químicamente antes del secado (Górnicki & Kaleta, 2007), con tal de modificar su estructura, ablandar el tejido y liberar compuestos bioactivos, con tal de promover cambios que determinan la respuesta al proceso de secado y la calidad del producto final. La molienda, la congelación, el escaldado, la fermentación o la aplicación de ultrasonidos, entre otros, son técnicas que pueden ser utilizadas como pretratamiento pudiendo aplicarse previamente o en combinación con la etapa de desestructuración del material vegetal. Debido a que el objetivo de la presente investigación es aportar valor añadido a residuos hortofrutícolas sin generar grandes costes de producción, el estudio de los pretratamientos se centró en tecnologías económicas y escalables tales como la desestructuración o disrupción del tejido, la congelación y la fermentación.

La disrupción del material vegetal como pretratamiento es un paso esencial para obtener polvos de alta calidad, con características nutricionales y fisicoquímicas adecuadas, además de mejorar el proceso de secado (Erenturk et al., 2005; Djantou et al., 2011). La intensidad del desestructurado puede tener un efecto sobre las propiedades fisiológicas y antioxidantes de los productos frescos (Dovene et al., 2019). La rotura de la matriz alimentaria influye en la velocidad de secado y en la liberación de

compuestos bioactivos, ya que factores como la estructura interna de la matriz generada, el tamaño de las partículas que conforman el lecho o la superficie de contacto con el aire de secado determinarán el proceso de deshidratación y, por consiguiente, las características de los polvos obtenidos (Ramos et al., 2003).

En cuanto a la congelación como pretratamiento, se ha visto que podría tener un efecto sobre la estructura de la matriz vegetal y, con ello, sobre la posterior etapa de deshidratación. La congelación implica la formación de cristales de hielo ocasionando un daño en la membrana celular y, tras la descongelación, estos cristales de hielo pueden dar lugar a espacios entre paredes celulares facilitando la transferencia de agua durante el secado reduciéndose así el tiempo necesario para secar el producto (Ando et al., 2016; Peng et al., 2018). Conocer el comportamiento del tejido vegetal frente a la congelación-descongelación es importante para garantizar la eficiencia del posterior proceso de deshidratación.

Por su parte, la fermentación se presenta como una alternativa sostenible y segura de pretratamiento para la valorización de residuos, que permitiría aprovechar el potencial nutricional de estos residuos vegetales (Di Cagno et al., 2013; Xing et al., 2020). Siendo una técnica que se remonta a siglos atrás, es considerada como una de las formas más efectivas de conservar los alimentos debido a la formación de ácidos orgánicos, alcoholes, bacteriocinas y otros compuestos antimicrobianos (Hutkins, 2018). La fermentación es un proceso que ocurre en presencia de microorganismos beneficiosos (levaduras, mohos y bacterias) que descomponen los azúcares y almidones en alcoholes y ácidos, aumentando el valor nutricional (Adebo et al., 2017) y mejorando las propiedades organolépticas (Xu et al., 2019a) de los alimentos. Las matrices fermentadas se presentan como ingredientes potenciales con propiedades mejoradas y gran aplicabilidad en la industria alimentaria, como por ejemplo, complementos alimenticios, condimentos para sopas, alimentos probióticos (Onimawo et al., 2003; Onweluzo et al., 2009; Reque & Brandelli, 2021), formulación de alimentos infantiles (Olagunju et al., 2013), productos enriquecidos a base de cereales (Xing et al., 2020) o para la síntesis de enzimas o metabolitos de interés (Vidhyalakshmi et al., 2012; Lekshmi et al.,

2020). Por ello, en los últimos años ha crecido el interés por estudiar la influencia de la fermentación sobre las propiedades funcionales, sensoriales (Wu et al., 2020) y fisicoquímicas (Xu et al., 2019b) de los alimentos, así como, su beneficio para la salud humana (Guan et al., 2020).

La fermentación puede realizarse de forma sumergida (Submerged Fermentation, SmF) o en estado sólido (Solid State Fermentation, SSF). Esta última consiste en el crecimiento de microorganismos en materiales sólidos no sumergidos y con baja disponibilidad de agua libre (Šelo et al., 2021), presentándose como una oportunidad de valorización de residuos agroindustriales al poder emplearse éstos como sustrato para el crecimiento microbiano. Varios estudios refieren la utilización de residuos hortofrutícolas como sustrato, por ejemplo, subproductos del procesado de piña (Rashad et al., 2015), cáscara de granada deshidratada (Lekshmi et al., 2020), peladura de patata (Vidhyalakshmi et al., 2012), piel de manzana liofilizada (Gulsunoglu et al., 2020), piel y bagazo de naranja deshidratada (Bier et al., 2019) o bagazo de caqui (Seguí et al., 2022). Entre las mejoras del perfil nutricional asociadas a la fermentación de residuos vegetales cabe destacar el aumento en la cantidad de compuestos antioxidantes (Gulsunoglu et al., 2020), ya sea por la producción de nuevos compuestos con actividad antioxidante por parte de los microorganismos fermentativos (Bei et al., 2017), como por la transformación de unos compuestos antioxidantes en otros con mayor actividad (Bier et al., 2019).

Entre los microorganismos implicados en los procesos fermentativos destacan las bacterias ácido-lácticas (BAL) pertenecientes a los géneros *Enterococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus* y *Pediococcus* (Roobab et al., 2020). Son considerados los principales microorganismos probióticos y se destaca su importancia industrial en alimentos de consumo habitual como yogur, queso, cerveza, vino o embutidos, entre otros (Gao et al., 2021). Aunque la industria está desarrollando alimentos probióticos distintos a los lácteos, como zumos, cereales, snacks o suplementos (Granato et al., 2010; Rivera-Espinoza & Gallardo-Navarro, 2010; Aspri et al., 2020), mantener la viabilidad del microorganismo probiótico en un nuevo producto es siempre un reto, ya que muchos factores pueden causar pérdida de viabilidad. La temperatura de almacenamiento, la humedad, el pH o las

condiciones de proceso son factores que disminuyen el crecimiento de las bacterias probióticas. Según la OMS, los probióticos son "microorganismos vivos que, cuando se consumen en cantidades adecuadas (10^8-10^9 UFC/día), aportan beneficios para la salud del hospedador". Por lo tanto, para designar un producto como probiótico se requiere no solo que contenga el microorganismo en una concentración adecuada en el momento del consumo (> 10^6-10^7 UFC/g), sino también que la cepa sea segura y beneficiosa para la salud (Betoret et al., 2012; Vesterlund et al., 2012).

Tras la deshidratación es necesaria una **etapa final de molienda** para obtener el tamaño de partícula deseado, el cual tiene un efecto sobre las propiedades fisicoquímicas y tecnológicas y sobre las posteriores aplicaciones del polvo como ingrediente alimentario. Estudios previos reportan la alternancia entre deshidratación y molienda con el fin de aumentar la efectividad de ambos tratamientos y disminuir los tiempos de exposición al secado. Un tratamiento previo de desestructuración mediante triturado o troceado implicaría mayor superficie de muestra expuesta a las condiciones de deshidratación, eliminándose más fácilmente la humedad de la muestra y disminuyendo los tiempos de secado. Y una segunda etapa de molienda posterior a la deshidratación será más efectiva a menor contenido de humedad y mayor fragilidad de la muestra, obteniéndose un polvo de granulometría más fina (Djantou et al., 2007 & 2011). Por lo tanto, el tamaño de partícula es el resultado de la molienda previa y posterior al secado, así como de la tecnología y las condiciones de secado que se utilicen, ya que condicionan la respuesta de la matriz alimentaria al secado y la estructura del material deshidratado (Djantou et al., 2011; Plazzota et al., 2018). Una selección adecuada de los parámetros durante el procesado es necesaria para preservar la calidad de los polvos obtenidos y optimizar sus propiedades funcionales y tecnológicas.

El almacenamiento también puede tener un impacto significativo en las características físicas, químicas y biológicas de los alimentos, provocando una reducción de la calidad del producto. Es por ello que durante su almacenamiento los productos deshidratados en polvo requieren protección frente al oxígeno, la humedad, las altas temperaturas o la luz, factores que pueden conllevar a la pérdida de aromas, color o capacidad antioxidante,

entre otros (Bernaert et al., 2013; Henríquez et al., 2013). Por lo tanto, determinar la estabilidad de los polvos durante el almacenamiento es crucial, ya que, sus propiedades pueden verse afectadas.

2.3. Respuesta de los productos en polvo al proceso de digestión simulada *in vitro*

Los objetivos de la nutrición son proporcionar nutrientes en la cantidad y calidad necesarias para satisfacer las necesidades del organismo. Una nutrición óptima debe incluir compuestos bioactivos como fibra, antioxidantes y fitoquímicos, entre otros, ya que estos tienen la capacidad de mejorar la salud, generar bienestar y son útiles para prevenir el riesgo de padecer enfermedades (Cilla et al., 2018). El potencial de estos compuestos fisiológicamente activos para ejercer efectos beneficiosos para la salud depende de varios factores, entre los que se incluyen su liberación de la matriz tras ser ingeridos, los cambios experimentados durante la digestión, la absorción, el metabolismo y la biodistribución, así como la dosis ingerida y la predisposición del hospedador (Bohn et al., 2015).

No existe una definición universalmente aceptada respecto al término biodisponibilidad. Desde el punto de vista nutricional, se define como la fracción del componente ingerido que está disponible para su uso en las funciones fisiológicas normales. Este concepto incluye dos términos adicionales, que son la bioaccesibilidad y la bioactividad. La bioaccesibilidad se define como la fracción de un compuesto que se libera de su matriz alimentaria en el tracto gastrointestinal y, por tanto, está disponible para la absorción intestinal. La bioactividad incluye los acontecimientos relacionados con la forma en que el compuesto bioactivo llega a la circulación sistémica y es transportado al tejido de destino, así como a la interacción con el metabolismo y toda la cascada de efectos fisiológicos que genera (Rodriguez-Mateos et al., 2014; Álvarez-Olguín et al., 2022).

Para que los compuestos bioactivos puedan ejercer su bioactividad, deben ser biodisponibles y mantenerse estables durante todo el proceso de digestión hasta ser absorbidos y distribuidos por el resto del organismo (Camargo et al., 2021). Una vez que los alimentos son ingeridos, sufren una

serie de transformaciones mecánicas y enzimáticas que conllevan su descomposición en sustancias más fácilmente asimilables por el organismo. La transformación mecánica ocurre principalmente en la boca y el estómago donde, mediante la masticación y los movimientos peristálticos, se reduce el tamaño de partícula del alimento. La transformación enzimática tiene lugar en mayor medida en el estómago y en los intestinos, promoviendo la hidrólisis de macromoléculas en componentes de menor tamaño (Guerra et al., 2012).

El diseño de un proceso para obtener ingredientes funcionales debe tener como objetivo preservar y, si es posible, mejorar las propiedades del material procesado, además de garantizar la funcionalidad de sus compuestos bioactivos, evaluando el impacto del proceso de digestión sobre la estabilidad y función de los mismos y sobre su bioaccesibilidad (Carbonell-Capella et al., 2014; Galanakis, 2021).

La mejor manera de determinar los beneficios derivados de la ingesta de alimentos, su biodisponibilidad y su bioaccesibilidad, es someter el producto a una digestión gastrointestinal *in vivo* (Martínez-Las Heras et al., 2017). No obstante, los métodos *in vivo* presentan una serie de limitaciones (económicas, éticas, complejidad y baja reproducibilidad) que hacen que se recurra a modelos *in vitro*. Estos simulan las condiciones fisiológicas y fisicoquímicas de las distintas etapas de la digestión *in vivo*, permitiendo un mayor control de las variables experimentales y facilitando la estandarización; siendo, además, métodos seguros, rápidos y que no presentan restricciones éticas (Minekus et al., 2014; Alegría et al., 2015). Generalmente, los métodos de digestión *in vitro* incluyen las fases oral, gástrica e intestino delgado y, en ocasiones, la fermentación en el colon (Minekus et al., 2014). Estos modelos pueden ser estáticos o dinámicos (figura 4). En los modelos estáticos, los productos de la digestión permanecen generalmente inmóviles y no imitan procesos físicos (corte, mezclado, hidratación, etc.). A pesar de ser menos reproducibles, son más utilizados por su sencillez y bajo coste. Los modelos dinámicos son más precisos, ya que tratan de incluir procesos físicos y mecánicos y cambios en las condiciones del lumen intestinal a lo largo del tiempo (Minekus et al., 2014). Los métodos *in vitro* pueden simular solo el proceso de digestión

(bioaccesibilidad) o los procesos de digestión y absorción (biodisponibilidad). Comúnmente, la absorción final se evalúa mediante el cultivo celular Caco-2 (Etcheverry et al., 2012; Álvarez-Olguín et al., 2022). Para estudiar la fermentación del intestino grueso se emplean modelos de colon *in vitro* que utilizan microbiota fecal humana como inóculo (Bazzocco et al., 2008).

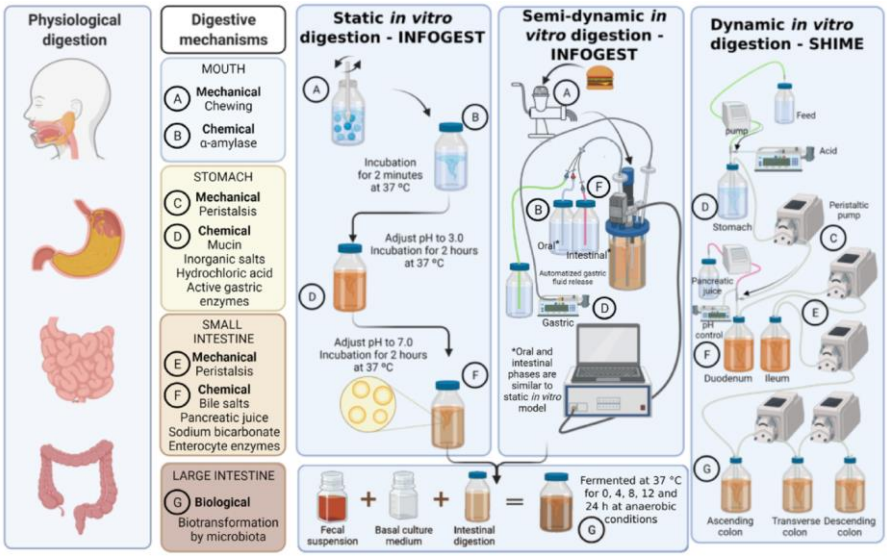

Figura 4. Modelos de digestión simulada *in vitro*. FUENTE: Brobowski Rodrigues et al. (2022).

Mediante los métodos de simulación *in vitro* es posible estudiar el efecto de las condiciones de digestión sobre la estructura y composición del alimento, además de los efectos provocados por los diferentes tratamientos de procesado o por la interacción entre diferentes componentes alimentarios (fibra, polifenoles, grasas, etc.) (Guerra et al., 2012; Minekus et al., 2014). Esta información resulta de gran importancia en el diseño y la formulación de alimentos funcionales (Lucas-González et al., 2018). Para garantizar la funcionalidad de los alimentos o ingredientes en polvo fabricados a partir de residuos de frutas y hortalizas es necesario estudiar el efecto de la digestión sobre los compuestos de interés, dilucidando así la relación entre la composición del producto y su estructura, su procesado y digestión. Tal y como se ha mencionado previamente, el procesado determina los compuestos bioactivos disponibles, así como la estructura del

polvo, que a su vez determina la bioaccesibilidad de los compuestos bioactivos (Gouw et al., 2017; Lucas-González et al., 2018; Bas-Bellver et al., 2020). Asimismo, en el proceso de digestión del ingrediente en polvo, solo o como parte de un alimento formulado, es importante evaluar interacciones entre ingredientes y/o entre un ingrediente y la matriz alimentaria de la que forma parte, es decir, entre compuestos bioactivos y otros componentes presentes en el producto completo (Etcheverry et al., 2012; Bas-Bellver et al., 2020). Según se reporta en la bibliografía revisada, los polvos de frutas y hortalizas se pueden digerir directamente en los fluidos gastrointestinales simulados (Gouw et al., 2017; Bas-Bellver et al., 2020), diluidos en agua o grasas (Gullon et al., 2015; Zhang et al., 2018) o como parte de un alimento formulado (modelo) (Sarvan et al., 2017).

2.4. Aplicación de los productos en polvo en la industria alimentaria y otras aplicaciones

La valorización integral de los residuos hortofrutícolas como ingredientes en polvo, se presenta como una alternativa interesante y de variada aplicación en la industria alimentaria. La bibliografía recoge ejemplos en los que ese han propuesto como conservantes y aditivos alimentarios (colorantes, saborizantes, etc.), habiéndose incorporado a productos cárnicos (Neacsu et al. 2015; Mokhtar et al., 2018; Lucas-González, et al. 2019; Nieto et al., 2021), productos de panadería y repostería (Olawuyi et al., 2019; Kirbas et al., 2019; Bianchi et al., 2021), a zumos y bebidas (Sagar et al., 2018), o a alimentos preparados como sopas instantáneas (Karam et al., 2016) o fórmulas infantiles (Mokhtar et al., 2018). En estas industrias se han propuesto no sólo como aditivos, sino también como nuevos ingredientes alimentarios, con la finalidad de enriquecer nutricionalmente los productos o mejorar sus propiedades tecnológicas (Elleuch et al., 2011; Ferreira et al., 2015; Plazzota et al., 2018).

Por lo que respecta a la industria panadera, el enriquecimiento de sus productos con ingredientes funcionales es una práctica cada vez más habitual, dada la creciente demanda del consumidor por este tipo de productos. Los productos horneados tales como panes, rosquilletas, galletas

o bizcochos, son una fuente importante de calorías, pero son alimentos pobres desde el punto de vista nutricional (Ranasinghe et al., 2022). Por lo tanto, la adición de ingredientes funcionales a las masas permite mejorar las características nutricionales de estos productos tan habitualmente consumidos como snack o tentempié, lo que los convierte en productos de consumo habitual. Diversos estudios han empleado frutas, vegetales y sus subproductos para la elaboración de productos horneados mejorados, en los que se han constatado una mejora del valor nutricional de los mismos (Olawuyi et al., 2019; Kirbas et al., 2019; Bianchi et al., 2021). La adición de harinas de origen vegetal contribuye a disminuir el índice glucémico de los productos horneados, fundamentalmente por su mayor contenido en fibra, pero también contribuye a reducir el riesgo de otras enfermedades no trasmisibles tales como el cáncer, los desórdenes gastrointestinales o enfermedades coronarias (Ranasinghe et al., 2022). Por otro lado, la presencia de gluten en las harinas de trigo causa reacciones de alergia e intolerancia en pacientes celíacos o sensibles al gluten. Estos individuos deben consumir productos libres de gluten a lo largo de su vida para prevenir estos síntomas y alteraciones (Kirbas et al., 2019; Olawuyi & Lee, 2019). En este contexto, existe una necesidad de llevar al mercado productos sin gluten que permitan que estas personas puedan consumir productos de panadería sin cambiar su patrón dietético (Gularte et al., 2012). Las harinas de origen vegetal constituyen un complemento a otras harinas sin gluten empleadas habitualmente en la formulación de estos alimentos, como es el caso de la harina de arroz.

Por su parte, en la industria agrícola, la preocupación por la inocuidad de los alimentos y su sostenibilidad ha llevado a los productores a explorar nuevos métodos ecológicos para reemplazar o complementar las actuales prácticas basadas en el empleo de plaguicidas químicos. Así pues, el uso de compuestos naturales como son los fitoquímicos ha suscitado interés como bioplaguicidas (Walia et al., 2017). Se trata de metabolitos producidos por las plantas en respuesta al estrés o al daño celular, y que forman parte de sus sistemas de defensa frente a agentes patógenos (Cluzet et al., 2020). En particular, las brassicas se caracterizan por ser ricas en glucosinolatos e isotiocianatos, compuestos bioactivos que además de sus beneficios para la

salud, también presentan propiedades antimicrobianas y antifúngicas, lo que ha impulsado el estudio de su aplicación frente al control de plagas (hongos, insectos, plantas arvenses…) (Borges et al., 2014; Popova et al., 2017). De este modo, los polvos procedentes de residuos de brassicas tales como la col o el brócoli, podrían plantearse como alternativa potencial para combatir plagas y malas hierbas y prevenir enfermedades en los cultivos (Yu et al., 2019; Liu et al., 2022), con el fin de evitar, o al menos reducir, el uso de plaguicidas sintéticos. En este sentido, a lo largo del proyecto FUNBIOPEST (AGCOOP_A/2021/020 "Obtención de productos en polvo a partir de las líneas de confección de col blanca para su uso como ingrediente funcional sostenible y para la gestión integrada de plantas arvenses") se colaboró con el Instituto Agroforestal Mediterráneo de la Universitat Politècnica de València (IAM-UPV) para evaluar el potencial efecto de los polvos sobre la inhibición del desarrollo de plantas arvenses. Polvos obtenidos a partir de residuo de col blanca y brócoli, seleccionados por su contenido en isotiocianatos, fueron aplicados en invernadero y campo mediante diferentes métodos de incorporación (mezclado con suelo o *mulching*), obteniéndose resultados prometedores como recurso natural y sostenible para el control integrado de plantas arvenses. Aunque los resultados de esta parte de la investigación no constituyeron parte de la presente tesis doctoral, sí se participó en el desarrollo del proceso de fabricación de polvos ricos en isotiocianatos, y la selección de los polvos con mejores propiedades para esta finalidad.

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3. OBJETIVOS Y PLAN DE TRABAJO

3.1. Objetivos

El **objetivo general** de la presente tesis consistió en desarrollar un proceso tecnológico de valorización integral de residuos de hortalizas procedentes de las líneas de confección en cooperativa, para la obtención de productos en polvo que puedieran ser reintroducidos en la cadena alimentaria como ingredientes funcionales, con especial atención al impacto del procesado, almacenamiento y digestión simulada *in vitro* sobre las propiedades de los productos en polvo obtenidos.

Para la consecución del objetivo general, se definieron los siguientes objetivos específicos (OE):

OE1. Llevar a cabo una revisión bibliográfica exhaustiva del estado del arte que permitiese identificar, en función de su viabilidad y de su impacto sobre las propiedades de los productos obtenidos, las operaciones unitarias y las condiciones de proceso más adecuadas para transformar el material vegetal.

OE2. Estudiar el efecto de diversos pretratamientos tales como la congelación, desestructuración y fermentación, sobre las cinéticas de secado por aire caliente de residuos de apio, ajo puerro, zanahoria, col y brócoli, a diferentes temperaturas.

OE3. Estudiar el efecto de los pretratamientos (disrupción, congelación, fermentación) y de la operación de deshidratación (secado por aire caliente, liofilización), sobre las propiedades fisicoquímicas, funcionales y tecnológicas de los productos en polvo, así como su estabilidad durante el almacenamiento.

OE4. Evaluar la respuesta de los productos en polvo frente a la digestión simulada *in vitro* en cuanto a la liberación de antioxidantes y otros compuestos bioactivos (carotenoides, isotiocianatos), en función de las condiciones de procesado y, en el caso de los carotenoides, de la composición del medio en el que se dispersan los polvos.

OE5. Emplear los ingredientes en polvo obtenidos a partir de residuos de col blanca y zanahoria en la formulación de nuevos productos de panadería sin gluten (magdalenas y rosquilletas), y evaluar el impacto del porcentaje de reemplazo de la harina de arroz sobre las propiedades de los productos obtenidos.

3.2. Plan de trabajo

Para la consecución de los objetivos anteriormente planteados, se proponee el siguiente plan de trabajo:

Para la consecución del OE1:

1. **Revisión bibliográfica acerca del impacto del procesado sobre las características de los productos vegetales en polvo.**

Para la consecución de los OE2 y OE3:

2. **Estudio del efecto de los pretratamientos y de las condiciones de secado sobre las propiedades de los productos en polvo obtenidos a partir de residuos de confección de hortalizas (col, apio, ajo puerro, zanahoria y brócoli).**
    2.1. Estudio del efecto de los **pretratamientos congelación y disrupción previa** (congelado *vs*. fresco, triturado *vs.* troceado), sobre las cinéticas de secado por aire caliente (SAC) y las **características fisicoquímicas y antioxidantes** de los productos en polvo obtenidos mediante SAC y liofilización (LIO), a partir de apio, col, ajo puerro (partes blanca y verde) y zanahoria (Figura 5).
        2.1.1. Recepción de la materia prima (residuos de col, apio, ajo puerro, zanahoria) desde Agrícola Villena Coop. V.
        2.1.2. Desestructuración de los residuos en procesador de alimentos hasta obtener trozos < 10 mm (troceado) o < 5 mm (triturado), los cuales serán procesados en fresco o congelados a –22 °C para procesarse después de haber sido descongelados.
        2.1.3. Secado por aire caliente a 70 °C o liofilización hasta reducir la a_w por debajo de 0,3. Estudio de las cinéticas de secado: curvas de secado (humedad vs. tiempo) y de velocidad de secado (velocidad de secado vs. humedad).
        2.1.4. Caracterización de los productos frescos y en polvo: a_w, x_w, x_{ss}, tamaño de partícula, fenoles totales, flavonoides totales, y actividad antioxidante (métodos DPPH y ABTS).

2.2. Estudio del **impacto del procesado** (disrupción previa y SAC a 60 y 70 °C, y LIO) sobre las cinéticas del secado por aire caliente y las **características fisicoquímicas, antioxidantes y tecnológicas** de los productos en polvo obtenidos a partir de residuos de apio, col, ajo puerro y zanahoria, y de su **estabilidad a lo largo de 4 meses de almacenamiento** (Figura 6).

2.2.1. Recepción de la materia prima (residuos de col, apio, ajo puerro, zanahoria) desde Agrícola Villena Coop. V.

2.2.2. Desestructuración del tejido (troceado vs. triturado) y deshidratación (SAC 60 y 70 °C, LIO). Estudio de las cinéticas de SAC a ambas temperaturas de secado.

2.2.3. Determinación de las propiedades a_w, x_w, x_{ss}, tamaño de partícula, color CIEL*a*b*, y propiedades antioxidantes (fenoles y flavonoides totales, actividad antioxidante DPPH y ABTS) y propiedades de interacción con el agua y con el aceite.

2.2.4. Estudio de la evolución de las propiedades a_w, x_w, x_{ss}, color CIEL*a*b* y propiedades antioxidantes (fenoles y flavonoides totales, actividad antioxidante DPPH y ABTS), a lo largo de 4 meses de almacenamiento en ausencia de luz y a temperatura ambiente.

2.3. Estudio de la **fermentación en estado sólido** de tallos de brócoli como pretratamiento a la deshidratación (SAC a 60 y 70 °C, y liofilización) y posterior molienda, y su **efecto sobre las propiedades fisicoquímicas y antioxidantes** de los productos en polvo y su **potencial efecto probiótico** (Figura 7).

2.3.1. Preparación de la materia prima (separación de tallos de brócoli).

2.3.2. Ensayos preliminares de fermentación. Disrupción previa (triturado vs. troceado) y fermentación con *Lactobacillus plantarum* spp. CECT 749. Estudio del crecimiento microbiano y propiedades antioxidantes del residuo fermentado.

2.3.3. Deshidratación (SAC a 60 o 70 °C o liofilización). Estudio de las cinéticas de secado por aire caliente y caracterización de los productos en polvo fermentados y sin fermentar (propiedades fisicoquímicas, antioxidantes y recuento microbiano).

Para la consecución del OE4:

3. Estudio del impacto del procesado sobre los compuestos bioactivos de interés y su bioaccesibilidad a lo largo de la digestión simulada *in vitro*, en función de las características fisicoquímicas de los productos en polvo obtenidos.

3.1. Efecto del procesado sobre las propiedades fisicoquímicas, funcionales y el **contenido en carotenoides** de los polvos de residuos de zanahoria, así como su influencia sobre la **respuesta a la digestión gastrointestinal simulada *in vitro*** (Figura 8).

3.1.1. Recepción de residuos de zanahoria procedentes de la línea de producción de palitos de zanahoria de IV gama para su procesado en fresco.

3.1.2. Transformación de los residuos en productos en polvo según los procesos definidos: disrupción (troceado vs. triturado), secado (SAC 60, 70 °C o liofilización) y molienda fina.

3.1.3. Estudio del impacto del procesado sobre las propiedades antioxidantes (fenoles y flavonoides totales, capacidad antioxidante DPPH y ABTS), así como del contenido en carotenoides (α-caroteno, β-caroteno, luteína, licopeno) de los productos en polvo.

3.1.4. Evaluación del efecto del procesado y de las características fisicoquímicas del polvo sobre las propiedades antioxidantes y el contenido en carotenoides durante la digestión simulada *in vitro*.

3.1.5. Evaluación de la dispersión de los polvos en agua, aceite o emulsión de aceite en agua sobre la liberación de carotenoides durante la digestión simulada *in vitro*.

3.2. Efecto del procesado sobre las propiedades fisicoquímicas, incluidas antioxidantes, y tecnológicas de productos en polvo obtenidos a partir de residuos de brassicas, con especial atención al **contenido en sulforafano**, y evaluación de la liberación de antioxidantes a lo largo de la **digestión simulada *in vitro*** en función del procesado previo (Figura 9).

3.2.1. Recepción de los residuos de col y separación de los tallos de brócoli.

3.2.2. Transformación de los residuos en productos en polvo según los procesos definidos: disrupción (troceado vs. triturado), secado (SAC 50, 60, 70 °C o liofilización) y molienda fina.

3.2.3. Estudio del impacto del procesado sobre las propiedades antioxidantes (fenoles y flavonoides totales, capacidad antioxidante DPPH y ABTS), así como del contenido en sulforafano.

3.2.4. Evaluación del efecto del procesado y las características fisicoquímicas del polvo sobre la liberación de antioxidantes durante la digestión simulada *in vitro*.

Para la consecución del OE5:

4. Aplicación de los productos en polvo obtenidos a partir de residuos de zanahoria y col como ingredientes funcionales en la formulación de alimentos horneados (Figura 10).

4.1. Preparar mezclas de harinas sustituyendo parcialmente harina de arroz por los ingredientes en polvo (zanahoria o col blanca), obtenidos mediante secado por aire caliente a 70 °C, en diferentes proporciones (5, 10, 20 y 30%).

4.2. En las mezclas de harinas, determinar la humedad, propiedades tecnológicas de interacción con agua y aceite y de *pasting*, y propiedades antioxidantes (fenoles totales, azúcares reductores y capacidad antioxidante por el método DPPH).

4.3. Elaboración de diferentes formulaciones de magdalenas y rosquilletas utilizando las combinaciones anteriores de harina de arroz con polvo de zanahoria o polvo de col, respectivamente.

4.4. Caracterización del comportamiento viscoelástico de las masas crudas de las diferentes formulaciones de magdalenas y rosquilletas, mediante un ensayo dinámico oscilatorio.

4.5. Evaluación de la calidad de las magdalenas y rosquilletas, tras su horneado, mediante su caracterización fisicoquímica (actividad del agua, análisis de color (CIEL*a*b*) y textura) y funcional (propiedades antioxidantes: DPPH, contenido en fenoles totales y contenido en azúcares reductores).

4.6. Estudio de la estabilidad de las propiedades fisicoquímicas y antioxidantes tras 7 días de almacenamiento en bolsas herméticas y en condiciones de refrigeración (4 °C).

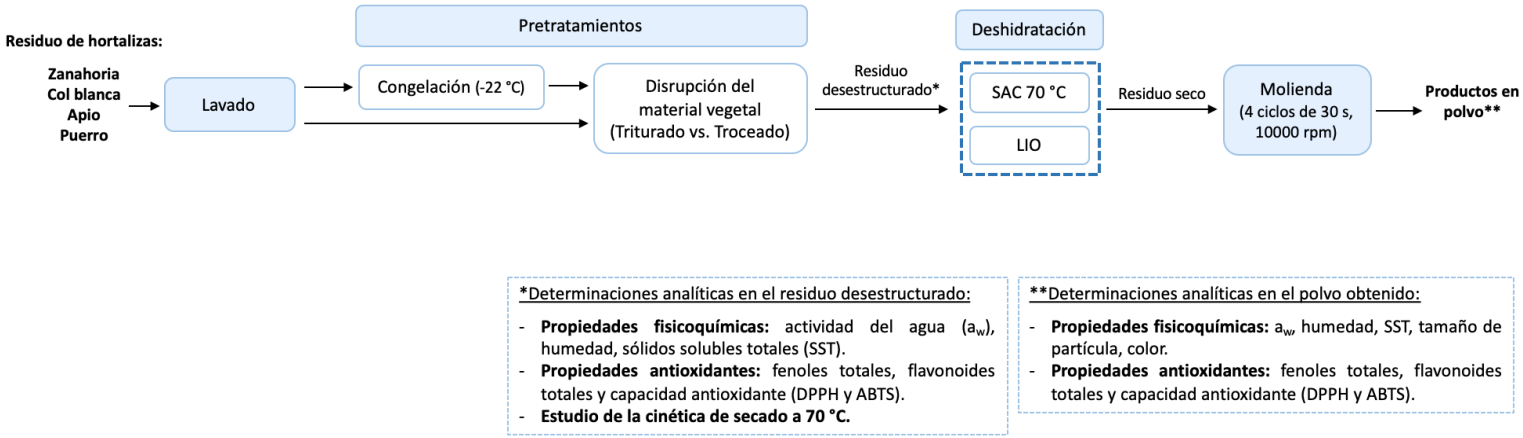

Figura 5. Diagrama de flujo del proceso de obtención de polvos a partir de residuos de zanahoria, col blanca, apio y puerro. Evaluación del efecto de los pretratamientos congelación y disrupción del material vegetal sobre las cinéticas de secado por aire caliente (SAC) y las características fisicoquímicas y antioxidantes de los productos en polvo obtenidos mediante SAC y liofilización (LIO).

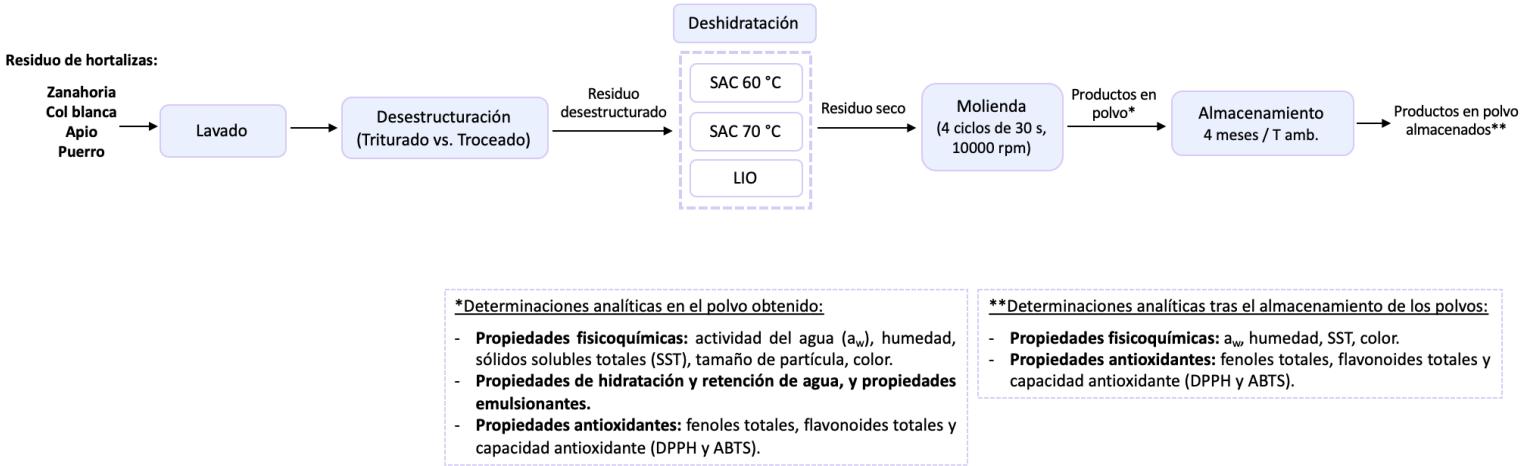

Figura 6. Diagrama de flujo del proceso de obtención de polvos a partir de residuos de zanahoria, col blanca, apio y puerro. Evaluación del efecto de la disrupción previa del material vegetal y del método de deshidratación empleado, es decir, secado por aire caliente (SAC) a 60 o 70 °C o liofilización (LIO), sobre las propiedades fisicoquímicas, antioxidantes y tecnológicas de los productos en polvo obtenidos. Y estudio de su estabilidad a lo largo de 4 meses de almacenamiento.

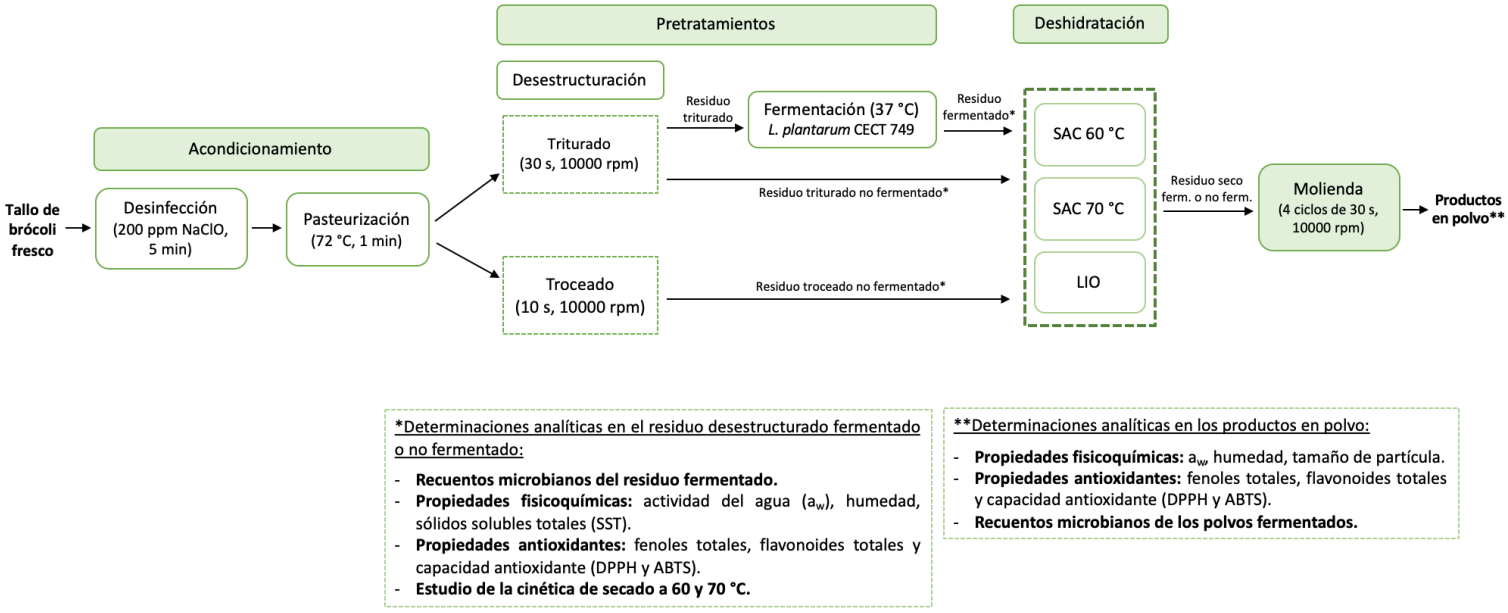

Figura 7. Diagrama de flujo del proceso de obtención de polvos a partir de tallos de brócoli con o sin fermentación con *Lactobacillus plantarum*. Evaluación del efecto de la fermentación como pretratamiento a la deshidratación (secado por aire caliente (SAC) a 60 y 70 °C, y liofilización (LIO)) sobre las cinéticas de secado y sobre las propiedades fisicoquímicas y antioxidantes de los productos en polvo y su potencial efecto probiótico.

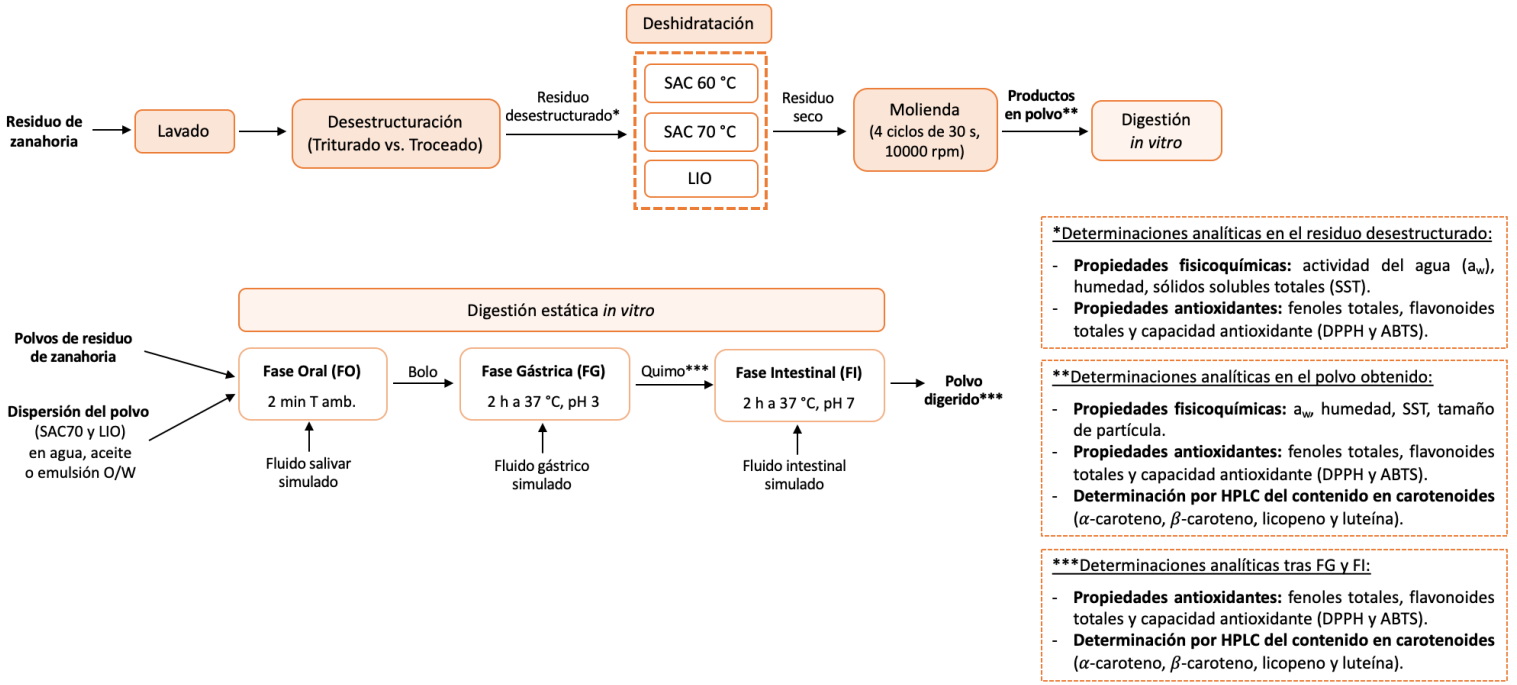

Figura 8. Diagrama de flujo del proceso de obtención de productos en polvo a partir de residuos de zanahoria. Y evaluación del efecto del procesado sobre las propiedades fisicoquímicas, funcionales y el contenido en carotenoides de los polvos obtenidos; y su influencia sobre la respuesta a la digestión gastrointestinal simulada *in vitro.* SAC: secado por aire caliente; LIO: liofilización.

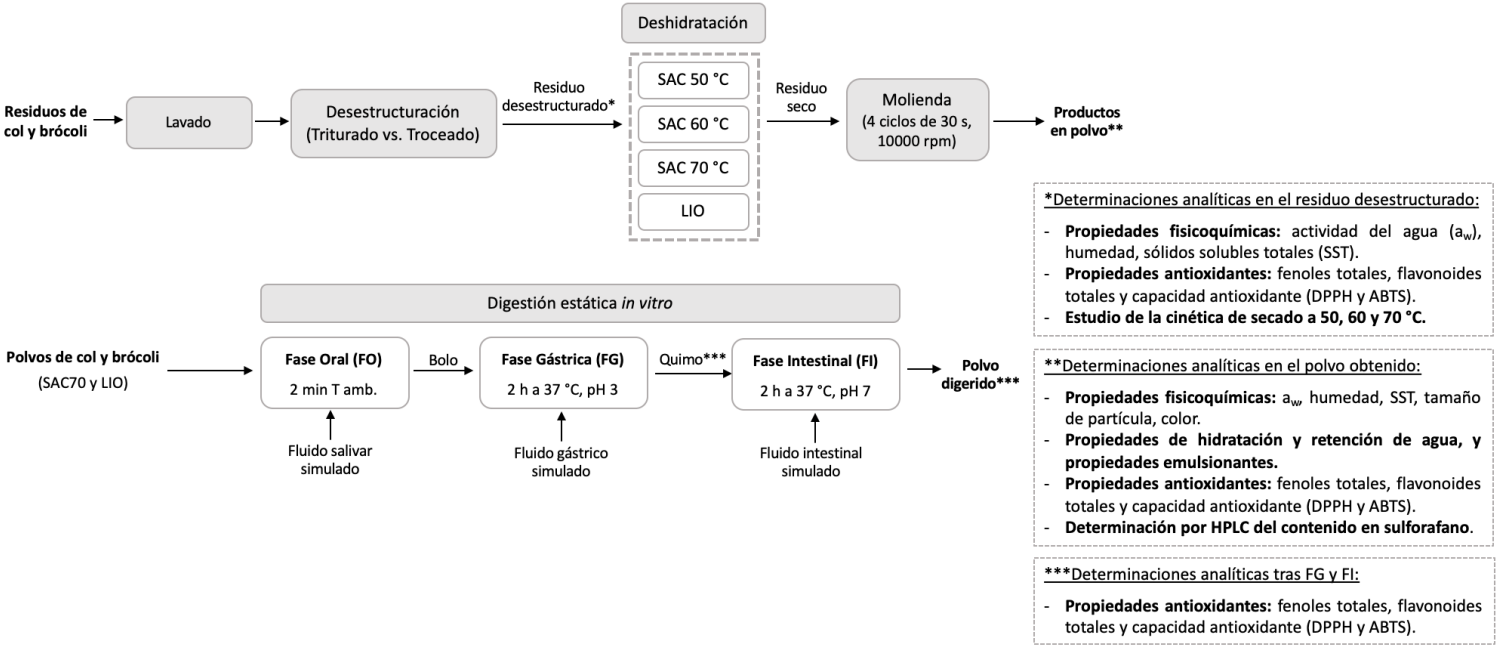

Figura 9. Diagrama de flujo del proceso de obtención de productos en polvo a partir de residuos de col blanca y tallos de brócoli. Evaluación del efecto del procesado sobre las propiedades fisicoquímicas, antioxidantes y tecnológicas de los productos en polvo obtenidos, con especial atención al contenido en sulforafano. Y estudio del efecto de la digestión gastrointestinal simulada *in vitro* sobre la liberación de antioxidantes en polvos seleccionados. SAC: secado por aire caliente; LIO: liofilización.

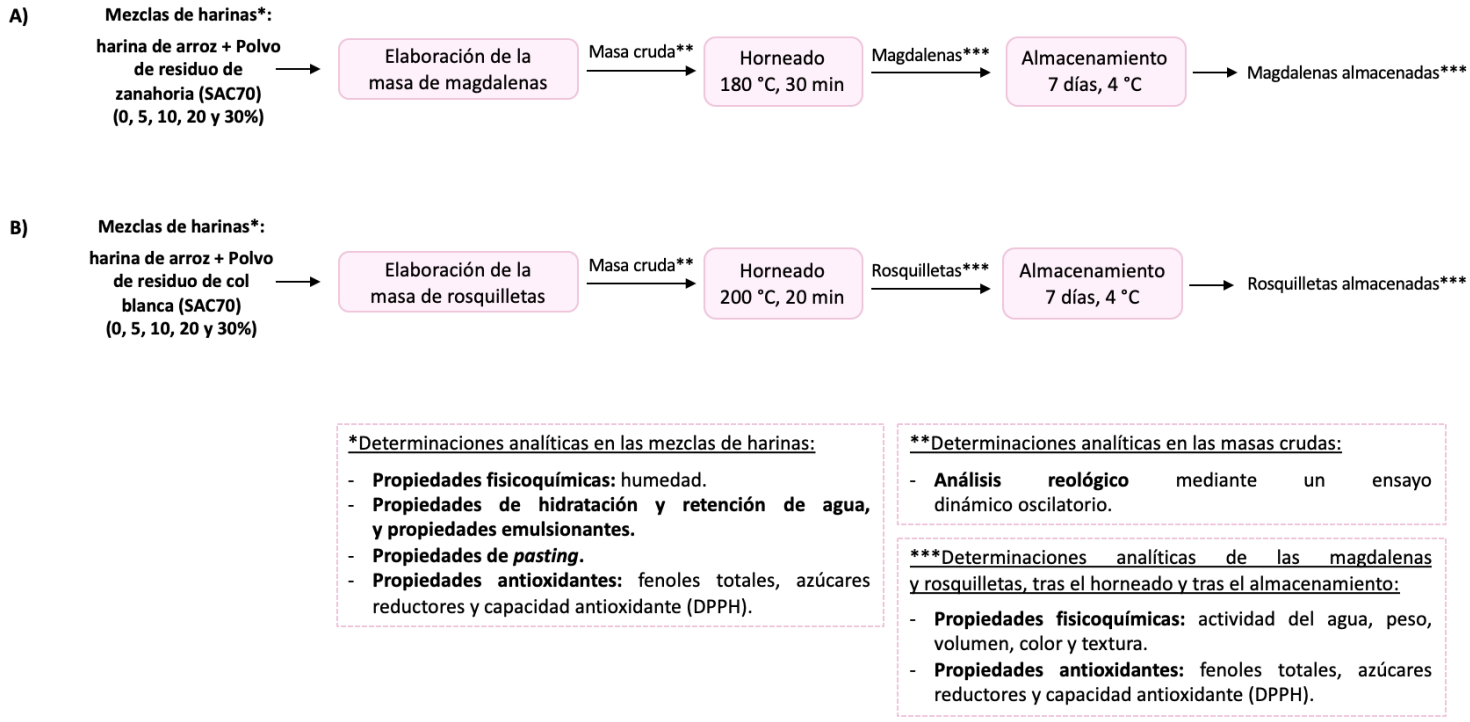

Figura 10. Diagrama de flujo seguido para la aplicación de los productos en polvo obtenidos a partir de residuos de zanahoria (A) y col (B) como ingredientes funcionales en la formulación de magdalenas y rosquilletas, respectivamente.

4. RESULTADOS

CAPÍTULO 1. Antecedentes del impacto del procesado sobre las características de los productos vegetales en polvo

Ramírez-Pulido, B., Bas-Bellver, C., Betoret, N., Barrera C., Seguí, L. Valorization of Vegetable Fresh-Processing Residues as Functional Powdered Ingredients. A Review on the Potential Impact of Pretreatments and Drying Methods on Bioactive Compounds and Their Bioaccessibility. *Frontiers in Sustainable Food Systems* 2021, 5.

RESUMEN DEL CAPÍTULO

Una gran cantidad de residuos son generados por la industria de transformación de frutas y hortalizas, con el consiguiente impacto sobre el medio ambiente. La eliminación de estos residuos mediante incineración o su almacenamiento en vertederos no son una opción sostenible, por lo que la industria debe dirigir sus esfuerzos tanto a reducir su generación como a reintroducirlos en la cadena agroalimentaria. Los subproductos hortofrutícolas son considerados una fuente rica en compuestos bioactivos con potencial para ser valorizados, pudiendo ser reintroducidos en la cadena de producción, contribuyendo de este modo a la circularidad y a un sistema de producción más sostenible.

En este primer capítulo se presenta una revisión sobre las tecnologías disponibles para la transformación de estos residuos en productos deshidratados en polvo, centrándose en el efecto de las técnicas de pretratamiento y de secado sobre las propiedades de los productos, sus características fisicoquímicas y funcionales, así como sobre la biodisponibilidad de los compuestos bioactivos que contienen. Con ello, se pretende identificar las tecnologías de procesado que resulten más adecuadas para la valorización de los residuos por medio de un proceso que permita su aprovechamiento integral y que sea viable en el contexto en el que se desarrollan los proyectos en colaboración con la Cooperativa Agrícola Villena Coop.V.

Valorization of Vegetable Fresh-Processing Residues as Functional Powdered Ingredients. A Review on the Potential Impact of Pretreatments and Drying Methods on Bioactive Compounds and Their Bioaccessibility

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Abstract

Food waste is a worldwide concern as it represents a constant threat to the environment and a serious operational problem for the food industry. The by-products of fruits and vegetables being a valuable source of bioactive compounds have the potential to be reused and reintroduced in the agri-food chain. This circular approach contributes to a sustainable production system. In this context, a collaborative project with the primary sector for the integral valorization of the waste generated in the fresh-processing vegetable lines of an agricultural cooperative is currently being developed, particularly focused on cabbage, carrot, celery, and leek. The objective of this project is to transform vegetable wastes into functional powdered ingredients and be able to use them in food formulations in order to improve the nutritional profile of foods, contributing to the development of sustainable healthy diets. Through an exhaustive bibliographic review, this research studies the influence of pretreatments, drying and *in vitro* digestion on the bioactive compounds of vegetable residues, with the aim of identifying the appropriate production parameters to achieve an adequate functional and physicochemical profile of the final powders.

Keywords: vegetables residues, bio-waste valorization, functional ingredients, pretreatments, drying, bioaccessibility, *in vitro* digestion.

1. Introduction

Food loss and food waste (FLW) impacts negatively on society, the environment and the economy. It represents a threat to the environment and a serious operational problem for the production plants (Goula and Lazarides, 2015). Environmental issues related to FLW are a matter of increasing concern since food production is resource-intensive and the growing population and incomes is expected to increase the demand of agricultural products of 50% by 2050 if there is no change in dietary habits and reduction of food waste (Food2030 Pathways for Action). FLW also participates from a societal paradoxin a world in which food insecurity and malnutrition continues to increase, while a substantial amount of food that could have been consumed is lost or ends up as residues. Therefore, reducing FLW in the next decade is a priority, which is expected to have an impact on all of the four Food 2030 priorities: nutrition, climate, circularity, and communities.

The Food and Agriculture Organization of the United Nations (FAO, 2019) has been working toward the harmonization of the concept of Food loss and Food waste, whereas EU legislation refers to food waste (FW) as the concept which covers all stages from farm to fork (except for the crops plowed in/not harvested) (Food2030 Pathways for Action). FW is generated throughout the whole supply chain (Mirabella et al., 2014). Primary production (25%) and processing (24%) accounts for about 50% of the total losses, fruit and vegetables being the products which participate most from this percentages (Food2030 Pathways for Action). In the first processing stages, these losses are mainly due to discards produced as a consequence of high commercial standards, as well as to edible parts which are eliminated by peeling or cutting in the fresh-processing lines (outer leaves, stems, damaged parts). Surpluses and spoilage may also contribute to these numbers. Although most of these wastes have the potential to be reused and possess high biological and nutritional value, they are usually underused and considered as low-value material. According to the Food and Agriculture Organization (FAO) of the United Nations, approximately a third of the edible parts of food produced for human consumption are lost or wasted globally. It has been estimated that the food currently wasted in Europe could feed

200 million people. In 2016 it was estimated that FWL in the EU was around 88 million tons representing an associated cost of 143 billion euros (Stenmarck et al., 2016). Furthermore, the respective consumption of resources and the emission of pollutants implies an increase in the environmental burden.

It is necessary to focus production systems in terms of minimizing wastes, and reintroducing them into the production chain. In this sense, concepts such as “circular economy,” “industrial ecology,” and “zero waste economy,” among others, have arisen to orient eco-innovation toward the use of waste as raw material for the development of new products and applications (Mirabella et al., 2014). The reduction of food waste (FW) has an enormous potential for lowering the resources used to produce it, saving money and decreasing the environmental impact (Stenmarck et al., 2016). This also represents an important contribution to the Circular Economy Action Plan adopted by the European Commission within the European Green Deal, which promotes sustainable initiatives along the entire life cycle of products. A circular approach benefits sustainable food production (European Commission, 2019).

As defined by the Food and Agriculture Organization of the United Nations (FAO, 2019), a sustainable food system must be able to ensure sustainable consumption and production patterns, as well as deliver safe, healthy, and nutritious diets. Accordingly, the food industry must incorporate processes and products which provide both a lower environmental impact and an increased positive impact on diets and health. Reformulation of processed foods provides a genuine opportunity to improve people's health by modifying the nutritional characteristics of commonly consumed processed foods (Bas-Bellver et al., 2020b), therefore addressing the FAO sustainable development goal “Ensure healthy lives and promote well-being for all at all ages.” This is in accordance with the definition of a sustainable healthy diet, which states that food consumption should be adequate in energy and nutrients for growth and development, meeting the needs of an active and healthy life. This concept states that dietary patterns should promote all dimensions of individuals' health and

well-being, while having low environmental impact and reducing food loss (FAO and WHO, 2019).

Increased awareness of the association between improper diet and health disorders has led to the development of a healthier food industry. Recommendations based on scientific evidence include increasing the consumption of plant-based foods, basically fruits and vegetables, as they are excellent sources of fiber and other bioactive compounds (Rao and Rao, 2007). These are perishable commodities which deteriorate in a short period of time if not handled properly, for which dehydration is a suitable method to extend their shelf life. Dehydration and milling can be combined to obtain fruit and vegetable powders. The current food industry shows a growing interest in the application of dried fruits and vegetables in powder form (Karam et al., 2016). In 2006, Zhang et al. (2006) already mentioned the growing increase of this market. Applications of fruit and vegetable powders include their use as ingredients in the confectionery, bakery, sweet, and distilling industries to improve the nutritional value of foodstuff (Camire et al., 2007), and include other applications such as dressings, soups or ready-to-eat products. Applications of fruit and vegetable powders is not limited to the use of the whole food, but the waste generated in the transformation industry is also a valuable source of bioactive compounds such as fiber, antioxidants and other phytochemicals (Tseng and Zhao, 2013) that can be transformed into powdered ingredients. Fruit and vegetables waste powders can also be proposed as new food ingredients, used to fortify food products or improve their technological properties (Elleuch et al., 2011; Ferreira et al., 2015; Plazzota et al., 2018; Bas-Bellver et al., 2020b). In this way, FW can be considered a source of valuable components at a low cost.

In this context, an eco-innovation project in collaboration with an agricultural cooperative (producers and fresh-processing industry) is currently being developed (Bas-Bellver et al., 2020b). The project aims to recover and reuse the waste generated in the vegetables fresh-processing lines (cabbage, carrot, celery and leek wastes) to obtain powdered functional ingredients to be used in the food industry. The annual production of these vegetable residues as estimated by the cooperative is ~180 tons in the case of leeks (some of the white part and the green leaves on top), ~250 tons in

the case of cabbage (outer leaves), and between 2,000 and 3,000 tons in the case of carrots and celery, since most of these residues comes from the ready-to-eat lines in which the discard percentages increase substantially. This project seeks to reach an integral use of the by-products by applying different dehydration technologies, and it is not focused on extracting the bioactive compounds, thus avoiding solvent extraction and the generation of other residues. Processing conditions, including pretreatments, determine the physicochemical and functional properties of the powders, and thus their applications, as well as the bioaccesibility of their bioactive compounds. Thus, the purpose of this review is to provide scientific evidence for the optimal integral recovery of vegetable residues by reviewing drying techniques and pretreatments and their impact on the product characteristics and compounds bioavailability, to contribute to food chain circularity and the development of sustainable food diets.

The present review has been divided into three categories which allows the objectives to be addressed effectively. First, the current context of obtaining functional powder ingredients from fruit and vegetable wastes is presented, with special attention to the vegetables involved in the project. Then, the effects of processing on the functional and physicochemical profile of fruit and vegetable powders are exposed to identify the drying methodologies and possible pretreatments or additional steps that could increase the biological or nutritional value and define appropriate production parameters. Finally, the response to *in vitro* digestion of the various bioactive compounds of interest and their effects on human health is detailed. Considering the above, bibliographic research will help define the process conditions for producing vegetable waste powders of high quality, with a physicochemical profile suitable for subsequent application in food formulations and with functional properties capable of exerting beneficial effects on consumer's health. Hence, food ingredients and products contributing to the principles of sustainable healthy diets could be developed.

2. Methodology

Selected keywords were entered into the most important databases of scientific journals, such as Science Direct, Scopus, Emerald Insight, Springer Link, Taylor & Francis Group, and Wiley Online Library. Additionally, tools such as Google Scholar and technical dictionaries such as IATE (Terminology of the European Union) and CAB Thesaurus were used. The titles and abstracts of more than 120 publications were selected and examined. Subsequently, the most relevant documents were chosen based on the previously defined criteria. In short, a total of 98 documents and investigations were selected and are the basis of the present bibliographic review. These works come from a total of 18 scientific journals, classified in the fields of food research, biotechnology, chemistry, and waste management. No geographic or time restrictions were applied in the initial search process. Once selected, temporality of the selected studies was determined, resulting in 80% of the investigations to have been published in the last 10 years (2011–2020) and 45% belonging to the last 5 years (2016–2020). Therefore, recent and updated information related to the subject of study is presented.

3. Current Context of Fruit and Vegetable Wastes as a Source for Food Ingredients

Figure 1 shows the evolution in the last 20 years of the number of published papers dealing with functional food development from food wastes (retrieved from ScienceDirect). For this search, the terms “functional food” or “functional ingredient” were combined with “food waste” or “by-product.” Other terms used in the literature to define food waste such as “residue” or “co-product” were not specific and retrieved a significant number of papers not dealing with the topic of interest, for which they were neglected for this analysis. According to the results plotted in Figure 1, the research interest in developing functional foods and obtaining functional ingredients from food waste has increased dramatically in the last years, the total number of published articles doubling every 5 years.

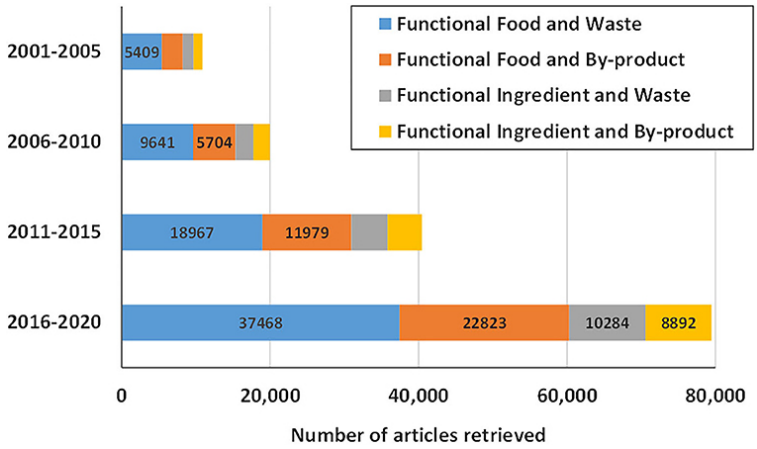

Figure 1. Evolution in the number of published articles (retrieved from Science Direct) using the combination of keywords "*Functional Food"* and "*Waste," "Functional Food"* and "*By-Product," "Functional Ingredients*" and "*Waste," "Functional Ingredient"* and "*By-product."*

At the beginning of the 21st century food industry and researchers were already aware of the need for developing valorization processes which reduced the environmental impact of the food industry and contributed to food industry sustainability. Nevertheless, it is the recent definition of the FAO sustainable goals for development that have boosted this research trend which contributes to the idea of sustainable food systems from a social, economic, and environmental point of view. The definition of this concept states that a sustainable food system must ensure sustainable consumption and production patterns, as well as deliver safe, healthy and nutritious diets Food and Agriculture Organization of the United Nations (FAO, 2019). Concerns related to FW have been presented in the scientific community since the 90s (Mirabella et al., 2014), such is the case of the research carried out by Kroyer (1995), who studied the impact of food processing on the environment highlighting the importance of waste treatment efficiently, reusing those by-products that are unavoidable to produce and turning them into new edible foods, thus rescuing biomass and valuable nutrients while reducing environmental burdens caused by inadequate management of waste.

In parallel, health benefits associated to dietary fibers (DF) has led to the development of a large and potential market for fiber-rich products and

ingredients, thus boosting the search for new sources of DF. This is one of the reasons why the by-products of fruits and vegetables that have been traditionally devalued are today considered a valuable source of this compound of interest (Rodríguez et al., 2006). This increasing attention to DF thanks to its beneficial physiological effects on human beings and its contribution to several functional and technological properties when incorporated into various food products, has led to the development of functional powdered ingredients obtained from pomaces or bagasse generated in the beverage and juice industries. In fact, studies dealing with this topic were found to be related to the levels of beverages consumption, as well as to DF content. Therefore, fruits like orange, apple, or grape (Figuerola et al., 2005; Aguedo et al., 2012; Tseng and Zhao, 2013), and vegetables such as carrot and cabbage retrieved more papers (Stoll et al., 2003; Chantaro et al., 2008; Nilnakara et al., 2009; Baljeet et al., 2014; Nouri et al., 2017), while other fruits or vegetables such as raspberry, mango or leek have been studied to a lesser extent (Ajila et al., 2010; Górecka et al., 2010; Wang et al., 2020).

The scientific literature contains many reports on DF additions obtained from by-products of fruits and vegetables, in various food products such as baked goods, beverages, confectionery, dairy, frozen dairy, meat, pasta and soups (Elleuch et al., 2011). Figuerola et al. (2005) used apple and citrus residues to obtain fiber concentrates with functional properties and use them in food fortification. Physicochemical characteristics of the powder, and particularly particle size, provided these powders high water retention and fat adsorption capacity. The powders also had a high DF content and a relatively low caloric value. Regarding the effectiveness of its incorporation in the development of foods reduced in calories and rich in dietary fiber, it was found to depend not only on the characteristics of each concentrate, but also on the way of adding them to the food matrix (particle size, temperature, ionic strength). Authors also stated that the characteristics of each concentrate determine the volume replacement and their thickening or texturizing effect.

Other authors (Tseng and Zhao, 2013) used grape pomace from the wine industry to produce antioxidant DF. Results showed that the content of

phenolic compounds was affected by many factors such as grape variety, growth climate and location, harvest time, processing and storage conditions or extraction procedure. Wine grape pomace was reported to contain 61% of DF which, together with its content of antioxidant compounds, remained stable after 16 weeks of storage, as a prove of the stability of these kind of products. The application of this powder as a food ingredient to increase the nutritional value and improve the storage capacity of a yogurt and a salad dressing were investigated. Results showed that the addition of wine grape pomace powder resulted in a 35–65% reduction in peroxide values in all samples and that the products had a DF content of 0.94–3.6% (w/w).

Lulo fruit is a tropical species rich in antioxidant compounds, minerals, and fiber. In a previous study (Hinestroza-Córdoba et al., 2020) lulo fruit bagasse, a by-product generated during lulo fruit juice manufacturing, was proposed to obtain a functional powdered ingredient. The obtained lulo bagasse powder was rich in fiber, phenols, and flavonoids with antioxidant capacity, which makes it an interest functional ingredient to enrich different types of food matrices. Blueberry bagasse and persimmon wastes have also been proposed for similar purposes (Bas-Bellver et al., 2020a) due to their fiber content and bioactive compounds: anthocyanins in the case of blueberry and carotenoids for persimmon. Overproduction and growing industrialization of the latter generates huge amounts of discards and residues, for which its valorization is gaining importance in recent years (Conesa et al., 2020). Ajila et al. (2010) obtained powders from mango peels and applied it to improve the antioxidant properties of macaroni preparations.

Regarding vegetables, Plazzota et al. (2018) obtained flours from fresh-cut salad waste to be used as food ingredients rich in antioxidants and with high solvent loading capacity. Their study concluded that drying is a feasible strategy to give value to these discards and obtain products with tailored physicochemical properties, as a function of the drying methods being used and mechanisms involved. Ferreira et al. (2015) proposed the integral exploitation of several fruits and vegetables (orange, passion fruit, watermelon, lettuce, courgette, carrot, spinach, mint, taro, cucumber, and rocket) by obtaining an isotonic beverage and a functional flour from the

generated wastes. The residues flours showed good water holding capacity, associated to their fiber content, and were used to formulate biscuits and cereal bars rich in fiber and minerals.

Focusing on the vegetables of interest for the project being developed (cabbage, carrot, leek, celery), the literature evidenced more interest in obtaining powders or flours from cabbage and carrot residues as compared to celery and leek, possibly due to their production and consumption levels. Cabbage is rich in sulforaphane, a product of hydrolysis of glucosinolates that is known to be a potent food-derived anticancer substance (Tanongkankit et al., 2011). Carrots are one of the most important root crops and a rich source of α- and β-carotene (Hiranvarachat et al., 2011; Chen et al., 2016), which are important precursors of vitamin A in the human metabolism (Barzee et al., 2019). It is also characterized by being rich in dietary fiber (Chau et al., 2004). Celery stands out for its high apigenin content, a known flavone which has been claimed to exert remarkable health benefits (Yusni et al., 2018). Finally, leek is an important source of sulfides (Luo et al., 2014).

Nilnakara et al. (2009) evaluated the production of antioxidant DF powder from the outer leaves and kernel of cabbage. This specific by-product represents the 40% of the total weight and it is often used as fertilizer or animal feed. The researchers studied the effects of blanching in hot water and hot air drying at different temperatures on the quality of the final product. As for carrot, a large proportion of this root is consumed as juice (Chau et al., 2004), which generates thousands of tons of carrot pomace which are generally disposed of as animal feed. It has been reported that ~80% of the carotenes remain in this by-product (Baljeet et al., 2014). Based in the previous, Nouri et al. (2017) sought to reuse it and develop a functional powder ingredient to be used in the formulation of a fried pastry product with the aim of improving its nutritional profile by producing low-fat donuts with high fiber content.

In contrast, publications related to celery and leek were very limited. Specific research dealing with the recovery of by-products of these respective vegetables and their reuse for the development of functional foods was scarce. Researchers, Wang et al. (2020) studied the effects of

wheat flour replacement by celery powder (CP) at different levels (1, 2, 3, and 5/100 g of flour) on bread quality. Results showed that the higher CP content, the greater the water absorption of the dough; however, the protein network, the maximum viscosity and the crystallinity of the starch decreased with an increase on the CP content. On the other hand, a higher level of CP increased the hardness and chewiness of the bread. It is important to highlight that the addition of CP significantly increased the total phenol content of the bread and, therefore, caused a significant improvement of its antioxidant properties. Regarding leek, Ozgur et al. (2011) studied the changes in some physicochemical properties and the variations in the antioxidant compounds of leek caused by the drying process. Leek drying resulted in a loss of ascorbic acid and phenolic compounds, so that the antioxidant capacity was reduced by more than 50% after drying.

This section has evidenced that FW can be transformed into functional food ingredients which, in fact, have been proved to increase the nutritional profile of several formulated foods contributing to the concept of sustainable diets and participating of circularity and food systems sustainability.

4. Influence of Drying Methods and Pretreatments on the Quality and Characteristics of Vegetables and Fruit Wastes Powders

Processing is one of the main factors affecting bioactive compounds, by increasing or decreasing their content, and having an impact on their bioaccessibility (Cilla et al., 2018). Focusing on vegetables and fruit waste powders, it is crucial to study the impact of different available technologies on the functional properties of the obtained powders, given that functional foods are those that, in addition to providing nutrients, are capable of positively affect specific biological functions and thus improving the general state of people's health (Rodríguez et al., 2006). Food processing techniques are increasingly sophisticated and diverse in response to the growing demand for quality food with functional properties, for which emerging technologies are proposed for the development of functional foods (Galanakis, 2021). In addition to processing stages, characteristics of the food powders also depend on the composition and physicochemical properties of

the raw materials, which in turn need to be considered when choosing the appropriate processing parameters. Therefore, to maximize the value of the waste, processing and pre-processing stages must be well-defined.

Dehydration or drying is a fundamental stage for the production of powdered ingredients. It involves the transient transfer of heat and mass accompanied by physical, chemical and phase change transformations. These transformations have a significant impact on heat and mass transfer mechanisms during drying, but they also impact the product quality and determine its technological properties, such as water and oil interaction properties. Dehydration is a widely studied operation of great application in the food industry. Figure 2 summarizes the evolution in the number of papers which have studied the application of different dehydration techniques to obtain functional foods or ingredients, and their impact on the bioactive compounds present in the food material. Results evidence that dehydration is a common technique applied to obtain functional food ingredients. Research interest on the impact of drying on the development of ingredients and functional foods, and its effects on their bioactive compounds is patent in the last decade.

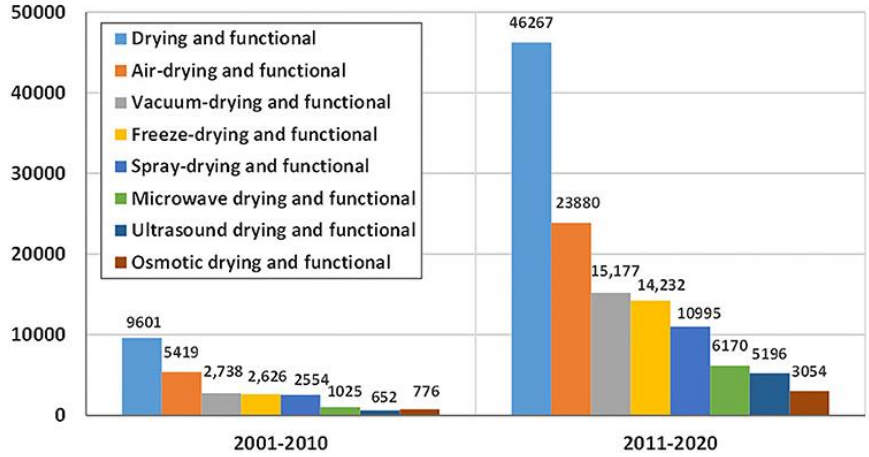

Figure 2. Dehydration techniques applied in the process of functional food or functional ingredients obtention. Average number of articles retrieved when using each technique combined with the terms "*functional food powder," "functional ingredient,"* or "*bioactive compound."*

In order to select the most suitable dehydration technique to obtain functional powders it is necessary to detail the different drying methods available and analyze their advantages, disadvantages and limitations. It is also relevant to compare different studies and analyze the impact of each dehydration technique on the bioactive compounds present in different fruit and vegetable matrices and the technological properties of the ingredients.

Table 1 gathers the main dehydration techniques applied to develop functional ingredients and foods from vegetables and fruit wastes and summarizes some advantages and disadvantages of their use. Air-drying (AD), vacuum-drying (VD), freeze-drying (FD), and spray-drying (SD) are all dehydration technologies commonly applied to obtain powdered ingredients, alone or in combination with others (Zhang et al., 2006; Sagar and Suresh Kumar, 2010). On the one hand, hot air drying (HAD) is commonly applied in the food industry due to its lower processing and investment costs. From an industrial point of view, it is a suitable technology for the valorization of solid wastes from the fruit and vegetable sectors since it allows the integral recovery of the biowastes. VD is a technique similar to air-drying, with the advantage of increasing mass transfer rates due to the low pressure generated in the drying chamber, while reducing the damage since the food is not exposed to a high temperature and in the absence of air oxidation reaction does not occur (Maisnam et al., 2017). Indeed, Wu et al. (2007) reported higher effective moisture diffusivity values of vacuum-dried vegetables as compared to hot-air dried samples. On the other hand, freeze-drying (FD) could also be used for an integral valorization approach, and it provides high-quality products and powders which strongly preserve their functional properties; however, this technology has limited industrial applications due to its high operating costs, which are 4–8 times higher compared to air-drying (Ratti, 2001).

Other drying techniques such as spray-drying (SD) have gained importance for the development of functional foods or preservation of bioactive constituents since it consists of generating small droplets from which water is evaporated nearly instantaneously, in an almost adiabatic process (Patel et al., 2009). The thermal energy carried by the air is mostly used to evaporate water from the droplets while the sample remains at a

moderate temperature, close to the wet bulb temperature of the air, thus helping preserve bioactivity. However, SD is suitable for liquids, emulsions and suspensions, for which it cannot be considered a viable option for the integral valorization of fruit and vegetables wastes. In order to obtain powders from biowastes by SD, it would be necessary to perform a previous solid-liquid extraction and a further drying of the extracts, thus not allowing the integral valorization of the wastes.

As for the other techniques being presented in Table 1, such as osmotic dehydration (OD) and microwave (MD) or ultrasound (UD) assisted drying, this consist of combinations of this technique with one of the previous, as a previous stage or as assisted drying in order to reduce energy consumption and/or heat damage (Sagar and Suresh Kumar, 2010). OD has the advantage of being a non-thermal process that requires little energy, but its effectiveness in reducing the water content of foods depend on many variables, such as the osmotic agent and concentration, temperature, sample to solution ratio, agitation, sample size and shape, other pre-treatments applied, etc. As an example, Prosapio and Norton (2017) reported a significant reduction in the processing time (8–2 h) in strawberry cubes which had been previously subjected to OD. Singh et al. (2006) applied OD combined with HAD to produce a value-added ingredient from carrot pomace to be used in the formulation of a dairy-based product, obtaining good acceptability results. Partial dehydration by osmosis has also been proposed prior to microwave drying in order to reduce processing time, limit energy consumption and improving the product quality (Al-Harahsheh et al., 2009).

Table 1. Main dehydration techniques available for the development of functional ingredients and foods.

Dehydration Technique	Product	Impact on bioactive compounds	Advantages	Disadvantages	Limitations	References
Air-drying (AD)	Chopped cabbage outer leaves and fresh carrot cubes; several vegetable wastes	High temperatures can decrease the content of thermolabile substances. Antioxidant activity may increase or decrease, depending on the raw material and temperature.	Most widely used drying technique as it is inexpensive and easy to operate. It allows integral recovery of vegetable waste	Long drying times and surface overheating cause color darkening, loss of flavor, increased compactness, decreased rehydration capacity	Heat sensitive bioactive compounds are exposed to high temperatures which may cause loss of nutritional value	Ratti, 2001; Nilnakara et al., 2009; Hiranvarachat et al., 2011; Bas-Bellver et al., 2020a
Vacuum-drying (VD)	Carrot slices; eggplant	Beneficial to improve the quality and nutritional value of the powder. Appropriate for thermal and/or oxygen sensitive materials	Higher drying rate, lower drying temperature, and oxygen-deficient processing environment which prevent oxidation	Batch process, has very small throughput, involves high residence times (20–100 h)	Limited energy transmission because the transfer of thermal energy to the workload becomes difficult since convection is ineffective at low pressure	Wu et al., 2007; Ghandi et al., 2013; Jiang et al., 2013; Chen et al., 2016
Freeze-drying (FD)	Several vegetable wastes; fresh cut salad wastes; grape pomace; apple pomace; apple slices	Negligible loss of nutrients, retention of original color and hence higher quality, high porosity of the dried material, reduced particle size once milled	Less damage in the product due to oxygen limitation and low temperatures. Allows to preserve to a greater extent the properties of the product	Long drying time (24–72 h) required and its batch nature. High costs. Lower potential for commercial application	Expensive process due to the cost of maintaining the pressure and temperature conditions at which it takes place	Ratti, 2001; Huang et al., 2009; Jiang et al., 2013; Tseng and Zhao, 2013; Rana et al., 2015; Plazzota et al., 2018; Bas-Bellver et al., 2020a
Spray-drying (SD)	Fruit and vegetable waste extracts; blueberry waste extracts; bayberry juice; orange juice	Product deterioration can occur due to the combined effects of dehydration, thermal, and oxygen stresses imposed on bioactive components	Widely used technique. Fast, continuous, reproducible, single-step. Scalable without major modifications	The yield strongly depends on the work scale. Low yield is due to the loss of product in the walls of the drying chamber	Only applicable to suspensions, solutions or emulsions. It does not allow an integral use of solid waste, generally applied to extracts.	Goula and Adamopoulos, 2010; Fang and Bhandari, 2011; Waterhouse et al., 2017; Santos et al., 2018; de Sá Mendes et al., 2020
Microwave drying (MD) (Assited)	Orange peel; tomato pomace; persimmon	Even though this method might not be better than others in keeping composition and nutritional capacities of the food products, their functional properties could be maintained more effectively	Faster reduction in moisture content. A reduction in drying time of up to 90% has been reported. Reduced processing time and processing costs.	Non-uniform temperature distribution. The product must be in constant movement within the cavity to avoid hot spots. Internal temperature can increase to values which produce calcinations	Relatively slow development of equipment capable of operating efficiently at large scales	Al-Harahsheh et al., 2009; Menéndez and Moreno, 2017; Talens et al., 2017; Celen, 2019
Ultrasound drying (UD) (Assited)	Passion fruit peel; Red beetroot; carrot slices	Reduce damage of heat-sensitive components.	Better quality of the final powder. It can reduce energy consumption. Faster and more powerful.	The effectiveness of the treatment depends on the correct intensity applied.	More effective in porous materials, in conditions of low temperatures and low air speeds.	Jiang et al., 2013; do Nascimento et al., 2016; Szadzińska et al., 2016; Guo et al., 2020
Osmotic drying (OD)	Tomato pomace; carrot pomace; white cabbage; strawberry	Loss of most nutrients is avoided. It also brings the maximum stability for phytochemicals	Non-thermal process that can significantly reduce the drying time and therefore achieve a higher food quality. It does not have a high energy requirement	Partial dehydration of food. Results depend on the osmotic solution composition	It applies to foods with an organized cellular structure, compartmented by semipermeable membranes.	Singh et al., 2006; Al-Harahsheh et al., 2009; Prosapio and Norton, 2017; Cvetković et al., 2019

Studies focusing on obtaining food ingredients from fruit and vegetables wastes are prioritized.

Drying with microwave energy (MW) differs significantly from conventional drying methods since the electromagnetic field implies a volumetric heating and causes the water molecules in the material to vibrate millions of times every second, thus allowing the moisture in the material to evaporate fast. When applied to drying, MW are usually coupled with drying in order to increase the dehydration rates. Talens et al. (2017) produced an orange peel fiber powder by combining MW and HAD in a process which allowed reducing time and energy consumption. Their results revealed that coupling both technologies allowed to positively modify de physicochemical properties of the fiber powder so that swelling capacity of orange fiber was improved. Regarding ultrasounds (US) application to drying, it has been proved to be an effective aid for fruit and vegetable preservation. Indeed, the mechanical and cavitation effects of ultrasound are responsible for the reduction of both internal and external diffusion resistances of materials in mass transfer during drying, and as a result, drying rate could be improved (Guo et al., 2020). do Nascimento et al. (2016) used an ultrasonic assisted drying to study the effect of temperature and ultrasound application on drying kinetics and antioxidant potential of passion fruit peel. According to their results, US reduced both, the internal and external mass transfer resistance. Antioxidant potential of passion fruit peels were found to be affected by drying conditions, best results being obtained in samples dried at 40°C when US were applied.

HAD has been proved to be a suitable technique for the integral valorization of the vegetable wastes generated in the cooperative. In the following paragraphs, information on air drying applied to the vegetables of interest (cabbage, carrot, leek, and celery), and how processing variables impact the bioactive compounds and determines the product quality is addressed.

Brassica vegetables such as cabbage are known to be rich in glucosinolates and isothiocyanates, which have been proved to act as antimicrobial, antioxidant and anticarcinogenic agents (Deng et al., 2015). Glucosinolates are degraded into isothiocyanates, specially to mention sulphoraphane, by means of the enzyme myrosinase upon tissue disruption (Mawlong et al., 2017). Therefore, the impact of the drying variables on

myrosinase activity is of great importance when designing the drying stage. In addition, cabbage contains other interesting compounds from the nutritional point of view such as fiber or antioxidants (Nilnakara et al., 2009). Myrosinase thermal stability depends on the enzyme source. According to Ghawi et al. (2012), thermal stability of cabbages myrosinase ranges between 40 and 60°C. Tanongkankit et al. (2012) studied the effect of HAD on cabbage and its glucosinolates content and found that a temperature range between 60 and 69°C produced a significant reduction on their content. It was also reported that the formation of sulforaphane occurred when the temperature of the vegetable was in the range of 25–53.5°C, and that thermal degradation occurred when this temperature was exceeded (Tanongkankit et al., 2011). Lekcharoenkul et al. (2014) proposed a multi-stage drying process in which drying temperature is modified in order to achieve a greater retention of sulphoraphane in fiber-rich powders produced from the outer leaves of cabbage. Hence, in the early drying period the heating rate should be high to rapidly increase the temperature of the material to a value above 40°C and, after on, the temperature of the material must be controlled so that it does not exceed 50°C. According to Gamet-Payrastre et al. (2000), to ensure the functional properties of the powder it should contain a minimum sulforaphane content of ~2.7 mg/L.

In the case of carrot, air drying has been used alone or combined with other technologies to obtain value added products, focusing on dietary fiber content and carotenoids content. Carrot peels have been used as a raw material to produce antioxidant dietary fiber powder by means of air drying (Chantaro et al., 2008). The authors studied the impact of air drying (60–80°C), alone or in combination with blanching (90°C), on the physicochemical properties of the powders. Fiber content and related properties such as water retention and swelling capacities, were significantly affected by blanching; in contrast, drying did not significantly influence hydration properties. Thermal degradation of bioactive compounds such as β-carotene and phenolic compounds occurred both during air drying and blanching. This study demonstrated the feasibility of obtaining powders from carrot wastes to be used as functional ingredients, and demonstrated that the processing stages had a significant impact on the powder properties. In addition,

particle size was found to be closely related to the physicochemical and hydration properties of the fiber powders, and their subsequent application. In fact, particle size has been demonstrated to be a critical parameter when developing powders and both pre-drying and post-drying milling stages determine powder properties (Bas-Bellver et al., 2020b). Milling will be discussed at the end of the present section as an additional stage of the powder manufacturing process. Nevertheless, it is worth mentioning at this point that not only milling but drying itself has a great impact on particle size and, hence, on powder physicochemical and hydration properties. The different structure generated during drying implies a different behavior during milling. Thus, air-drying usually generates harder materials which are more difficult to grind, leading to powders which usually show poorer hydration properties as compared to freeze-dried. On the contrary, freeze-drying leads to smaller particle sizes due to the increased porosity and brittleness of the dried material (Martínez-Las Heras et al., 2017; Plazzota et al., 2018; Bas-Bellver et al., 2020a).

As for thermal degradation of carotenoids, Mayer-Miebach et al. (2005) reported a 20% of β-carotene breaks down at 90°C. Hiranvarachat et al. (2011) tested air-drying at 70, 80, and 90°C during 5, 4, and 3 h, respectively, and concluded that β-carotene was better preserved at the lower temperature assayed. The most stable individual carotenoid of this vegetable is lutein, because its content is reduced by only 15.1% at 80°C. In contrast, under the same conditions β-carotene is heavily degraded. On the other hand, combining ultrasound and vacuum processes to dehydrate carrot slices can decrease the drying time in 41–53%. This novel technique called ultrasonic vacuum drying can also improve the rehydration potential, nutritional value, color, and texture properties of the samples (Chen et al., 2016).

As indicated previously, less research effort has been focused on leek and celery. Regarding leek, Ozgur et al. (2011) investigated changes in some physicochemical properties and antioxidant compounds caused by HAD at 63 ± 2°C for 3 h with an air velocity of 2.5 m/s. They observed that the antioxidant capacity decreased by more than 50%, there was a loss of ascorbic acid and the dehydrated samples showed a significant color

difference mainly due to the effect of temperature on heat sensitive compounds. However, the α and β chlorophyll content was higher in dried leeks than in fresh ones. Regarding celery, no detailed studies were found on the impact of HAD on their properties. Priecina et al. (2018) investigated the effect of convective and microwave-vacuum drying on the bioactive compounds of celery roots and found that carotenoids and ascorbic acid were sensitive to thermal processing, while phenolic compounds increased with drying process.

One way to alleviate the adverse effect of HAD is to pretreat a product, either physically or chemically, before drying (Górnicki and Kaleta, 2007). The main purpose of pretreating the sample prior to drying is generally to inactivate enzymes such as polyphenoloxidase, peroxidase, and phenolase, as well as to inhibit some undesirable chemical reactions, which may cause adverse changes in a product. In addition, pretreatments modify structures, soften the tissue, and release bioactive constituents, promoting changes which determine the response to the drying process and the quality of the dried product. Table 2 summarizes some of the pretreatments applied to obtain functional powders, according to the literature reviewed. In this table, information on process conditions and the potential impact on the fruit or vegetable matrix, as well as on the bioactive compounds is given. Milling, freezing or (steam) blanching are common pre-treatments which can easily be scaled up. Many pretreatment techniques have been used in the food industry, including blanching and the use of weak acids (Prakash et al., 2004; Górnicki and Kaleta, 2007). Novel technologies such as electric pulses or ultrasounds may be an interesting option thanks to their positive effects, but their industrial application is more limited.

Used as a pretreatment, milling is a key step to achieve the desired results and improve the drying process. Food matrix breakage has an impact on drying rates and on the release of bioactive compounds. When producing powders, a final milling stage is needed to obtain the desired particle size; nevertheless, particle size is also determined by the grinding applied prior to drying since it conditions the response of the food matrix to the drying step and, therefore, the structure of the dried material, which determines the grinding results. Therefore, milling as a pretreatment is an essential step to

obtain high-quality powders, with proper nutritional and physicochemical characteristics (Erenturk et al., 2005; Djantou et al., 2011; Bas-Bellver et al., 2020b). As for freezing, Ando et al. (2016) investigated the impact of the freeze-thaw process on the drying rates of carrot roots, obtaining that the frozen-thawed samples damaged by ice crystals had the highest drying rate. However, Bas-Bellver et al. (2020b) reported an increased resistance to drying due to a more compacted bed during drying, making it more difficult to reach low moisture content and water activity values in frozen samples *vs*. fresh ones. In brassica vegetables, such as cabbage, pretreatments contributing to cell disruption such as milling or freeze-thawing are expected to promote sulforaphane formation and its bioaccessibility (Pongmalai et al., 2018).

Table 2. Pretreatments discussed in the literature and potential impact on fruit and vegetable tissue and its bioactive compounds.

Pre-treatments	Process Conditions	Outstanding results	References
Milling	Chopping or grinding fresh vegetables	It determines particle size characteristics; the size reduction improves the drying process. It is an essential step to obtain high-quality powders, with proper nutritional and physicochemical characteristics.	Erenturk et al., 2005; Djantou et al., 2011; Bas-Bellver et al., 2020a
Freezing	Vegetable wastes stored in freezing conditions prior to processing	Different impacts on water activity values, depending on the food matrix. Maintains nutritional and sensory quality of products. When frozen and later HAD, an increased resistance to drying was found in some cases.	Ando et al., 2016; Peng et al., 2018; Bas-Bellver et al., 2020b
Steam cooking	From 1 to 20 min in a closed bath and a single layer of vegetable waste	In the case of brassica vegetables, it does not produce a significant loss of glucosinolates. The content of lutein, α-carotene, β-carotene, total carotenoids, and vitamin A remain high. Heating alters plant tissues, making bioactive compounds more available. A long steam cooking time prior to HAD can reduce nutrient content.	Tanongkankit et al., 2012; dos Reis et al., 2015
Blanching	Cooking in water at 90, 93 and 95 ± 2 °C during 1 or 2 min with a 1:7 ratio (vegetable waste vs. water). Immediate cooling in cold water at 4 °C	It reduces the drying time, due to the softening of the structure which facilitates water removal. The WRC and SWC can be improved. The retention of TPC can also be improved and avoids enzymatic browning	Ismail et al., 2004; Chantaro et al., 2008; Nilnakara et al., 2009
Acid pretreatment	Blanching vegetable waste with 0,7% (w/v) of citric acid during 2,5 min (pH 4–5)	It improves product quality by inactivating enzymes and modifying the texture. The color can also be maintained due to the chelating properties of the acids. Better water rehydration capacities, and a redder color can be obtained	Branem et al., 2002; Zhu et al., 2007; Hiranvarachat et al., 2011
Microwaves	Fresh vegetables samples are pretreated with MW at different powders and in a variable time	It increases the nutritional value of the dry samples. It leads to the maximum content of extractable phenols. Better functional properties can be obtained. Particular case of the cabbage: the content of sulforaphane increased by 6.23 times compared to the fresh samples	Bejar et al., 2011; Nieto Calvache et al., 2015; Pongmalai et al., 2018
Osmotic pretreatment	Immersion of fruit or vegetables in salt or sucrose hypertonic solution.	It reduces nutritional losses due to the decrease of the drying time. It also inhibits enzymatic browning, allows to achieve higher volatile compounds during further dehydration and the natural color can be maintained	Nimmanpipug et al., 2013; Mosquera-Vivas et al., 2019; Bozkir and Ergün, 2020
Ultrasounds	Ultrasonic parameters: sonication time, amplitude, and ultrasound power.	Non-thermal technology that has the potential to greatly decrease the drying time, it increases the drying kinetics, decreases the water activity, improves the product color and reduces nutrient loss.	Xu et al., 2009; Huang et al., 2020
Electric pulses	Sample subjected to an intermittent application (<300 Hz) of electric fields at moderate-high intensity (0.1–20 kV/cm) and short duration (from a few μs to several ms)	Great potential to improve the recovery of compounds quickly, economically and environmentally sustainable. Causes electroporation which involves the formation of localized pores in the cell membranes, improving subsequent stages.	Vorobiev and Lebovka, 2012; Barba et al., 2015; Puértolas and Barba, 2016

SWC, Swelling capacity; WRC, Water retention capacity; TPC, Total phenolic content.

Blanching is another pretreatment generally applied prior to drying to preserve the quality of the obtained product (Chantaro et al., 2008; Nilnakara et al., 2009). The main purpose of blanching is the inactivation of enzymes that cause quality losses such as browning and nutrient loss, but it has also been proved to be effective for increasing the drying rate of vegetables by softening the tissue and modifying its structure (Ando et al., 2016). Blanching is generally performed by submerging samples in hot water, although it is also possible to apply steam (Priecina et al., 2018). Nilnakara et al. (2009) applied blanching in hot water as a pretreatment to HAD to obtain fiber powders from cabbage residues. Their study revealed no significant differences with regard to antioxidant properties of samples; however, blanching provided a better appearance in terms of color due to the inhibition of enzymes responsible for enzymatic browning reactions.

Chantaro et al. (2008) combined blanching and air drying to obtain powders rich in fiber from carrot peels. Their findings were that blanching had a significant effect on the fiber contents and compositions, as well as on water retention and swelling capacities of the fiber powder. Although some antioxidants degraded during blanching and drying, there were no significant differences in terms of the total phenols, β-carotene and antioxidant activity of blanched and unblanched carrot peel powders. As for total DF, blanching improved its yield as well as the insoluble to soluble fiber ratio. Water retention and swelling capacities, which are relevant for food formulations, were favored by the blanching pretreatment. Ando et al. (2016) also assayed blanching as a pretreatment to air drying and compared it to the freeze-thawing process. In their study, drying rates of blanched carrots were significantly slower than those of freeze-thawed and non-pretreated ones. Priecina et al. (2018) combined steam-blanching with convective air drying and microwave-vacuum drying of celery, and reported that both drying and steam-blanching processes may help improve the bioactive constituents, but their amount can decrease or increase differently depending on processing or drying time and the applied temperature.

With regard to post-treatments applied to obtain powdered ingredients, it is worth mentioning the importance of milling and its role in determining

particle size, which has a relevant impact on physicochemical and technological properties and subsequent applications of the powder as a food ingredient. Researchers such as Chantaro et al. (2008) and Tanongkankit et al. (2012), ground the dehydrated carrot and cabbage, respectively, to obtain a specific particle size for particular applications. As introduced previously, particle size is the result of both pre- and post-drying milling, as well as the drying technology and conditions being used, and it has been proved to be related to several quality parameters of powders (Djantou et al., 2011; Plazzota et al., 2018; Bas-Bellver et al., 2020a,b). Sieving is an additional post-treatment which has served to obtain a specific desired particle size. At this point, rehydration is an additional parameter to be considered as an indicator of quality (Hiranvarachat et al., 2011; Ozgur et al., 2011; Chen et al., 2016).

Thus, it is deduced that a proper selection of processing parameters which include conditions of pretreatments and drying stages, are critical to preserve the quality of the obtained powders and optimize their functional and technological properties.

5. Response of Vegetables and Fruit Wastes Powders to the *In Vitro* Digestion Simulation Process

The goals of nutrition are to provide nutrients in such quantity and quality as are necessary to meet body requirements. Optimal nutrition should include bioactive compounds such as fiber, antioxidants, phytochemicals, among others, since these have the capacity to improve health, generate well-being and are useful to prevent the risk of suffering diseases (Cilla et al., 2018). The potential of these physiologically active components which are present in foods to exert beneficial effects depends on several factors, which include their release from the matrix, changes during digestion, absorption, metabolism and biodistribution. In order for these compounds to exert their bioactivity, they must be bioavailable. Other factors such as dose and host must also be considered (Bohn et al., 2015).

The term bioavailability has various working conditions and there is no universally accepted definition. From a nutritional point of view, it is defined as the fraction of ingested component that is available for use in normal

physiological functions (Wood, 2005). This concept includes two additional terms, which are bioaccessibility and bioactivity. Bioaccessibility is defined as the fraction of a compound that is released from its food matrix in the gastrointestinal tract and, therefore, is available for intestinal absorption. Bioactivity includes events related to the way in which the bioactive compound reaches the systemic circulation and are transported to the target tissue; it also refers to the interaction with the metabolism of biomolecules in these tissues and the entire cascade of physiological effects it generates.

Designing a process to obtain functional ingredients must aim to preserve and, if possible, improve the functional properties of the processed material, but it must also ensure the functionality of the bioactive compounds contained in the food ingredients by testing the impact of the digestion process on these constituents and, therefore, on their bioaccessibility. Potential health benefits of any functional ingredient require analyzing if the digestion process affects the stability and function of its bioactive compounds (Carbonell-Capella et al., 2014; Galanakis 2020). In the case of powdered ingredients, it has been previously mentioned that processing determines the available bioactive compounds as well as the powder structure, which in turn determines the bioactive compounds bioaccessibility (Gouw et al., 2017; Lucas-González et al., 2018; Bas-Bellver et al., 2020a). *In vitro* methods can evaluate the bioaccessibility of the compounds of interest by simulating gastrointestinal digestion (Cilla et al., 2018), and in this way helping design functional foods which health promoting effects can be claimed.

As shown in Figure 3, the interest in understanding the response of the bioactive compounds present in products or ingredients obtained from fruit or vegetable wastes has increased considerably in the last years. Main terms used by researchers are, again, “waste” and “by-product,” whereas the use of other keywords such as “pomace” or “bagasse” is marginal.

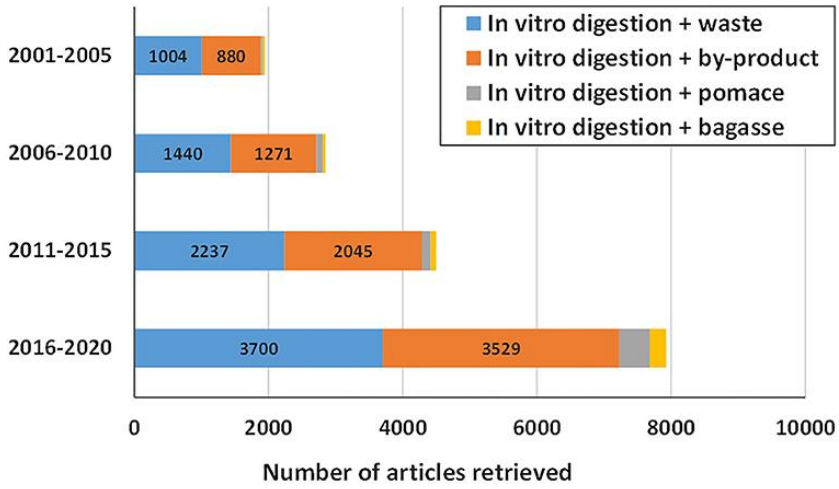

Figure 3. Evolution in the number of published articles (retrieved by Science Direct) which combine the keywords "*in vitro digestion"* and "*waste," "by-product," "pomace,"* or "*bagasse,"* in the last 20 years.

Regarding the development of functional food or ingredients from fruit and vegetables wastes (particularly cabbage, carrot, celery, and leek) research on the digestion of the powder ingredient, alone or as part of a formulated food, becomes essential. A method commonly applied for the study of the gastrointestinal digestion of food powders obtained from fruit and vegetable residues, is the practical static *in vitro* digestion methodology. It consists of mouth mastication, stomach digestion, and intestinal digestion of the food or ingredient in the presence of simulated gastrointestinal fluids and under controlled conditions. To this aim, Minekus et al. (2014) proposed a standardized method which is generally accepted and applied. In the revised literature, fruit, and vegetable powders are digested directly in the simulated intestinal fluids (Gouw et al., 2017; Bas-Bellver et al., 2020a), diluted in water or lipid (Gullon et al., 2015; Zhang et al., 2018), or as part of a (model) formulated food (Sarvan et al., 2017).

Gouw et al. (2017) used this method to study the impact of the digestion process on the bioactive compounds present in different dried fruit pomaces (apple, blueberry, raspberry, and cranberry). Results evidenced that bound polyphenols from food powders are liberated in the simulated gastrointestinal digestion and that phenolics can be released from the food

matrix by direct solubilization in the intestinal fluids and through the action of digestive enzymes. Low molecular weight polyphenols are partially absorbed in the small intestine, while high molecular weight can be fermented in the colon by the microbiota and partially absorbed to gut epithelial cells, acting as the counteract of prooxidants. Gullon et al. (2015) evaluated the *in vitro* gastrointestinal digestion of date seed meal and apple bagasse by diluting the powders in water, and studied further colonic fermentation. The powders were characterized by being rich in dietary fiber and exhibited high bioaccessibility values of phenolic compounds. Fermentation by colonic bacteria generated short chain fatty acids such as formate, succinate, acetate, propionate, and butyrate. Results of the *in vitro* simulated process confirmed that these by-products could be used to obtain functional food ingredients.

Zhang et al. (2018) applied an *in vitro* digestion model through which they evaluated the impact of digestion on carotenoids, in fresh and air-dried samples rich in these bioactive compounds such as carrots. Results obtained showed that the cell wall acts as the main natural structural physical barrier that governs the release of carotenoids. Pectin composition and the presence of other polysaccharides in the cell wall influence the bioacessibility of carotenoids by interacting differently with target compounds. Comparison of digested fresh and hot air-dried samples revealed that HAD promoted cell wall disruption and induced an anticipated release of carotenoids leading to higher bioaccessibility values. Addition of lipid to dried samples during digestion had a marked positive effect on the bioaccessibility of individual carotenoid. In this sense, Hornero-Méndez and Mínguez-Mosquera (2007) evaluated the effect of both processing and lipid content (cooking oil) in the bioaccessibility of carrot carotenoids. Although the heat treatment during cooking showed to have a negative impact on the carotenoid content, a positive effect was found on the micellarization of the carotenes and therefore on their bioaccessibility. Carotenoids bioaccessibility in fruit waste powders were also evaluated by Bas-Bellver et al. (2020a). In this study, blueberry and persimmon wastes were processed to obtain functional powders rich in anthocyanins and carotenoids, respectively. The study revealed that the release of antioxidant constituents

along the digestion process depended on several factors such as type of residue, drying process and fiber content and type. It was evidenced that the anthocyanin content after digestion was particularly affected by the dehydration technique being used, since despite providing a powder with a higher anthocyanin content, freeze-dried samples were more significantly affected by digestion. *In vitro* colonic fermentation was also carried out in this study, evidencing that the carotenoids and anthocyanins remaining in the digested samples after the intestinal phase can reach the colon to be fermented by gut microbiota and promote a benefit for human health.

Regarding glucosinolates and isothyocianates, Sarvan et al. (2017) studied the form of conversion of glucoraphanin and the bioaccessibility of the decomposition products released, evaluating the effect of steam cooking, as well as the composition of food (addition of proteins or lipids) through an *in vitro* digestion model. They were able to determine that the main formation of sulforaphane and sulforaphane nitrile occurred during *in vitro* chewing. The addition of proteins or lipids had no influence on the formation and bioaccessility of sulphoraphane, concluding that the main factor for the formation of sulphoraphane *in vitro* upper digestive tract is the presence of active myrosinase in the plant.

6.Conclusion

The development of functional food ingredients from vegetables and fruit wastes requires scalable processes which allow to preserve or enhance functional properties and bioactive compounds, as well as products that meet the standards of high quality, safety, and organoleptic characteristics demanded by consumers. Many studies have evidenced that agricultural fruits and vegetables residues are promising sources of dietary fibers and other functional compounds. The way of processing these wastes determines the characteristics and functionality of the final products, for which research must focus on the development of particularized processes.

Dehydration as a food preservation technology is a useful technique for the transformation of plant-based food residues into functional powdered ingredients. An integral recovery is important to avoid other residues and

rescue the main bioactive compounds. Hot air drying remains to be the dehydration technique most used in the food industry, mainly due to its lower processing and investment costs. Applied to vegetable wastes processing, it provides stable ingredients and allows the integral valorization of the biowastes, without the need for extraction and concentration stages. Nevertheless, drying technologies reported in the literature are increasingly sophisticated and diverse. Vacuum-drying and freeze-drying can be exploited by the food industry to integrally valorize these residues and produce functional powdered ingredients; microwaves or ultrasounds assisted procedures can be applied to optimize drying rates and improve the powder characteristics. However, these technologies imply higher costs and the need for specialized staff, for which their implementation is more limited, especially in the first stages of transformation. Alternatively, pretreatments can also be applied to improve drying rates and product properties. Available pretreatments include emerging technologies such as microwaves or ultrasounds, but also conventional ones such as blanching or milling. The recent literature reveals that well-established pretreatments such as blanching or milling may increase drying rates and reduce the adverse effects of dehydration, improve the efficiency of the process and enhance technological and functional properties of the powders produced. The selection of these widely known and scalable technologies is of particular relevance in early transformation processes.

The literature review has also evidenced that process conditions have an impact on the beneficial effect derived from consumption of vegetable powders. Selection of processing conditions must focus on the preservation of bioactive compounds but also on product structure, since it conditions their bioaccessibility throughout the digestion process, including their potential impact on gut microbiota.

As key indicators of the final quality of the vegetable powders, the physicochemical composition, the functional properties, and the potential physiological effects on human health through *in vitro* digestion must be evaluated, in order to guarantee the quality of the powders and their effective application in food formulations. A comprehensive approach should include particularized processes for specific applications and

preservation trials to ensure sustainable industrial production of functional powdered ingredients from vegetable wastes.

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CONCLUSIÓN

Esta revisión bibliográfica ha puesto de manifiesto que la selección de las condiciones de procesado define en gran medida las características de los productos en polvo y la bioaccesibilidad de los compuestos bioactivos que contienen. Para obtener polvos de calidad será necesario escoger tecnologías que permitan preservar los compuestos bioactivos y que garanticen la estabilidad de los productos, para su posterior utilización como ingredientes funcionales en el desarrollo de formulaciones alimentarias.

El secado o deshidratación es una técnica útil y ampliamente utilizada para la transformación de residuos vegetales, que en su forma original son propensos al deterioro microbiano. Dentro de las tecnologías de secado disponibles, destaca el secado por aire caliente por su bajo coste y porque da lugar a ingredientes estables, permitiendo además la valorización integral de los residuos. No obstante, el secado por aire caliente puede resultar en una disminución de la calidad del producto seco, por lo que respecta a sus propiedades funcionales. Otras tecnologías que también permitirían la valorización integral de los residuos vegetales para la obtención de productos en polvo serían el secado a vacío o la liofilización. Por otro lado, se podrían conseguir mejoras en los rendimientos gracias a procedimientos asistidos por microondas o ultrasonidos. No obstante, en comparación con el secado por aire caliente, el uso de estas otras tecnologías en la industria no es tan frecuente debido a su mayor complejidad o dificultad de escalado, y a mayores costes de inversión y operación.

En cuanto a los pretratamientos que pueden preceder al secado, se ha visto que influyen tanto en la etapa de deshidratación, reduciendo los efectos adversos de la misma, como en las propiedades funcionales y tecnológicas de los productos en polvo, mejorándolas. De entre los pretratamientos, la bibliografía destaca la molienda, congelación, escaldado y tecnologías emergentes como la aplicación de microondas o ultrasonidos.

De acuerdo a los resultados obtenidos mediante esta revisión biobliográfica, y atendiendo a la evolución del proyecto de tesis doctoral, las operaciones unitarias a estudiar para el proceso de obtención de productos

deshidratados en polvo se centrarán en aquellas que permitan la valorización integral del residuo y supongan un coste de inversión y operación adecuado al contexto de aplicación. Concretamente, la investigación abordará el estudio de pretratamientos tales como la molienda, la congelación y la fermentación, y en cuanto a técnicas de secado se optará por el secado por aire caliente a diferentes temperaturas, y la liofilización, empleado este último como método de referencia en cuanto a la conservación de las propiedades del producto fresco.

CAPÍTULO 2. Influencia del pretratamiento y las condiciones de secado sobre las propiedades de los productos en polvo obtenidos a partir de residuos de hortalizas generados en las líneas de confección de bandejas y productos de IV gama de Agrícola Villena Coop.V.

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RESUMEN DEL CAPÍTULO

En el presente capítulo se aborda el estudio del impacto que tienen los diferentes pretratamientos y técnicas de deshidratación seleccionados sobre las propiedades de los productos en polvo obtenidos a partir de los residuos generados en las líneas de confección de hortalizas de Agrícola Villena Coop.V. La congelación y la disrupción previa se estudiaron sobre los residuos de col, zanahoria, apio y ajo puerro (parte verde y parte blanca), en el marco del proyecto AGCOOP_D/2018/025 "Obtención de polvos de uso alimentario con propiedades funcionales a partir de residuos de las líneas de confección de hortalizas". Más adelante, se estudió también la fermentación con microorganismos probióticos como pretratamiento previo al secado, en este caso sobre col y brócoli, en el marco del proyecto AGCOOP_A/2021/020 "Obtención de productos en polvo a partir de las líneas de confección de col blanca para su uso como ingrediente funcional sostenible y para la gestión integrada de plantas arvenses".

En una primera aproximación al desarrollo de los procesos de obtención de residuos de hortalizas en polvo, se estudió el efecto de los pretratamientos y técnicas de secado seleccionadas sobre las propiedades fisicoquímicas y funcionales (antioxidantes) de los polvos. Como pretratamiento se estudiaron la congelación (fresco vs. congelado) y la intensidad de disrupción del tejido (triturado vs. troceado); y como técnicas de deshidratación el secado por aire caliente (SAC) a 70 °C y la liofilización (LIO). En el caso de los productos SAC, se estudió también la respuesta de las diferentes matrices vegetales pretratadas frente al SAC a través de la obtención de las curvas de secado y de velocidad de secado.

Los procesos de transformación aplicados permitieron transformar con éxito los residuos de hortalizas en productos en polvo, los cuales se caracterizaron con base a sus propiedades fisicoquímicas y antioxidantes. Los resultados obtenidos mostraron que los métodos de deshidratación empleados permitieron disminuir la humedad y la actividad del agua (a_w) del producto inicial alcanzándose el valor objetivo de $a_w < 0,3$ necesario para garantizar su estabilidad. Se demostró que la intensidad de triturado (triturado/troceado) previa a la etapa de secado condiciona el tamaño de

partícula del polvo final, obteniéndose menor tamaño cuando las muestras se sometieron a una mayor intensidad de desestructurado previo al secado (triturado) frente al troceado. Respecto a las propiedades antioxidantes analizadas (fenoles totales, flavonoides totales y capacidad antioxidante por los métodos DPPH y ABTS), las variables de los pretratamientos de disrupción previa del material vegetal y condiciones de almacenamiento del residuo (en fresco o congelado) influyeron de forma diferente según el material vegetal deshidratado. En términos generales, no se observó un efecto positivo del tratamiento de congelación previo. Esto último, unido a que esta etapa supondría un coste energético adicional, llevó a desestimar este tratamiento previo de cara a continuar la investigación. Finalmente, con el estudio de las curvas de secado y de velocidad de secado (a 70 °C) se confirmó que la matriz vegetal y el pretratamiento tienen un efecto significativo en el comportamiento de las muestras frente al proceso de secado por aire caliente.

Tras una primera aproximación del proceso de transformación de los residuos vegetales en polvo, se continuó trabajando con las mismas matrices vegetales (zanahoria, col, apio y puerro) únicamente en fresco, es decir, tras su recepción. En este segundo trabajo, se incorporó la temperatura de SAC a 60 °C, además de SAC a 70 °C y liofilización, y se amplió la caracterización de los polvos obtenidos, tanto en cuanto a su caracterización fisicoquímica como tecnológica. Adicionalmente, se estudiaron los cambios en las propiedades de los productos en polvo a lo largo de un periodo de almacenamiento de cuatro meses, en ausencia de luz y a temperatura ambiente. Así pues, tras el procesado y a lo largo de cuatro meses de almacenamiento, los productos en polvo se caracterizaron mediante el análisis de sus propiedades fisicoquímicas, antioxidantes y tecnológicas (propiedades de interacción con el agua y el aceite).

Tras el procesado se consiguió obtener polvos estables con bajo contenido en humedad y actividad del agua. Coincidiendo con el estudio anterior, la intensidad de desestructurado previo y el método de deshidratación aplicado tuvieron un impacto significativo sobre el tamaño de partícula de los productos en polvo, de modo que los polvos liofilizados y aquellos que se habrían triturado previamente al secado por aire caliente

mostraron menores tamaños de partícula. En cuanto a las propiedades antioxidantes, éstas se vieron favorecidas por el secado por aire caliente, en particular a 70 °C, frente a la liofilización, lo que podría atribuirse a la formación de nuevos compuestos antioxidantes debido a reacciones bioquímicas, como las reacciones de Maillard, o a un menor tiempo de exposición al secado. En cuanto a las propiedades tecnológicas evaluadas, los polvos mostraron resultados positivos en cuanto a sus propiedades de interacción con el agua, siendo generalmente mayores en los materiales liofilizados debido a su mayor porosidad. Por el contrario, los productos en polvo mostraron una pobre capacidad de interacción con el aceite.

A lo largo del almacenamiento se constató una reducción en la calidad de los productos en polvo. El contenido en humedad y la actividad del agua se incrementaron a lo a lo largo del periodo de almacenamiento, sugiriendo la necesidad de condiciones de almacenamiento más controladas para evitar la ganancia de humedad y la pérdida de estabilidad. También se constataron cambios en el color de los productos, y hubo una reducción generalizada de las propiedades antioxidantes. Esta parte del estudio reveló la importancia de estudiar, no solo las condiciones de proceso, sino también el impacto del almacenamiento sobre las características de los ingredientes en polvo.

Un tercer estudio incluido en este capítulo aborda la operación de fermentación como pretratamiento del material vegetal previo a la etapa de deshidratación. La fermentación se presenta como una alternativa segura y sostenible para obtener productos a partir de residuos vegetales, los cuales pueden mejorar su valor nutricional debido a las transformaciones sufridas durante esta etapa. Con este fin, se realizaron ensayos preliminares con los residuos de col blanca y brócoli, utilizando diferentes microorganismos, en particular *Lactobacillus salivarius*, *Lactobacillus plantarum* y *Lactobacillus reuteri*, aunque finalmente se decidió trabajar con tallos de brócoli utilizando *L. plantarum* para su fermentación, debido a los mejores resultados obtenidos.

En este trabajo se evaluó el efecto de la intensidad de desestructurado (triturado *vs.* troceado) y de la fermentación con *Lactobacillus plantarum* spp. CECT 749 como pretratamientos previos a la deshidratación mediante

secado por aire caliente (a 60 o 70 °C) o liofilización, y su efecto sobre las propiedades fisicoquímicas y antioxidantes de los polvos finales obtenidos. Según los resultados obtenidos, *L. plantarum* alcanzó el máximo crecimiento microbiano tras 24 h de fermentación, no observándose un efecto de la intensidad de triturado previo sobre el crecimiento del microorganismo probiótico. En las cinéticas de secado, estudiadas a 60 °C y 70 °C, no se observó un efecto del triturado o troceado previo de la muestra. No obstante, la fermentación previa y una mayor temperatura de secado incrementaron la velocidad de secado. Respecto a las propiedades antioxidantes, la diferente intensidad de triturado no tuvo un impacto significativo sobre las mismas; por el contrario, la etapa de fermentación y el secado por liofilización favorecieron las propiedades antioxidantes de los polvos de tallo de brócoli. Como era de esperar, las altas temperaturas de secado afectaron negativamente al recuento microbiano, mientras que la viabilidad microbiana en los polvos liofilizados fue significativamente mayor, demostrando un mayor potencial probiótico.

Turning vegetable waste into functional powdered ingredients for the food industry: a case study applied to agri-food cooperative residues in Spain

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Abstract

Current food transformation processes must face the food waste issue by developing valorization processes to reintroduce by-products in the economic cycle and contribute to circular economy, generating social and economic value, and ensuring permanence of agricultural and rural activities. In the present paper, the results of a collaboration project between a regional agri-food cooperative and university are summarized. The project aimed to revalorize a series of vegetable wastes (carrot, leek, celery, and cabbage) from the fresh and ready-to-eat lines of the cooperative, by producing functional powders to be used as functional food ingredients. Vegetables residues were successfully transformed into functional ingredients by hot air drying or freeze-drying, and variables such as storage conditions and grinding intensity prior to drying were considered. Twenty-five vegetable powders were obtained and characterized in terms of physicochemical and antioxidant properties. Results showed that drying (mainly hot air drying) allowed obtaining stable powders, with very low water activity values, and a significantly increased functionality. Vegetable waste powders could be used in the food industry as coloring and flavoring ingredients, or natural preservatives, or either be used to reformulate processed foods in order to improve their nutritional properties.

Keywords: vegetables waste, agri-food by-products, bio-waste valorization, bio-waste processing, food system sustainability, functional food ingredients

1. Introduction

Present food transformation processes need not only to focus on proper waste management, but they also need to be rethought to contribute to circular economy through the valorization of by-products, which are reintroduced in the economic cycle [1]. Continuity of agricultural and rural activities requires the development of new processes for the valorization of food industrial residues in order to generate social and economic value [2,3]. In this context, integral valorization, circular economy, process efficiency, biorefinery, or zero residue economy concepts contribute to describe present trends [4]. Valorization of food process by-products with the aim of contributing to food industry sustainability as well as developing circular economy systems cannot be considered an option but a must.

Agriculture is the world's most globalized industry. According to a recent publication by AgroPress and TOMRA sorting solutions foods [5], almost a third of worldwide food production is never eaten, which represents ~1.3 billion tons of food waste per year. In the particular case of fruits and vegetables the percentage of wastes is 45%, the United Nations Food and Agriculture Organization (FAO) having suggested values as high as 60%. More than a significant figure if considered that, in the particular case of Spain, over 13.4 million tons of vegetables and 18.4 million tons of fruit were produced in 2017 [6].

Fruits and vegetables are the most used commodities among horticultural crops, but their processing residues are still generally infra-utilized and considered a low value material. Fresh and processing industries generate significant losses and wastes which are becoming a serious nutritional, economical, and environmental problem [7]. The need for reducing the generation of food wastes and developing processes that allow their reuse by re-introducing the wastes or their components in the productive cycle has been discussed during years [8], but it still challenges all the agents involved. The sustainable development goals defined by the Food and Agricultural Organization of the United Nations especially focus on sustainability of food systems, as "sustainable consumption and production

patterns" must be ensured, and especially mentions the coordination of global initiatives, activities, and projects on food losses and waste reduction. Fruits and vegetables are consumed raw, minimally processed, or processed, because of their nutrients and health-promoting constituents [7]. In addition, agroindustrial wastes (such as seeds, peels, or pulp) generated in the different steps of the processing chains, present a high content of bioactive compounds, often even higher than that of the whole fruit [9,10]. Although the market currently offers a variety of foods which contain artificial preservatives, additives and/or taste improvers, modern consumers are more health-committed, buy food products more consciously, carefully read labels and decide in favor of natural foods, preferring low-processed foods without the addition of artificial preservatives or other additives. There is a continuous growing interest in such food, leading to new products permanently appearing on the market, including those which may contain fruit and vegetable industrial wastes [11]. This trend forces food producers to design and formulate foods containing natural additives, thus improving the nutritional value of juices, beverages, purees, or other food and, simultaneously, extending their shelf-life [7]. Nevertheless, the use of plant ingredients by the food and drink industry is still scarce, for which generating knowledge of their phytochemical properties is expected to be useful to increase their use as food ingredients.

Fruit and vegetable residues are perishable and have a very short shelf life. They are easily fermented while accumulated in the fresh processing plant facilities. For this reason, the preliminary treatment of the residues in the place where they are produced is expected to improve their durability as well as enable a better use and, additionally, help preserve the bioactive compounds it contains. The pretreatment (e.g., drying) of fresh residues immediately after processing to extend their durability could eliminate wastes and reduce environmental pollution.

On the other hand, reformulation of processed foods provides a genuine opportunity to improve people's health by modifying the nutritional characteristics of commonly consumed processed foods [12], therefore addressing another one of the FAO sustainable development goals: "Ensure healthy lives and promote well-being for all at all ages" [13]. According to

this goal, consumers must be encouraged to shift to nutritious and safe diets with a lower environmental footprint [13].

Fruit and vegetable powders have become a new way of consuming these products, which has gain importance along years [11,14,15]. Powdered foods are usually stable and presented in a concentrated and versatile form, for which they can be used in food formulation. Using food waste (vegetable waste in this case) to produce food powdered ingredients is an approach that contributes to the food chain sustainability and system circularity, by reusing a waste material and re-introducing it in the production cycle. In addition, vegetable waste powdered ingredients would have potential applications as coloring, savoring, or preservative agents, but they could also be used to increase the nutritional value of (processed) food, contributing to the development of nutritious and safe diets with a reduced environmental impact. Manufacturing powders from food waste may be a challenge considering the heterogeneity and structural differences among materials and edible/non-edible part of the same residue. From a technological point of view, parameters of unit operations such as cleaning, drying and milling (pre and post-drying) may have a relevant impact on the functional properties of the powder.

As described in the regional order "ORDEN 3/2018" (Conselleria de Agricultura, Medio Ambiente, Cambio Climático y Desarrollo Rural, Comunitat Valenciana, Spain) [16], the agricultural sector in the C. Valenciana bears a productive structure which is characterized by small, fragmented, nd disperse units. Agricultural cooperativism contributes to unify interests and improve the sector's competitiveness. Likewise, the Federation of Agri-Food Cooperatives of this region (Federació de Cooperatives Agroalimentàries de la Comunitat Valenciana) posts on the innovation and technologic development of this primary sector.

The present contribution summarizes a case study of collaboration of the Institute of Food Engineering for Development (IUIAD) of the Universitat Politècnica de València (UPV) and the Villena agrifood cooperative (Agrícola Villena, Coop. V), in the context of the Rural Development Program 2014–2020 of the C. Valenciana, funded by the European Agricultural Fund for

Rural Development. The aim of the project is to develop processes to obtain functional powders to be used as food ingredients, from the wastes generated in the manufacturing lines of four selected vegetables (celery, carrot, cabbage, and leek), prepared raw to be cooked or, in the case of celery and carrot, ready-to-eat formats. These particular vegetables were chosen considering the ease of obtaining a clean (or easy to wash) residue as well as the expected bioactive constituents' content in the food. Yearly production of these vegetables residues is estimated by Agrícola Villena, Coop.V. to be ~178 tons in the case of leek and ~250 tons in the case of cabbage. The amount of carrot residues generated is significantly higher (almost 3000 tons) since most of this residue proceeds from the ready-to-eat line, in which discarding percentages increase dramatically.

To develop the processes, the effect of different process variables (fresh/freezed, chopped/ground, air dried/freeze dried) on physicochemical and antioxidant attributes of powders, alongside with the study of the drying curves of the wastes, were studied. The present approach is a clear example of collaboration between primary sector-fresh vegetable industry and university to address the concept of global sustainability of food systems, focused not only on the environment, but also on food access and the development of technologies that increase bioavailability of bioactive compounds. Both functional food development and sustainability of the food system meet at this point.

2. Materials and Methods

2.1. Vegetables Waste Processing for Powder Manufacturing

In a first stage of the project, vegetable wastes were partially processed in the cooperative and further processed at the IUIAD facilities, as the objectives of the project included the study of drying conditions and drying curves, and the characterization of the powders obtained. In a second stage of the project, it is expected that the milling and drying stages are completed at the cooperative, once conditions of milling and drying are conveniently fixed and a solid recommendation for the equipment to be acquired is obtained as a result of the project.

2.1.1. Vegetables Waste Processing at the Cooperative Facilities

Among the different fresh products prepared and commercialized by Agrícola Villena, Coop. V, the ones identified as good candidates for the project were the cabbage and leek used for preparing combo vegetable trays (washed and cut, not chopped or minced) and the ready-to-eat lines (carrot and celery sticks). As introduced previously, the amount of wastes generated in the former is ~200 tons/year, whereas the amount of waste materials generated in the ready-to-eat lines can be higher than 2500 tons/year.

Leek and celery are washed with tap water and processed by hand (cutting with knife) to separate the edible part from the part that will be considered residue. In the particular case of cabbage, dirty or damaged leaves are removed by hand, and no washing is used. Carrots are processed by first washing with tap water, mechanically peeled, and cut in sticks in a mechanical cutter. Finally, carrot sticks undergo a second washing step with an ascorbic acid-water solution. Those sticks not meeting the quality standards are discarded and considered residue.

2.1.2. Vegetables Waste Processing after Reception at IUIAD Facilities

The vegetable wastes received were processed freshly (Fre) or stored in a freezer at −22 °C (Fro) until processing. When not washed at the cooperative facilities, residues were washed with chlorinated tap water before further processing. Prior to drying, particle size was reduced to pieces of medium size ≤ 10 mm, called hereinafter chopped (C), or ground to particles ≤ 5 mm (G). Particle size reduction was performed in a lab mill according to the conditions specified in Table 1.

Table 1. Conditions for particle size reduction before drying, for the different vegetable wastes being processed.

	Carrot	**Celery**	**White cabbage**	**Leek (w)**	**Leek (g)**
Ground(G)	10,000 rpm, 10s (350 g)	10,000 rpm, 10s (200 g)	10,000 rpm, 10s + 10s (150 g)	10,000 rpm, 10s + 20s + 10s (150g)	10,000 rpm, 10s + 10s (150g)
Chopped (C)	5000 rpm, 5s (350 g)	5000 rpm, 5s (200 g)	5000 rpm, 5s (150 g)	10,000 rpm, 5 s (150g)	5000 rpm, 10 s (150g)

Once milled, vegetable wastes were hot air dried (HAD) or underwent a freeze-drying process (FD). Air drying took place in a convective tray dryer (Pol-ekoAparatura, Katowice, Poland) at 70 °C, until water activity (aw) was reduced to values below 0.3. Freeze-drying took place in a freeze dryer (Lioalfa-6, Telstar) for 24 h under freezing conditions and sub-atmospheric pressure (P = 0.1 mbar), with a previous freezing of the samples at −40 °C during 24 h. After dehydration, representative samples of each dehydrated residue were crushed to a fine grain size (10,000 rpm, 2 min at 30 s intervals) using a food processor (Thermomix®, Vorwerk) to obtain the final powder. A total of 25 powders were obtained, 5 from each type of waste: Carrot (Ca), Celery (Ce), White cabbage (WC), Leek white portion (Lw), and Leek green portion (Lg). Powders were also identified by initial storing conditions: Fresh (Fre) or Frozen (Fro); the pretreatment applied: Ground (G) or Chopped (C); and the drying method used: Hot air drying (HAD) or Freeze-drying (FD).

2.1.3. Matrix Behavior during Air Drying: Drying and Drying Rate Curves

Kinetics of drying during the hot air-drying process are important as it is relevant to elucidate the duration of the constant drying rate period (CDRP), during which resistance for water transfer in the product is low as compared to evaporating rate and adiabatic conditions of drying prevail. It also allows estimation of the critical moisture content at which drying rate starts to decrease, indicating the beginning of the falling drying rate period (FDRP), when there is an internal control of mass (water) transfer. For this purpose, drying and drying rate curves of all the vegetable matrices were obtained during air drying at 70 °C. The analytical procedure consisted of placing each residue in two different trays for gravimetric determinations, and a third one for a_w measurements. Samples were weighed along the whole drying process (i.e., until reaching a water activity value below 0.3), at increasing time intervals, starting from 30 min. When drying finished, final moisture content of samples (x_{wf}) was determined by the following the official method established by the AOAC 934.06 [17], and the moisture content along the treatment was calculated (x_{wf}) by stating a dry matter balance (Equation (1)) along the drying process. Water mass fractions (x_w) were then transformed into wet basis (X_w) to plot both the drying curve (X_w/X_0 vs. time) and the

drying rate curve (−ΔXw/X0)/Δt) vs. Xw/X0. Drying curves were obtained for the chopped and ground residues directly processed after reception (fresh) at the IUIAD pilot plant.

$$M_t \cdot (1-x_{wt}) = M_f \cdot (x_{wf}) \quad (1)$$

2.2. Analytical Determinations of Raw Materials and the Obtained Powders

Powders were characterized in terms of physicochemical and antioxidant properties. Physicochemical characterization consisted of determining moisture content, water activity, total soluble solids, and particle size. Antioxidant properties of powders were measured by qualifying phenol and flavonoid compounds, as well as antioxidant activity by the DPPH and ABTS methods.

2.2.1. Physicochemical Properties of Vegetable Wastes and Powders

Water activity (a_w) was measured by means of a dewpoint hygrometer (Aqualab 4TE, Decagon devices, Inc., USA); moisture content (x_w) was determined following the official method established by the AOAC 934.06 [17], which consists of determining the weight loss of the samples by drying them in a vacuum oven (Vaciotem, JP Selecta) (P = 10 mm Hg) at 60 ◦C, until constant weight. Total soluble solids (x_{ss}) were estimated from the Brix degrees measurement performed at 20 ◦C, obtained with a thermostatic refractometer (ABBE ATAGO 3-T, Japan). When necessary, water extraction of soluble solids was performed adding water in a 1:10 (w/v) ratio, for Brix measurements. All three properties were measured in triplicate. Particle size distribution was determined by laser diffraction using a Malvern Mastersizer equipment (Model 2000; Malvern Instruments Limited, UK), in dry and wet conditions. For the dry method, a dispersion unit Sirocco 2000 with air as dispersant at 2.5 bar of pressure and 60% speed were used. For wet measurements, a 1.52 refraction index was used for the sample, whereas 1.33 was used for the dispersed phase (water), the particle absorption index being 0.1. Results are the mean of five replicates and are given as equivalent volume diameter D[4,3] and surface area mean diameter D[3,2], together with the distribution percentiles d10, d50, and d90.

2.2.2. Antioxidant Properties of Vegetable Wastes and Powders

Antioxidant properties of vegetable wastes and corresponding powders were evaluated by determining their phenol and flavonoid content, as well as their DPPH and ABTS antioxidant activities. Determinations were carried out on extract of samples, by using an 80% (v/v) methanol/water solution as solvent, and an extraction ratio of 0.5:10 (m/v). The mixture was continuously stirred during 1 h in a horizontal stirrer (COMECTA WY-100), and further centrifuged for 5 min at 10,000 rpm in a microcentrifuge (5804R, Eppendorf®). Measurements were performed on the separated supernatants (extracts).

Total phenolic content of samples was determined with the Folin–Ciocalteau method [18]. An aliquot of 0.125 mL of the previously prepared extract was mixed with 0.5 mL of distilled water and 0.125 of the Folin–Ciocalteau reagent (Sigma Aldrich). The mixture was allowed to react for 7 min in darkness before adding 1.25 mL of a 7% sodium carbonate solution to stop the reaction and 1 mL of distilled water until completing a volume of 3 mL. After 90 min in darkness, absorbance was measured at 760 nm with a spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, UK). Results are given in mg of Gallic Acid Equivalents (GAE) per g of dry matter, and are the average of three replicates.

The colorimetric method of aluminium chloride [19] was applied to determine total flavonoid content of samples. One-and-a-half milliliters of the extract was mixed with 1.5 mL of a 2% w/v aluminum chloride in methanol solution, and vigorously shaken after mixture. The absorbance was measured at 368 nm after 10 min of reaction, and compared to a standard curve of quercetin (purity ≥ 95%, Sigma-Aldrich). Flavonoid content is given in mg of quercetin equivalents (QE) per gram of product, in dry basis, as the average of three replicates.

Antioxidant activity (AO) of the obtained powders and vegetable wastes was measured as Trolox Equivalents, by the DPPH and ABTS methods. The former evaluates the radical scavenging ability of sample against 1,1-diphenyl-2-picryl hydrazyl (DPPH·), applying the method proposed by Brand-Williams et al. [20], which measures the change in color of a DPPH-methanol

solution when reacting with the sample. An aliquot of 100 µL of the extract was mixed with 200 mL of a 0.1 mM solution of DPPH in methanol and 900 µL of methanol. After 60 min in darkness, absorbance was measured at 517 nm in a spectrophotometer. Results were expressed in mg of Trolox Equivalent (TE) per gram of sample in dry basis. The ability to scavenge the ABTS+ cation (2,20-azobis-3-ethyl benzthiazoline-6-sulphonic acid) was measured as described in Re et al. [21]. The radical ABTS+ was released by reacting 7 mM of ABTS with potassium persulfate (2.45 mM) during 16 h at room temperature and darkness. ABTS+ was mixed with phosphate buffer (pH 7.4) to reach an absorbance of 0.70 ± 0.02 at 734 nm. An aliquot of 100 µL of the sample was added to 2900 µL of the solution ABTS+ in phosphate buffer with an absorbance of 0.70 ± 0.02 and the absorbance of samples was read after 7 min. Distilled water was used as a reference. Presented results are the average of three replicates and are expressed in mg of Trolox Equivalent (TE) per gram of dry matter.

2.3. Statistical Significance of Results

Results were statistically analyzed using Statgraphics Centurion XVI Centurion XVI.I, Statpoint Technologies, Inc.) with a confidence level of 95% (*p*-value ≤ 0.05). Data were processed by performing simple and multifactor ANOVA. All analytical determinations were performed at least in triplicate.

3. Results and Discussion

3.1. Physicochemical Properties of the Vegetable Waste Powders

Physicochemical parameters including water activity (a_w), moisture content (% grams of water/100 total grams), and soluble solids content (x_{ss}) of the raw waste materials and dried powders are summarized in Table 2. Before processing, all vegetable wastes showed very high a_w values, indicating their perishability and high risk of spoilage. Drying of these wastes to produce the powders allowed reduction of a_w values below the target (0.3), with few exceptions. Carrot and celery freeze-dried powders exhibited water activities slightly higher than the expected, but in any case, safe levels were reached. As for moisture content, similar results were obtained, and a higher moisture content was obtained for carrot and celery freeze-dried

products. On the other hand, more variability was observed among the different processing applied to the vegetable wastes, which suggested that pretreatments (freezing and milling) had different impacts on x_w values depending on the structure of the processed material. Raw vegetable wastes presented very high moisture contents (86–92%), which presumably contributed to the high a_w values and subsequent water availability to participate in spoiling reactions. Moisture content values were in the range of other fruit or vegetable (waste) powders such as blackcurrant, apple, or mango fruit pomace, as deduced from the literature [22–24]. Therefore, all the powders obtained were considered stable, since the amount of water present in the samples was very low, and not available to participate in spoilage reactions.

With regard to the drying process, air drying usually provided lower a_w and moisture content than freeze drying. This could be a consequence of air drying being prolonged until reaching the target a_w value, whereas a 24 h lyophilization cycle was applied in all cases, for which aw was not registered until the end of the process when releasing vacuum and opening the chamber. It is also postulated that freezing prior to sublimation could have produced a more compacted bed, from which water was more difficult to sublimate and desorb, when completing the freeze-drying cycle. A similar behavior was observed for the HAD wastes that had been previously stored at freezing conditions: final water content of frozen and further HAD powders was higher in most cases, except for celery, which showed almost no significant differences, and carrot, which behaved differently to the rest of vegetables. Freezing could have an impact on the sample density by reducing porosity and thus generating a compacted bed with increased resistance to drying. The different behavior shown by the carrot waste could be explained by the particular (micro)structural characteristics of carrots, which present a more rigid matrix, less susceptible to compact [25].

Table 2. Water activity (a_w), moisture content (x_w) and soluble solids content (xss) of carrot (Ca), celery (Ce), white cabbage (WC), leek-white (Lw), and leek-green (Lg) waste powders obtained. Fro: frozen, Fre: fresh; HAD: air dried, FD: freeze dried; C: chopped; G: ground. Mean ± standard deviation.

Waste/Powder	a_w	Moisture content (%)	x_{ss} (g_{ss}/g_{dm})
Carrot (Ca)	0.996±0.004^{c}	87.32±0.02^{f}	1.155±0.013^{c}
CaFD_FroG	0.358±0.013^{b}	5.04±0.06^{e}	0.645±0.007^{a}
CaHAD_FreG	0.190±0.006^{a}	2.40±0.18^{c}	0.647±0.017^{a}
CaHAD_FreC	0.204±0.013^{a}	3.2±0.5^{d}	0.694±0.007^{b}
CaHAD_FroG	0.20±0.02^{a}	0.84±0.10^{a}	0.665±0.017^{a}
CaHAD_FroC	0.18±0.03^{a}	1.25±0.06^{b}	0.658±0.017^{a}
Celery	0.994±0.008^{e}	91.7±0.4^{d}	0.522±0,007^{b}
CeFD_FroG	0.336±0.001^{d}	4.71±0.18^{c}	0.368±0.006^{a}
CeHAD_FreG	0.172±0.017^{b}	2.00±0.12^{b}	0.64±0.03^{d}
CeHAD_FreC	0.152±0.011^{a}	1.49±0.09^{a}	0.69±0.02^{e}
CeHAD_FroG	0.156±0.010^{ab}	1.1±0.3^{a}	0.579±0.017^{c}
CeHAD_FroC	0.208±0.005^{c}	1.26±0.17^{a}	0.546±0.017^{b}
White cabagge	0.999±0.003^{d}	90.2±0.2^{e}	0.725±0,007^{e}
WCFD_FroG	0.27±0.02^{c}	3.11±0.14^{d}	0.491±0.006^{a}
WCHAD_FreG	0.219±0.007^{b}	1.70±0.13^{c}	0.590±0.017^{b}
WCHAD_FreC	0.169±0.002^{a}	1.18±0.18^{b}	0.643±0.013^{c}
WCHAD_FroG	0.30±0.03^{c}	0.301±0.012^{a}	0.661±0.011^{c}
WCHAD_FroC	0.286±0.011^{c}	0.50±0.13^{a}	0.688±0.007^{d}
Leek white (Lw)	0.999±0.003^{d}	86.62±0.07^{c}	0.97±0.03^{e}
LwFD_FroG	0.088±0.013^{a}	1.3±0.3^{b}	0.48±0.02^{a}
LwHAD_FreG	0.200±0.019^{c}	1.02±0.11^{b}	0.549±0.011^{bc}
LwHAD_FreC	0.20±0.02^{c}	1.3±0.3^{b}	0.534±0.006^{b}
LwHAD_FroG	0.186±0.013^{c}	0.3±0.3^{a}	0.658±0.013^{d}
LwHAD_FroC	0.124±0.003^{b}	0.46±0.11^{a}	0.571±0.019^{c}
Leek green (Lg)	0.999±0.003^{d}	91.69±0.16^{c}	0.404±0.007^{b}
LgFD_FroG	0.079±0.003^{a}	1.6±0.5^{b}	0.34±0.02^{a}
LgHAD_FreG	0.264±0.009^{c}	1.38±0.10^{b}	0.501±0.013^{d}
LgHAD_FreC	0.261±0.013^{c}	1.6±0.5^{b}	0.432±0.017^{c}
LgHAD_FroG	0.14±0.02^{b}	0.51±0.16^{a}	0.435±0.017^{c}
LgHAD_FroC	0.100±0.014^{a}	0.28±0.09^{a}	0.446±0.013^{c}

$^{a,b,c\ldots}$Different superscript letters for a similar residue indicate statistical significant differences at the 95% confidence level (p-value < 0.05).

Particle size of powders was also determined, and characteristic parameters calculated, as previously described. This parameter was finally not obtained for the vegetable wastes stored at freezing conditions, as

during the course of the project, it was decided to proceed only with fresh samples according to the results obtained (previous physicochemical and next antioxidant), and the economic and technical convenience of processing the samples directly after being generated at the cooperative facilities. Characteristic parameter values of fresh processed samples are summarized in Table 3.

There were statistically significant differences among samples, with regard to particle size and particle distribution. In general terms, powders obtained from a vegetable matrix ground prior to dehydration exhibit a smaller particle size, as compared to chopped samples. These results confirm that not only milling after drying, but also grinding/chopping prior to drying, determines particle size characteristics. As indicated by Djantou et al. [26], elucidating the interdependence of drying and milling results essential to obtain high-quality powders, with proper nutritional and physicochemical characteristics. The green part of leek was an exception, since the previously ground vegetable waste led to a bigger particle size than the chopped one. As occurred with carrot before, structure of the green part of leek significantly differs from the rest of vegetable wastes being processed. As indicated later in this document (Section 3.3; drying curves), the green portion of leek dried significantly faster when chopped as compared to ground, which could have resulted in a more brittle structure, more easily milled after dehydration. Characteristic parameters obtained by the wet procedure were more heterogeneous among samples. In general, solubilization of soluble solids constituents in water during this determination led to a reduction in particle size parameters. Carrot powders were an exception. A possible explanation would be that smaller carrot powder particles would have completely solubilized, the bigger ones contributing more significantly to the average equivalent diameter calculated by the equipment. Graphs of particle size distributions are given as supplementary material in order to not to duplicate results; instead, percentiles d_{10}, d_{50}, and d_{90} are shown as an approximation of the distribution pattern. Distributions obtained by the wet procedure were more heterogeneous and, in occasions, a bimodal function was obtained after

soluble solids solubilization. This suggests the existence of two main groups of particles with two different (average) particle sizes.

Table 3. Particle size characteristic parameters obtained by the wet and dry procedures: equivalent volume diameter D[4,3], surface area mean diameter D[3,2] percentiles d_{10}, d_{50}, and d_{90}. Carrot (Ca), celery (Ce), white cabbage (WC), leek-white (Lw), and leek-green (Lg) waste powders obtained. Fre: fresh; HAD: air dried, FD: freeze dried; C: chopped; G: ground. Mean ± standard deviation.

		DRY PROCEDURE				
		D [4,3]	**D [3,2]**	**d_{10}**	**d_{50}**	**d_{90}**
Carrot	CaHAD_G	190±3^{d}	27.3±0.6^{b}	9.9±0.2^{a}	153±5^{d}	434±6^{ef}
	CaHAD_C	300±15^{g}	35±4^{d}	12.0±0.9^{bc}	260±12^{i}	660±35^{h}
White cabbage	WCHAD_G	190±6^{d}	37±4^{d}	14.6±1.3^{d}	165±6^{e}	407±11^{d}
	WCHAD_C	213±4^{e}	30.7±1.3^{bc}	11.3±0.7^{ab}	197±3^{g}	444±9^{f}
Celery	CeHAD_G	183±3^{d}	34.2±0.5^{cd}	14.9±0.2^{d}	136±3^{c}	428±8^{ef}
	CeHAD_C	266±25^{f}	60±8^{e}	26±5^{f}	235±28^{h}	553±40^{g}
Leek (White)	LwHAD_G	110.6±1.6^{a}	22.9±0.6^{a}	9.7±0.4^{a}	82.4±1.6^{a}	259±3^{a}
	LwHAD_C	156.0±1.8^{c}	34.8±0.4^{d}	16.7±0.3^{e}	128.1±1.5^{c}	339±4^{c}
Leek (Green)	LgHAD_G	207±3^{e}	63±2^{e}	32.0±1.5^{g}	182±3^{f}	416±7de
	LgHAD_C	137±4^{b}	30.6±1.0^{bc}	13.2±0.6^{cd}	107±4^{b}	312±8^{b}
		WET PROCEDURE				
		D [4,3]	**D [3,2]**	**d_{10}**	**d_{50}**	**d_{90}**
Carrot	CaHAD_G	165±26^{bc}	33±2^{a}	13.2±0.9^{a}	109±11^{bc}	398±68^{bc}
	CaHAD_C	147±6^{ab}	32.5±1.3^{a}	13.6±0.6^{a}	96±6^{ab}	364±18^{b}
White cabbage	WCHAD_G	285±27^{d}	55±3^{d}	22.9±1.8^{c}	242±20^{e}	610±61^{d}
	WCHAD_C	305±13^{d}	56.8±1.3^{d}	24.1±0.8^{c}	276±8^{f}	625±31^{d}
Celery	CeHAD_G	178±35^{c}	37±4^{b}	15.7±1.6^{b}	106±22^{bc}	447±82^{c}
	CeHAD_C	151±17^{b}	33.6±1.6^{a}	14±0.6^{a}	99±10^{ab}	369±45^{b}
Leek (White)	LwHAD_G	155±9^{bc}	38.8±1.3^{bc}	15.9±0.5^{b}	120±7^{c}	349±20^{ab}
	LwHAD_C	148±25^{b}	37.3±1.8^{b}	16.3±0.7^{b}	98±7^{ab}	334±52^{ab}
Leek (Green)	LgHAD_G	165±6^{bc}	40.4±0.9^{c}	17.2±0.5^{b}	139±6^{d}	353±20^{b}
	LgHAD_C	122±14^{a}	32±4^{a}	13.7±1.8^{a}	84±12^{a}	291±31^{a}

$^{a,b,c...}$Different superscript letters (dry or wet procedure) indicate statistical significant differences at the 95% confidence level (p-value < 0.05).

3.2. Antioxidant Properties of the Vegetable Waste Powders: Phenols, Flavonoids, DPPH and ABTS Antiradical Capacity

Vegetable processing, including not only drying, but also pretreatments or storage conditions, has an impact on the antioxidant properties of

vegetables. Processing conditions may promote the formation of compounds with novel antioxidant properties, thus maintaining or even enhancing the antioxidant potential of foods. In contrast, postharvest processing and storage can cause loss of antioxidants or formation of compounds with pro-oxidant action, which may also lower the antioxidant capacity [27].

Figure 1 and Figure 2 show the phenol and flavonoid content of the vegetable waste powder and their original waste, per gram of dry matter. White cabbage and leek (green portion) presented the highest phenolic concentration, calculated as gallic acid equivalents (GAE) per gram of dry matter. As compared to non-processed waste, total phenolic content usually increased when processed and, especially, when air drying was used to remove water (Figure 1). This was especially significant for white cabbage and carrot, and less significant for leek. The green part of leek and, especially, the celery, experimented a decrease in their phenolic content after processing. Phenolic constituents of celery were especially sensitive to high temperature, as this was the only case in which HAD produced a decrease in the phenolic content for all the powders, whereas FD resulted in an increased value. In this particular case, low temperature and vacuum conditions applied during FD would have better preserved phenolic constituents. The multifactor ANOVA analysis performed to the data obtained suggested that both pretreatment parameters (particle size reduction and storage conditions) significantly affected the results in a different way depending on the vegetable being dried (p-value of interaction ≤ 0.05). In the case of carrots, freezing had a significant negative impact on the phenolic content, whereas the effect of chopping vs. grinding was not significant. On the contrary, the white cabbage residue did not present significant differences with regard to storage conditions, but the chopped samples better preserved their phenolic content.

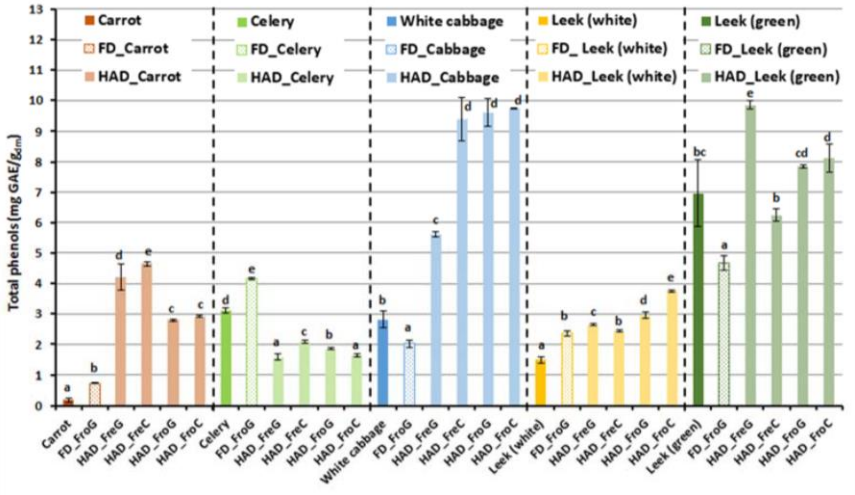

Figure 1. Total phenols content, expressed in mg of gallic acid equivalent (GAE) per gram of dry matter, in the raw bagasse (colored full bars) and vegetable waste powders. FD: freeze dried; HAD: air dried; Fro: frozen; Fre: fresh; G: ground; C: chopped. Mean values of three replicates. Bars indicate standard deviation. [a,b,c,d,e] Different superscript letters indicate statistical significant differences at the 95% confidence level (*p*-value < 0.05), for each vegetable waste.

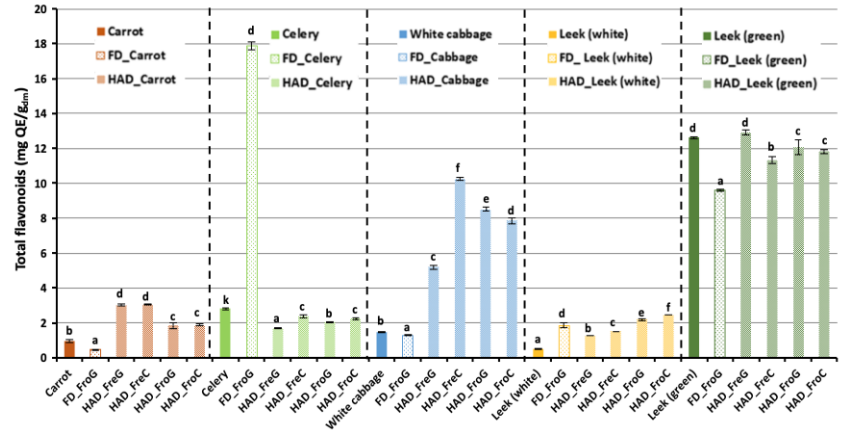

Figure 2. Total flavonoid content, expressed in mg of quercetin equivalents (QE) per gram of dry matter, in the raw bagasse (colored full bars) and vegetable waste powders. FD: freeze dried; HAD: air dried; Fro: frozen; Fre: fresh; G: ground; C: chopped. Mean values of three replicates. Bars indicate standard deviation. [a,b,c,d] Different superscript letters indicate statistical significant differences at the 95% confidence level (*p*-value < 0.05), for each vegetable waste.

As for flavonoid content (Figure 2), results were more heterogeneous. Flavonoids were especially present in the green part of leek and white cabbage, as in the case of phenols, the other residues and corresponding powders presenting a lower content. The effect of the variables analyzed was not statistically significant. This spectrophotometric procedure is especially influenced by the difference in absorbance between the major flavone in the sample and that one use as a reference value [28], although it is useful for comparison purposes.

DPPH and ABTS antioxidant (AO) activities are summarized in Figure 3 and Figure 4. Again, AO properties of cabbage and green leek vegetable wastes and powders were significantly higher. In this case, also carrots, mainly non-processed, exhibited significant antioxidant properties. This difference with the previous analysis (phenols and flavonoids) was most probably due to carotenoids, one of the main bioactive compounds in carrots that exhibited significant AO activity, but are not detected by the previous methods [29,30]. The effect of processing variables depended again on the vegetable matrix being analyzed. In most cases, processing resulted in an increased AO capacity of samples. Storing in freezing conditions did not significantly affect leek and cabbage, although a negative effect was evidenced for carrot and celery. Except for celery, freeze-drying did not improve the AO capacity of powder, thus suggesting that, more than preserving the bioactive compounds present in the raw wastes, hot air drying would be inducing the formation of new antioxidant compounds. Grinding or chopping did not significantly affect the AO of powders in most cases. This implies that conditions of this stage will be selected as a function of drying behavior patterns, rather than functional properties of powders. Nevertheless, it must be taken into account that other important functional parameters such as fiber content may be affected by particle size [31], for which this parameter needs to be known before making a decision. In addition, more detailed information on specific bioactive compounds potentially present in the vegetable wastes and their powders, such as carotenoids in carrots [29], apigenin in celery [32], glucosinolates in white cabbage, [33] or sulfoxides in leek [34], would be convenient to know, to discriminate more precisely which components are negatively affected by

processing and which others enhanced. For this purpose, High-Performance Laser Chromatography (HPLC) analyses are planned to be performed in the following months.

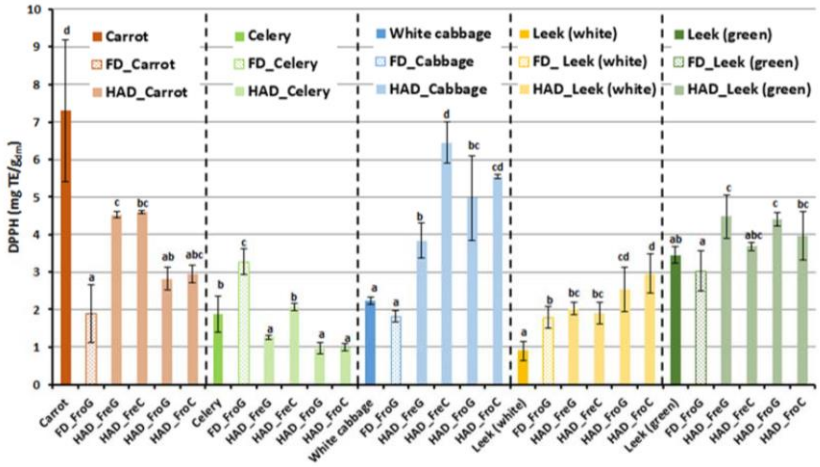

Figure 3. Results of antioxidant activity by the DPPH method, expressed in mg of trolox equivalent (TE) per gram of dry matter, of the raw bagasse (colored full bars) and vegetable waste powders. FD: freeze dried; HAD: air dried; Fro: frozen; Fre: fresh; G: ground; C: chopped. Mean values of three replicates. Bars indicate standard deviation. ^{a,b,c,d} Different superscript letters indicate statistical significant differences at the 95% confidence level (p-value < 0.05), for each vegetable waste.

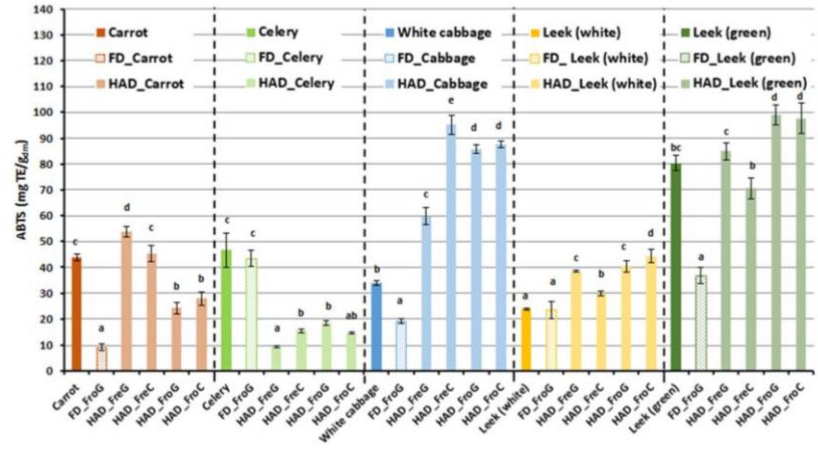

Figure 4. Results of antioxidant activity by the ABTS method, expressed in mg of trolox equivalent (TE) per gram of dry matter, of raw bagasse (colored full bars) and vegetable waste powders. FD: freeze dried; HAD: air dried; Fro: frozen; Fre: fresh; G:

ground; C: chopped. Mean values of three replicates. Bars indicate standard deviation. [a,b,c,d,e] Different superscript letters indicate statistical significant differences at the 95% confidence level (p-value < 0.05), for each vegetable waste.

3.3. Drying and Drying Rate Curves of Vegetable Wastes, As Affected by Each Vegetable Matrix

Figure 5 shows the drying curves obtained, i.e., the variation in water content (dry basis) in the reduced form (X_w/X_0) against processing time, during air drying at 70 °C of a 10 mm thick layer of the vegetal wastes subjected to different pretreatments which affect the structure matrix. Drying curves provide information about the exposure time to air under certain conditions that is required to reach a specific moisture content. Obviously, samples requiring less time would dry faster. In all the analyzed products, except for carrot waste, chopping instead of grinding was recommended to shorten the drying time. A more compact structure might have resulted after crushing, so that the superficial area in direct contact with the air stream would be reduced as compared to the respective chopped sample. This effect was particularly evident in the green portion of leek, in which the drying time required to reduce the initial moisture content by 80% increased from 260 min (4.3 h) for chopped samples to 470 min (7.8 h) for ground samples. However, these differences were much less pronounced in the case of white cabbage residue and the white portion of leek residue, thus suggesting that the structure of chopped and ground samples were more similar for those products. As for carrot residue, the time required to reduce the initial moisture content by 80% decreased from 420 min (7 h), for chopped samples, to 350 min (5.8 h), for ground samples. Carrot residue is known to have a considerable rigid structure, as indicated previously, for which grinding may have favored the breakage of such structures and the release of the water retained inside [25].

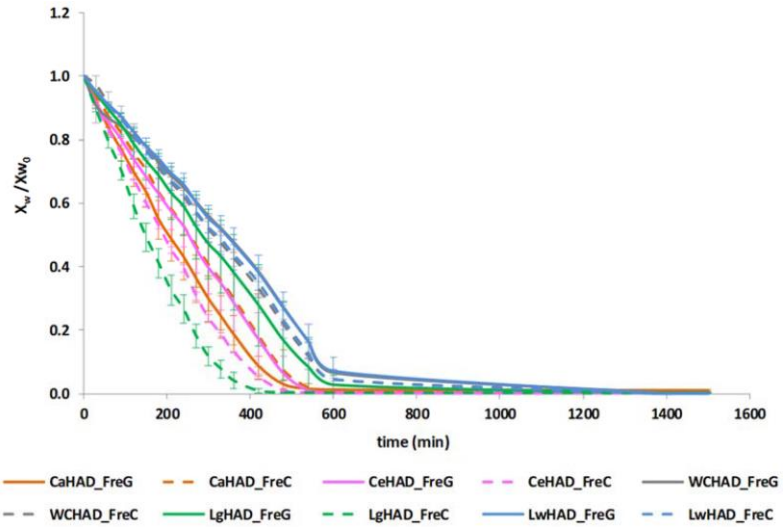

Figure 5. Drying curves (moisture content in g_w/g_{dm}) of chopped (C) and ground (G) vegetable wastes. Ca: carrot; Ce: celery; WC: white cabbage; Lg: leek green; Lw: leek white.

Drying rate curves (Figure 6) not only confirmed the already mentioned results with regard to matrix and pretreatment impact on drying behavior of samples, but also evidenced that most of the water in the residues was eliminated at the constant drying rate period (CDRP) under constant drying conditions. Drying rate during the CDRP ranged between 0.019 $g_w \cdot g_{dm}^{-1} \cdot min^{-1}$, for both chopped and ground white portions of leek residue, and 0.053 $g_w \cdot g_{dm}^{-1} \cdot min^{-1}$, for chopped celery. The study of drying kinetics is also relevant because it allows discriminating between the CDRP in which resistance to water transfer in the product is low as compared to evaporating rate, for which there is external control of mass transfer and adiabatic conditions of drying prevail, from the falling drying rate period (FDRP) in which there is internal control of mass (water) transfer. The inflection point between both periods indicates the critical moisture content, at which drying rate starts to decrease. In the vegetable wastes studied, critical moisture content was in most cases below 80% of the initial one. As long as the water content of the solid is higher than the critical one, the product could be dried at high temperature without significantly affecting their functional properties; thus, temperature effect on dried matrices would become more

important during the FDRP. According to these results, if thermolabile compounds are to be preserved, the drying process could be improved by reducing the dryer temperature when reaching critical moisture content.

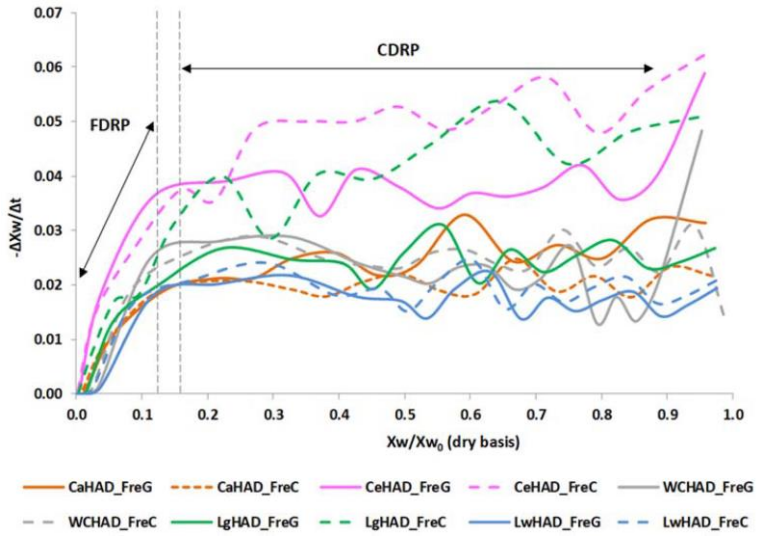

Figure 6. Drying rate curves (moisture content in g_w/g_{dm}) of chopped (C) and ground (G) vegetable wastes. Ca: carrot; Ce: celery; WC: white cabbage; Lg: leek green; Lw: leek white. CDRP: constant drying rate period; FDRP: falling drying rate period.

4. Conclusions

The sustainable development goals defined by the Food and Agricultural Organization of the United Nations especially focus on the sustainability of food systems and especially mentions the coordination of global initiatives, activities, and projects on food losses and waste reduction. Fruit and vegetables industries (fresh and processed) significantly contribute to food waste numbers, participating of an increasing nutritional, economical, and environmental problem. There is urgent need for the development of processes which allow their reuse by re-introducing the wastes in the productive cycle, contributing to the circular economy, engaging all the agents involved.

The present study is an example of successful collaboration between primary producers (agri-food cooperative) and university, together with

regional government as facilitators of European funds for rural development and sustainable development. Vegetables residues have been successfully transformed into functional ingredients by a series of processes involving pretreatments and drying stages, aimed at maximizing bioactive compounds and their antioxidant activity. Results showed that drying allowed obtaining stable powders, with very low water activity values and a significantly increased functionality. The powders obtained could be used in the food industry as coloring and flavoring ingredients, or either as natural preservatives. In addition, they also have application in the reformulation of processed foods to improve their nutritional properties, and thus participate of another of the FAO goals: Ensure healthy lives and promote well-being for all at all ages. At present, the project continues on evaluating the effect of processing parameters on the concentration of specific bioactive constituents and dietary fiber, both being important phytochemicals in the fight against non-communicable diseases such as obesity and related disorders, which are becoming an epidemic and a major public health concern. A proposal of pilot plant is also being developed so as to manufacture the powders at the cooperative facilities, where wastes are produced, to evaluate economic feasibility of the processes.

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Physicochemical, technological and functional properties of upcycled vegetable waste ingredients as affected by processing and storage

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Under review

Abstract

Vegetable wastes are generated during harvesting, processing and distribution, and imply a wastage of nutrients which evidence inefficiencies in present food systems. Vegetable residues are a rich source of bioactive compounds for which their valorisation and reintroduction into the food chain may play a key role towards circular economy and more sustainable food systems. In the present work, upcycled powdered ingredients were obtained from vegetables wastes (carrot, white cabbage, celery and leek) through a feasible disruption, dehydration and milling process. Disruption pre-treatment at different intensities was followed by a freeze-drying or hot-air drying (at 60 and 70 °C) step, and subsequent milling for the production of fine powders. Powdered products were characterized in terms of physicochemical, antioxidant and technological properties (water and oil interaction properties), just after processing and during four months of storage. Antioxidant properties were generally favoured by hot-air drying, particularly at 70 °C, which could be due to new compounds formation combined to less exposure time to drying conditions. The powders showed interesting water interaction properties, especially freeze-dried ones. During the storage period the quality of the powders decreased, moisture migration increased, and antioxidant compounds generally reduced. Changes in colour properties were also evidenced. Upcycled vegetable waste powders are proposed as ingredients to fortify foods, both processing and storage conditions determining their properties.

Keywords: vegetables wastes, by-products valorisation, upcycled ingredients, bio-waste processing, storage stability, functional ingredients, functional properties.

1. Introduction

Intensive food production, including fruits and vegetables processing, implies the use of energy and other resources, for which food discard and food waste generation is a matter of great concern that should be urgently addressed. Fruit and vegetables production generate remarkable amounts of residues due to both processing wastes and discards due to high commercialization standards. However, this plant material is rich in nutrients and has a potential to improve the diets of people suffering from nutritional disorders. The reintroduction of these residues into the food chain is a step towards circular economy and more sustainable food systems [1], thus contributing to the achievement of the Sustainable Development Goals approved by FAO [2]; among others, Goal 3 on ensuring healthy lives and promoting well-being for all at all ages; and Goal 12 on responsible production and consumption [3]. FAO encourages a shift to nutritious and safe diets with a lower environmental footprint.

Fresh vegetables perish rapidly after harvesting due to their high moisture content and water activity values, which makes them susceptible to microbial spoilage [4,5]. Drying is a preservation technique commonly applied to reduce water content to safe levels [6], thus minimizing microbial spoilage and other deterioration reactions, extending shelf life of food products and making them suitable for safe storage [5]. Hot-air drying is the most extended drying method used in the food industry, and characterizes by lower production and investment costs; in contrast, freeze-drying is a more expensive method which requires the need for qualified staff, but yields highest quality final products [4,6]. Dried powders obtained from fruit or vegetables are versatile products, physicochemically and microbiologically stable. In addition, powdered fruits and vegetables may be used as ingredients in functional food development [7] since they can be used to reformulate products to obtain healthier alternatives. Similarly, most fruit and vegetable wastes (discards, by-products) consist of edible parts that can also be transformed into powdered products rich in bioactive compounds, thus integrally valorizing these wastes.

Processing parameters in powder manufacturing, including pretreatments, drying stage and milling conditions, determine the functional and technological properties of the product [8]. The effect of drying on powders characteristics has been evaluated on several fruits and vegetable crops, revealing that heat treatment can produce physical, structural, chemical, and biological changes on the raw material, as well as induce a loss of nutrients and phytochemicals unstable to heat [5,9,10]. The impact of the drying stage depends on the product, the technique, and the drying conditions applied. For instance, high drying temperatures can improve the antioxidant properties of the product by activating enzymes that act in the conversion of phenolic compounds releasing products with improved antioxidant capacity [9,11]; but high temperatures can also have a negative impact on the antioxidant properties of the dried product [4]. On the other hand, milling conditions (pre- or post-drying) determine particle size characteristics and thus, powders' properties [1,12]. Both drying and milling are interdependent [12,13], size reduction prior to drying modifies drying behavior as it determines mass transfer mechanisms; whereas the structure generated during drying affects the result after milling.

Storage and distribution may also have a significant impact on physical, chemical and biological characteristics of foods, causing a reduction in product quality [6]. In this sense, powdered dehydrated products require protection against oxygen, moisture, high temperature or light, since postharvest processing and storage may induce reaction mechanisms leading to food degradation with the loss of volatile flavorings and color as well as antioxidants [5], or formation of compounds with pro-oxidant action which may lower the antioxidant capacity [14]. Therefore, determining the product stability during storage is crucial, since both the physicochemical and antioxidant status may be affected.

The aim of this research was to obtain new powdered ingredients from the vegetable wastes generated at the early stages of processing in an agricultural cooperative, and evaluate the impact of processing and storage on powders properties. To this end, carrot, white cabbage, celery and leek wastes followed a disruption, drying and milling process to integrally valorize their constituents in powdered form. Then, physicochemical, antioxidant

and technological properties of the upcycled vegetable waste ingredients were assessed just after processing and during a four-month storage period.

2. Materials and methods

2.1. Vegetable wastes used as raw materials

Raw materials (vegetable wastes) were generated in the processing lines of the agricultural cooperative Agrícola Villena, Coop. V. (Alicante, Spain). These consisted of wastes of the ready-to-eat lines in the case of carrot (*Daucus carota*, L.) and celery (*Apium graveolens*, L.); and wastes of the fresh pre-packed vegetables lines, in the case of cabbage (*Brassica oleracea* var. *capitata*, L.) and leek (*Allinum porrum*, L.).

2.2. Powders manufacturing

Once received at the laboratory facilities, vegetable wastes were processed freshly so that the plant material was disrupted with a food processor (Thermomix® TM6, Vorwerk, Madrid, Spain) to reduce particle size to pieces of ≤ 10 mm diameter (chopped, C), or pieces of ≤ 5 mm diameter (grounded, G). Conditions for tissue disruption were set according to previous experiences [1]. Disruption was followed by a dehydration treatment, either hot-air drying (HAD) or freeze-drying (FD). HAD was conducted in a convective tray dryer (Pol-eko Aparatura, Katowice, Poland) until water activity (a_w) was reduced below 0.3. To this aim, ground and chopped residues were distributed on the dryer trays (~200 g of residue/tray) in 10 mm thick layers, and dried at 60 °C or 70 °C of temperature with an air stream velocity of 2 m/s. FD was carried out in a freeze dryer (Lioalfa-6, Telstar, Terrasa, Spain) for 24 h under freezing conditions (-45 °C) and sub-atmospheric pressure (P = 0.1 mbar), with previous sample grinding and freezing in a deep freezer (Matek CVN-40/105) at −40 °C during 24 h. After drying processes, dried materials were milled (10,000 rpm for 2 min at 30 s intervals) (Thermomix® TM6, Vorwerk, Madrid, Spain) to obtain the final fine-grained powder. Powders were packed into glass jars in a light-free environment and stored during 4 months at room temperature (24-27 °C). Particle size characteristics and technological properties (water and oil interaction properties) were measured after

powder manufacturing. Physicochemical characteristics (including water activity, moisture content, total soluble solid content and optical properties) as well as antioxidant properties (total phenol and flavonoid content and antioxidant capacity) were measured both, just after being processed and after 2, 3 and 4 months of storage.

The experimental design yielded 20 types of powders, which will be identified next by type of waste: Carrot (Ca), Celery (Ce), White cabbage (WC) and Leek (L); the pre-treatment applied: Ground (G) or Chopped (C); and the drying method used: Hot-air drying at 60 °C (HAD60) or at 70 °C (HAD70), or Freeze-drying (FD).

2.3. Analytical determinations

2.3.1. Physicochemical and antioxidant properties

Moisture content (x_w) was measured according to the official method 934.06 of the AOAC [15], based on water removal of samples during vacuum drying (Vaciotem, JP Selecta) (P = 10 mmHg) at 60 °C until constant weight. *Water activity* (a_w) was obtained with a dewpoint hygrometer at 25 °C (Aqualab 4TE; Decagon devices Inc., USA). *Total soluble solids content* (x_{ss}) was determined by a thermostatic refractometer (Abbe Atago 3-T, Japan) through the measurement of Brix degrees at 20 °C, according to the ISO 1743:1982 method. When necessary, Brix measurements were obtained from an aqueous extract of soluble solids in a 1:10 (w/v) ratio. *Particle size distribution* was determined in dry and wet conditions, using a Malvern Mastersizer equipment (Model 2000; Malvern Instruments Limited, UK). For the dry method, the equipment was coupled to a dispersion unit Scirocco 2000 with air as dispersant at 2.5 bar of pressure and 60% speed. For the wet method, the equipment was coupled to a unit Hydro 2000, setting the particle absorption index at 0.1, and using refractive indexes of 1.52 and 1.33 for the sample and for the dispersed phase (deionized water), respectively. Results were obtained as equivalent volume mean diameter D[4,3] and surface area mean diameter D[3,2], as well as the distribution percentiles d_{10}, d_{50}, and d_{90}. Optical properties were measured with a spectrocolorimeter (Minolta CM 3600D, Konica Minolta Sensing, Inc, Japan), using the illuminant D65 and an observer angle of 10° as reference. *Color coordinates* of the

CIEL*a*b* color space, and resultant C_{ab}* (chroma) and hab (hue), were obtained by reflectance from the absorption spectrum provided by the equipment in the 380-770 nm range. Readings were made on a black background and placing samples in standardized size plastic cuvettes (37 × 50 × 22 mm). Color changes during powders storage was calculated by means of Equation 1 [16].

$$\Delta E = \sqrt{(L_i^* - L_n^*)^2 + (a_i^* - a_n^*)^2 + (b_i^* - b_n^*)^2} \quad (1)$$

where L_i*, a_i*, and b_i* are the color parameters of the powders after processing and L_n*, a_n*, and b_n* are color parameters of the stored powder at month n.

Antioxidant properties of vegetable wastes powders were measured by determining phenol and flavonoid compounds, and antioxidant activity by the DPPH and ABTS methods. Determinations were accomplished on extracts of samples, using an 80% (v/v) methanol/water solution as the extracting solvent, and an extraction ratio of 1:20 (w/v). Extracts were obtained by stirring the powder and solvent during 1 h in a horizontal stirrer (COMECTA WY-100, Comecta, Barcelona, Spain), and then centrifuged for 5 min at 10,000 rpm (Eppendorf Centrifuge 5804/5804R, Eppendorf SE, Hamburg, Germany). Measurements were carried out on the separated supernatants (extracts). An 80% (v/v) methanol/water solution replacing the extract was used as a blank in all analyses.

Total phenolic content was determined using the modified method of Folin-Ciocalteu [17,18]. For the analyses, 0.125 mL of the extract were mixed with 0.5 mL of bidistilled water and 0.125 mL of the Folin-Ciocalteu reagent (Sigma Aldrich). The mixture was kept 6 min in darkness, followed by the addition of 1.25 mL of sodium carbonate solution (7%) and 1 mL of bidistilled water. After 90 min in darkness, absorbance was measured at 760 nm with a spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, UK). Results were expressed in mg of Gallic Acid Equivalents (GAE) per g of dry matter. *Total flavonoid content* was measured following the modified colorimetric method of aluminium chloride [19]. Accordingly, 1.5 mL of the extract were mixed with 1.5 mL of a 2% w/v aluminium chloride in methanol solution.

After reaction for 10 min in darkness, absorbance was measured at 368 nm. Results were expressed in mg of Quercetin Equivalents (QE) per g of dry matter.

Antioxidant activity was measured by the DPPH and ABTS radical methods. The ability to scavenge the DPPH radical was determined applying Brand-Williams et al. [20] method, with some modifications. Thus, 0.1 mL of the extract were mixed with 2 mL of a 0.1 mM solution of DPPH (2,2-diphenyl-1-picryl hydrazyl) in methanol and 0.9 mL of methanol. The mixture reacted during 60 min in darkness and the absorbance was measured at 575 nm in a spectrophotometer (Helios Zeta UV/Vis, Thermo Scientific, UK). The ability to scavenge the ABTS radical was measured following the method described by Re et al. [21]. $ABTS^{+}$ free radical (2,2-azobis-3-ethyl benzthiazoline-6-sulphonic acid) was obtained by preparing a solution of 7 mM of ABTS and 2.45 mM of potassium persulfate and left to react during 16 h in darkness at room temperature. $ABTS^{+}$ solution was mixed with phosphate buffer (pH 7.4) until an absorbance of 0.70 ± 0.02 at 734 nm was reached. Measurements were performed by mixing 0.1 mL of the extract and 2.9 mL of the ABTS+ solution. Absorbance was measured at 734 nm after 7 min of reaction. Regardless of the method used, the antioxidant activity was expressed in mg of Trolox Equivalent (TE) per g of dry matter.

2.3.2. Water interaction and oil emulsifying properties

Specific volume of powders was determined by measuring the volume of 5 g of sample in a 10 mL test tube. *Solubility*, which is the mass fraction of dissolved solids (DS) in the hydrated sample, was obtained following the method described by Mimouni et al. [22] as the ratio between the total soluble solids content (x_{ss}) and the total solids content ($1-x_w$). *Hygroscopicity* was determined by the method proposed in Cai & Corke [23], based on water gain when the product is kept inside an airtight container with a saturated solution of sodium sulphate at room temperature (25 °C) during one week. Results were expressed in g of water/100 g of sample. *Wettability* was defined as the time in which 2 g of powder in 20 mL of distilled water get fully wet [24]. *Swelling capacity* (SC) was calculated following the Raghavendra et al. [25] method, as the ratio between the volume of the

sample when immersed in water excess after 18 h at 25 °C, and the initial weight of the sample. Results were given in mL/g. *Water holding capacity* (WHC) was determined as the amount of water retained by the sample without the application of any external force, except gravity and atmospheric pressure [25]. WHC was calculated following equation 2, which calculates the ratio between the amount of water contained in the hydrated powder (0.2 g of powder hydrated with 10 mL of water, during 18 h at 25 °C) (HR) and the dry weight of the powder after freeze-drying (DR). *Water retention capacity* (WRC) was obtained as the amount of water retained by the sample when subjected to an external force such as pressure or centrifugation [25]. Around 1 g of powder was hydrated with 10 mL of water during 18 h at 25 °C. Then, the mixture was centrifuged at 2000 rpm for 30 min, discarding the supernatant and obtaining the weight of the decanted residue (W), which was then freeze-dried and weighed (R). WRC was calculated as the ratio between the water retained by the powder (W) and the dry weight of the residue (DR).

$$WHC = \frac{HR\text{-}DR}{DR} \quad (2)$$

$$WRC = \frac{W}{DR} \quad (3)$$

Oil holding capacity (OHC) was obtained according to Garau et al. [26]. Around 0.2 g of powder were mixed with 1.5 g of sunflower oil and kept overnight at room temperature. Mixture was centrifuged at 1500 x g for 5 min, discarding the supernatant and obtaining the weight of the residue. Results were expressed in g of oil absorbed per g of powder. *Emulsifying activity* (EA) was carried out following Yasumatsu et al. [27] method. A 2% (w/v) aqueous powder solution was mixed with sunflower oil and homogenised with a vortex (Reax top, Heidolph, Germany) during 5 min at 2400 rpm; then, the resulting emulsion was centrifuged at 10,000 rpm for 5 min. Volume of emulsion formed was calculated according to equation 4, where VEL is the emulsion volume (mL) and V is the total fluid volume (mL). *Emulsion stability* (ES) was determined by the modified method of Yasumatsu et al. [27]. A 2% (w/v) aqueous powder solution was mixed with sunflower oil and vortexed at 2,400 rpm during 5 min. The emulsion was heated up to 80 °C for 30 min, tempered at room temperature, and then

centrifuged at 2,000 rpm for 5 min. ES was calculated by means of equation 5, where VREL is the volume of the emulsion layer (mL) and V is the total fluid volume (mL).

$$\%EA = \frac{V_{EL}}{V} \cdot 100 \quad (4)$$

$$\%ES = \frac{V_{REL}}{V} \cdot 100 \quad (5)$$

2.4. Statistical analysis

All analytical determinations were determined at least in triplicate. Results were statistically analysed using Statgraphics Centurion software (Centurion XVII.I, StatPoint Technologies, Inc.). One-way ANOVA and Multifactor ANOVA were carried out to analyse statistical significance of results, at the 95% confidence level. SPSS 16.0 statistics software (IBM SPSS) was used for principal Component Analyses (PCA).

3. Results and discussion

3.1. Impact of processing conditions on the vegetable powders properties

3.1.1. Impact of processing on particle size

Particle size characteristic parameters D [4,3], D [3,2], d_{10}, d_{50} and d_{90} of the powders are summarized in Table 1, whereas distributions are shown in Figure 2. Both, previous milling intensity and the dehydration method applied had an impact on powders' particle size, so that statistically significant differences were observed among samples. In line with previous studies, it was confirmed that chopping before drying leads to coarser particle sizes than grinding [1,28]. Even if chopping usually implies shorter drying times, due to a less compacted bed which facilitates water migration to the surface through the inter-particle spaces, faster drying rates and larger particles being dried have also been related to case-hardening phenomena. Case-hardening makes it more difficult to reach low moisture content in the core of the particle, thus leading to rubbery materials [29] which are less crispy and more difficult to mill [30] and, consequently, coarser particles are obtained after milling. According to Jung et al. [31], average particles moisture content influences grinding properties, particle size distribution, grinding energy and product yield; that is, wet materials imply larger average

particle sizes, whereas low moisture content materials are generally more brittle, and more easily powdered.

Regarding the dehydration technique applied, freeze-drying implied finer particle size powders than air-drying. One possible reason for that is that the porous structure generated during freeze-drying facilitates milling [32]. On the other hand, no clear trend was observed regarding air temperature (60 °C or 70 °C) on particle size. While drying at 70 °C implied coarser particles in the case of celery and leek residues, the opposite behavior was observed in carrot and white cabbage, although differences were not always significant in the latter. This could be attributed to differences among products with regard to matrix characteristics and their response to drying. In Bas-Bellver et al. [28] it was observed that the effect of temperature on particle size was more evident in broccoli than in white cabbage, as a consequence of the different drying behavior of both residues. As for particle size distribution patterns (figure 1), results obtained using the wet procedure slightly shifted to the right side, as compared to the dry procedure ones. This increase in the particle size could be attributed to both, the solubilisation of small particles, and the formation of aggregates when powders were dispersed in water.

Table 1. Particle size characteristic parameters obtained by the wet and dry procedures: equivalent volume diameter D[4,3], surface area mean diameter D[3,2], percentiles d_{10}, d_{50}, and d_{90}. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying. Mean and standard deviation of three replicates.

	Particle size characteristics using the dry procedure				
	D [4,3]	**D [3,2]**	**d_{10}**	**d_{50}**	**d_{90}**
Ca G_HAD60	171±6^{g}	34.6±1.3^{g}	11.6±0.3^{h}	137±7^{i}	391±10^{g}
Ca C_HAD60	210±6^{j}	51.2±1.5^{i}	17.8±0.5^{k}	190±7^{l}	442±12^{i}
Ca G_HAD70	155±3de	26.0±0.6^{d}	8.6±0.2^{cde}	126±5^{g}	358±5^{cd}
Ca C_HAD70	200±6^{i}	50±3^{i}	17.36±0.7^{jk}	180±7^{k}	423±9^{h}
Ca FD	124±3^{b}	33.1±1.7^{g}	12.5±0.8^{i}	107±3^{f}	258±6^{a}
WC G_HAD60	161±4^{ef}	24.8±0.7^{cd}	8.5±0.3^{bcd}	134±4^{hi}	362±6de
WC C_HAD60	214±7^{j}	48±2^{h}	17.0±0.9^{j}	197±7^{l}	435±12^{hi}
WC G_HAD70	167±6^{fg}	28.1±0.7^{e}	9.6±0.2^{g}	140±7^{i}	370±13^{def}
WC C_HAD70	183±5^{h}	26.1±0.3^{d}	9.2±0.2^{g}	165±5^{j}	388±9^{fg}
WC FD	102±3^{a}	18.6±1.3^{a}	6.7±0.5^{a}	72±4^{b}	246±5^{a}
Ce G_HAD60	129±8^{bc}	23.2±1.3^{bc}	9.2±0.4^{fg}	71±6^{b}	341±18^{c}
Ce C_HAD60	262±9^{k}	60±2^{j}	24.5±0.5^{l}	220±7^{m}	567±21^{j}

Ce G_HAD70	148±27^{d}	23.3±0.9^{bc}	9.1±0.3^{efg}	75±6^{bc}	372±51^{efg}
Ce C_HAD70	280±16^{l}	69±3^{k}	28±2^{m}	240±15^{n}	594±32^{k}
Ce FD	100±4^{a}	19.5±1.3^{a}	7.9±0.6^{b}	58±4^{a}	262±11^{a}
L G_HAD60	125±2^{bc}	22.7±0.2^{b}	8.23±0.09^{bc}	89±2^{d}	300±5^{b}
L C_HAD60	158±3^{ef}	30.8±0.6^{f}	11.8±0.3^{h}	125±3^{g}	359±6^{cd}
L G_HAD70	134±2^{c}	24.0±0.3^{bc}	9.07±0.14^{efg}	97.3±1.4^{e}	319±4^{b}
L C_HAD70	163±4^{efg}	29.6±0.7^{ef}	11.3±0.4^{h}	128±5^{gh}	369±7de
L FD	109±2^{a}	22.5±0.9^{b}	9.0±0.4^{efg}	82±3^{c}	256±3^{a}

Particle size characteristics using the wet procedure

	D [4,3]	**D [3,2]**	**d_{10}**	**d_{50}**	**d_{90}**
Ca G_HAD60	245±24^{efg}	53±3^{f}	20.6±1.2^{hi}	209±20^{cd}	530±54^{fg}
Ca C_HAD60	348±37^{i}	79±2^{h}	35.4±1.1^{l}	292±16^{gh}	723±97^{i}
Ca G_HAD70	228±8^{def}	48±2^{e}	18.8±1.1^{gh}	194±9^{c}	500±23^{efg}
Ca C_HAD70	273±18^{gh}	67±2^{g}	28.8±0.8^{j}	249±16^{ef}	562±37^{gh}
Ca FD	156±2^{a}	39.0±0.4^{bcd}	15.8±0.3^{cdef}	123.6±1.4^{ab}	350±6^{ab}
WC G_HAD60	218±9de	41.5±1.0^{d}	14.8±0.4^{bcde}	190±8^{c}	471±21^{def}
WC C_HAD60	300±34^{h}	52±3^{ef}	18.9±1.4^{gh}	257±19^{fg}	626±78^{h}
WC G_HAD70	228±12^{def}	37.9±1.0^{bcd}	12.9±0.4^{ab}	192±9^{c}	505±32^{efg}
WC C_HAD70	271±18^{gh}	48±2^{e}	17.8±0.8^{fg}	243±14^{def}	569±46^{gh}
WC FD	171±6^{abc}	40.8±0.4^{d}	16.23±0.14^{ef}	138±3^{b}	376±15^{abc}
Ce G_HAD60	207±21^{cd}	35.9±1.1^{bc}	13.7±0.3^{abc}	129±10^{ab}	508±57^{efg}
Ce C_HAD60	381±85^{ij}	69±10^{g}	30±5^{k}	294±58^{h}	824±132^{j}
Ce G_HAD70	164±14^{ab}	30.8±1.4^{a}	11.8±0.4^{a}	896±71^{i}	418±33^{bcd}
Ce C_HAD70	391±66^{j}	75±7^{h}	34±4^{l}	312±32^{h}	933±150^{k}
Ce FD	154±4^{a}	38.4±0.6^{bcd}	15.9±0.2^{def}	111±3^{ab}	362±9^{ab}
L G_HAD60	195±10^{bcd}	39.8±1.0^{cd}	14.9±0.5^{bcde}	146±4^{b}	450±30^{cde}
L C_HAD60	260±42^{fg}	54±5^{f}	22±3^{i}	212±34^{cde}	564±87^{gh}
L G_HAD70	200±7^{bcd}	38.1±0.6^{bcd}	14.1±0.3^{bcd}	146±4^{b}	470±19^{def}
L C_HAD70	250±9^{efg}	54±2^{f}	22.2±1.3^{i}	214±12^{cde}	538±15^{fg}
L FD	139±4^{a}	35.0±0.7^{b}	14.1±0.3^{bdc}	99±3^{a}	330±11^{a}

$^{a,b,c...}$Different superscript letters in the same column for a similar residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

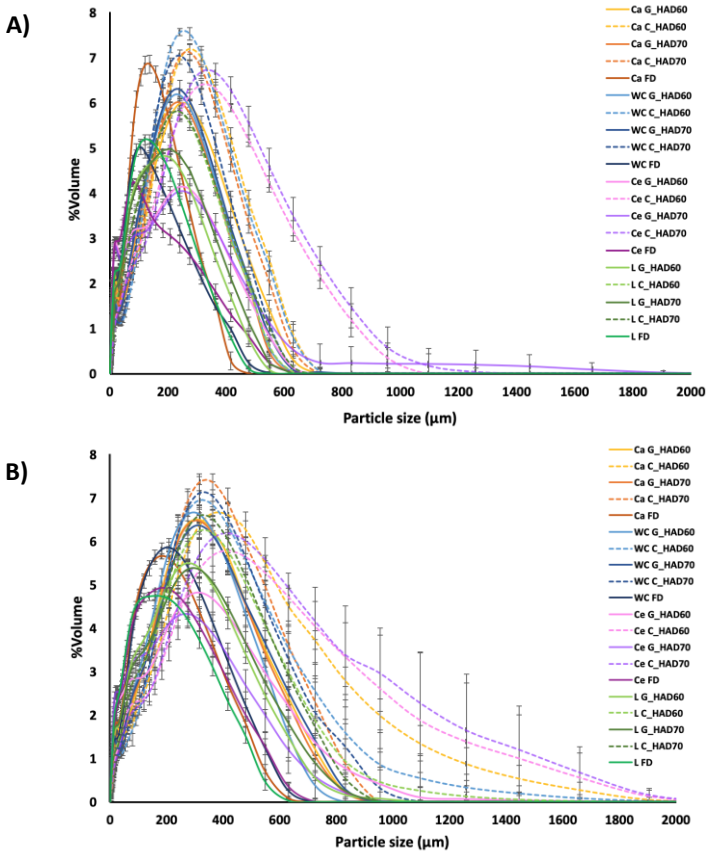

Figure 1. Particle size distribution of vegetable waste powders. A) Determination by the dry procedure. B) Determination by the wet procedure. Error bars represent the standard deviation of five replicates. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying.

3.1.2. Physicochemical and antioxidant properties of vegetable waste powders as affected by processing conditions

Water activity (a_w), moisture content (grams of water/100 total grams), and total soluble solids content (x_{ss}) of the powders obtained are shown in Table 2.

Table 2. Water activity (a_w), moisture content (g_{water}/100 g) and soluble solids content ($g_{soluble\ solids}/g_{dry\ matter}$) of vegetables waste powders after processing. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot air-drying; FD: freeze-drying. Mean and standard deviation of three replicates.

	a_w	Moisture (%)	x_{ss} (g/g_{dm})
Ca G_HAD60	0.254 ± 0.008^{b}	2.9 ± 0.4^{b}	0.667 ± 0.017^{a}
Ca C_HAD60	0.239 ± 0.010^{ab}	2.96 ± 0.10^{b}	0.659 ± 0.017^{a}
Ca G_HAD70	0.236 ± 0.011^{a}	1.62 ± 0.32^{a}	0.685 ± 0.011^{ab}
Ca C_HAD70	0.240 ± 0.005^{ab}	3.26 ± 0.12^{b}	0.709 ± 0.012^{bc}
Ca FD	0.236 ± 0.007^{a}	2.80 ± 0.11^{b}	0.724 ± 0.018^{c}
WC G_HAD60	0.223 ± 0.003^{c}	2.95 ± 0.02^{c}	0.565 ± 0.017^{b}
WC C_HAD60	0.192 ± 0.006^{b}	2.55 ± 0.15^{b}	0.591 ± 0.017^{b}
WC G_HAD70	0.223 ± 0.024^{c}	1.6 ± 0.3^{a}	0.512 ± 0.017^{a}
WC C_HAD70	0.176 ± 0.008^{b}	2.27 ± 0.05^{b}	0.491 ± 0.013^{a}
WC FD	0.121 ± 0.006^{a}	2.33 ± 0.07^{b}	0.58 ± 0.02^{b}
Ce G_HAD60	0.181 ± 0.008^{b}	1.45 ± 0.10^{ab}	0.531 ± 0.006^{c}
Ce C_HAD60	0.232 ± 0.007^{d}	2.7 ± 0.2^{c}	0.49 ± 0.03^{ab}
Ce G_HAD70	0.205 ± 0.010^{c}	1.10 ± 0.17^{a}	0.505 ± 0.011^{abc}
Ce C_HAD70	0.217 ± 0.005^{c}	1.9 ± 0.6^{b}	0.51 ± 0.02^{bc}
Ce FD	0.150 ± 0.009^{a}	1.9 ± 0.3^{b}	0.47 ± 0.03^{a}
L G_HAD60	0.229 ± 0.009^{b}	1.34 ± 0.04^{b}	0.620 ± 0.017^{b}
L C_HAD60	0.260 ± 0.003^{c}	1.85 ± 0.08^{c}	0.66 ± 0.02^{c}
L G_HAD70	0.230 ± 0.021^{b}	1.0 ± 0.3^{a}	0.479 ± 0.006^{a}
L C_HAD70	0.261 ± 0.006^{c}	1.6 ± 0.3^{bc}	0.598 ± 0.013^{b}
L FD	0.157 ± 0.006^{a}	1.36 ± 0.06^{b}	0.59 ± 0.03^{b}

$^{a,b,c,\ldots}$Different superscript letters in the same column for a similar residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

The drying methods applied allowed to reduce aw values below the target (0.3), which implies and increased shelf life and stability [33]. With regard to moisture content, both the drying technique applied and temperature as well as the disruption intensity had an impact on the moisture content of powdered ingredients. Disruption intensity had a different effect depending on the drying temperature applied, and the material being dried. Ground samples dried at the highest temperature (70 °C) presented lower moisture content than chopped samples, for all the vegetables studied. In contrast, in vegetables dried at 60 °C, only celery and leek samples followed this trend. This result confirms that faster drying rates when large particles are dried may lead to case-hardening phenomena and a difficulty to reach low moisture content values in the core of the particles, thus yielding powders with a higher average moisture content [29], as discussed in the previous section. These phenomena are significantly influenced by the vegetable matrix structure.

Carrot powders, particularly FD ones, characterized by their higher soluble solids content (table 2). In fact, sugar content in carrots is relatively high among vegetables, in the range 5.4 to 7.5% [34]. With regard to FD, the more porous and brittle structure generated after processing [32], may have intensified breakage of fibres during the final milling step and released more soluble sugars. As for the rest of powders, no specific trend was observed regarding drying technique and temperature, or disruption intensity.

Antioxidant properties of the powdered products (total phenols, total flavonoids and the antioxidant capacity measured by the DPPH and ABTS methods) are given in Table 3.

Table 3. Antioxidant properties of vegetables waste powders: total phenols (mg GAE/g_{dm}), total flavonoids (mg QE/g_{dm}), antioxidant capacity by the DPPH and ABTS methods (mg TE/g_{dm}). Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot air-drying; FD: freeze-drying. Mean and standard deviation of three replicates.

	Total phenols (mg GAE/g_{dm})	**Total flavonoids (mg QE/g_{dm})**	**DPPH (mg TE/g_{dm})**	**ABTS (mg TE/g_{dm})**
Ca G_HAD60	1.53 ± 0.12^{b}	1.24 ± 0.06^{a}	1.90 ± 0.12^{bc}	55 ± 7^{b}
Ca C_HAD60	2.06 ± 0.16^{c}	1.464 ± 0.003^{b}	2.1 ± 0.2^{c}	57.5 ± 1.4^{b}
Ca G_HAD70	2.004 ± 0.013^{c}	1.27 ± 0.03^{a}	1.69 ± 0.10^{b}	62 ± 3^{c}
Ca C_HAD70	2.42 ± 0.15^{d}	1.45 ± 0.02^{b}	2.65 ± 0.11^{c}	64.8 ± 1.7^{c}
Ca FD	0.74 ± 0.14^{a}	1.26 ± 0.03^{a}	1.01 ± 0.11^{a}	16.9 ± 0.3^{a}
WC G_HAD60	4.69 ± 0.12^{b}	5.6 ± 0.3^{b}	2.39 ± 0.14^{b}	101 ± 5^{b}
WC C_HAD60	4.71 ± 0.06^{b}	6.8 ± 0.2^{c}	2.9 ± 0.3^{c}	105 ± 3^{bc}
WC G_HAD70	6.3 ± 0.3^{c}	7.4 ± 0.3^{d}	3.11 ± 0.12^{c}	110 ± 3^{c}
WC C_HAD70	6.22 ± 0.12^{c}	7.8 ± 0.3^{d}	3.08 ± 0.10^{c}	109.0 ± 0.3^{c}
WC FD	2.79 ± 0.07^{a}	3.25 ± 0.05^{c}	1.15 ± 0.13^{a}	34.4 ± 0.5^{a}
Ce G_HAD60	2.26 ± 0.02^{b}	6.9 ± 0.6^{ab}	1.50 ± 0.16^{c}	63.4 ± 0.3^{b}
Ce C_HAD60	2.40 ± 0.15^{b}	7.8 ± 0.8^{bc}	1.08 ± 0.12^{ab}	68 ± 3^{c}
Ce G_HAD70	3.25 ± 0.16^{d}	8.2 ± 0.8^{c}	1.21 ± 0.08^{b}	77.5 ± 1.0^{e}
Ce C_HAD70	2.70 ± 0.13^{c}	6.29 ± 0.13^{a}	0.9 ± 0.2^{a}	72 ± 2^{d}
Ce FD	1.88 ± 0.15^{a}	9.72 ± 0.13^{d}	1.13 ± 0.13^{ab}	8 ± 2^{a}
L G_HAD60	3.32 ± 0.16^{b}	7.5 ± 0.2^{b}	1.6 ± 0.2^{b}	98.9 ± 1.4^{b}
L C_HAD60	3.26 ± 0.17^{b}	7.3 ± 0.4^{b}	1.6 ± 0.3^{b}	99 ± 2^{b}
L G_HAD70	4.34 ± 0.12^{d}	7.3 ± 0.3^{b}	1.9 ± 0.4^{b}	112 ± 5^{c}
L C_HAD70	3.8 ± 0.4^{c}	6.4 ± 0.7^{a}	2.4 ± 0.3^{c}	111 ± 4^{c}
L FD	2.52 ± 0.11^{a}	6.78 ± 0.16^{ab}	0.72 ± 0.03^{a}	13.8 ± 1.1^{a}

$^{a,b,c...}$Different superscript letters in the same column for a similar residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Phenolics resulted higher in cabbage powders, whereas carrot powders exhibited the lowest values. Regarding the dehydration technique applied, HAD produced powders with higher phenolic content, as compared to FD ones. This was confirmed by the multifactor ANOVA analysis, which revealed that drying temperature had a positive and significant effect (p-value < 0.05) on total phenolic content. On the one hand, increasing temperature may reduce the activity of enzymes capable of degrading phenolic compounds [35]; on the other, the use of higher temperatures implies a reduction in the time of exposure to drying conditions, thus reducing phenols degradation. In line with this, it has been previously evidenced that high-temperature and short times may favour antioxidant properties [10]. Disruption intensity prior to drying showed no clear trend and its impact on phenolic content depended on both the drying temperature and the product structure. In general, ground samples dried at 70 °C exhibited the highest phenolic content, whereas chopped samples resulted in lower values. This fact could be related to the reduced cell tissue damage in chopped samples, so that phenolics remain trapped by the structure during drying, thus being less susceptible to oxidation during this stage of processing. Regarding total flavonoid content, carrot powders presented the lowest values and celery the highest ones. Generally, HAD powders contained more flavonoids than FD ones, except for FD celery powders. Neither the drying temperature nor the previous disruption had a statistically significant effect on total flavonoid content, although general trends were similar to that of phenols.

Antioxidant activities (DPPH and ABTS methods) were the highest in cabbage and leek powders. Previous disruption hardly affected the antioxidant activity, whereas drying had a significant impact. Results of antioxidant activity were in line with the obtained for phenols and flavonoids. HAD, especially at 70 °C, favoured antioxidant capacity of the powders as compared to FD in all cases. This could be explained by the formation of new compounds with antioxidant properties like Maillard reaction products, or the incidence of other biochemical reactions which are favoured by high exposure temperature [33,36]. Activity of enzymes with pro-oxidant action may have also been reduced at higher temperatures. In addition, the use of lower temperatures during air drying implies lengthening

the treatment, thus leading to an increased exposure time to oxygen, and an increased incidence of oxidative reactions. As compared to other values reported in the literature, antioxidant capacity of the powders obtained in the present work were in the range of the reported by Bernaert et al. [14] for freeze-dried leek powders, and higher than the obtained by Márquez-Cardozo et al. [37] for pumpkin powders.

3.1.3. Impact of processing on the optical properties of vegetables waste powders

Figure 2 shows the color CIE L*a*b* coordinates distribution of the vegetables waste powdered products. Luminosity (L*) of powders was in the range 60 to 70, carrot waste powders having the highest values. In general, luminosity was slightly higher in FD samples than in HAD powders, as reported by other authors in *Carica papaya* L. leaf powder [38], or cabbage and broccoli powders [39,40]. In fact, FD is characterized by providing whitish appearance to the dried product due to the reduced incidence of oxidative reactions and the increased porosity of FD samples. The a* coordinate allowed to discriminate between HAD and FD powders, as well as between carrot waste powders and other vegetables'. HAD samples concentrate around zero a* values, whereas FD samples exhibited more negative a* values. In contrast, carrot powders showed higher positive a* values (in the range 6-8) approximating to redness.

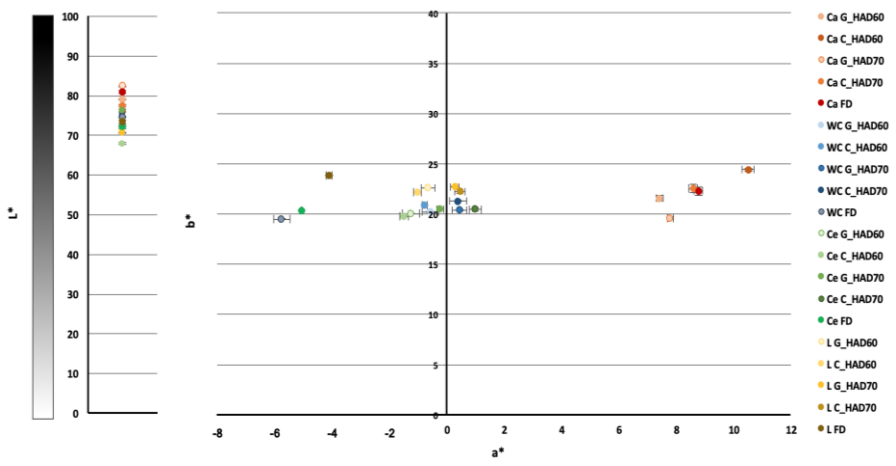

Figure 2. CIEL*a*b* coordinates of vegetables waste powders. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying. Error bars represent the standard deviation of three repetitions.

3.1.4. Impact of processing on water and oil interaction properties

Table 4 shows the results of solubility, specific volume, hydration and water retention properties, as well as emulsifying properties of the vegetable powdered ingredients obtained.

Table 4. Results of solubility, specific volume, hydration and water retention properties and emulsifying properties of vegetable powders. SC: swelling capacity (mL/g); WHC: water holding capacity (g/g); WRC: water retention capacity (g/g); OHC: oil holding capacity (g/g). Mean ± standard deviation of three repetitions.

	Specific Volume (mL)	Wettability (min)	Hygroscopicity (%)	SC (mL/g)	WHC (g/g)	WRC (g/g)	Solubility (%)	OHC(g/g)
Ca G_HAD60	1.51±0.04^{b}	33±4^{bc}	49.8±1.4^{ab}	8.64±0.04^{b}	5.1±0.5^{a}	6.8±0.2^{b}	59±7^{a}	2.24±0.10^{b}
Ca C_HAD60	1.65±0.05^{c}	29±2^{b}	53±5^{bc}	9.8±0.3^{d}	7.4±1.2^{ab}	6.8±0.5^{b}	58±3^{a}	2.13±0.11^{ab}
Ca G_HAD70	1.39±0.03^{a}	32±3^{bc}	56±3^{c}	7.65±0.19^{a}	6.8±0.5^{ab}	6.4±0.4^{a}	62±6^{a}	2.16±0.18^{ab}
Ca C_HAD70	1.55±0.03^{b}	8.5±0.4^{a}	48±3^{a}	9.2±0.3^{c}	9.2±3.3^{b}	7.6±0.5^{c}	60±4^{a}	1.99±0.13^{a}
Ca FD	1.37±0.02^{a}	35±3^{c}	55.2±0.4^{c}	10.1±0.4^{d}	28.6±1.8^{c}	9.00±0.11^{d}	57±6^{a}	2.24±0.09^{b}
WC G_HAD60	1.47±0.03^{b}	11.6±1.4^{b}	31.1±1.3^{b}	9.7±0.6^{c}	5.7±0.7^{a}	5.8±0.5^{a}	45±2^{b}	1.92±0.04^{a}
WC C_HAD60	1.67±0.06^{c}	5.5±0.6^{a}	23.6±1.9^{a}	8.88±0.17^{b}	5.2±0.4^{a}	6.0±1.2^{a}	41.9±1.7^{b}	2.04±0.05^{b}
WC G_HAD70	1.45±0.06^{b}	21±2^{c}	25.5±1.3^{a}	9.2±0.3^{bc}	5.6±0.9^{a}	6.4±0.2^{a}	45±4^{b}	2.16±0.08^{c}
WC C_HAD70	1.27±0.06^{a}	29±3^{d}	33±3^{b}	7.8±0.4^{a}	6.3±0.7^{a}	8.0±0.7^{b}	40±3^{b}	1.91±0.05^{a}
WC FD	1.60±0.04^{c}	14.2±1.7^{b}	55.17±0.15^{c}	9.2±0.2^{bc}	27.8±0.8^{b}	10.2±0.7^{c}	30±3^{a}	2.15±0.08^{bc}
Ce G_HAD60	1.71±0.03^{b}	5±2^{ab}	41.3±0.9^{c}	5.4±0.2^{a}	4.7±0.6^{a}	6.2±0.4^{ab}	44±5^{a}	2.39±0.09^{b}
Ce C_HAD60	1.64±0.05^{a}	6.0±0.9^{bc}	40.4±1.7^{bc}	6.58±0.18^{bc}	7.1±1.0^{c}	5.8±0.7^{a}	43±8^{a}	2.36±0.12^{b}
Ce G_HAD70	1.82±0.03^{c}	11.9±1.2^{d}	37±3^{ab}	6.0±0.3^{b}	5.3±0.3^{ab}	6.6±0.3^{b}	40.7±1.9^{a}	2.46±0.03^{b}
Ce C_HAD70	1.69±0.03^{ab}	7.4±1.1^{c}	40±4^{bc}	6.78±0.10^{c}	6.1±0.5^{bc}	6.12±0.08^{ab}	40±10^{a}	2.13±0.09^{a}
Ce FD	1.81±0.03^{c}	3.3±0.3^{a}	33.9±0.3^{a}	9.1±0.6^{d}	22.5±0.2^{d}	9.7±0.6^{c}	36±5^{a}	2.92±0.04^{c}
L G_HAD60	1.27±0.04^{a}	9±3^{cd}	50±3^{c}	9.5±0.4^{b}	4.6±0.7^{a}	7.6±0.5^{b}	47±7^{a}	1.910±0.019^{a}
L C_HAD60	1.33±0.03^{b}	11±3^{d}	44.5±1.8^{b}	9.08±0.15^{a}	5.1±0.3^{a}	6.9±0.5^{ab}	46±5^{a}	1.91±0.11^{a}
L G_HAD70	1.45±0.04^{c}	5.6±1.2^{b}	56±3^{d}	9.55±0.09^{b}	4.4±0.3^{a}	6.4±0.5^{a}	44±8^{a}	2.11±0.14^{a}
L C_HAD70	1.293±0.012^{ab}	6.2±0.8^{bc}	45±2^{b}	10.56±0.18^{c}	5.0±0.6^{a}	8.77±0.07^{c}	45±9^{a}	1.86±0.12^{a}
L FD	1.72±0.02^{d}	1.6±0.3^{a}	26.03±0.13^{a}	9.06±0.13^{a}	18±3^{b}	9.5±0.5^{c}	38±6^{a}	2.8±0.3^{b}

$^{a,b,c...}$Different superscript letters in the same column for a similar residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Regarding hydration and water retention properties, statistically significant differences were found between the different types of powders for a similar residue. In contrast, specific volume values resulted more similar among powders. *Hygroscopicity* is related with the ability of a product to absorb water from the environment and determines its stability during storage period [41]. It is an important evaluation criterion from which the possibility of stickiness in powders during subsequent storage can be assessed. According to the literature, this parameter is related to saccharides content of powders [42]. In fact, results were higher in carrot powders (table 4), which could be a consequence of their higher soluble solids content. This fact was reported by Si et al. [42] on raspberry powders, where the highest hygroscopicity was found in the powders with the highest soluble solids content. In contrast, lower hygroscopicity values are related to insoluble fibre components and larger particle sizes, resulting in less surface area for water adsorption. The ANOVA analysis did not allow to identify any specific trend regarding the effect of processing conditions on hygroscopicity; likewise, no trend was identified as for wettability. In the literature, however, it has been reported that the larger the particle size, the shorter the wettability time, since coarser particles imply a more porous structure and, consequently, higher wettability [43].

Swelling capacity (SC), *water retention capacity* (WRC) and *water holding capacity* (WHC) were, in general, favored when FD was used as the dehydration technique, as compared to HAD. The increased ability to incorporate water may be explained by the porous structure of freeze-dried materials [44]. Martínez-Las Heras et al. [45] found similar trends in persimmon fibres. Results obtained for HAD powders were in the same range than the reported for apple pomace powder, carrot pomace powder, beetroot pomace powder [46] or pumpkin powder [37]; and higher than in goldenberry waste powder [47] or fig pulp powder [48]. Hydration properties were found to be influenced by porosity and particle size, since an improvement of SC, WRC and WHC was obtained as particle size decreased [45].

Solubility is an important physical parameter determining the functional properties of powdered dried products, since it is mainly connected with the presence of small hydrophilic molecules and their ability to interact with water [49]. Statistically significant differences were obtained between powders within the same product. In agreement with Si et al. [42], powders solubility decreased as did particle size, being the values slightly lower for the FD powders than for their respective HAD powders. Among vegetables wastes, white cabbage, celery and leek powders showed similar results; while carrot powders showed the highest solubility index, as expected from their higher soluble solids content, and in line with the values reported for products richer in sugars such as persimmon pulp powders (52-77% [50]) or mango peels powders (50-70% [43]).

No results were obtained for emulsifying activity and emulsifying stability, although powders exhibited a certain *oil holding capacity* (OHC). Highest values were obtained for carrot and celery powders. These results were in the range of other reported products such as carrot pomace powder (2.442 ± 0.067 g/g), apple pomace powder (2.241 ± 0.068 g/g) or beetroot pomace powder (2.206 ± 0.064 g/g), in Sahni & Shere [46]. Values obtained were higher than other by-products powders, like peel and pulp fig powders (0.75-0.90 g/g) [48] or pulp pumpkin powders (1.01-1.30 g/g) [37].

In order to statistically evidence the relationships between powders physicochemical characteristics and technological properties, a Principal Component Analysis (PCA) was applied (Supplementary materials). Three components were required to explain 75% of the variance, whereas 2 components explained 60% of the total variance. The PCA evidenced a close relationship between soluble solids content and water interaction properties such as solubility, wettability and hygroscopicity, whereas other water interaction properties such as WHC and WRC were more significantly related to particle size, having an inverse relationship with that parameter. On the other hand, OHC seemed to be more explained by the specific volume of the samples. Plotting the powders according to the components which explain their variance allow to concentrate FD powders on the left side of the plot, where properties such as specific volume, OHC and water retention and hydration properties are located; whereas HAD powders accumulate on the

right side. As for the different wastes, carrot powders accumulate in the region which is explained by soluble solids content and related technological parameters such as solubility, wettability and hygroscopicity.

3.2. Evolution of vegetables waste powders properties during storage

3.2.1. Water activity, moisture content and total soluble solids

Water activity (a_w), moisture content (grams of water/100 total grams), and total soluble solids content (x_{ss}) of powders after processing and along four months of storage are presented in Figures 3 to 5, respectively.

During storage, both moisture content and water activity increased, more markedly in the case of celery and cabbage waste powders. There was significant variability among the different milling pre-treatments and the drying techniques applied, this suggesting a different impact of storage on a_w and x_w values depending on the structure of the processed material. Overall, there was about 2.4% ± 1.5% moisture gain and about 0.14 ± 0.08 a_w gain during 4 months of storage at room conditions. Similar results were reported by other authors in several products such as orange juice powders during the 10 months of storage, independently of packaging time and storage conditions (moisture increase of 1.5% during the first 6 months and aw gain from 0.264 to 0.448) [51]; instant soup mix from dehydrated pumpkin powder (moisture increase from 4.91 to 5.18% and aw from 0.341 to 0.342) [52], apricot fruit bar with 3% moisture gains during 6 moths [53], soursop fruit powder stored over 91 days in different conditions [16], or apple peel powders with moisture increases depending on the temperature, time and packaging storage conditions [5]. Results suggested the need to store in suitable conditions and packaging to avoid moisture gain and, consequently, loss of stability during powder storage.

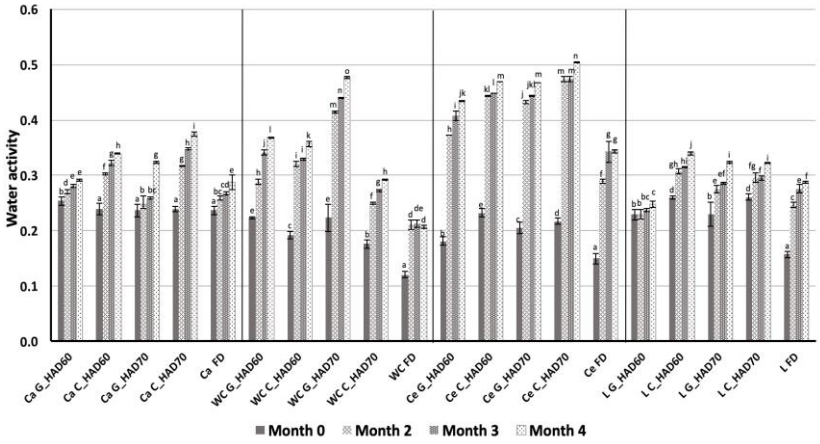

Figure 3. Results of water activity of vegetable waste powders during the four-months storage period. Different letters within the same residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05). Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying. Error bars represent the standard deviation of three replicates.

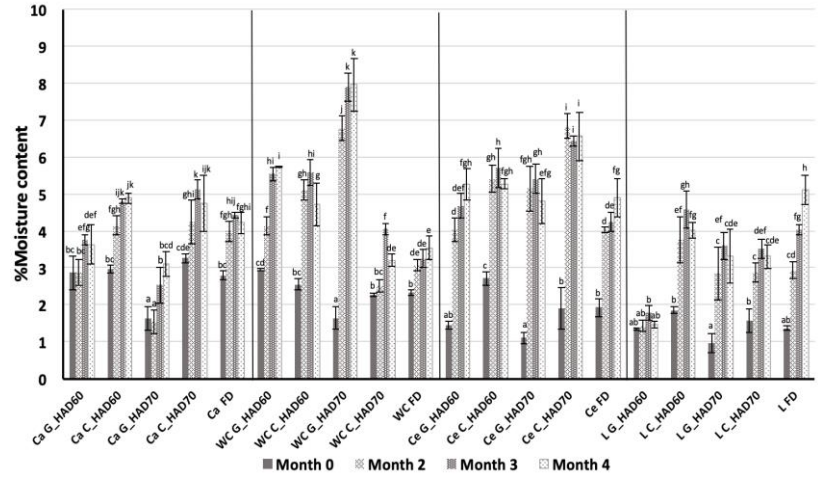

Figure 4. Results of moisture content (%) of the vegetable waste powders during the four-months storage period. Different letters within the same residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05). Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying. Error bars represent the standard deviation of three replicates.

Evolution of soluble solids content during storage did not follow a consistent trend. For some powders, there was a slight increase in soluble solids over time, whereas others exhibited a decrease. Simple sugars may experiment variations during storage since sucrose may invert to glucose and fructose, and fructose may be consumed in Maillard reactions [54]. Liu et al. [54] also observed fluctuations in tomato powders soluble solids content, with no significant changes along 5 moths of storage at different temperatures.

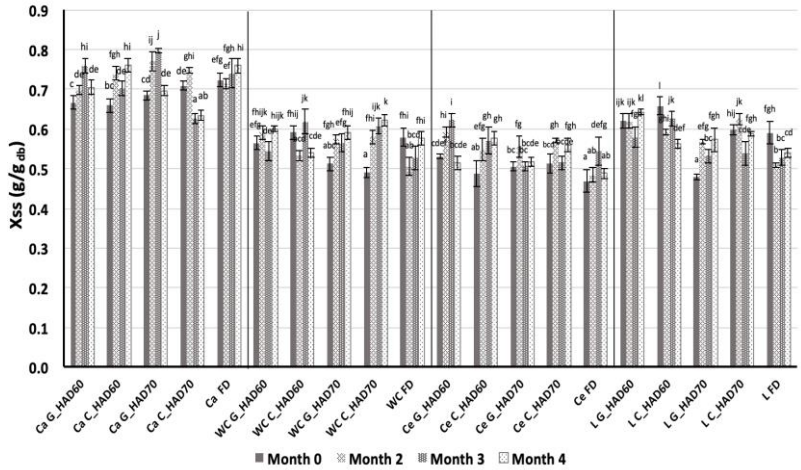

Figure 5. Total Soluble Solids content (x_{ss} in g/g_{db}) of vegetable waste powders along the four-moths storage period. Different letters within the same residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05). Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying. Error bars represent the standard deviation of three replicates.

3.2.2. Evolution of optical properties during storage

Color is a very important quality factor in fruit and vegetable products, since it influences consumer acceptability. Figure 6 (A-D) shows color differences (ΔE) during the storage period, with respect to initial values. Color changes during powders storage have been extensively reported. These changes are normally attributed to chemical and physical reactions such as non-enzymatic browning [16,51,55,56], and related to water activity, moisture or sugar content in the stored food products [16]. Among the

vegetable wastes studied, the lowest ΔE was obtained for celery powders, while carrot and white cabbage powders showed the highest color differences. Despite a stabilization of color changes was observed after two or three months of storage, the fourth month implied a significant change. In some powders, particularly carrot waste ones, color differences decreased or maintained during the fourth month. In general, color changes were less significant in FD powders than in HAD ones.

Chroma (C_{ab}*) and hue (hab) parameters are summarized in Table 3. In carrot and celery waste powders, the purity of color decreased slightly during storage; whereas cabbage and leek waste powders experimented a slight increase. Regarding hue, carrot waste powders exhibited an orange hue which increased during storage. In contrast, in cabbage, celery and leek powders, with a tendency towards green, the hue generally decreased along storage. Hue decrease was also observed by Tavares et al. [57] with jambolan juice powder stored at 25 °C and 35 °C, although in Fernández-López et al. [51] values remained unchanged throughout the storage period.

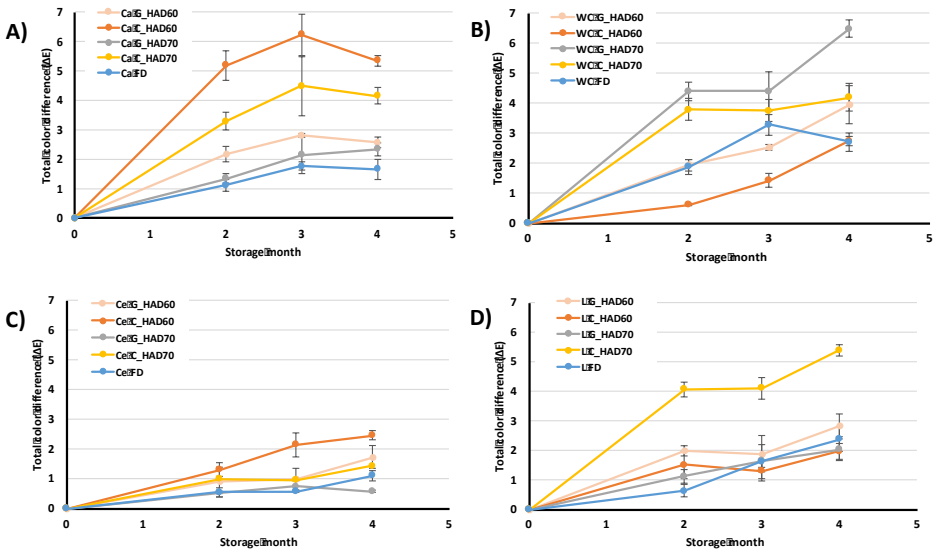

Figure 6. Total color difference of (A) carrot, (B) white cabbage, (C) celery and (D) leek waste powders stored over 4 months, with respect to time zero values. Error bars represent the standard deviation of three replicates.

Table 3. Color parameters C_{ab}* (chroma) and h_{ab} (hue) of powders along four months of storage. Mean ± standard deviation of three replicates.

	Chroma (C_{ab}*)				Hue (h_{ab})			
	Month 0	**Month 2**	**Month 3**	**Month 4**	**Month 0**	**Month 2**	**Month 3**	**Month 4**
Ca G_HAD60	25.8±0.3^{cd}	24.69±0.08^{bc}	23.7±0.7^{ab}	25.8±1.3^{d}	71.7±0.5^{de}	75.93±0.17^{fgh}	77.3±0.5^{ghi}	75±6^{fg}
Ca C_HAD60	30.4±0.3^{h}	26.4±0.7^{de}	26.1±0.5^{de}	28.6±0.5^{g}	67.7±0.3^{a}	75.4±0.5^{fg}	77.9±0.8^{hi}	78.3±0.3^{i}
Ca G_HAD70	23.4±0.2^{a}	23.1±0.4^{a}	23.0±1.4^{a}	24.1±0.5^{ab}	69.0±0.4^{ab}	71.67±0.16^{de}	73.9±1.0^{ef}	74.8±0.5^{f}
Ca C_HAD70	27.2±0.6^{ef}	26.5±0.2^{de}	26.7±1.3^{de}	28.0±0.7^{fg}	69.9±0.3^{abcd}	75.8±0.3^{fgh}	78.5±1.3^{i}	78.9±0.3^{i}
Ca FD	26.8±0.5^{de}	26.4±0.8^{de}	28.2±0.7^{fg}	28.3±0.5^{fg}	69.2±0.7^{abc}	71.3±0.3^{cd}	71.2±0.8^{bcd}	71.71±0.09^{de}
WC G_HAD60	23.5±0.2^{a}	24.74±0.04^{bc}	24.85±0.12^{c}	25.40±0.15^{d}	91.5±0.2^{i}	88.88±0.02^{f}	87.7±0.3^{e}	86.6±0.2^{c}
WC C_HAD60	24.55±0.11^{bc}	24.8±0.2^{bc}	24.6±0.2^{bc}	25.4±0.3^{d}	92.01±0.09^{j}	90.88±0.10^{h}	89.56±0.09^{g}	88.66±0.09^{f}
WC G_HAD70	23.5±0.3^{a}	27.0±0.2^{efg}	27.2±0.4^{g}	27.15±0.08^{fg}	88.7±0.3^{f}	86.1±0.3^{b}	86.00±0.12^{b}	84.51±0.05^{a}
WC C_HAD70	24.3±0.5^{b}	26.7±0.2^{efg}	26.7±0.3^{ef}	26.6±0.2^{e}	89.0±0.2^{f}	87.01±0.06^{d}	86.9±0.2^{cd}	86.7±0.3^{c}
WC FD	23.4±0.5^{a}	25.4±0.2^{d}	26.6±0.4^{e}	25.5±0.4^{d}	105.95±0.19^{n}	103.41±0.09^{m}	101.72±0.12^{l}	101.1±0.5^{k}
Ce G_HAD60	23.4±0.4^{cde}	23.5±0.4^{def}	22.3±0.2^{b}	21.2±0.2^{a}	93.42±0.14^{g}	92.41±0.14^{ef}	92.5±0.2^{f}	93.06±0.05^{g}
Ce C_HAD60	24.2±0.4^{h}	23.1±0.3^{cd}	22.25±0.15^{b}	21.33±0.10^{a}	94.05±0.19^{h}	92.0±0.3^{e}	92.1±0.3^{ef}	92.27±0.15^{ef}
Ce G_HAD70	23.5±0.2^{cdef}	23.5±0.2^{def}	23.3±0.2^{cde}	23.05±0.12^{c}	90.6±0.2^{d}	89.84±0.18^{c}	89.77±0.15^{c}	89.6±0.4^{c}
Ce C_HAD70	24.1±0.3^{gh}	23.8±0.2^{efg}	23.9±0.5^{fgh}	23.3±0.4^{cde}	87.9±0.6^{b}	88.1±0.4^{b}	87.4±0.8^{a}	87.9±0.4^{ab}
Ce FD	24.78±0.06^{i}	24.2±0.3^{h}	24.15±0.06^{gh}	23.1±0.4^{cd}	103.45±0.05^{j}	103.09±0.06^{ij}	102.62±0.04^{i}	102.86±0.06^{i}
L G_HAD60	26.7±0.3^{bc}	27.5±0.5^{de}	27.04±0.13^{cd}	27.5±0.4^{de}	91.55±0.10^{i}	90.31±0.08^{g}	90.2±0.4^{g}	89.53±0.18^{f}
L C_HAD60	26.45±0.05^{bc}	24.6±0.4^{a}	25.1±0.4^{a}	26.4±0.3^{bc}	92.50±0.12^{k}	92.12±0.07^{j}	91.62±0.19^{i}	91.05±0.13^{h}
L G_HAD70	27.01±0.06^{cd}	27.8±0.2^{ef}	27.8±0.7^{ef}	28.03±0.04^{efg}	89.31±0.08^{f}	88.6±0.4^{de}	88.48±0.15^{cd}	88.23±0.13^{c}
L C_HAD70	26.1±0.3^{b}	28.1±0.9^{efg}	27.9±0.4^{ef}	28.2±0.2^{fg}	88.8±0.4^{e}	86.94±0.13^{b}	86.86±0.15^{b}	86.01±0.14^{a}
L FD	28.54±0.05^{gh}	27.9±0.3^{efg}	29.1±0.4^{h}	28.9±0.3^{h}	99.35±0.10^{o}	98.93±0.07^{n}	97.68±0.05^{m}	97.03±0.05^{l}

$^{a,b,c\cdots}$Different letters in the same column for a similar residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

3.2.3. Evolution of antioxidant properties during storage

Powders' antioxidant properties (total phenols, total flavonoids and the antioxidant capacity measured by the DPPH and ABTS methods) during four months of storage are shown in Figures 7-10, respectively. The evaluation of postharvest processing and the impact of subsequent storage on vegetable antioxidants properties is of great practical importance [14]. Maximizing nutrients and bioactive compounds retention not only during processing but also during storage is a prevailing matter. Nonetheless, antioxidants naturally present can be significantly reduced as a consequence of processing and storage [4,5].

Storage negatively affected phenol and flavonoid content of the vegetable wastes powdered ingredients, particularly after the third month. A similar trend has been reported by Henríquez et al. [5] on dehydrated apple peel powders which experimented a phenolic content decrease during the storage period, independently on the packaging and storage conditions. Similar results have been stated by Michalczyk et al. [58] for air-dried (40 °C) and freeze-dried berries during a 10-month storage period in similar conditions than in the present study.

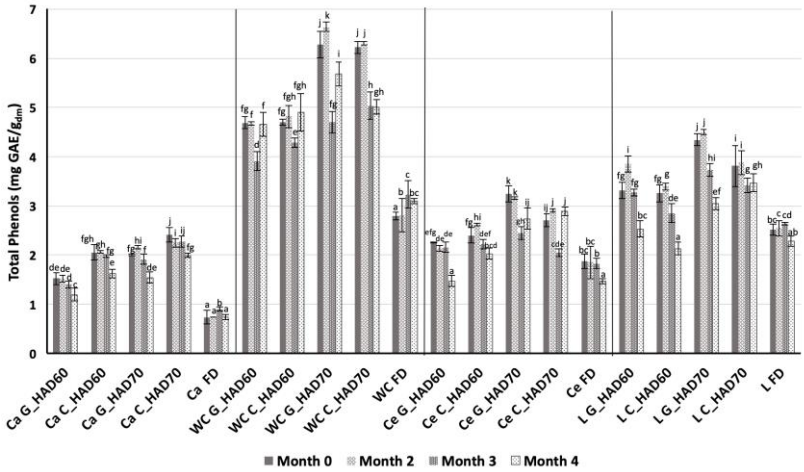

Figure 7. Total phenol content (mg GAE/g_{dm}) of vegetable powders during four months of storage. Different letters within the same residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05). Error bars represent the standard deviation of three replicates.

As occurred with total phenol and flavonoid content, storage did have a negative effect on the antioxidant capacity measured with the ABTS method, which was more noticeable in air dried powders. FD celery and leek powders were an exception, since antioxidant capacity slightly increased during storage. This decrease in the ABTS antioxidant capacity was also observed in [4] on FD and HAD kale leaves. In contrast, DPPH antioxidant activity evolved differently during storage depending on the dehydration technique used. On the one hand, FD powders increased their AO during storage; in contrast, HAD ones decreased DPPH AO capacity at the end of the storage period, although a slight increase could be observed in the second month. This fact also was observed by del Caro et al. [59] on hot-air dried prunes. They attributed the increase observed in antioxidant activity during storage to the formation of Maillard reaction new compounds with antioxidant activity that continue to be formed even after long storage periods. Similar results have been obtained by other authors [58] who reported a slight increase in the DPPH antioxidant activity during 10 months of storage (in glass jars and at room temperature) in air-dried (40 °C) and freeze-dried strawberry and raspberry.

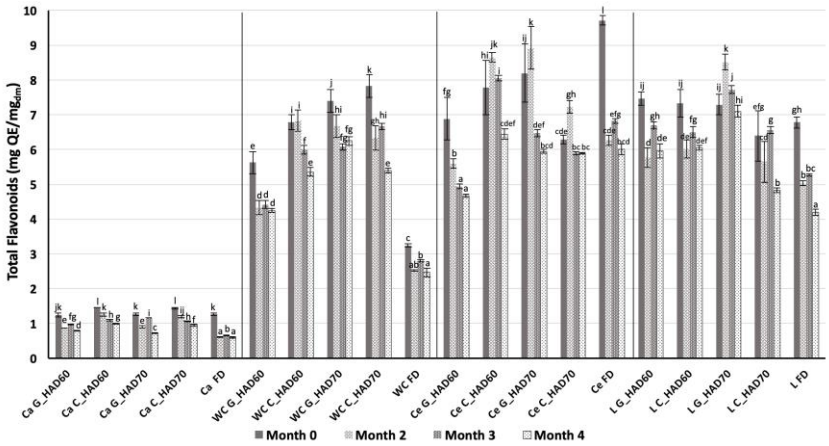

Figure 8. Total flavonoid content (mg QE/g_{dm}) of vegetable powders along four months of storage. Different letters within the same residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05). Error bars represent the standard deviation of three replicates.

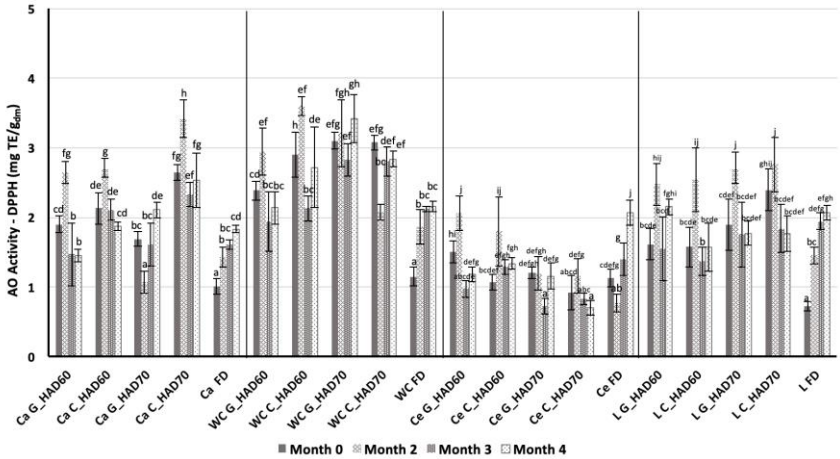

Figure 9. Antioxidant activity of vegetable waste powders measured by the DPPH method (mg TE/g_{dm}) along four months of storage. Different letters within the same residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05). Error bars represent the standard deviation of three replicates.

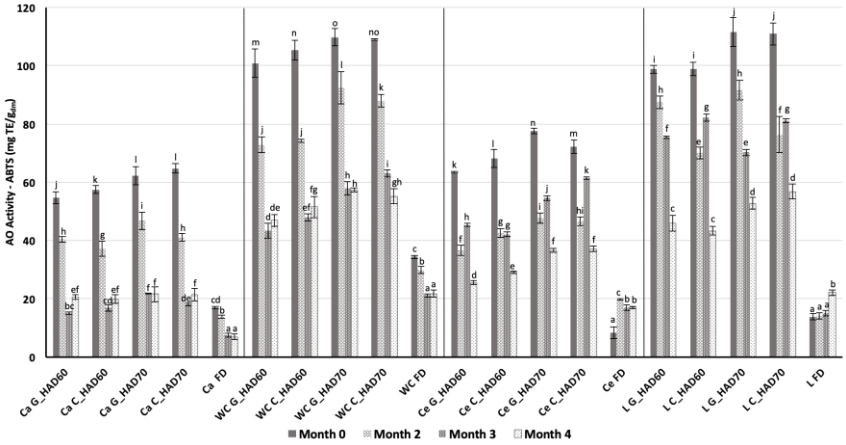

Figure 10. Antioxidant activity of vegetable waste powders measured by the ABTS method (mg TE/g_{dm}) along four months of storage. Different letters within the same residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05). Error bars represent the standard deviation of three replicates.

4. Conclusions

The present study has applied an economical and feasible approach to integrally valorise vegetable wastes, thus extending the shelf-life of these perishable plant materials and obtaining new upcycled ingredients.

Vegetable wastes were successfully transformed into powdered products through a simple but efficient transformation process involving a disruption pre-treatment, a dehydration step and final milling. The process described could be easily adopted by industry to upcycle these wastes and obtain new ingredients which could be used in the food sector to improve the nutritional value of foods.

The study has demonstrated that both processing conditions and storage imply quality changes in the powdered ingredients. Processing parameters have conditioned physicochemical, antioxidant and technological properties of vegetable waste powders. In addition, physicochemical attributes such as soluble solids content, particle size or specific volume have been related with technological characteristics such as hydration and oil interaction properties. During the storage period, physical and chemical properties significantly varied. Moisture migration, color changes and a general decrease in the antioxidant properties of powdered products was evidenced. FD powders characterized by increasing their antioxidant activities along storage, in contrast to HAD powders.

In conclusion, it has been evidenced that processing conditions must be chosen considering not only their impact on product characteristics, but also their influence on storage stability. Control of storage conditions is also crucial for preserving powders properties. Nevertheless, capital investment and production costs are critical issues when developing waste valorisation processes. According to the latter, HAD can be considered the most suitable drying technique for upcycling these vegetable wastes.

Supplementary Materials

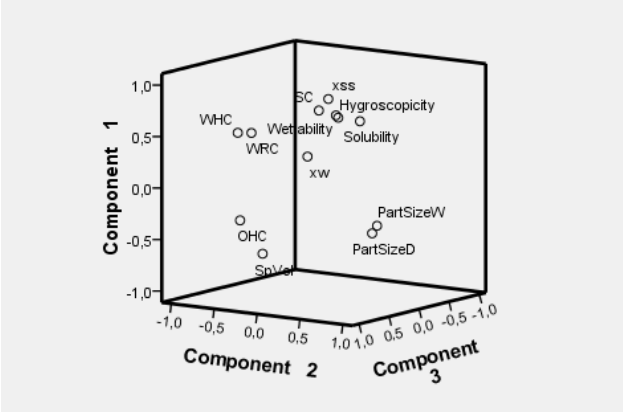

Figure S1. 3D graph of the PCA (Principal Component Analysis) performed on vegetable waste powder properties plotting 3 components (75% of the total variance explained). WHC: water hydration capacity; WRC: water retention capacity; SC: Swelling Capacity; OHC: oil holding capacity; SpVol: Specific volume; PartSizeW: particle size by the wet method; PartSizeD: particle size by the dry method; xw: moisture content; xss: soluble solids content.

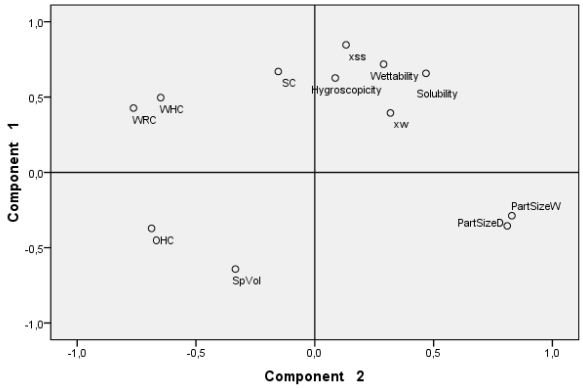

Figure S2. 2D graph of the PCA (Principal Component Analysis) of vegetable waste powder properties plotting 2 components (60% of the total variance explained). WHC: water hydration capacity; WRC: water retention capacity; SC: Swelling Capacity; OHC: oil holding capacity; SpVol: Specific volume; PartSizeW: particle size by the wet method; PartSizeD: particle size by the dry method; xw: moisture content; xss: soluble solids content.

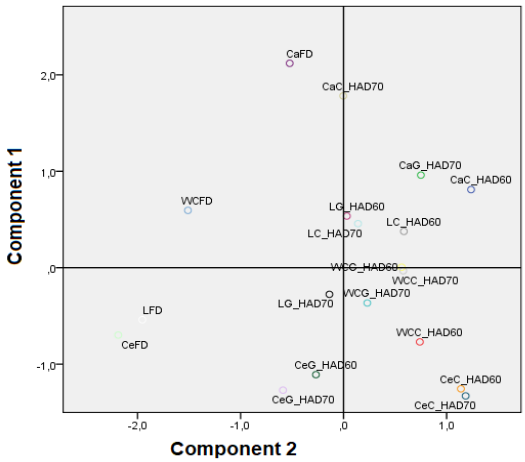

Figure S3. 2D graph of the PCA (Principal Component Analysis) of vegetable waste powders as described by components 1 and 2. Ca: carrot; WC: white cabbage; Ce: celery; L: leek; G: ground; C: chopped; HAD: hot-air drying; FD: freeze-drying. Error bars represent the standard deviation of three replicates.

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Effect of fermentation and processing conditions on drying kinetics and antioxidant properties of broccoli stems

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Abstract

Food loss and waste in the fruit and vegetable industry represent an environmental, economic, and social problem. In the specific case of broccoli, much of its weight is made up of the stem, which is often discarded despite its content in a great variety of bioactive compounds. An alternative for its valorization consists of transforming it into powders with functional properties, which will depend on the conditions applied during processing. In the present work, disruption intensity (grinding or chopping) and fermentation with *Lactobacillus plantarum* spp. CECT 749 were assayed as pretreatments for improving the antioxidant properties of broccoli stems powders. Fermented and non-fermented stems were dried (freeze-dried or air-dried at 60 or 70 °C) and milled to obtain fine powders, and characterized in terms of physicochemical and antioxidant properties. Disruption intensity did not have a significant impact on the drying rate and the antioxidant properties of the broccoli stems powders. As a pretreatment, fermentation increased total phenols and flavonoids content of broccoli stems and favoured the water removal taking place during the drying step. Increasing the drying temperature shortened the drying process and increased the antioxidant activity of the powders. However, freeze-drying allowed to obtain a powder with better antioxidant properties and higher microbial content. In general terms, the powdered products developed in this research would contribute to reducing broccoli stem waste and could be used as a potential ingredient in the development of new functional foods.

Keywords: broccoli, fermentation, hot-air drying, freeze-drying, waste recovery, antioxidant properties, probiotic, functional powders.

1. Introduction

Food waste is an issue of major global concern due to the associated social, economic and environmental consequences [1]. Specifically, in vegetable distribution channels around 30% of the total (mainly inedible parts) becomes waste or by-product [2,3]. Efforts to combat this issue are emphasised in the 2030 Sustainable Development Goals, which prioritise continuous and sustainable improvement of the food system, while meeting the food growing demand and driving climate action [1]. In this context, it is necessary to search for new recovery strategies that contribute to the circular economy of food waste and, specifically, for plant waste before it is completely discarded, as these residues are rich in nutrients and bioactive compounds, such as polysaccharides, proteins, dietary fibers and antioxidant compounds among others [4,5].

Broccoli (*Brassica oleracea L.* var. *italica*) is one of the most widely consumed vegetable of the Brassicaecea family, both in fresh and processed form [6], due to its health benefits. Several epidemiological studies have identified an inverse correlation between broccoli consumption and the risk of diseases such as cancer, cardiovascular diseases, neurological diseases or diabetes [7,8]. Bioactive compounds in broccoli include isothiocyanates, glucosinolates, phenols, flavonoids or vitamins [2,9], that are involved in antioxidant activity, enzyme regulation or control of apoptosis and cell cycles [6]. Currently, around 600,000 tonnes of broccoli are produced annually in Spain [10], of which only 10-15% are intended for human consumption [11]. The parts of broccoli consumed as food are mostly florets, which constitute only 15% of the total plant weight. Leaves and stems, which respectively represent approximately 47% and 38% of the total weight, are usually discarded [12].

In the last years, manufacturing dehydrated powders from vegetable wastes has arisen as a useful and effective valorisation alternative to take advantage of their nutritional potential; since powders are stable and versatile products that can be consumed directly or as an ingredient in food formulation. The combination of drying and milling is an economical and environmentally friendly alternative for the full valorization of these wastes

[13]. Dehydration aims to minimise spoilage by reducing water content [7,8], as well as to reduce storage and transport costs. Among the dehydration techniques, hot-air drying is the most widely used; however, drying conditions (temperature and relative humidity of the air, load density, type of contact between the air and the material to be dried, etc.) must be selected properly in order to minimise thermal degradation of bioactive compounds and to preserve the nutritional value of foods [6]. Hot-air drying is a complex process involving simultaneous mass and heat transfer [14]. Thus, modeling of drying process is required to predict optimum drying parameters and the drying behavior of the material [14,15]. Several mathematical models are proposed to describe the drying kinetics of food, being the thin-layer drying models (e.g., Page, Henderson-Pabis, Lewis, Linear…) the most widely used [16], to fit the drying curves and estimate the drying time for a particular layer of sample or food slices [14]. On the other hand, freeze-drying is presented as a promising drying technique that maintains the appearance, shape, taste and biological activity of foods [7], but it is an expensive technique with a more difficult implementation. Pre-drying treatments, including sample milling, also play a decisive role on the functional properties of dehydrated products. The intensity of milling directly affects the drying time or the airstream exposure, and thus the content of biologically active compounds [17–19]. Furthermore, milling facilitates the extraction of bioactive compounds and increases their bioavailability, which has been observed in the content and activity of antioxidant compounds present in different plant tissues [9,20].

For its part, fermentation is an ancient technique, considered one of the most effective ways of preserving food due to the formation of organic acids, alcohols, bacteriocins and other antimicrobial products [21,22]. Therefore, fermented matrices are potential ingredients with improved properties and high applicability in the food industry, e.g., food supplements, soup seasonings or probiotic foods [23,24], formulation of baby food [25] or fortified cereal-based products [26]. This is why in recent years there has been a growing interest in studying the influence of fermentation on foodstuffs and its benefits for human health [27], as well as on the improvement of functional, sensory [28] and physicochemical properties

[29]. Among the microorganisms involved in fermentation processes, lactic acid bacteria (LAB) stand out, belonging to the genera *Enterococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus* and *Pediococcus* [30]; whose industrial importance is highlighted in commonly consumed foods such as yoghurt, cheese, beer, wine or meat products, among others [31]. According to the WHO, probiotics are "live microorganisms that, when consumed in adequate amounts (10^8-10^9 CFU/day), bestow health benefits to the host". Therefore, to designate a product as a probiotic requires not only to contain the microorganism in adequate concentration at the time of consumption (> 10^6-10^7 CFU/g), but also evidence that the strain is safe and beneficial to health. During fermentation, LAB bacteria produce primary and secondary metabolites, such as organic acids, CO_2, hydrogen peroxide and antimicrobial peptides that inhibit the growth of pathogenic microorganisms [32,33]. Acid-lactic fermentation also improves the nutritional and health attributes of foods by bioconverting phytochemical compounds, such as polyphenols, into their more bioactive and bioaccessible forms [34]. Moreover, the fermentation process has been reported to modify raw material structure, that is loosening of cell wall structure and increasing the size of tissue pores [35], which would determine the fermented material behaviour during the subsequent drying process. *L. plantarum* has been reported as the most widely used for fermenting plant substrates and a versatile strain that enhances fermented products properties [36,37].

In this context, the present work aims to evaluate drying operation and the effect of processing variables, including disruption intensity (grinding or chopping) and fermentation with *Lactobacillus plantarum* spp. CECT 749, on the drying process kinetics, as well as, on the antioxidant properties of different broccoli stem powders obtained. In fermented samples, the survival of the microbial strain to the dehydration step was also assessed.

2. Material and methods

2.1. Raw material and preconditioning

Broccoli used in this study was purchased from a local supermarket in Valencia (Spain) and the stems were manually separated in the laboratory using a knife. To reduce microbial load and avoid possible substrate

competition during fermentation with the inoculated microorganism, chemical and thermal treatments were carried out. On one hand, fresh broccoli stems were washed by immersion in sodium hypochlorite (200 ppm) solution in water for 5 min. After disinfection, the stems were disrupted in a Thermomix® food processor (Vorwerk, Madrid, Spain) for 30 s at 10,000 rpm (if ground samples, G) or 10 s at 10,000 rpm (if chopped samples, C). Disruption conditions were established according to previous research [18]. Next, the disrupted tissue was distributed among 250 mL sterile glass containers with twist-off closure (200 g of sample per jar) and blanched by immersion in a water bath until the centre of the pot was summited to 72 °C for 1 min. After this, samples were cooled with running water until they reached 40 °C.

2.2. Microbial strain and fermentation procedure

Lactobacillus plantarum spp. (CECT 749) strain, acquired from the Colección Española de Cultivos Tipo (CECT, Valencia, España), was used for broccoli stem fermentation. Reactivation of the freeze-dried strain was carried out in Man, Rogosa and Sharpe (MRS) broth (Scharlab, Barcelona, Spain) at 37 °C for 24 h. Then, the starter culture was obtained with a concentration of 8.6 ± 0.3 log CFU/mL (measured by plate count) and inoculation was performed by adding 2 mL of it to each jar containing the blanched and cooled plant material. Fermentation tests were carried out in duplicate in an oven (Incudigit-TFT, J.P.Selecta, Barcelona, Spain) at 37 °C for 96 h. Microbial counts were made at different times (0, 7, 24, 48, 72 and 96 h) to identify the fermentation time resulting in the highest microbial concentration.

2.3. Dehydration and powders manufacturing

To reduce the water content and increase their stability, ground or chopped and fermented or non-fermented broccoli stems were either freeze-dried (FD) or hot-air dried (HAD) at 60 or 70 °C until reaching water activity (a_w) values below 0.3, which is known to ensure the stability of the final product [38]. For FD, ground samples were deep-frozen at –40 °C for 24 h in a CVN-40/105 freezer (Matek, Barcelona, Spain) prior to sublimation of

the frozen water for 24 h under freezing conditions (-45 °C) and sub-atmospheric pressure (P = 0.1 mbar) in a LyoQuest-55 laboratory freeze-dryer (Telstar, Terrassa, Spain). For HAD, a convective CLW 750 TOP+ transverse flow tray dryer (Pol-Eko-Aparatura SPJ, Katowice, Poland) with air at 2 m/s and at 60 or 70 °C was used. Samples (ground or chopped) were distributed in 1 cm-thick layers on perforated dryer trays with a load of 200 g of residue per tray. After dehydration, dried samples were milled using a Thermomix® food processor (Vorwerk, Madrid, Spain) at 10,000 rpm for 2 min (at 30 s intervals) to obtain a fine powder, as described previously [18]. Powders obtained were packed into glass jars in a light-free environment until analysis.

2.4. Drying and drying rate curves. Modelling of the thin-layer drying curves

Weight variation was registered every 30 min during the first 6 hours and every hour until 24 h of drying treatment. Water activity was also measured every hour until a target value below 0.3 was obtained. This procedure was carried out as explained elsewhere [18,19] and allowed to obtain the drying curves and drying rate curves of broccoli stems as a function of the different pretreatments applied (fermented/non-fermented, chopped/ground). This experimental procedure also allowed to determine the time needed to reach the target a_w value for each pretreated residue, which resulted as follows: 10 h for non-fermented chopped or ground broccoli residue dried at 60 °C (NF_C_HAD60 and NF_G_HAD60), 7 h for fermented ground broccoli residue dried at 60 °C (F_G_HAD60), 7 h for non-fermented chopped broccoli residue dried at 70 °C (NF_C_HAD70), 6 h for non-fermented ground broccoli residue dried at 70 °C (NF_G_HAD70) and 5 h for fermented ground broccoli residue dried at 70 °C (F_G_HAD70).

Modelling the drying curves is necessary to understand the drying characteristics of disrupted broccoli stems. For this purpose, experimental data of the drying process of broccoli samples corresponding to the falling drying rate period (FDRP) were fitted to the 5 commonly used thin-layer drying models listed in Table 1 [39]. In these models, MR represents the dimensionless moisture ratio or reduced driving force (equation 1).

$$MR = \frac{X_t^w - X_{eq}^w}{X_c^w - X_{eq}^w} \quad (1)$$

where:

X^w_t is the moisture content of the product on a dry basis at a given time; X^w_c is the critical moisture content of the product expressed on a dry basis, which coincides with the moisture content at the initial time of the FDRP; and X^w_{eq} represents the equilibrium moisture content of the product on a dry basis.

Table 1. Models used to analyse the drying kinetics of broccoli stems during the falling drying rate period.

Model	Equation
Lewis	$MR = \exp(-k \cdot t)$
Henderson & Pabis	$MR = a \cdot \exp(-k \cdot t)$
Linear	$MR = -k \cdot t + a$
Page	$MR = \exp(-k \cdot t^n)$
Diffusional	$MR = \frac{8}{\pi^2} \cdot \exp\left(-\frac{D_{ef} \cdot \pi^2 \cdot t}{4 \cdot l^2}\right)$

a and n are the constants of the kinetic models, k is the rate coefficient, D_{ef} is the effective or apparent diffusivity and l is the half thickness.

The goodness of fit of the selected kinetic models to the experimental data was evaluated with the sum squared error (SSE) and the correlation coefficient (R^2).

2.5. Analytical determinations

2.5.1. Physicochemical properties

Water activity (a_w) was obtained with an Aqualab® 4TE dew point hygrometer (Decagon Devices Inc., Pullman, Washington, USA) at 25 °C. **Moisture content (x_w)** was determined following the gravimetric double weighing procedure (AOAC 934.06, 2000) [40], based on the removal of the water present in a known amount of sample by drying in a vacuum oven (Vaciotem-T, JP Selecta, Barcelona, Spain) (P = 10 mmHg) at 60 °C until reaching constant weight. **Total soluble solids content (x_{ss})** was calculated from the measurement of the Brix degrees obtained at 20 °C by a thermostated Abbe refractometer NAR-3T (Atago, Tokyo, Japan). In

powders, measurements were performed in an aqueous extract 1:10 (w/v) ratio. **pH** was measured with a digital pH-meter S20 SevenEasy™ (Mettler-Toledo Inlab, Ohio, USA), previously calibrated with the pertinent buffer solutions at pH 7 and 4. **Particle size distribution** was determined, in both dry and wet conditions, using a Mastersizer 2000 laser diffraction equipment (Malvern Panalytical Lt, Malvern, UK). For the dry method, the equipment was coupled to a dispersion unit Scirocco 2000, using air as dispersing agent at 2.5 bar of pressure and 60% speed. For the wet method, the equipment was coupled to a unit Hydro 2000. The analysis was carried out with a particle absorption index at 0.1 and refraction indexes of 1.52 and 1.33 for the sample and the dispersed phase (deionized water), respectively. Results of particle size measurements are the mean of five replicates and are given in terms of equivalent volume mean diameter D[4,3], surface area mean diameter D[3,2] and distribution percentiles d_{10}, d_{50} and d_{90}.

2.5.2. Antioxidant properties

Antioxidant compounds were extracted by mixing the samples with an 80% (v/v) methanol/bidistilled water in a 4:10 (w/v) or 1:10 ratio (w/v) for raw material or powders, respectively. The mixture was stirred in darkness for 1 h in a horizontal stirrer (Magna Equipments S. L., model ANC10, Barcelona, Spain), and then centrifuged at 10,000 rpm for 5 min (Eppendorf Centrifuge 5804/5804R, Madrid, Spain). When necessary, dilutions of extracts were carried out for the analysis below.

Total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method [41,42]. 0.125 mL of the extract were mixed with 0.5 mL of bidistilled water and 0.125 mL of Folin-Ciocalteu (Scharlab S.L., Barcelona, Spain) reagent. After 6 min of reaction in darkness, 1.25 mL of 7% (w/v) sodium carbonate solution and 1 mL of bidistilled water were added. This preparation was kept in darkness for 90 min and the absorbance was measured at 760 nm in a Helios Zeta UV/Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Results were expressed as mg of Gallic Acid Equivalents (GAE) per g of dry matter.

Total flavonoid content was determined using the modified aluminium chloride colorimetric method [43]. 1.5 mL of the extract was mixed with 1.5

mL of a 2% (w/v) aluminium chloride solution (Thermo Fisher Scientific Inc., Waltham, MA, USA). After 10 min of reaction in darkness, absorbance was measured at 368 nm with a Helios Zeta UV/Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Results were expressed as mg of Quercetin Equivalents (QE) per g of dry matter.

Antioxidant activity was evaluated following the DPPH (1,1 diphenyl-2-picryl hydrazyl) and ABTS (2,20-azobis-3-ethyl benzothiazolin-6-sulphonic acid) methods. For the DPPH method [44], 0.1 mL of the extract and 2.9 mL of a 0.1 mM DPPH (Merck KGaA and affiliates, Darmstadt, Germany) solution in methanol were mixed. After 60 min of reaction in darkness, absorbance was measured at 575 nm. Results were expressed as mg of Trolox Equivalent (TE) per g of dry matter. The ABTS method was applied according to the procedure described by Re et al. [45]. 0.1 mL of the extract was mixed with 2.9 mL of $ABTS^{+}$ (VWR International LLC, Radnor, PA, USA) solution in phosphate buffer with an absorbance of 0.70 ± 0.02 at 734 nm. After 7 min of reaction the absorbance was measured at 734 nm. Results were expressed as mg of Trolox Equivalent (TE) per g of dry matter.

2.6. Microbial counts

Viable number of *Lactobacillus plantarum* was estimated by serial dilution from 10^{-1} to 10^{-8} g/L with buffered peptone water (Scharlab, Barcelona, Spain), seeding on MRS agar (Scharlab, Barcelona, Spain) and incubation at 37 °C for 24 h. First dilution (10^{-1} g/L) was obtained by mixing 3 g of solid sample with 27 mL of sterile buffered peptone water in a stomacher bag and homogenizing for 2 min. Finally, colonies present on the plates were counted.

2.7. Statistical analysis

Statistical analysis was carried out with Statgraphics Centurion XVII software (Statpoint Technologies, Virginia, USA), applying simple and multifactorial analysis of variance (ANOVA) with a confidence level of 95% (p-value < 0.05).

3. Results

3.1. Fermentation preliminary study

Figure 1 shows the results of the microbial counts of *Lactobacillus plantarum* (LP) along the fermentation of ground (G) or chopped (C) broccoli stems. The initial microbial count was 6.6 ± 0.3 log CFU/g and increased significantly during the first hours of fermentation until reaching a maximum of 9.0 ± 0.4 log CFU/g after 24 h. From this moment on, viability started to decrease, probably due to nutrients depletion. Also, pH decreased from 6.3 to 4.1 after 96 h of fermentation as a result of the formation of organic acids such as lactic, acetic and propionic acids [27]. This is of great importance for inhibiting the growth of spoilage microorganisms and extending fermented product shelf-life during storage [46]. From the microbial counts of the fermented product and considering that the recommended dose for probiotics to exert a beneficial health effect is 10^9 CFU/day [13,47], an ingesta between 1 and 5 g of 24 h-fermented broccoli stems would provide the expected benefit (figure 1).

Multifactorial analysis of variance confirmed that the intensity of disruption prior to fermentation did not isignificantly nfluence the microbial growth. In chopped samples, the microorganism grew slightly faster and died more slowly between 24 and 96 h than in ground samples. This could be due to a lower release of polyphenols with an antimicrobial effect, when chopping, thus compromising microbial viability to a lesser extent. Other authors, however, reported that a smaller particle size would increase the release of nutrients into the medium, thus favoring fermentation [48,49]. Further drying study on fermented samples was performed on ground (G) samples fermented during 24 h, since in a previous study a similar behaviour in drying kinetics was obtained for both chopped and ground broccoli stems [19]; and, in addition, better charactistics and bioavailability of nutrients were expected to be obtained in the final powder manufactured with a grinding step, rather than chopping.

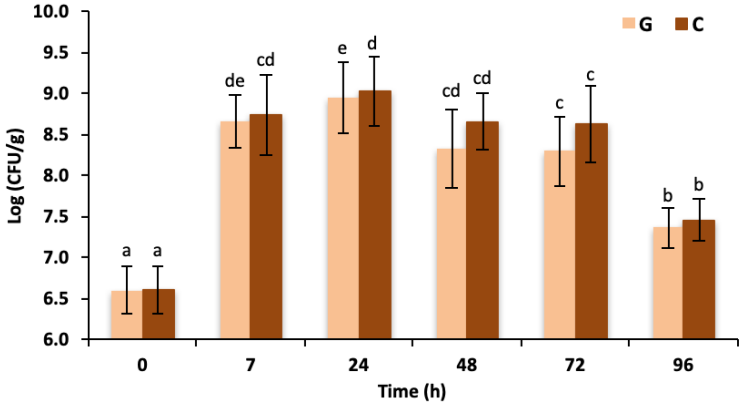

Figure 1. Viable counts of *L. plantarum* during fermentation of chopped (C) and ground (G) broccoli stems. Error bars represent the standard deviation of eight replicates. Different letters in the same series indicate statistically significant differences at the 95% confidence level (*p-value* < 0.05).

3.2. Physicochemical characteristics of unfermented and fermented broccoli stems

Table 2 summarizes physicochemical and antioxidant properties of the disrupted and blanched broccoli stems before and after fermentation during 24 h with *Lactobacillus plantarum* (CECT 749). Moisture content, water activity and soluble solid content were similar to the reported in previous studies [19,50]. As expected, moisture and water activity (a_w) values before fermentation were considerably high, thus confirming that the broccoli residue represents a good environment for bacterial growth. Fermentation had no effect on moisture content, a_w and soluble solid content since no statistically significant differences were obtained between fermented and non-fermented broccoli stems. However, in the case of soluble solid content, Tkacz et al. [51] reported a decrease in sea buckthorn-apple juices fermented with *L. plantarum*, changes attributed to malic acid and lactic acid generated during fermentation process.

According to antioxidant properties, total phenols content was similar to values previously reported for raw broccoli stems (3.8 ± 0.2 mg GAE/g_{dm}) [19], but flavonoid content presented lower values than in other studies (4.6 ± 0.7 mg QE/g_{dm} or 2.4 ± 0.1 mg QE/g_{dm}) [19,52]. Likewise, the antioxidant capacities obtained by the DPPH and ABTS methods were lower than the

values reported in other studies [19,53] with values between 3.7 ± 0.3 mg TE/g_{dm} [19,53] and 3.0 ± 0.8 mg TE/g_{dm} [53] in DPPH; and ranging from 9 ± 4 mg TE/g_{dm} [53] to 34.0 ± 1.6 mg TE/g_{dm} [19,53] in ABTS. These differences might be attributed to different broccoli varieties, culture conditions or other agronomic factors [53]. As reported in bibliography, broccoli stems antioxidant content is lower than in other parts of the plant like leaves (with a total phenol content of 24 ± 5 mg GAE/g_{dm} and an overall antioxidant capacity of 2.9 ± 0.8 mg TE/g_{dm} and 8 ± 4 mg TE/g_{dm} for radicals DPPH and ABTS, respectively) or florets (with a total phenol content in the range of 6-10 mg GAE/g_{dm}, a total flavonoid content of 5.4 ± 0.6 mg QE/g_{dm} and an antioxidant capacity of 5.7 ± 1.5 mg TE/g_{dm} and 16 ± 4 mg GAE/g_{dm} for radicals DPPH and ABTS, respectively) [52,53].

Regarding the impact of fermentation on the antioxidant properties, there were statistically significant differences between fermented and non-fermented samples, fermentation generally having a positive impact on antioxidant characteristics, excepting in phenols analysis where no effect was observed. Also, in Tkacz et al. [51] fermentation by using *L. plantarum* enhanced flavonols and antioxidant activity of sea buckthorn berries mixed with apple juices. Lactic acid bacteria have been reported to increase antioxidant properties in fermented plant-based foods due to a decrease in pH and enzyme hydrolysis during fermentation [54]. Fermentation mainly improves the release of antioxidant compounds via microbial hydrolysis, since microbial enzymes produced can hydrolyse phenolic and flavonoid compounds releasing other compounds with antioxidant activity. Also, fermentation could induce structural breakdown of plant cell wall liberating and/or synthesising antioxidant compounds; as well as phytochemicals structural changes enhancing or modifying antioxidant activity [55]. However, fermentation can affect in a positive or negative way the release and production of antioxidant compounds, depending on the stating microorganism, fermentation conditions (pH, temperature, water content, etc.) or type of vegetable matrix [54–56].

Table 2. Moisture content (x_w), water activity (a_w), soluble solids content (x_{ss}), total phenol content, total flavonoid content and antioxidant capacity measured by DPPH and ABTS methods of non-fermented and fermented broccoli stems.

Properties	Non-fermented	Fermented
x_w(%)	90.5 ± 1.0[a]	92 ± 2[a]
a_w	0.989 ± 0.005[a]	0.991 ± 0.003[a]
x_{ss} (g/g)	0.696 ± 0.062[a]	0.615 ± 0.035[a]
Total Phenol Content (mg GAE/g_{dm})	3.62 ± 0.17[a]	3.7 ± 0.4[a]
Total Flavonoid Content (mg QE/g_{dm})	0.75 ± 0.15[a]	1.2 ± 0.3[b]
DPPH (mg TE/g_{dm})	0.46 ± 0.16[a]	1.1 ± 0.3[b]
ABTS (mg TE/g_{dm})	3.66 ± 0.06[a]	5.46 ± 0.11[b]

[a,b,c…]different superscripts in the same line indicate significant differences at 95% confidence level (*p*-value < 0.05).

3.3. Drying curves and drying rate curves

Drying rate curves, obtained by the incremental method, and drying curves corresponding to the different processing conditions tested are plotted in Figure 2. Drying curves represent the evolution over time of the quotient between the moisture at each instant of the process (X^w_t) and the initial moisture (X^w_0), both expressed on a dry matter (kg w/kg ss). Drying rate curves represent the rate at which moisture content decreases along drying with respect to the amount of water present in the sample; they are helpful to identify the response to drying regarding the different drying periods which may be identified.

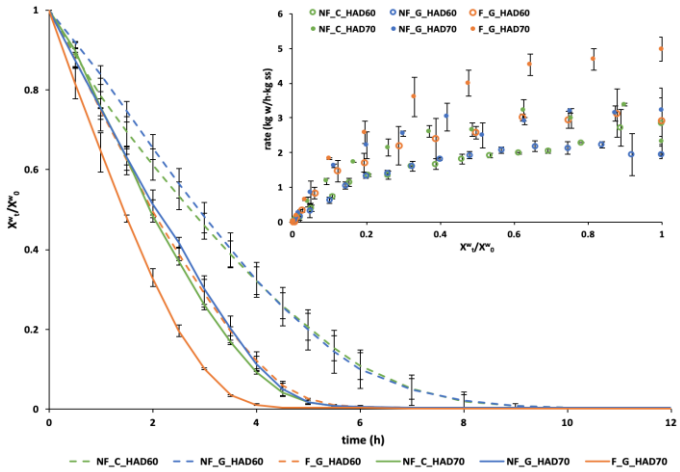

Figure 2. Drying and drying rate curves of chopped (C) and ground (G), fermented (F) and non-fermented (NF) broccoli stems, hot air-dried (HAD) at 60 and 70 °C.

As observed, time required to reduce the water content of the samples subjected to a similar pre-treatment decreased as the drying air temperature increased from 60 to 70 °C. Regarding disruption intensity prior to drying, no remarkable differences were observed between the drying curves of chopped and ground broccoli stems dried at the same temperature. In contrast, fermentation of the ground stems decreased significantly the time needed to reduce their moisture content. This effect of fermentation on drying behaviour could be attributed to microorganisms' metabolism and the ability of lactic acid bacteria to produce enzymes, such as cellulases and glycosidases, which degrade cell wall polysaccharides, thereby reducing the tissue resistance to water transport [57].

Drying curves were used to determine the moisture samples in equilibrium with the airstream (X^w_{eq}). No significant differences were found between ground and chopped samples, whereas fermentation and drying temperature implied a reduction in equilibrium values so that it deceased from 0.027 to 0.018 kg w/kg ss for samples air-dried at 60 °C and from 0.018 to 0.011 kg w/kg ss for samples air-dried at 70 °C.

As indicated previously, drying periods are better identified in the drying rate curves. As deduced from their shape (figure 2) drying of disrupted broccoli stems started with a constant drying rate period (CDRP) in which drying is controlled by the rate at which water is removed from the surface of the product. In this period, free or loosely bound water is removed and the risk of sample damage by exposure to high temperatures is low since the temperature of sample is close to the wet bulb temperature of the drying air [19,58,59]. As drying proceeds, replacement of water evaporated from the surface of the product by water from the inside becomes more difficult, so that dried regions start to appear on the product surface. As the wetted surface area diminishes, there is less water available to evaporate. In consequence, less energy is used to evaporate water from the surface and part of the energy is used to heat up the sample. In this period, the drying rate decreases progressively so it is called the falling drying rate period (FDRP), during which the risk of thermolabile compounds degradation is higher. The moment which defines the transition from CDRP to FDRP is known as the critical time (t_c) and the water content of the solid at this time

is called critical moisture content (X^w_c). Drying rate during the CDRP and critical moisture content for the different conditions assayed are summarized in Table 3. In general terms, the duration of the CDRP decreased with increasing the drying temperature and when fermentation was applied as a pre-treatment. No significant differences (*p*-value < 0.05) were found between drying rate values in the CDRP for ground and chopped samples dried at a given temperature. However, the critical moisture content was significantly affected by the intensity of the previous disruption, but to a different extent depending on the drying temperature.

Table 3. Values of critical time (t_c), critical moisture content (X^w_c) and drying rate in the constant drying rate period ($rate_{CDRP}$) of disrupted broccoli stems as a function of pre-treatment (G: ground; C: chopped; F: fermented; NF: non-fermented) and temperature of drying air (HAD60: 60 °C; HAD70: 70 °C).

	t_c (h)	**X^w_c (kg w/kg ss)**	**$rate_{CDRP}$ (kg w/h · kg ss)**
NF_G_HAD60	2.5	6.8 ± 0.3^b	2.08 ± 0.12^a
NF_C_HAD60	3	5.81 ± 0.05^a	2.2 ± 0.4^a
F_G_HAD60	1.5	7.4 ± 0.2^b	3.00 ± 0.10^b
NF_G_HAD70	2.5	5.4 ± 0.2^a	3.0 ± 0.3^b
NF_C_HAD70	1.5	7.3 ± 0.2^b	3.0 ± 0.5^b
F_G_HAD70	1	8.7 ± 0.6^c	4.7 ± 0.2^c

$^{a,b,c,\ldots}$different superscripts in the same column indicate significant differences at 95% confidence level (*p*-value < 0.05).

3.4. Modelling of the drying of disrupted broccoli stems in thin layers

Experimental data corresponding to the FDRP drying kinetics were fitted to the mathematical models listed in the Materials and Methods section, and parameters obtained are shown in Table 4, including drying model coefficients and the comparison criteria R^2 and SSE used to evaluate goodness of fit.

Table 4. Parameters resulting from fitting mathematical models to broccoli stem drying data during the falling drying rate period (FDRP). SSE: sum squared error. HAD: hot-air drying at 60 or 70 °C; C: chopped, G: ground; F: fermented.

Model	Parameters	NF_G_HAD60	NF_C_HAD60	F_G_HAD60	NF_G_HAD70	NF_C_HAD70	F_G_HAD70
Page	**k (h^{-1})**	0.35 ± 0.05^{a}	0.36 ± 0.04^{a}	0.503 ± 0.005^{ab}	0.8 ± 0.2^{d}	0.57 ± 0.02^{bc}	0.73 ± 0.08^{cd}
	n	1.30 ± 0.08^{a}	1.31 ± 0.06^{a}	1.420 ± 0.005^{ab}	1.31 ± 0.10^{ab}	1.41 ± 0.02^{ab}	1.46 ± 0.05^{b}
	SSE	0.0057	0.0597	0.0089	0.0060	0.0080	0.0069
	R^2	0.992	0.992	0.984	0.979	0.988	0.990
Diffusional	**D_{ef} x 10^{-9} (m^2/s)**	1.5 ± 0.4^{a}	1.6 ± 0.6^{a}	2.35 ± 0.02^{b}	3.0 ± 0.2^{cd}	2.58 ± 0.07^{bc}	3.34 ± 0.14^{d}
	SSE	0.045	0.049	0.052	0.028	0.050	0.053
	R^2	0.902	0.901	0.879	0.935	0.903	0.919
Lewis	**k (h^{-1})**	0.6 ± 0.2^{a}	0.61 ± 0.12^{a}	0.80 ± 0.13^{b}	1.14 ± 0.08^{cd}	0.985 ± 0.010^{bc}	1.26 ± 0.06^{d}
	SSE	0.0294	0.0318	0.0379	0.0266	0.0362	0.0393
	R^2	0.939	0.940	0.911	0.947	0.931	0.941
Henderson & Pabis	**k (h^{-1})**	0.7 ± 0.2^{a}	0.71 ± 0.15^{a}	1.07 ± 0.02^{b}	1.20 ± 0.04^{b}	1.155 ± 0.003^{b}	1.45 ± 0.05^{b}
	a	1.5 ± 0.2^{a}	1.5 ± 0.2^{a}	1.74 ± 0.06^{a}	1.2 ± 0.2^{a}	1.77 ± 0.07^{a}	1.72 ± 0.03^{a}
	SSE	0.0497	0.0519	0.0791	0.0265	0.0755	0.0822
	R^2	0.965	0.968	0.946	0.951	0.960	0.957
Linear	**k (h^{-1})**	0.154 ± 0.003^{a}	0.167 ± 0.002^{b}	0.224 ± 0.007^{c}	0.282 ± 0.009^{d}	0.282 ± 0.002^{d}	0.334 ± 0.003^{e}
	a	0.83 ± 0.03^{a}	1.36 ± 0.02^{c}	0.860 ± 0.005^{ab}	0.83 ± 0.05^{a}	1.329 ± 0.005^{c}	0.90 ± 0.02^{b}
	SSE	0.0315	0.1612	0.0289	0.0625	0.1228	0.0624
	R^2	0.894	0.912	0.925	0.880	0.960	0.949

$^{a,b,c\ldots}$Different superscript letters in the same line indicate statistical significant differences at the 95% confidence level (p-value < 0.05).

Statistically significant differences ($p < 0.05$) of k, n and a parameters were observed between samples in all models tested. Generally, kinetic parameters results evidenced a higher drying rate and mass transfer coefficient (k) when the broccoli stem was fermented prior to drying compared to non-fermented; as well as, when temperature increased from 60 to 70 °C, which is in agreement with previous reports [39,60] since at higher temperatures the energy available for water mobility and evaporation rises increasing drying rate [60]. Not a clear effect was observed regarding disruption intensity, obtaining generally similar values in ground and chopped non-fermented broccoli stem for the same drying temperature.

According to R^2 and SSE test applied to support kinetic models, results stated that the proposed models fitted the data adequately, showing R^2 values generally higher than 0.90 and low SSE values. Among models, Page resulted in the best fit for all samples, with the highest R^2 values ($R^2 \geq 0.98$) and the lowest SSE values varying from 0.0057 to 0.0089. The Page model has shown good applicability for both dehydration and rehydration processes of several food products, subjected to different pretreatments and drying methods [15,61]. Analysing characteristic parameters of this model, it is observed that k, related to the diffusion coefficient and the geometry of the sample [62], and n values, related to the type of diffusion and the microstructure of the food [62], are of the same order as those obtained by Salim et al. [14] during the drying of broccoli stems cut into 6 mm thick slices at 40 °C ($k = 0.12\ h^{-1}$ and $n = 1.205$), 50 °C ($k = 0.138\ h^{-1}$ and $n = 1.230$) and 60 °C ($k = 0.144\ h^{-1}$ and $n = 1.288$). As previously mentioned, the value of k increased with the temperature and in fermented samples. Although the intensity of the disruption pretreatment did not significantly affect the value of the parameter k in the samples dried at 60 °C, at 70 °C grinding resulted in significantly higher k values than chopping and closer to values obtained for fermented samples. Since the values obtained for n are higher than 1, it can be stated that drying broccoli stems under the conditions studied is a super-diffusion process [62]. For a similar temperature, the value of n was not significantly affected by the intensity of previous disruption but, increased significantly with fermentation.

To confirm the goodness of fit of Page's model, figure 3 plots the predicted MR values against experimental ones. The closeness of the plotted data to the straight line represents the good correlation between calculated and experimental results. Likewise, the drying curves predicted with the Page model and experimental ones are also plotted.

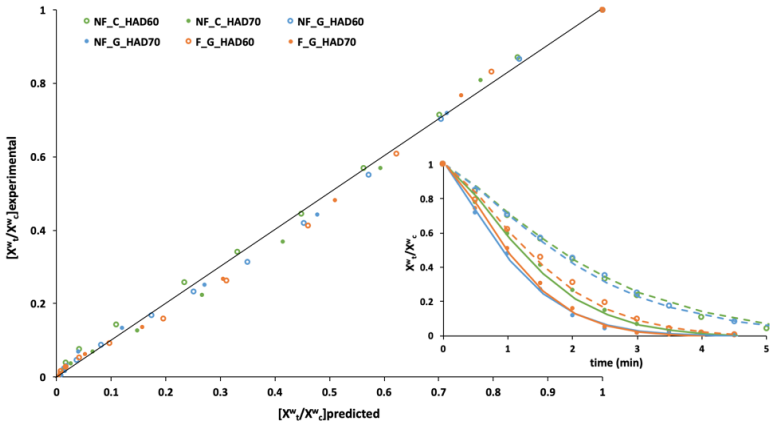

Figure 3. Goodness of fit of Page's model: comparison between experimental and predicted reduced moisture values and comparison between experimental (filled and unfilled markers) and predicted drying curves (solid and dashed lines).

Several empirical equations are usually used to evaluate and model the drying kinetics of food, such as Page, Lewis, Henderson-Pabis and Linear models studied in this work, among others. All of these models are ultimately governed by the diffusional model of Fick's second law, which is the most widely studied theoretical model in thin-layer drying of different food products [15,63]. Diffusivities obtained from the first term of the analytical solution to Fick's second law of diffusion proposed by Crank [64], for an infinite layer geometry and long treatment times, were of the same order as those obtained by other authors for similar products, e.g., effective diffusivity of fresh broccoli florets ranged from 2.82 x 10^{-10} to 2.00 x 10^{-9} m^2/s in Mahn et al. [65] or even increased to an average value of 4.9 x 10^{-9} m^2/s in Reyes et al. [66], and in the case of blanched broccoli taking values between 1.99 x 10^{-8} and 3.56 x 10^{-8} m^2/s [16]. Regarding the effect of the process variables, the statistical analysis confirmed that increasing the drying air temperature from 60 to 70 °C or applying a fermentation step prior to drying

significantly increased the value of the effective water diffusivity. Higher effective diffusivity values represent a faster drying process, which can be positive for the dried probiotic product development, although the effect of temperature must be evaluated to determine the optimal process conditions to ensure probiotic viability in the final product [60]. No significant differences were found between the effective water diffusivity values of chopped and ground samples dried at the same temperature.

3.5. Characterization of broccoli stem powdered products

3.5.1. Moisture content, water activity and particle size characteristics

Table 5 shows the values of moisture content and water activity (a_w) of the different powders obtained. Although freze-drying is an expensive technique with a more difficult implementation than hot-air drying, it is a technique known to better preserve the nutritional properties of agri-food products, so it was also employed to stabilized fermented and non-fermented broccoli stems. Results indicate that drying by either of the two techniques significantly decreased the moisture and a_w values of the raw material (table 2). The a_w indicates the availability of water to participate in reactions responsible for food spoilage, so values below 0.3, as obtained, guarantee the powder's stability [38].

Table 5. Moisture content (x_w) and water activity (a_w) of broccoli stem powders. HAD: hot-air drying at 60 or 70 °C, FD: freeze-drying; C: chopped, G: ground; NF: non-fermented, F: fermented. Mean ± standard deviation of three independent measurements.

Treatment	Moisture content (%)	a_w
NF_G_HAD60	3.7 ± 0.2^{g}	0.226 ± 0.003^{a}
NF_C_HAD60	3.82 ± 0.08^{g}	0.234 ± 0.004^{b}
F_G_HAD60	2.56 ± 0.04^{c}	0.253 ± 0.004^{c}
NF_G_HAD70	2.44 ± 0.05^{bc}	0.254 ± 0.004^{c}
NF_C_HAD70	3.00 ± 0.07^{e}	0.234 ± 0.004^{b}
F_G_HAD70	3.197 ± 0.011^{f}	0.237 ± 0.007^{b}
NF_G_FD	2.76 ± 0.04^{d}	0.289 ± 0.005^{e}
NF_C_FD	2.40 ± 0.03^{b}	0.262 ± 0.005^{d}
F_G_FD	2.096 ± 0.006^{a}	0.2490 ± 0.0007^{c}

$^{a,b,c,\ldots}$Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Figure 4 shows the particle size distribution curves obtained, by both the dry and wet methods, for the different powders obtained. Curves of the powders obtained by HAD showed a greater span, i.e., a greater variability in the size of the particles. In the case of FD, curves showed a narrower distribution characteristic of a more homogeneous particle size. This could be due to the fact that during freezing and subsequent sublimation, the food matrix is more intensively broken and a more porous structure is obtained, which favours the formation of finer particles after final milling [38,67]. By the dry method, chopping and fermentation pre-treatments prior to dehydration resulted in powders with a more homogeneous particle size distribution than those obtained by grinding. Also, it is observed that the powders obtained with a chopping pre-treatment prior to HAD, presented a larger particle size than pre-grinding and a lower homogeneity for the same drying temperature. This result is in line with previous studies [18–20]. In wet measurement, larger particle size was obtained because hydration may induce the formation of particle aggregates. Increasing air-drying temperature produced a significant decrease in the mean particle size, regardless of the measuring method used. The values of the characteristic particle size parameters shown in Table 6 elucidated the statistically significant differences between samples.

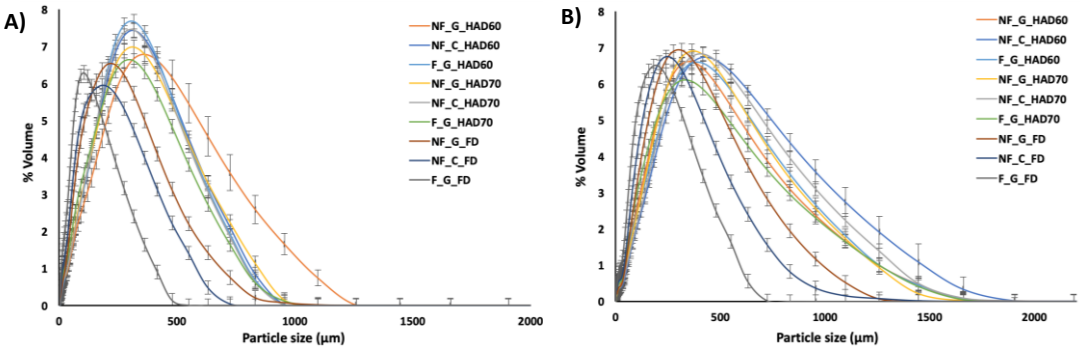

Figure 4. Particle size distribution of the broccoli stem powders. A) Determination by the dry method. B) Determination by the wet method. Error bars are the standard deviation of five replicates. HAD: hot-air drying at 60 or 70 °C, FD: freeze-drying; C: chopped, G: ground; NF: non-fermented, F: fermented.

Table 6. Particle size characteristic parameters obtained by the dry and wet procedures: equivalent volume diameter D[4,3], surface area mean diameter D[3,2], percentiles d_{10}, d_{50}, and d_{90}. HAD: hot-air drying at 60 or 70 °C, FD: freeze-drying; C: chopped, G: ground; NF: non-fermented, F: fermented. Mean ± standard deviation of five replicates.

	DRY PROCEDURE								
	NF_G_HAD60	**NF_C_HAD60**	**F_G_HAD60**	**NF_G_HAD70**	**NF_C_HAD70**	**F_G_HAD70**	**NF_G_FD**	**NF_C_FD**	**F_G_FD**
D[4,3]	333 ± 19^{f}	282 ± 10^{e}	281 ± 16^{e}	282 ± 10^{e}	276 ± 16^{e}	254 ± 16^{d}	215 ± 8^{c}	176 ± 4^{b}	120 ± 2^{a}
D[3,2]	96 ± 8^{e}	98 ± 2^{e}	114 ± 7^{f}	84 ± 3^{d}	97 ± 6^{e}	76 ± 3^{c}	84 ± 2^{d}	57.1 ± 0.9^{b}	42 ± 2^{a}
d_{10}	50 ± 6^{e}	51 ± 2^{e}	57 ± 5^{f}	44 ± 3^{d}	50 ± 4^{e}	36 ± 2^{c}	44.1 ± 0.6^{d}	28.3 ± 0.5^{b}	19.1 ± 0.2^{a}
d_{50}	283 ± 22^{f}	250 ± 10^{e}	251 ± 16^{e}	244 ± 10^{e}	245 ± 16^{e}	216 ± 13^{d}	175 ± 4^{c}	140 ± 2^{b}	95.2 ± 0.9^{a}
d_{90}	691 ± 31^{g}	562 ± 18^{ef}	549 ± 28de	575 ± 19^{f}	548 ± 27de	532 ± 34^{d}	443 ± 18^{c}	379 ± 9^{b}	258 ± 5^{a}
	WET PROCEDURE								
	NF_G_HAD60	**NF_C_HAD60**	**F_G_HAD60**	**NF_G_HAD70**	**NF_C_HAD70**	**F_G_HAD70**	**NF_G_FD**	**NF_C_FD**	**F_G_FD**
D[4,3]	378 ± 18de	453 ± 24^{g}	390 ± 34^{e}	388 ± 17^{e}	419 ± 21^{f}	362 ± 17^{d}	312 ± 9^{c}	255 ± 12^{b}	187 ± 3^{a}
D[3,2]	104 ± 4^{f}	115 ± 4^{h}	101 ± 5^{e}	109 ± 3^{g}	104 ± 3^{f}	89 ± 2^{d}	82 ± 2^{c}	63 ± 2^{b}	49.0 ± 0.4^{a}
d_{10}	66 ± 3^{e}	82 ± 3^{g}	62 ± 4^{d}	74 ± 3^{f}	73 ± 3^{f}	56 ± 2^{c}	66.0 ± 1.2^{e}	46 ± 2^{b}	28.2 ± 0.4^{a}
d_{50}	306 ± 9^{e}	374 ± 12^{h}	323 ± 20^{f}	324 ± 12^{f}	350 ± 11^{g}	280 ± 9^{d}	260 ± 5^{c}	210 ± 5^{b}	155 ± 2^{a}
d_{90}	798 ± 54^{d}	945 ± 65^{f}	821 ± 90de	802 ± 44^{d}	869 ± 56^{e}	797 ± 46^{d}	637 ± 23^{c}	527 ± 29^{b}	393 ± 7^{a}

$^{a,b,c...}$Different superscript letters in the same line indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

3.5.2. Antioxidant properties of broccoli stem powders

Table 7 shows the total phenols and flavonoids content, as well as the antioxidant capacity measured by ABTS and DPPH methods, of the different powders obtained from broccoli stems. In all cases, a positive effect of processing was observed, since the antioxidant properties of the powders were better as compared to non-processed stems (table 3).

Powders fermented with *Lactobacillus plantarum* spp. (CECT 749) generally presented higher total phenols and flavonoids content, but one of the lowest antioxidant capacities measured by both DPPH and ABTS. These results are consistent with other studies, like that of Cai et al. [46] in which fermentation significantly increased total phenol content, but to a lesser extent the antioxidant capacity of broccoli puree. This could be explained by microbial enzymes which degrade cell wall polysaccharides, mainly cellulases and glycosidases, favouring the release of phenolic compounds [57,68], as well as to the fermentative microorganism production of new antioxidant compounds [69], and the transformation of some antioxidant compounds into others with higher activity [57]. Moreover, fermented powders required less drying time, which decreased the degradation of phenolic compounds by oxidation.

If comparing between processing methods, statistically significant differences (p-value < 0.05) were found among powders regarding total phenols and flavonoids. Freeze-dried powders presented the highest values, as a result of the use of vacuum and the low temperatures which prevent their degradation. During freezing and subsequent sublimation, cell walls and membranes are broken, obtaining a porous structure that after milling results in a finer powder with a larger surface area in contact with solvent, which increases extraction efficiency [38,70]. FD ground broccoli stems exhibited better phenol and flavonoid content compared to chopped, which may be attributed to an intensified structure disruption before drying and while freezing. Comparing among HAD samples, the reduction in drying time could have favoured the increase in phenols and flavonoids content when drying at 70 °C, but similar results were obtained in non-fermented ground and chopped samples. In powders obtained by HAD at 60 °C, differences

found between the ground and chopped samples could be attributed to the different duration of the CDRP, which was longer in the case of the chopped samples. Considering that during this period the temperature of the solid being dried remains closer to the saturation temperature of the air in contact with it, the exposure time to elevated temperatures of the chopped samples was shorter, so their total phenol and flavonoid content was significantly higher. This trend that has also been observed in a previous study [19]. Regarding the antioxidant capacity, in all cases, values obtained by the ABTS method were higher than by DPPH, which may be due to a greater affinity of the antioxidant compounds present in the broccoli stem with this free radical. Powders obtained by freeze-drying generally showed higher values than HAD samples. Not a clear trend was observed according to hot-air drying temperature applied. Regarding the disruption intensity prior to HAD, grinding significantly reduced (p-value < 0.05) the ability to inhibit the ABTS radical, but slightly increased the ability to inhibit the DPPH radical. On the contrary, grinding prior to freeze-drying resulted in a higher capacity to inhibit the ABTS radical and a lower capacity to inhibit the DPPH radical. To explain this, it should be considered that antioxidant capacity could be affected by the presence of other compounds with antioxidant activity which have not been quantified in this study, but could have a different behavior under the processing conditions used.

Table 7. The processing effect on total phenol content (mg GAE/g_{dm}), total flavonoid content (mg QE/g_{dm}) and antioxidant activity measured by DPPH and ABTS methods (mg TE/g_{dm}) of the different powders obtained. HAD: hot-air drying at 60 or 70 °C, FD: freeze-drying; C: chopped, G: ground; NF: non-fermented, F: fermented. Mean ± standard deviation of six replicates. $^{a,b,c,\cdots}$different superscripts in the same column indicate significant differences at 95% confidence level (p-value < 0.05).

	Total Phenol Content (mg GAE/g_{dm})	**Total Flavonoid Content** (mg QE/g_{dm})	**DPPH** (mg TE/g_{dm})	**ABTS** (mg TE/g_{dm})
NF_G_HAD60	4.1 ± 0.3^{a}	2.9 ± 0.4^{a}	3.1 ± 0.2^{c}	12.1 ± 1.1^{a}
NF_C_HAD60	5.1 ± 0.3^{b}	4.7 ± 0.2^{b}	2.7 ± 0.2^{ab}	26.7 ± 1.5^{d}
F_G_HAD60	6.4 ± 0.3^{d}	4.5 ± 0.5^{b}	3.0 ± 0.5^{bc}	19.2 ± 0.8^{c}
NF_G_HAD70	5.6 ± 0.6^{c}	4.31 ± 0.11^{b}	3.2 ± 0.2^{c}	14.5 ± 0.9^{b}
NF_C_HAD70	5.4 ± 0.4^{bc}	4.4 ± 0.4^{b}	3.0 ± 0.4^{bc}	15.3 ± 0.8^{c}
F_G_HAD70	8.0 ± 0.3^{f}	5.7 ± 0.4^{c}	2.48 ± 0.11^{a}	13.0 ± 1.1^{b}
NF_G_FD	8.4 ± 0.3^{g}	8.71 ± 0.13^{e}	4.9 ± 0.2^{e}	25 ± 4^{d}
NF_C_FD	7.0 ± 0.4^{e}	5.8 ± 0.4^{c}	5.9 ± 0.4^{f}	18.9 ± 0.5^{c}
F_G_FD	11.8 ± 0.2^{h}	6.8 ± 0.5^{d}	3.9 ± 0.3^{d}	25.4 ± 1.1^{d}

3.5.3. Microbial count in broccoli stems powders

Figure 5 shows the results of microbial counts on fresh broccoli stem fermented for 24 h with *Lactobacillus plantarum* spp. CECT 749 and powders obtained from fermented broccoli stem by different dehydration techniques.

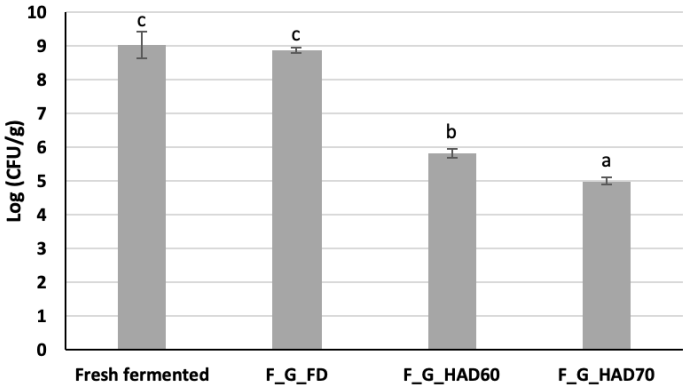

Figure 5. Viable counts (*L. plantarum*) in powders obtained by pre-fermentation of broccoli stems. Error bars represent the standard deviation of four replicates. HAD: hot-air drying at 60 or 70 °C, FD: freeze-drying; C: chopped, G: ground; F: fermented. [a,b,c…]different letters in the same series indicate significant differences at the 95% confidence level.

As expected, increasing from 60 to 70 °C the drying temperature significantly reduced the microbial content, from 1.54 x 10^9 CFU/g to 7.18 x 10^5 CFU/g and 1.01 x 10^5 CFU/g, respectively. These values are below those typically proposed for probiotic foods, which contain more than 10^6 CFU per g of product [47,71]. However, freeze-drying treatment permitted a final concentration of viable colonies near to 10^7 CFU/g, allowing sufficient cell viability to be considered as probiotic powder.

Similar tendency was reported in a previous study about persimmon powders inoculated with *L. salivarius* [13], in which FD powder presented a final concentration of 10^7 CFU/g being considered probiotic, and bacterial viability were affected by HAD in a greater extent. Also, in a study [72] about dried apple snacks enriched with *L. plantarum*, bacterial counts were affected by drying methods tested, but with FD a concentration higher than 10^8 CFU/g was reached. However, hot-air drying at low temperatures, particularly 40 °C, has been demonstrated to retain 10^6 CFU/g of *L. casei* in

dried murta berries, value adopted by food industry to be considered as probiotic product [15]. Moreover, the use of a pretreatment like ultrasound-assisted air drying has been reported to improve *L. casei* viability in apple snacks dried at 10, 40 and 60 °C, obtaining in the last one a concentration of 10^6 CFU/g due to drying time reduction when applying ultrasound which resulted in higher viable counts [60].

In this sense, although in the present work FD has been demonstrated to preserve better the bacterial viability, it is an expensive process and difficult to implement at an industrial level. So, it should be considered for future studies to diminish air temperature and/or design a multi-stage air-drying process that may result in increased viability.

4. Conclusions

This research showed that fermentation is a simple and economic method that increases the nutritional value of broccoli stem residue. Different processing alternatives have been demonstrated to transform broccoli stem residues into stable powdered products, which could be used as an ingredient in food formulation.

On the one hand, based on the microbial growth kinetics, the fermentation time to reach a maximum content of *Lactobacillus plantarum* CECT 749 was established at 24 h; and regarding the intensity of pre-destructuring, both levels tested had a similar effect on the microbial content and antioxidant properties of the broccoli stem, which were significantly improved after fermentation. On the other hand, as deduced from drying and drying rate curves and the kinetic parameters resulting from fitting different empirical models to the experimental data, the intensity of pre-destructuring hardly affected the dehydration process duration, whereas fermentation of the residue with *Lactobacillus plantarum* spp. and the increase of drying temperature (from 60 to 70 °C) shortened it significantly. These variables during hot-air drying had direct consequences on the total phenol and flavonoid content of the powders, with the higher results obtained by the fermented residue dried at 70 °C. However, the powders obtained by freeze-drying showed the best antioxidant properties, as well as a smaller and more homogeneous particle size. Freeze-drying also proved to

be the best technique to preserve the viability of the microorganism used in the fermentation process.

In general terms, it is concluded that the powdered products developed in this research would contribute to reducing broccoli stem waste and could be used as a potential ingredient in the development of new functional foods.

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CONCLUSIÓN

Estas investigaciones permitieron obtener productos en polvo estables a partir de los residuos de confección de bandejas y productos de IV gama de zanahoria, col, apio, puerro y brócoli. Se prevé que este tipo de aproximaciones pueda contribuir de manera efectiva al desarrollo de sistemas alimentarios más saludables y sostenibles. Las diferentes matrices vegetales fueron transformadas mediante una serie de procesos que alternaban etapas de pretratamientos y de secado, con la finalidad de maximizar las propiedades funcionales de los productos en polvo. Se demostró que tanto las variables del proceso como el tipo de matriz vegetal tienen un efecto diferente en las características de los productos obtenidos. Además, se comprobó que la fermentación es un método sencillo y económico con capacidad para aumentar el valor nutricional de los residuos vegetales, permitiendo obtener polvos probióticos.

Escoger las etapas de proceso adecuadas, así como realizar estudios de estabilidad durante el almacenamiento es crucial para obtener polvos funcionales de calidad, que luego puedan ser incorporados a la cadena alimentaria. En términos generales, se concluye que los productos en polvo desarrollados contribuirían a reducir los residuos de las hortalizas seleccionadas, y podrían aplicarse en la industria alimentaria como colorantes, aromatizantes o conservantes naturales, o bien utilizarse como ingredientes en el desarrollo de nuevos alimentos con el fin de mejorar sus propiedades nutricionales.

CAPÍTULO 3. Impacto del procesado sobre los compuestos bioactivos de interés y evaluación de la respuesta de los residuos en polvo a la digestión simulada *in vitro* en función de las variables de proceso y de las características de los productos en polvo obtenidos.

Bas-Bellver, C., Barrera, C., Betoret, N., Seguí, L. Effect of Processing and In Vitro Digestion on Bioactive Constituents of Powdered IV Range Carrot (*Daucus carota*, L.) Wastes. *Foods* 2023, 12(4), 731.

Bas-Bellver, C., Barrera, C., Betoret, N., Seguí, L. Impact of Disruption and Drying Conditions on Physicochemical, Functional and Antioxidant Properties of Powdered Ingredients Obtained from Brassica Vegetable By-Products. *Foods* 2022, 11(22), 3663.

ANNEX: *In vitro* digestion effect on antioxidant properties of selected powdered ingredients obtained from brassica vegetable by-products.

RESUMEN DEL CAPÍTULO

Como se ha mencionado a lo largo del documento, los residuos vegetales, aunque tradicionalmente infrautilizados y considerados material de bajo valor, son ricos en compuestos bioactivos, lo que los hace susceptibles de ser valorizados. Además, para garantizar que estos compuestos ejerzan un efecto beneficioso en la salud, se requiere conocer su bioaccesibilidad tras la digestión. Por ello, este tercer capítulo recoge los estudios relacionados con la influencia de las operaciones unitarias establecidas anteriormente sobre las propiedades de los polvos, con especial atención sobre los compuestos bioactivos específicos. Concretamente, las investigaciones se centraron, por un lado, en la evaluación de carotenoides en polvos obtenidos a partir de residuos de zanahoria, y por otro, en la determinación de glucosinolatos e isotiocianatos en polvos de residuos de col blanca y brócoli; así como en el estudio de la respuesta a la digestión gastrointestinal simulada *in vitro* (fases oral, gástrica e intestinal) de aquellos polvos de residuos de zanahoria, col y brócoli seleccionados como más prometedores para ser propuestos como ingrediente funcional.

En primer lugar, la zanahoria (*Daucus carota* L.) es una hortaliza ampliamente consumida en todo el mundo y es una fuente rica en compuestos como vitaminas, minerales, antioxidantes y principalmente carotenoides. Los carotenoides son pigmentos lipofílicos sintetizados principalmente por las plantas, aunque también por algunos microorganismos. Se ha visto que son beneficiosos para la salud humana, puesto que reducen el riesgo de padecer ciertas enfermedades como son las cardiovasculares, inflamatorias, ciertos tipos de cáncer, y además favorecen el sistema inmune. No obstante, se trata de compuestos muy inestables a la luz, temperatura, oxidación o procesado entre otros, sobre todo una vez liberados de la matriz vegetal. Además, la biodisponibilidad de los carotenoides es muy baja, principalmente cuando se consumen crudos. Se ha visto que cuando el material vegetal es cocinado, procesado o consumido junto con lípidos, la biodisponibilidad de los carotenoides puede verse favorecida.

Este capítulo incluye un primer trabajo en el que se evaluó el efecto de las variables del proceso sobre las propiedades fisicoquímicas, funcionales y el contenido en compuestos bioactivos específicos de los polvos de residuos de zanahoria, así como su respuesta a la digestión gastrointestinal simulada *in vitro*. Los residuos de zanahoria se transformaron en polvo siguiendo el proceso descrito en investigaciones previas, consistente en una primera disrupción del residuo (triturado vs. troceado), seguido del secado (liofilización o secado por aire caliente a 60 °C o 70 °C) y una molienda final para obtener el polvo definitivo. Antes y después de la digestión *in vitro* se evaluó el efecto de estas operaciones aplicadas sobre las propiedades fisicoquímicas, antioxidantes y el contenido en carotenoides. Además, se estudió el efecto de dispersar los polvos en diferentes matrices (agua, aceite o emulsión de aceite en agua) sobre la liberación de carotenoides durante la digestión *in vitro*. Según los resultados obtenidos, tanto la intensidad de la desestructuración previa como el método de secado influyeron significativamente en las propiedades de los polvos. Generalmente, el secado por aire caliente y, particularmente, tras un troceado previo, dio lugar a polvos con mejores propiedades antioxidantes, mientras que con la liofilización se consiguieron polvos más finos y con mayor contenido en carotenoides. Por su parte, la digestión *in vitro* favoreció la liberación de carotenoides. La respuesta a la digestión varió en función de las condiciones de procesado que implicaron diferentes propiedades físicas de los polvos, así como de la co-ingestión de polvos con aceite, obteniéndose mayores índices de recuperación en los polvos dispersados en aceite.

Por otro lado, las brassicas tienen la particularidad de sintetizar unos metabolitos secundarios llamados glucosinolatos que, al producirse un daño en el tejido de la planta, son hidrolizados por la enzima mirosinasa produciendo una serie de compuestos bioactivos conocidos como isotiocianatos. Estos compuestos bioactivos juegan un papel importante en la respuesta al estrés biótico, especialmente en la defensa contra herbívoros y microorganismos. El interés por los efectos beneficiosos para la salud humana de los glucosinolatos y sus productos de hidrólisis (isotiocianatos) es cada vez mayor, especialmente el sulforafano, uno de los más abundantes en las brassicas. Varios estudios señalan su función protectora frente a

ciertas enfermedades como la diabetes, enfermedades cardiovasculares o el efecto antiinflamatorio y anticancerígeno.

Es por esto que también se llevó a cabo una segunda investigación con el propósito de desarrollar un proceso de obtención de productos en polvo a partir de residuos de brócoli (*Brassica oleracea* var. *italica*) y col blanca (*Brassica oleracea* var. *capitata*), evaluándose el efecto del procesado sobre las propiedades fisicoquímicas, tecnológicas y antioxidantes, principalmente sobre el contenido en sulforafano. Además, se evaluó la respuesta a la digestión simulada *in vitro* sobre las propiedades antioxidantes en polvos seleccionados. Los polvos se obtuvieron siguiendo el proceso establecido en previas investigaciones que consta de una primera etapa de disrupción del tejido vegetal (triturado vs. troceado), seguido del secado (secado por aire caliente a 50, 60 o 70 °C, o liofilización) y de la molienda final para obtener los polvos.

Los resultados obtenidos demostraron que las propiedades de los polvos quedaron definidas por las técnicas y condiciones empleadas. La liofilización conservó mejor las propiedades antioxidantes de las materias primas, aunque el secado por aire caliente, en particular a mayores temperaturas, favoreció las propiedades antioxidantes debido a la generación de nuevos compuestos antioxidantes por reacciones de Maillard o al menor tiempo de exposición a las altas temperaturas y oxígeno. La intensidad del desestructurado previo también afectó a las propiedades de los polvos, obteniéndose en el caso del troceado mejores propiedades antioxidantes, además de facilitar el posterior secado.

Las propiedades tecnológicas de interacción con el agua y el aceite también quedaron determinadas por el método de deshidratación empleado. Los polvos de brassicas exhibieron mejores propiedades de hidratación que emulsionantes. El contenido en sulforafano, analizado en el material vegetal fresco y en los polvos, resultó más elevado en las muestras de brócoli que en las de col. Se observó un impacto negativo del procesado atribuido principalmente a la etapa de deshidratación; no obstante, para ambos residuos, la liofilización preservó mejor el contenido en sulforafano que el secado por aire caliente.

Finalmente, se realizaron ensayos de digestión simulada *in vitro* en aquellos polvos de col y brócoli que presentaron las mejores propiedades en la caracterización previa, esto es, los obtenidos mediante liofilización y mediante secado por aire caliente a 70 °C con previa etapa de troceado. La digestión resultó en un efecto positivo sobre la liberación de compuestos antioxidantes, obteniéndose una mayor extracción y solubilización de los mismos según avanzaba el proceso de digestión.

Effect of Processing and In Vitro Digestion on Bioactive Constituents of Powdered IV Range Carrot (*Daucus carota*, L.) Wastes

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Abstract

Daucus carota L. is an important food crop utilized worldwide and a rich source of bioactive compounds. Carrot processing generates residues which are discarded or underused, for which using them as a source for obtaining new ingredients or products is an opportunity for the development of healthier and more sustainable diets. In the present study, the impact of different milling and drying procedures and in vitro digestion on the functional properties of carrot waste powders was evaluated. Carrot waste was transformed into powders by disruption (grinding vs. chopping), drying (freeze-drying or air-drying at 60 or 70 °C) and final milling. Powders were characterized in terms of physicochemical properties (water activity, moisture content, total soluble solids and particle size) nutraceuticals (total phenol content, total flavonoid content antioxidant activity by DPPH and ABTS methods, as well as carotenoid content (α-carotene, β-carotene, lutein, lycopene). Antioxidants and carotenoid content during in vitro gastrointestinal digestion were also evaluated; the latter in different matrices (directly, in water, in oil, and in oil-in-water emulsion). Processing allowed to reduce water activity of samples and obtain powders rich in antioxidant compounds and carotenoids. Both disruption and drying had a significant impact on powders' properties freeze-drying led to finer powders with higher carotenoid content but lower antioxidant values, whereas air-drying implied chopped air-dried powders exhibited higher phenols content and improved antioxidant activity. Simulated in vitro digestion studies revealed that digestion helps release bioactive compounds which are bound to the powder structure. The solubilization of carotenoids in oil was low, but

fat co-ingestion notably increased their recovery. According to the results, carrot waste powders containing bioactive compounds could be proposed as functional ingredients to increase the nutritional value of foods, thus contributing to the concepts of more sustainable food systems and sustainable healthy diets.

Keywords: carrot; waste recovery; carotenoids; antioxidant properties; functional powders; in vitro digestion

1. Introduction

Carrot (*Daucus carota* L.) is a root crop of the Umbelliferae family. From a nutritional point of view, it is a rich source of bioactive compounds such as vitamins, minerals, antioxidant compounds and dietary fibre [1,2]. Orange carrots are a well-known source of phenolics and α- and β-carotene, which impart its characteristic colour and account for about half of the provitamin A carotenoid found in the food supply [1–4]. *Daucus carota* is an important food crop utilized worldwide which can be consumed raw, in juice or drinks, cooked as a savoury dish or in sweet dishes [2]. However, as it is the case of most vegetables, carrot processing generates by-products, such as culled carrots and carrot waste.

A variety of technologies are aimed at adding value to vegetables and fruit residues and reduce their environmental impact [5]. These include chemical, physical, and biological transformations applied alone or combined to produce functional components, novel foods, extracted chemicals, biofuels or pomace powder, among others [2]. Carrot residues can be successfully stored in their dried form, which can be used in the development of bakery products and extrudates [2], and also as an ingredient in prepared foods such as instant soups or healthy snack foods [6]. When properly managed, dehydration can be applied to obtain powders from vegetable wastes, greatly preserving the bioactive compounds of interest, thus broadening the benefits these by-products can offer as a source of antioxidants, antimicrobial compounds and other bioactive substances [7,8]. This makes it really interesting to obtain powdered products based on carrots to use them as functional ingredients in food formulation. Among dehydration techniques, hot-air drying (HAD) and

freeze-drying (FD) are usually applied in the food industry to reduce water activity to levels that guarantee product stability and extend shelf life [9], in addition to obtaining a final product that is easier to store, transport, dose and mix with other foods [1]. HAD is simpler and more economic, but implies exposure to conditions which promote oxidation, crusting and loss of organoleptic and functional properties [10]; in contrast, FD prevents oxidation during processing, minimizes compositional changes and largely preserves volatile and soluble compounds, yielding a final porous structure with a minimum moisture content, but at a higher cost and energy requirements [10].

Carrots contain a variety of bioactive compounds, including flavonoids, phenolic acids, carotenoids, polyacetylenes, and ascorbic acid. Among the bioactive constituents of carrots, carotenoids are probably the most important and the most studied ones, although polyacetylenes have also raised significant interest in recent years due to their anti-tumour and anti-inflammatory properties [11]. Carotenoids are lipophilic pigments synthesized mainly in plants, and also microorganisms, but not by animals [12,13]. They are a group of yellow, orange, and red phytochemicals [3], which are classified as nonpolar carotenes (composed exclusively of carbon and hydrogen) and less nonpolar xanthophylls (with at least one functional group containing oxygen), based on structural differences [12]. Carotenoids are very unstable compounds, especially once released from the food matrix, since they are highly sensitive to light, oxidation, acid pH and temperature [14]. Hence, harvesting, storage and processing conditions are decisive to preserve them [4].

In addition to the provitamin A activity attributed to some carotenes (β-carotene, αcarotene, β-cryptoxanthin), carotenoids have an important physiological activity contributing to good human health [15]. Thus, they exhibit antioxidant activity, enhance the immune system, protect against solar radiation or help maintain the visual system [13,15,16]. Moreover, carotenoids may reduce the risk of cardiovascular disease, certain types of cancer, and age-related macular degeneration and cataracts [17]. Since humans are not capable of synthesizing them, we can only obtain them through our diets [15,18]. Carotenoids are mainly obtained from fruits and

vegetables and are also added to food as colorants. The most widely distributed ones are β-carotene (e.g., carrots, spinach, banana, mandarins) and lutein (mostly obtained from green vegetables) [19,20].

Despite plant foods containing high levels of carotenoids, their bioavailability is rather low, particularly when foods are consumed raw and unprocessed. To exert their health-promoting properties, carotenoids must be released from the food matrix after ingestion and remain stable during the process until being absorbed and finally distributed throughout the body [12,18]. Therefore, in order to improve their health-beneficial effects, vegetables and fruits are better consumed after being cooked or processed, e.g., mechanically and thermally treated, and also co-consumed with lipids [20]. Regarding the latter, the addition of fat plays an important role in carotenoid bioavailability since, besides stimulating the release of bile salts and the enzymes responsible for the micellarization of fat soluble compounds, it increases the size and number of micelles in the medium, favouring carotenoids solubilization and their absorption by the intestinal cells [21,22]. The type and amount of fat added during the digestion process determine the results.

In vitro methods simulating digestion processes are widely used to study the gastrointestinal behaviour of food and pharmaceuticals [23]. Simulated digestion methods generally include the oral, gastric and small intestinal phases, and occasionally large intestinal fermentation. Digestion models are used to assess digestibility and bioaccessibility of macro and micronutrients: i.e., the amount of compound that is released from the matrix and is considered to be available for absorption. I*n vitro* simulated digestion methods are used to study the matrix release of micronutrients such as phenolics or carotenoids [24]. The aim of the present work was to evaluate the effect of the process variables on the bioactive constituents' content of carrot waste powders and their response to in vitro gastrointestinal digestion. Impact of previous disruption (grinding or chopping) and the different dehydration methods and conditions applied (hot-air drying at 60 or 70 °C or freeze-drying) on physicochemical properties, phenol and flavonoid contents, antiradical capacity and carotenoids content, were evaluated before and after *in vitro* digestion. Moreover, the effect of

dispersing powders in different matrices (water, oil, or oil-in-water emulsion) on the release of individual carotenoids from carrot powders during in vitro digestion was also assessed.

2. Materials and Methods

2.1. Raw Material

Discards from the ready-to-eat carrot sticks processing line were provided by the agricultural cooperative Agrícola Villena, Coop. V. (Alicante, Spain At the cooperative's facilities), whole carrots (Daucus carota, L.) were washed, mechanically peeled and cut into sticks. Sticks not meeting the quality standards in terms of size and shape, as well as small pieces, were discarded, which constituted the raw material for the present study. Discards were shipped to the IIAD (UPV) facilities and stored at 4 °C until their use in the experiments described next. This IV-range carrot-processing line produces around 3000 tons per year of discarded product.

2.2. Powders Manufacturing

Carrot discards were disrupted to obtain different particle sizes in a food processor (Thermomix® TM6, Vorwerk, Madrid, Spain). Rotating speed and time were adjusted to obtain chopped (C) or medium size pieces of ≤10 mm diameter (350 g, 5 s at 5000 rpm) and ground (G) or small size pieces of ≤5 mm diameter (350 g, 10 s at 10,000 rpm). Chopped and ground carrot discards were then dehydrated by hot-air drying (HAD) or freeze-drying (FD). HAD was carried out in a convective tray dryer with transversal flux (Pol-eko Aparatura, Katowice, Poland) at 60 or 70 °C until reaching a water activity (aw) below 0.3. For that purpose, disrupted wastes were distributed in layers of approximately 1 cm thickness on perforated trays (200 g of sample per tray). Drying at 60 °C implied 16 and 17 h for ground and chopped samples, respectively, whereas drying at 70 °C took 12 h in both cases. As for FD, it was conducted in ground samples, and it required a previous freezing step in a deep freezer (Matek model CVN-40/105) at −40 °C for 24 h). Then, samples were lyophilized in the freeze dryer equipment (LyoAlfa 6-80, Telstar, Terrasa, Spain) for 24 h at 0.1 mbar of pressure and −45 °C (condenser temperature). Once dehydrated, freeze-dried or air-dried samples were

milled at 10,000 rpm for 2 min at 30 s intervals (Thermomix® TM6, Vorwerk, Madrid, Spain) in order to obtain a fine-grained powder. The powders obtained were stored in closed and opaque glass jars at room temperature to ensure their conservation until analysis. Conditions for air-drying, freeze-drying and disruption were established according to preliminary tests and previous published research [25].

As a result, 5 different powders were obtained, which will be identified hereinafter by the disruption pre-treatment as G, ground and C, chopped, and by the dehydration technique and conditions applied (HAD60 for hot-air drying at 60 °C, HAD70 for hot-air drying at 70 °C, and FD for freeze-drying).

2.3. In vitro Digestion Method

The *in vitro* simulation of gastrointestinal digestion (oral, gastric and intestinal phases) was performed according to the standardized INFOGEST method proposed by Minekus et al. [23]. Accordingly, simulated digestive fluids, Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF), were reproduced. To simulate the oral phase, samples were mixed in a 1:1 ratio (w/v) with the simulated salivary fluid (SSF) and vortexed (Reax top, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) for 2 min at 37 °C. To simulate the gastric phase, the oral bolus was mixed in a 1:1 (v/v) ratio with the simulated gastric fluid (SGF) and kept in constant stirring at 55 rpm (Intell-Mixer RM-2, Elmi Ltd., Riga, Latvia) and at 37 °C (JP Selecta SA, Barcelona, Spain) for 2 h. For the intestinal phase, the chyme was mixed in a 1:1 (v/v) ratio with the simulated intestinal fluid (SIF) and stirred at 55 rpm (Intell-Mixer RM-2, Elmi Ltd., Riga, Latvia) and 37 °C (JP Selecta SA, Barcelona, Spain) for 2 h more. Sampling for subsequent analysis of total phenols, total flavonoids, total antioxidant activity and carotenoids content was performed at the end of both the gastric and the intestinal phases. Antioxidant properties were determined on the whole digested sample, and on the supernatant and pellets collected after centrifugation at 10,000 rpm for 5 min (Eppendorf® Centrifuge 5804R, Hamburg, Germany). Carotenoid content was exclusively analysed on the whole digested sample, as explained later. When appropriate, the bioaccessibility index (BI) and recovery index (RI) were calculated as follows (Equations (1) and (2)).

$$BI\ (\%) = \frac{A}{B} \cdot 100 \quad (1)$$

$$RI\ (\%) = \frac{C}{B} \cdot 100 \quad (2)$$

where:

A is the amount (µg) of the compound of interest in the soluble fraction after the intestinal phase of digestion; B is the amount (µg) of the compound of interest in the undigested powder; and C is the amount (µg) of the compound of interest in the total digest after the gastric or the intestinal phase.

BI refers to the percentage of compounds which remain solubilized in the chyme after the intestinal phase with respect to the undigested sample, which approximates the proportion of bioactive compounds available for absorption by the intestinal cells. RI refers to the percentage of compounds present in the total digested sample after the gastric or the intestinal phase of digestion [26,27].

The effect of different dispersing media on the response to simulated in vitro digestion was evaluated by simulating the same digestion procedures with powders dispersed in a 1:1 ratio (v/v) with the following media: water, sunflower oil, and a 10% (v/v) oil-inwater emulsion. The latter was obtained by dispersion (Ultra-Turrax® T25D, IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 10,000 rpm for 10 min. This part of the experiment was conducted on ground air-dried samples at 70 °C (G_HAD70) and freeze-dried ones (FD), in view of powder characterization results.

2.4. Analytical Determinations

2.4.1. Water Activity, Moisture Content, Total Soluble Solids, and Particle Size

Water activity (a_w) was measured at 25 °C with a dew point hygrometer (Aqualab 4TE, Decagon devices, Inc., Pullman, Washington, WA, USA). Moisture content (xw) was calculated from the weight loss undergone by a specific amount of sample during its drying in a vacuum oven (Vaciotem, JP Selecta SA, Barcelona, Spain) at 10 mm Hg and 60 °C until constant weight

[28]. The total soluble solids content (xss) was estimated from the Brix degrees measurement obtained at 20 °C with a thermostated Abbe refractometer NAR-3T (Atago, Tokyo, Japan). When needed, water was added in a 1:10 (w/v) ratio. Particle size distribution of the powders was obtained by laser diffraction using a Mastersizer 2000 equipment (Malvern Panalytical Ld., Malvern, UK). For the dry method determination, a dispersion unit Sirocco 2000 with air as dispersant at 2.5 bar of pressure and 60% speed was used. For the wet method, the particle absorption index was set at 0.1 and refractive indexes of 1.52 and 1.33 were applied to the sample and the dispersed phase (deionized water), respectively. From the particle size distribution curves, the volume moment mean diameter D [4,3] and the surface area moment mean diameter D [3,2], together with the distribution percentiles d10, d50 and d90, were obtained.

2.4.2. Antioxidant Properties of Carrot Wastes and Powders

Total phenol content, total flavonoid content, and antioxidant activity using the DPPH and ABTS methods were determined. Determinations were carried out on sample extracts obtained from 1 g of fresh carrot waste or 0.5 g of non-digested powder or digested precipitate, with 10 mL of an 80% (v/v) methanol/water solution. Extraction was performed using a horizontal stirrer (Magna Equipments S. L., model ANC10, Barcelona, Spain) in dark conditions during 1 h [25]. Then, the mixture was centrifuged for 5 min at 10,000 rpm in an Eppendorf centrifuge 5804/5804R (Eppendorf SE, Hamburg, Germany). Measurements were also performed on the supernatants separated after digestion, with no solvent addition. Bidistilled water replacing the extract was used as a blank.

Total phenolic content was spectrophotometrically determined following the FolinCiocalteau method [29]. An aliquot of 0.125 mL of the previously obtained extract was mixed with 0.5 mL of bidistilled water and 0.125 of the Folin–Ciocalteau reagent (Scharlab S.L., Barcelona, Spain). The mixture was kept in darkness for 6 min, and then 1.25 mL of a 7% sodium carbonate solution and 1 mL of double distilled water were added. After 90 min in darkness, absorbance was measured at 760 nm with a spectrophotometer (Helios Zeta UV/Vis, Thermo Fisher Scientific Inc.,

Waltham, MA, USA). Results were expressed in mg of gallic acid equivalents (GAE) (purity ≥ 95%, Sigma-Aldrich, St. Louis, MO, USA) per g of dry matter (dm).

Total flavonoid content was determined by following the colorimetric method of aluminium chloride [30]. In total, 1.5 mL of the extract was mixed with 1.5 mL of 2% (w/v) aluminium chloride (Scharlab S.L., Barcelona, Spain) in a methanol solution. After 10 min of reaction in darkness, the absorbance was measured at 368 nm in a spectrophotometer (Helios Zeta UV/Vis, Thermo Fisher Scientific Inc., Waltham, MA, USA). Flavonoid content was expressed in mg of Quercetin Equivalents (QE) (purity ≥ 95%; Sigma-Aldrich, St. Louis, MO, USA) per g of dry matter (dm).

Antioxidant activity (AO) was measured using the DPPH and ABTS methods, which measure the radical scavenging ability of samples against the free radicals DPPH+ (2,2- diphenyl-1-picryl hydrazyl) and ABTS+ (2,2-azinobis-3-ethyl benzthiazoline-6-sulphonic acid). Following the method proposed by Brand-Williams et al. [31], 0.1 mL of the extract were made to react with 2 mL of a 0.06 mM solution of DPPH (purity ≥ 98%; Merck KGaA and affiliates, Darmstadt, Germany) in methanol and 0.9 mL of methanol. After 60 min in darkness, absorbance was measured at 517 nm in a spectrophotometer (Helios Zeta UV/Vis, Thermo Fisher Scientific Inc., Waltham, MA, USA). Following the method described by Re et al. [32], ABTS+ free radical was first released by mixing a 7 mM solution of ABTS (purity ≥ 98%; VWR International LLC, Radnor, PA, USA) with a 2.45 mM solution of potassium persulfate for 16 h in darkness and at room temperature. Then, the resulting solution was mixed with phosphate buffer (pH 7.4) until reaching an absorbance of 0.70 ± 0.02 at 734 nm. Measurements were carried out by mixing 0.1 mL of the extract with 2.9 mL of the ABTS solution, and the absorbance was read at 734 nm after 7 min of reaction. Antioxidant activities were given in mg of Trolox Equivalent (TE) (purity ≥ 97%; Sigma-Aldrich, St. Louis, MO, USA) per g of dry matter (dm).

2.4.3. Carotenoids Content

The identification and quantification of specific carotenoids were performed using high-performance liquid chromatography (HPLC) following

the procedure described by Bunea et al. [33] with some modifications. For this purpose, 1 g of the sample (non-digested or digested powder) was extracted with 25 mL of a 1:1:1 (v/v/v) methanol/ethyl acetate/petroleum ether solution. The mixture was then homogenized for 1 min at 11,000 rpm with a T25D Ultra-Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany), vortexed for 1 min (Reax top, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) and centrifuged (Eppendorf® Centrifuge 5804R, Hamburg, Germany) at 10,000 rpm for 10 min at 10 °C. The supernatant was introduced in a separation funnel. This process was repeated as many times as necessary in order to recover all the carotenoids from the samples, i.e., until a colourless supernatant was obtained. Then, total collected supernatant was washed several times with 100 mL of saturated NaCl saline solution, until both the aqueous and etheric phases were clear. The carotenoid-rich phase (etheric) was dried over anhydrous sodium sulphate and evaporated at 35 °C under vacuum in an Hei-VAP Core rotary evaporator (Heidolph Instruments GmbH & Co., Schwabach, Germany). Finally, the residue was resuspended in 1.5 mL of ethyl acetate.

HPLC analyses were performed with an Alliance 2995 system with DAD detector (Waters, Milford, MA, USA). The chromatographic separation of the compounds was achieved by using a reverse phase Luna® C18 column (250 × 4.6 mm, 5 µm) (Phenomenex, Torrance, CA, USA). Elution was carried out at 25 °C with a mobile phase consisted of mixtures of acetonitrile: water (9:1, v/v) with 0.25% triethylamine (phase A) and ethyl acetate with 0.25% triethylamine (phase B). The gradient was generated by decreasing phase A from 90 to 50% from 0 to 10 min, to continue decreasing it from 50 to 10% at 20 min, with a flow rate of 1 mL/min. Individual carotenoids selected for their identification and quantification were the most abundant in orange carrot: α-carotene (purity ≥ 95%), β-carotene (purity ≥ 95%), lutein (purity ≥ 96%) and lycopene (purity ≥ 85%), all of them from Merck KGaA and affiliates (Darmstadt, Germany) [3,13,34]. Calibration curves of external standards were prepared in order to identify and quantify the corresponding retention times in the chromatograms, monitored at 450 nm.

2.5. Statistical Analysis

The results were statistically analysed using Statgraphics Centurion XVI (Centurion XVII.I version, StatPoint Technologies, Inc., Warrenton, VA, USA) with a confidence level of 95% (p-value ≤ 0.05). One-way ANOVA and Multifactor ANOVA were performed to the processing of data. Variables relationships were assessed by means of a Pearson's product moment correlations test. All the analytical tests described were carried out at least in triplicate.

3. Results and Discussion

3.1. Physicochemical Properties of Carrot Waste Powder

Water activity, water content and the total soluble solids content of carrot waste powders are summarized in Table 1. Moisture content (87.32 ± 0.02 g_w/100 g) and water activity (0.996 ± 0.004) of the raw materials were in a range which implied high perishability. Dehydration allowed to reduce carrot water activity below the target value of 0.3, thus ensuring stability [9]. Pre-processing stages may have different impact on drying behaviour and final moisture content depending on the structure of the raw material [25]. Compared to chopping, when drying was conducted at 70 °C, grinding prior to air-drying resulted in a greater moisture content drop for a similar decrease in the water activity. Similar results had been previously obtained by Bas-Bellver et al. [25]. This would indicate that water outflow would have been favoured by the more intense rupture of structures and an increased exchange surface between the solid and the drying air [35]. In contrast, this difference was not observed at 60 °C, when similar final moisture contents were reached for both chopped and ground samples. Case-hardening, which might have occurred at higher temperatures, could have originated an increased internal resistance to water transport [36], this being more significant on larger pieces.

FD powders showed the highest soluble solids content. This is in line with previous results [25], and might be due to the more porous structure resulting from this dehydration process, which, indeed, favours further milling. According to Xiao et al. [37], HAD promotes a more rigid and compact structure, which is especially evident in the outermost layers of the solid. Conversely, ice crystals formation during the freezing process and

subsequent sublimation leads to a more porous and fragile structure [38], which increases the surface exposed to extraction and leads to greater fibre breakage [39]. These facts agree with the observed by Yi et al. [40] in mango, pitaya and papaya slices dehydrated by HAD and FD, or Owusu et al. [41] in tomato slices dried at different temperatures. Presumably, a more intense breakage of fibres into simpler units might also contribute to higher soluble solids values. The interdependence between grinding and drying processes and their relationship with the final characteristics of the powders have been previously reported [25,35,42].

Table 1. Water activity (a_w), moisture content (x_w) and soluble solids content (Xss) of carrot residue powders. G: ground, C: chopped; HAD: hot-air drying at 60 and 70 °C, FD: freeze drying. Mean ± standard deviation of three independent measurements.

Sample	a_w	x_w (g_w/100 g)	X_{ss} (g_{ss}/g_{dm})
G_HAD60	0.254 ± 0.008^{b}	2.9 ± 0.4^{b}	0.667 ± 0.017^{a}
C_HAD60	0.239 ± 0.010^{ab}	2.96 ± 0.10^{b}	0.659 ± 0.017^{a}
G_HAD70	0.236 ± 0.011^{a}	1.6 ± 0.3^{a}	0.685 ± 0.011^{ab}
C_HAD70	0.240 ± 0.005^{ab}	3.26 ± 0.12^{b}	0.685 ± 0.012^{bc}
FD	0.236 ± 0.007^{a}	2.80 ± 0.11^{b}	0.724 ± 0.018^{c}

$^{a,b,c...}$ Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Particle size characteristics of powders, as obtained by the wet and dry procedures, are summarized in Table 2. Applying the dry procedure is interesting when incorporating the powder to a solid formulation; in contrast, the wet procedure provides useful information when powders are included to a liquid formulation or solubilized in the gastrointestinal fluids during digestion.

Table 2. Particle size characteristic parameters obtained by the wet and dry procedures: equivalent volume diameter D [4,3], surface area mean diameter D [3,2], percentiles d10, d50, and d90. HAD: hot-air drying at 60 and 70 °C, FD: freeze-drying; C: chopped; G: ground. Mean ± standard deviation of five replicates.

	DRY PROCEDURE				
	D[4,3]	**D[3,2]**	**d_{10}**	**d_{50}**	**d_{90}**
G_HAD60	171 ± 6^{c}	34.6 ± 1.3^{b}	11.6 ± 0.3^{b}	137 ± 7^{c}	391 ± 10^{c}
C_HAD60	210 ± 6^{e}	51.2 ± 1.5^{c}	17.8 ± 0.5^{d}	190 ± 7^{e}	442 ± 12^{e}
G_HAD70	155 ± 3^{b}	26.0 ± 0.6^{a}	8.6 ± 0.2^{a}	126 ± 5^{b}	358 ± 5^{b}
C_HAD70	200 ± 6^{d}	50 ± 3^{c}	17.36 ± 0.7^{d}	180 ± 7^{d}	423 ± 9^{d}
FD	124 ± 3^{a}	33.1 ± 1.7^{b}	12.5 ± 0.8^{c}	107 ± 3^{a}	258 ± 6^{a}
	WET PROCEDURE				
	D[4,3]	**D[3,2]**	**d_{10}**	**d_{50}**	**d_{90}**
G_HAD60	245 ± 24^{b}	53 ± 3^{c}	20.6 ± 1.2^{b}	209 ± 20^{b}	530 ± 54^{b}
C_HAD60	348 ± 37^{d}	78.8 ± 1.9^{e}	35.4 ± 1.1^{d}	292 ± 16^{d}	723 ± 97^{c}
G_HAD70	228 ± 8^{b}	48 ± 2^{b}	18.8 ± 1.1^{a}	194 ± 9^{b}	500 ± 23^{b}
C_HAD70	273 ± 18^{c}	67.1 ± 1.9^{d}	28.8 ± 0.8^{c}	249 ± 16^{c}	562 ± 37^{b}
FD	156 ± 2^{a}	39.0 ± 0.4^{a}	15.8 ± 0.3^{a}	123.6 ± 1.4^{a}	350 ± 6^{a}

$^{a,b,c...}$ Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Both the previous disruption intensity and the drying technique applied had a significant impact on the particle size of powders. Grinding prior to drying led to finer powders than chopping. This fact could be due to a more brittle dried product because of a more homogenous water outflow during HAD. When very low moisture contents are reached, more homogeneous drying rates imply the transition of the entire material into the glassy state; in contrast, fast drying rates causing crusting phenomena (case-hardening) implies rubbery cores with an increased moisture content, which reduces milling efficiency [36]. These results are in line with other studies in which it has been evidenced that milling prior to drying determines particle size characteristics [25,35].

Particle size distribution curves are shown in Figure 1. As observed, FD powders exhibited smaller particle sizes, which could be related to the higher brittleness of the porous structure generated in FD products, which facilitates milling as compared to the more compact structure of HAD products [38]. As deduced from the span and the particle size distribution patterns, the wet procedure distributions shift to the right and widen compared with the dry procedure distributions, which indicates the solubilization of finer particles in the water used as the dispersing agent.

Additionally, when dispersed in a liquid medium, aggregates may be formed, and thus particle size increase.

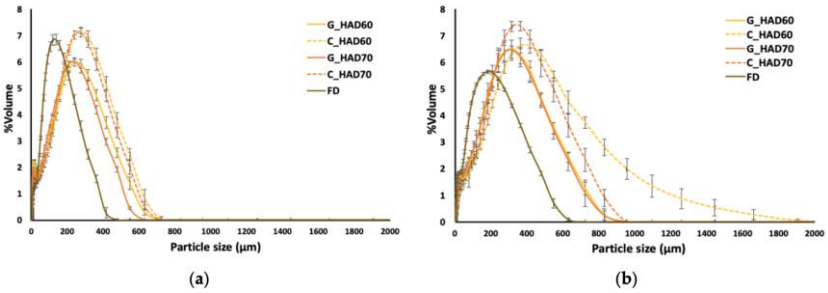

Figure 1. Particle size distribution of carrot residue powders. (a) Determination by the dry method. (b) Determination by the wet method. Error bars are the standard deviation of five replicates. HAD: hot-air drying at 60 and 70 °C, FD: freeze-drying; C: chopped; G: ground.

3.2. Antioxidant Properties of Fresh and Powdered Carrot Waste

Table 3 shows total phenol and flavonoid content, as well as antioxidant capacity (DPPH and ABTS methods) of carrot waste powders and raw carrot waste.

Table 3. Total phenol content, total flavonoid content and antioxidant capacity by the DPPH and ABTS methods. HAD: hot-air drying at 60 and 70 °C, FD: freeze-drying; C: chopped, G: ground. Mean ± standard deviation of the three measurements.

Sample	Total phenols (mg GAE/g_{dm})	Total flavonoids (mg QE/g_{dm})	DPPH (mg TE/g_{dm})	ABTS (mg TE/g_{dm})
Carrot waste	2.03 ± 0.09^{c}	1.210 ± 0.012^{a}	4.28 ± 0.04^{e}	14.0 ± 0.6^{a}
G_HAD60	1.53 ± 0.12^{b}	1.24 ± 0.06^{ab}	1.90 ± 0.12^{b}	55 ± 2^{b}
C_HAD60	2.06 ± 0.16^{c}	1.464 ± 0.003^{c}	2.1 ± 0.2^{c}	57.5 ± 1.4^{b}
G_HAD70	2.004 ± 0.013^{c}	1.27 ± 0.03^{b}	1.69 ± 0.10^{b}	62 ± 3^{c}
C_HAD70	2.42 ± 0.15^{d}	1.45 ± 0.02^{c}	2.65 ± 0.11^{d}	64.8 ± 1.7^{c}
FD	0.74 ± 0.14^{a}	1.26 ± 0.03^{ab}	1.01 ± 0.11^{a}	16.9 ± 0.3^{a}

$^{a,b,c...}$ Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Different processing treatments had a different impact on total phenolics (Table 3). In the available literature, there is evidence that dehydration may have a negative impact on total phenolics, as in Macura et

al. [43] who reported a decrease in phenolics in hot-air dried purple carrot samples. Other authors [44], however, have reported an increase in phenolics in hot-air dried and freeze-dried products. In the present study, significant losses of phenolic compounds in FD samples have been observed. This could be attributed to the fact that, once produced, low-moisture FD products are generally more susceptible to oxidation because of their porous structure [45]. In HAD samples, however, losses due to temperature or oxygen exposure during treatment could have been balanced by different biochemical reactions taking place during dehydration and the consequent formation of new antioxidant compounds, which modify the overall antioxidant activity of the product [46]. For instance, Maillard compounds formed during HAD, together with other compounds such as glucose, fructose, tertiary aliphatic amines, tryptophan and other reducing agents [47], are capable of reacting with the Folin–Ciocalteu reagent [48]. A multifactor ANOVA analysis revealed that both air temperature and grinding prior to drying had a significant effect on total phenolic content. Grinding prior to dehydration causes greater damage to vegetal tissue than chopping, releasing more phenolic compounds which would then be more exposed to oxygen, light, or temperature, and thus degraded [47]. On the other hand, a higher drying temperature might increase the phenol content by reducing the activity of certain enzymes capable of degrading phenolic compounds [49], or as a consequence of the generation of new phenolic substances. Moreover, drying at 70 °C shortens processing times and thus the oxidation risk due to airflow exposure. Regarding total flavonoid content, the only factor having a statistically significant effect was the size of the particles to be dried, with chopping leading to a higher flavonoid content, possibly combined with a decreased in the exposed surface to drying conditions.

Antiradical properties of powders showed a different trend depending on the method used. Dehydration of carrot waste led to a significant decrease in its ability to reduce the DPPH radical, but to a significant increase in the antiradical activity measured by the ABTS method (Table 3). These results are in line with those obtained in a previous study [25]. Regardless of the method used, air-drying, especially at 70 °C and after chopping, led to the highest antioxidant activity values, whereas FD led to the lowest ones.

This could be, again, evidencing the release or formation of other compounds with antioxidant properties, or products from Maillard reactions during air-drying [47,48]. In addition, the longer exposure time needed to achieve the target aw when dried at 60 °C would imply a higher incidence of oxidative reactions and lower antioxidant capacity as compared with samples dried at 70 °C. Chopping before air-drying had better results than grinding in terms of antioxidant activity values, although not always significant. Similarly, in brassica powders, regarding disruption pre-treatments, chopping generally led to better antioxidant properties than grinding due to the lower cellular damage, so that phenols and flavonoids remain more protected and less susceptible to oxidation during drying [50].

Correlation tests (Pearson's product moment correlations) were carried out to unveil the relationship between processing conditions and antioxidant properties measured on the powdered products (Table S1). The analysis evidenced that both pretreatment and drying conditions were significantly correlated with the antioxidant properties of powders. The intensity of the disruption is significantly ($p < 0.01$) and negatively correlated with all the antioxidant properties measured, which confirmed a preference for chopping before drying. Regarding drying conditions, a positive significant correlation was evidenced between drying temperature and phenolics and antioxidant activities using the DPPH and ABTS methods. Particle size was also found to be correlated to processing conditions and antioxidant properties.

3.3. Carotenoid Content of Carrot Waste Powders

Table 4 shows the results of the individual and total carotenoid content of the carrot waste powders. The most abundant carotenoid in the products obtained was β-carotene followed by α-carotene, which agrees with previous reports [3,13,34]. As compared with FD powders, HAD showed significantly lower carotenoid content, which had been previously evidenced in carrot samples [4,43], but also in other carotenoid-rich wastes such as tomato peels [51] or lulo bagasse [52]. This might be due to the carotenoids' sensitivity to heat, oxygen, light and degradation by various enzymes such as lipoxygenase, whose activity is reduced during FD, but favoured with the

exposure to oxygen and high temperature, conditions which are characteristic of HAD treatments [14]. The susceptibility of carotenoids to the dehydration techniques applied was different for each individual carotenoid. α-carotene and β-carotene were more sensitive to HAD, whereas lycopene was affected to a lesser extent. On the other hand, FD did not prevent the degradation of lutein, as compared with the other carotenoids analysed. This result agrees with the findings of Zhang et al. [53], who concluded that, among the carotenoids present in carrots, lutein would be more resistant to air-drying treatments.

Table 4. Carotenoid content of carrot waste powders. HAD: hot-air drying at 60 and 70 °C, FD: freeze-drying; C: chopped, G: ground. Mean ± standard deviation.

Sample	Lycopene ($\mu g/g_{dm}$)	Lutein ($\mu g/g_{dm}$)	β-carotene ($\mu g/g_{dm}$)	α-carotene ($\mu g/g_{dm}$)	Total ($\mu g/g_{dm}$)
G_HAD60	3.19 ± 0.02^{c}	1.29 ± 0.04^{c}	59 ± 15^{c}	9.99 ± 0.10^{b}	73 ± 15^{c}
C_HAD60	2.64 ± 0.07^{b}	0.92 ± 0.15^{a}	29.1 ± 0.2^{a}	4.08 ± 0.04^{a}	37.0 ± 0.6^{a}
G_HAD70	3.08 ± 0.03^{c}	1.036 ± 0.011^{ab}	42.2 ± 1.7^{b}	9.4 ± 1.2^{b}	56 ± 3^{b}
C_HAD70	2.517 ± 0.011^{a}	1.142 ± 0.006^{bc}	52.5 ± 0.8^{bc}	12.04 ± 0.05^{b}	67.8 ± 0.6^{c}
FD	4.77 ± 0.09^{d}	1.147 ± 0.005^{bc}	221 ± 4^{d}	59 ± 2^{c}	286 ± 2^{d}

$^{a,b,c...}$ Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

In air-dried powders, an interaction between temperature and disruption conditions was observed. Except for lycopene, total and individual carotenoid content of samples dried at 70 °C was higher in chopped than in ground samples, but the opposite was observed at 60 °C. A possible explanation for this is that, as evidenced for total phenolic content, grinding releases a greater number of compounds of interest, but implies higher degradation due to an enhanced exposure to drying conditions [54]. In addition, chopped samples underwent longer drying treatments compared with ground when dried at 60 °C, whereas processing time was similar for ground and chopped samples dried at 70 °C. Pearson's product moment correlations (Table S1) displayed a negative relationship between drying temperature and carotenoid content. Particle size was also correlated with carotenoid content, with the smallest particles giving rise to higher levels of carotenoid content.

3.4. Simulated In vitro Digestion of Carrot Waste Powders

3.4.1. Antioxidant Properties along Simulated In Vitro Digestion

Response of antioxidants contained in carrot waste powders to the simulated in vitro digestion process is summarized in Table 5. Total phenols, total flavonoids and antioxidant capacity (DPPH and ABTS) were measured before digestion (BD) and after the gastric (GP) and intestinal phases (IP). After digestion, antioxidant properties were measured both in the supernatant (S) and the precipitate (P), as described before.

As observed, phenol and flavonoid contents generally decreased after the gastric stage and increased after the intestinal one. As for antioxidant capacities, the ability to react with DPPH sharply increased after the gastric phase and decreased during the intestinal one, but still the DPPH antioxidant activity after digestion was about 1.5–2-fold higher than before digestion. In contrast, ABTS reactivity decreased significantly in the gastric phase, and slightly increased after the intestinal one. Variability in AO assays results during in vitro digestion studies has also been observed by other authors, who attributed these differences to both the different chemical principles in which AO methods are based and the pH of the digestion phases [55].

Regarding the response of phenolic constituents during digestion, simulated in vitro digestion has been reported to increase the release of phenolics so that, as digestion progresses, compounds become more available. For instance, Gouw et al. [56] and Nayak et al. [57] attributed the increase in the amount of phenolics after in vitro digestion to the untangling of these constituents from dietary fibres due to enzymes action and gastric fluids conditions. Similar results had been evidenced for some fruit pomaces in Nayak et al. [58], in which the higher availability was attributed to the continuous liberation of phenolic compounds from macromolecules during the digestion process, together with polyphenol hydrolysis reactions during digestion. Chen et al. [59] also reported that gastric conditions might improve the extractability of phenolic compounds from fruit pomaces. Changes in the pH of the medium and the interaction with the enzymes involved in digestion promote the release by hydrolysis of compounds bound to the food matrix or the formation of new phenolic compounds which

implies changes in the antiradical activities measured [27,48]. Accordingly, recovery indexes after the intestinal phase of in vitro digestion have been reported to be higher than 100% for most antioxidant properties in dried vegetables [27,55].

However, other authors have evidenced significant losses in phenols and flavonoids after oral and gastric phases, whereas biochemical conditions in the intestinal phase imply an increase. Conditions in the small intestine (pH and pancreatin) would promote the solubilization of certain phenolic compounds that would be previously linked to macromolecules or present in a reduced form [60]. Moreover, the interactions of phenolic compounds with sugars or other dietary compounds released during digestion could play a protective role in their changes during the digestion process, affecting their solubility and potential bioavailability [42]. The results of the present work are in line with these results, since antioxidant constituents present in the supernatant after the intestinal phase increased in all cases, thus suggesting an increased solubilization of phenolics and other compounds during this stage. In the gastric phase, however, antioxidant compounds were more abundant in the precipitate, which suggests that most of them remained bound to the structure. Thus, during the intestinal phase, the enzymatic activity promoted the breakage of structures and the release of part of the liquid phase trapped in the precipitate, it being then collected as supernatant. Antioxidant compounds released to the liquid phase are considered to be more available; however, those present in the precipitate could also be further liberated continuously and slowly and metabolised by the action of colonic microorganisms [61,62]. In addition, the precipitate also retains part of the liquid phase, these compounds being more accessible than the bound ones.

With regard to the differences in DPPH and ABTS antioxidant assays results during in vitro digestion, it has been mentioned that differences between both methods could be explained in terms of the different chemical principles in which they are based and the pH of the digestion phases [55]. Nevertheless, differences between DPPH and ABTS assays can also be attributed to the main phenolic constituents in the sample. According to Platzer et al. [63], the AO activity measured depend on multiple criteria and

differ between methods: for instance, results in the DPPH assay depend mainly on the number of OH groups and Bors 1 and 3 criteria, whereas there is no clear relationship between the results in the ABTS assay and the number of OH groups and the Bors criteria are much less important. These authors evidenced the variability in the ability to react of the different phenolic constituents so that hydroxycinnamic acids, phenolic acids most abundant in carrot waste [64], achieved higher values than hydroxybenzoic acids in the ABTS assay, whereas this was not the case in the DPPH assay. This could justify the lower values obtained with the DPPH method in the present work. On the other hand, the ABTS assay is very sensitive to pH changes [65], such as the ones occurring during in vitro digestion, which could explain the sharp reduction observed in the results after the oral and gastric phases, as well as the different trends observed between the DPPH and ABTS methods. Moreover, regarding antioxidant reactivity, the pH of a substance might modify the compounds' reactivity and alter their biological reactivity, which could be different in the gastric and duodenal phases.

Focusing on the drying treatments applied, FD powders showed the lowest values at the end of both the gastric and the intestinal phases, for all the antioxidant properties analysed, as they presented the lowest initial values too. However, recovery indexes were very high for freeze-dried powders, suggesting an increased extractability of antioxidant constituents due to simulated in vitro digestion. In fact, FD, G_HAD60 and G_HAD70 powders exhibited the highest BI and RI after the intestinal stage. One main reason for this could be the smaller particle size of these powders, which implied a greater contact surface between the sample and the intestinal fluid and an enhanced action of pH and enzymes, thus increasing the extraction and solubilisation of antioxidant compounds [42,66]. Differences in antioxidant properties between both methods used could be explained in terms of the different chemical principles in which they are based and the pH of the digestion phases [55].

Table 5. Total phenol content, total flavonoid content and antioxidant capacity (DPPH and ABTS) of carrot waste powders before digestion (BD), after the gastric phase (GP) in the supernatant (S) and precipitate (P), and after the intestinal phase (IP) in the supernatant (S) and precipitate (P) of the in vitro digestion process. Results are given per gram of non-digested sample. Recovery (RI) and bioaccesibility (BI)

indexes are expressed in percentage. Mean ± standard deviation of three measurements.

	Sample	BD	Gastric Phase (GP)			Intestinal Phase (IP)			%BI
			S	P	TOTAL (%RI)	S	P	TOTAL (%RI)	
Total phenol content (mg GAE/g)	**G_HAD60**	1.48±0.11^{b}	0.24±0.02^{b}	0.68±0.10^{b}	0.93±0.10^{b} (63% ±7^{c})	1.01±0.04^{b}	0.35±0.02^{a}	1.37±0.05^{b} (92% ±3^{b})	100±4^{c}
	C_HAD60	2.00±0.15^{c}	0.259± 0.011^{b}	0.7±0.2^{b}	0.96±0.18^{b} (48% ±9^{b})	1.22±0.04^{c}	0.42±0.05^{a}	1.64±0.04^{c} (82% ±2^{ab})	82±3^{b}
	G_HAD70	1.972± 0.013^{c}	0.49±0.02^{d}	1.41±0.08^{c}	1.89±0.10^{c} (96% ±5^{d})	2.05±0.10^{d}	0.85±0.12^{b}	2.9±0.2^{d} (147% ±10^{c})	140±7^{d}
	C_HAD70	2.34±0.14^{d}	0.46±0.02^{c}	1.8±0.2^{d}	2.2±0.2^{d} (95% ±8^{d})	0.84±0.05^{a}	0.96±0.15^{b}	1.81±0.10^{c} (77% ±4^{a})	66±4^{a}
	FD	0.72±0.13^{a}	0.157± 0.005^{a}	0.052±0.01 4^{a}	0.21±0.02^{a} (29% ±3^{a})	0.81±0.03^{a}	0.33±0.03^{a}	1.14±0.6^{a} (158% ±8^{c})	158±5^{e}
Total flavonoid content (mg QE/g)	**G_HAD60**	1.20±0.06^{a}	0.44±0.05^{b}	0.245±0.00 8^{b}	0.69±0.05^{b} (57% ±4^{c})	0.210± 0.005^{a}	1.22±0.08^{d}	1.43±0.09^{d} (118% ±7^{e})	25.5± 0.6^{a}
	C_HAD60	1.421± 0.003^{c}	0.24±0.08^{a}	0.272±0.00 9^{b}	0.51±0.09^{a} (36% ±6^{a})	0.279± 0.013^{ab}	0.47±0.04^{b}	0.75±0.04^{b} (53% ±2^{b})	26.4± 1.2^{a}
	G_HAD70	1.25±0.03^{b}	0.39±0.04^{b}	0.73±0.05^{c}	1.13±0.06^{c} (90% ±5^{d})	0.83±0.03^{d}	0.462± 0.009^{b}	1.30±0.02^{c} (104% ±2^{d})	90±3^{c}
	C_HAD70	1.40±0.02^{c}	0.3813± 0.0011^{b}	1.137± 0.011^{d}	1.518±0.010^{d} (108.6%±0.7^{e})	0.59±0.04^{c}	0.658± 0.012^{c}	1.25±0.04^{c} (89% ±3^{c})	77±5^{b}
	FD	1.23±0.03^{ab}	0.44±0.05^{b}	0.090±0.01 3^{a}	0.53±0.04^{a} (44% ±3^{b})	0.28± 0.074^{b}	0.27±0.05^{a}	0.55±0.05^{a} (45% ±4^{a})	33±8^{a}
DPPH (mg TE/g)	**G_HAD60**	1.85±0.12^{b}	0.25±0.03^{c}	7.7±0.9^{b}	7.9±0.9^{b} (428% ±49^{a})	0.64±0.07^{a}	3.1±0.4^{b}	3.3±0.7^{b} (181% ±38^{ab})	51±6^{a}
	C_HAD60	2.1±0.2^{c}	0.180± 0.003^{b}	8.5±0.6^{bc}	8.6 ± 0.6^{bc} (417% ±28^{a})	0.79±0.05^{b}	2.9±0.4^{b}	3.7±0.3^{bc} (179% ±16^{a})	51±3^{a}
	G_HAD70	1.6 ±0.10^{b}	0.319± 0.003^{d}	8.3±0.3^{bc}	8.6±0.4^{bc} (519% ±21^{b})	1.37±0.05^{c}	2.90±0.13^{b}	4.27±0.08^{c} (256% ±5^{c})	111±4^{c}
	C_HAD70	2.56±0.10^{d}	0.318± 0.004^{d}	8.8±0.5^{c}	9.1±0.5^{c} (356% ±19^{a})	0.63±0.06^{a}	3.3±0.8^{b}	3.9±0.8^{bc} (153% ±31^{a})	45±4^{a}
	FD	0.99±0.11^{a}	0.100± 0.004^{a}	6.1±0.6^{a}	6.3±0.6^{a} (634% ±64^{c})	0.53±0.10^{a}	1.61±0.11^{a}	2.1±0.2^{a} (217% ±20^{b})	76±14^{b}
ABTS (mg TE/g)	**G_HAD60**	53±2^{b}	2.25±0.03^{d}	2.6±0.9^{a}	4.9±0.9^{a} (9.2% ±1.7^{a})	8.88±0.09^{c}	4.0±0.5^{a}	12.9±0.4^{b} (24.4% ±0.8^{b})	24.4± 0.2^{b}
	C_HAD60	55.8±1.3^{b}	1.45±0.02^{b}	4.6±1.3^{b}	6.0±1.3^{a} (11% ±2^{a})	11.0±0.2^{d}	6.8±0.6^{bc}	17.8±0.8^{cd} (31.9% ±1.4^{c})	26.5±0.5^{c}
	G_HAD70	61±3^{c}	2.06±0.03^{c}	3.6±0.9^{ab}	5.7±0.9^{a} (9.3% ±1.4^{a})	12.3±0.3^{e}	6.3±1.4^{b}	18.54±1.13^{d} (30.3% ±1.8^{c})	27.0±0.6^{c}
	C_HAD70	62.6±1.6^{c}	2.01±0.02^{c}	3.4±0.4^{ab}	5.4±0.4^{a} (8.6% ±0.6^{a})	7.1±0.3^{a}	3.7±1.2^{a}	10.8±1.6^{a} (17% ±2^{a})	20.8± 0.9^{a}
	FD	16.4±0.3^{a}	1.06±0.05^{a}	15.05±1.12^{c}	16.11±1.09^{b} (98% ±7^{b})	7.5±0.2^{b}	8.4±0.9^{c}	15.9±0.9^{c} (97% ±6^{d})	64.1± 1.4^{d}

$^{a,b,c...}$ Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

3.4.2. Carotenoid Release along Simulated *In vitro* Digestion

Individual and total carotenoid contents of raw wastes and powders, determined at the end of each stage of the in vitro digestion process, as well

as the corresponding Recovery Index (RI) values are shown in Table 6. According to the RI values, *in vitro* simulated digestion made both individual and total carotenoids more available.

Table 6. Total and individual carotenoid content (µg/g of non-digested sample) of carrot waste and carrot powders after the gastric phase (GP) and the intestinal phase (IP). Recovery index in percentage (%RI) after GP and IP. HAD: hot-air drying at 60 and 70 °C, FD: freeze-drying; C: chopped, G: ground. Mean ± standard deviation of four replicates.

			Carrot	G_HAD60	C_HAD60	G_HAD70	C_HAD70	FD
Lycopene	**GP**	**µg/g**	12.96±0.05^{e}	11.2±0.2^{b}	10.54±0.06^{a}	12.53±0.08^{d}	13.50±0.04^{f}	11.76±0.07^{c}
		%RI	241.2±0.9^{a}	362±7^{c}	411±2^{d}	414±3^{d}	554.3±1.7^{e}	253.3±1.5^{b}
	IP	**µg/g**	22.22±0.07^{c}	21.02±0.09^{a}	21.43±0.05^{b}	23.13±0.17^{e}	22.6±0.3^{d}	22.8±0.5^{de}
		%RI	413.7±1.3^{a}	679±3^{c}	836.7±1.9^{e}	764±6^{d}	929±12^{f}	490±10^{b}
Lutein	**GP**	**µg/g**	4.93±0.03^{e}	3.2±0.2^{c}	2.66±0.02^{b}	3.09±0.06^{c}	3.68±0.08^{d}	2.48±0.02^{a}
		%RI	40.5±0.2^{a}	258±19^{c}	297±2^{d}	303±6^{d}	333±7^{e}	222±2^{b}
	IP	**µg/g**	5.91±0.10^{c}	4.83±0.07^{b}	4.84±0.10^{b}	4.78±0.14^{b}	4.72±0.11^{ab}	4.61±0.09^{a}
		%RI	48.5±0.9^{a}	385±6^{b}	540±12^{e}	469±14^{d}	428±10^{c}	414±8^{c}
β-carotene	**GP**	**µg/g**	611±15^{f}	182±10^{b}	138±3^{a}	415±12^{d}	443±14^{e}	331±10^{c}
		%RI	109±3^{a}	318±17^{c}	490±12^{d}	1000±30^{f}	878±28^{e}	154±5^{b}
	IP	**µg/g**	350±40^{b}	436±14^{c}	100±4^{a}	422±5^{c}	456±10^{c}	528±55^{d}
		%RI	62±7^{a}	764±25^{d}	355±13^{c}	1017±11^{f}	905±21^{e}	246±26^{b}
α-carotene	**GP**	**µg/g**	157±9^{e}	37±3^{c}	17.5±0.3^{a}	27.2±0.5^{b}	14.2±0.5^{a}	92±5^{d}
		%RI	98±6^{a}	383±27^{e}	441±7^{f}	294±6^{d}	122±4^{b}	160±9^{c}
	IP	**µg/g**	128±15^{d}	52.2±0.9^{c}	30±2^{b}	37.6±0.7^{b}	16.2±0.8^{a}	166±8^{e}
		%RI	80±9^{a}	537±10^{e}	752±62^{f}	406±7^{d}	139±7^{b}	291±13^{c}
Total	**GP**	**µg/g**	787±27^{e}	223±12^{b}	169±3^{a}	458±13^{d}	474±14^{d}	437±11^{c}
		%RI	106±3^{a}	328±17^{c}	470±10^{d}	835±23^{f}	723±22^{e}	157±4^{b}
	IP	**µg/g**	505±27^{b}	514±15^{b}	156.1±1.6^{a}	488±4^{b}	500±11^{b}	722±61^{c}
		%RI	68±4^{a}	723±22^{d}	435±4^{c}	890±8^{f}	762±17^{e}	260±22^{b}

$^{a,b,c...}$ Different superscript letters in the same column for the same carotenoid indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Throughout the powders' digestion, an increased release of individual carotenoids was generally observed after IP as compared with GP. This had been also evidenced in persimmon peel powders by Bas-Bellver et al. [42], and in seed-used pumpkin powders by Lyu et al. [67]. In contrast, the

opposite was found in raw carrot for β-carotene and αcarotene content, which had also been corroborated by other authors in blended fruit juice [68], or raw carrot juice, but not in raw grated carrot [69]. These results would suggest that matrix transformations during powder manufacturing, pre-treatment and drying, would have favoured the release and solubilisation of carotenoids otherwise linked to the food matrix [18]. In fact, raw samples showed the lowest RI values, which suggests a poorer release of carotenoids during the simulated in vitro digestion process or degradation of part of the carotenoids originally present in the raw carrot waste.

Multifactor ANOVA performed on total carotenoid content of HAD powders after in vitro digestion revealed that both the drying temperature and the milling intensity had a statistically significant effect ($p < 0.05$). Increasing the drying temperature had a positive impact on total carotenoid release, which could be due to its effects on the dried carrot structure and consequent impact of powder particle size characteristics. Other authors [21] have evidenced a positive effect of cooking on the release of carrot carotenes during digestion and attributed this improvement to structural modifications which occurred during cooking. On the other hand, ground samples exhibited higher total carotenoid release than chopped ones, which became more evident when drying at 60 °C than at 70 °C. Again, the degree of cellular rupture could be involved in an increased carotenoids release. In fact, other authors have reported that mechanical disruption of vegetal matrix could be the main factor for carotenoid release and accessibility to digestion fluids [18,70,71]. The cell wall acts as the main structural physical barrier that drives the release of carotenoids [53], for which disruption of the food matrix is the first step in the absorption process [21,70]. Cell wall integrity is related to particle size, and it is also influenced by interactions between the structural compounds that conform the food matrix [71]. Therefore, disruption favours the release of a greater number of bioactive compounds, allowing their extraction from the food matrix [54]. Among air-dried samples, recovery indexes were higher for those powders obtained at 70 °C. Recovery indexes reveal that a more intense disruption pre-treatment, together with dehydration, and especially at the highest temperature assayed, improves carotenoid extraction during in vitro digestion.

In contrast, FD powders presented the lowest RI values for total carotenoids at the end of the intestinal stage, in spite of them having the highest total carotenoid content in the final digestion process. FD powders initially presented higher carotenoid content than HAD ones, most likely due to treatment conditions and the resulting structure and particle size, which would have facilitated carotenoids extraction. The lower temperature and less exposure to oxygen during FD could have also contributed to the preservation of the carotenoids. On the contrary, HAD powders presented lower initial carotenoid content, either because they had been degraded or because they remained trapped in the structure, so that the action of digestion fluids and their enzymes was more relevant for their release and bioaccessibility. Additionally, the presence of antioxidant compounds, which was higher in HAD powders than in FD, might have prevented carotenoid degradation during digestion, as suggested in the literature [18].

3.4.3. Interaction with Lipids during In vitro Digestion

Processing and interaction with other compounds present in the food are considered determining factors for carotenoids release and bioaccessibility. Mechanical disruption, the application of heat treatments and the addition of fat during the processing of fruit and vegetables have been reported to play a significant role in the release of carotenoids [72,73]. Due to its lipophilic character, the amount of fat, as well as the type of fat consumed, influences the bioavailability of carotenoids from natural sources [73–75]. The effect of dispersing the powders in water, an oil-in-water emulsion (10% oil), or sunflower oil (1:1 v/v ratio), on carotenoid release during the in vitro digestion process was evaluated.

The different media in which powders were dispersed had a statistically significant impact on carotenoids measurements (Figure 2). It was evidenced that, as compared to powders alone, the addition of water and the oil-in-water emulsion implied an increased carotenoid content identified in non-digested samples. This fact could suggest that incorporating the powders into a liquid matrix would favour the solubilization and extraction of carotenoids otherwise bound to the powder structure. In contrast, when powders were mixed with oil with no water addition, the number of

determined carotenoids decreased significantly, especially α- and β-carotene, and more remarkably in the FD powder. Similar results have been reported by other authors, who have found lower carotenoid content when increasing the amount of oil, and have attributed this result to limitations in the method used [21]. In our case, poor recovery of carotenoids when powders were dispersed in the oil matrix was also evidenced.

Focusing on the digestion process, the total carotenoid content in the digest of powders dispersed in water or oil-in-water emulsion were in the range of the determined powders that were directly digested, not dispersed in any media other than digestion fluids. When dispersed in the emulsion, there was an increase in some individual carotenoids during digestion, especially in FD powders, a fact which has also been observed by other authors [48,67,71,75]. Other carotenoids, however, decreased when powders were dispersed in the emulsion, which was even more remarkable in the case of powders dispersed in oil. As explained in the previous paragraph, limitations in the methodology used could be partially responsible for this result; nevertheless, our results also evidence poor solubilization of carotenoids in the sunflower oil used as the dispersing medium. However, the recovery indexes obtained after digestion of the powders dispersed in oil suggest that the *in vitro* digestion process favours the micellarisation of carotenoids, as compared with the non-digested samples. In fact, in vitro digestion significantly increased the number of carotenoids quantified, compared with the initial (non-digested) samples when adding oil, which suggests that the digestion fluids together with the stirring provided during the digestion procedure favours carotenoids micellarisation and quantification, and thus, carotenoids' bioaccessibility [53,67].

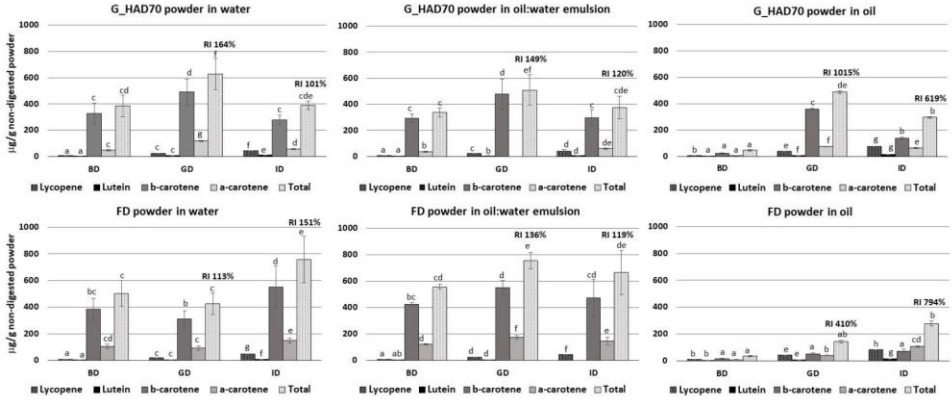

Figure 2. Total and individual carotenoid content (µg/g of non-digested powder) of selected powders mixed with water, 10% oil-in-water emulsion, or oil. Values of undigested samples (before digestion (BD)) and digested ones (after the gastric (GP) and after the intestinal phase (IP)) are given. Recovery index (RI) for total carotenoids after the gastric and intestinal phases are given in percentage. G_HAD70: ground and air-dried at 70 °C, FD: ground and freeze-dried. Values plotted correspond to mean ± standard deviation of four measurements. ^{a,b,c,d,e,f} Different superscript letters for a similar carotenoid and treatment (G_HAD70 or FD) indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Recovery indexes for total carotenoid content showed an increased release of carotenoids during digestion, for all three dispersing media analysed. Drying technique, either air-drying or freeze-drying, did have an impact on carotenoid release during digestion. As deduced from the RI, in G_HAD70 powders, the level of carotenoids released increased after the GP and decreased after the IP, for the three matrices tested. In contrast, carotenoids in FD powders were released throughout the process and increased after both GP and IP phases, exhibiting, in this case, a higher amount at the end of the process, especially in powders mixed with water or emulsion. This progressive release of carotenoids in FD samples during the in vitro digestion process has also been observed in powders digested with no medium addition (Table 6), and has been related to particle size and powder structure. Other authors have evidenced that a smaller particle size improves carotenoid release, such as in the study of Lyu et al. [67] on pumpkin seed powders; Zhang et al. [53] on dried carrot, sweet potato, yellow bell pepper and broccoli; or Moelants et al. [71] on carrot- and tomato-derived particles.

4. Conclusions

The valorisation of carrot residues and discards as functional powdered ingredients is an interesting alternative towards the development of more sustainable food systems and the concept of sustainable healthy diets. As evidenced, disruption and drying can be combined to obtain powders with appropriate aw characteristics and interesting nutritional properties. Both dehydration and disruption treatments influenced physicochemical and antioxidant properties of the powders obtained, as well as carotenoid content. In general, air-drying produced powders with improved antioxidant properties, whereas freeze-drying yielded finer powders with a higher carotenoid content.

Simulated *in vitro* digestion studies revealed that digestion helps to release bioactive compounds which are bound to the powder structure. The response to digestion is greatly influenced by processing conditions, which implied different physical characteristics of powders; finer ones such as freeze-dried powders were found to release more bioactive constituents during digestion. In addition to processing conditions, fat co-ingestion also influenced the powders' response to digestion, recovery indexes being increased in the case of powders dispersed in oil. As simulation digestion proceeds, digestion fluids together with stirring promote the breakage of structures as well as carotenoid release and micellarisation.

Future research should explore ensuring emulsification to better assess the impact of fat co-ingestion on the bioaccessibility of carotenoids. Hence, other emulsions (different fats, different fat concentrations, other emulsification procedures) should be tested. In addition, it would be convenient to modify the digestion protocol by increasing the amount of bile salts, thus simulating the increased level of bile salt secretion during the co-ingestion of fats. This is expected to increase the number and size of micelles, facilitating the solubilisation of carotenoids.

Supplementary Materials

Table S1. Pearson's product moment correlations matrix. Processing variables: disruption intensity, drying temperature; measured variables: particle size, total

phenols, total flavonoids, DPPH antioxidant capacity, ABTS antioxidant capacity and total carotenoid content.

	Disruption intensity	*Drying temperature*	*Particle size*	*Phenols*	*Flavonoids*	*DPPH*	*ABTS*	*Carotenoids*
Disruption intensity	1							
Drying temperature	-0.3932	1						
Particle size	-0.8626**	0.6942**	1					
Phenols	-0.6792**	0.9122**	0.8117**	1				
Flavonoids	-0.9572**	0.3509	0.8183**	0.6156*	1			
DPPH	-0.7632**	0.7921**	0.8782**	0.8972*	0.6841**	1		
ABTS	-0.4598**	0.9905**	0.7553**	0.9257**	0.4002**	0.8245**	1	
Carotenoids	0.4575	-0.9441**	-0.7911**	-0.8670**	-0.4249	-0.7708**	-0.9636**	1

*Indicates significant correlation at the 95% confidence level (*p*-value < 0.05). **Indicates significant correlation at the 99% confidence level (*p*-value < 0.01).

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Impact of Disruption and Drying Conditions on Physicochemical, Functional and Antioxidant Properties of Powdered Ingredients Obtained from Brassica Vegetable By-Products

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Abstract

Reintroducing waste products into the food chain, thus contributing to circular economy, is a key goal towards sustainable food systems. Fruit and vegetable processing generates large amounts of residual organic matter, rich in bioactive compounds. In Brassicaceae, glucosinolates are present as secondary metabolites involved in the biotic stress response. They are hydrolysed by the enzyme myrosinase when plant tissue is damaged, releasing new products (isothiocyanates) of great interest to human health. In this work, the process for obtaining powdered products from broccoli and white cabbage by-products, to be used as food ingredients, was developed. Residues produced during primary processing of these vegetables were transformed into powders by a process consisting of disruption (chopping or grinding), drying (hot-air drying at 50, 60 or 70 °C, or freeze drying) and final milling. The impact of processing on powders' physicochemical and functional properties was assessed in terms of their physicochemical, technological and antioxidant properties. The matrix response to drying conditions (drying kinetics), as well as the isothiocyanate (sulforaphane) content of the powders obtained were also evaluated. The different combinations applied produced powdered products, the properties of which were determined by the techniques and conditions used. Freeze drying better preserved the characteristics of the raw materials; nevertheless, antioxidant characteristics were favoured by air drying at higher temperatures and by applying a lower intensity of disruption prior to drying. Sulforaphane was identified in all samples, although processing implied a

reduction in this bioactive compound. The results of the present work suggest Brassica residues may be transformed into powdered ingredients that might be used to provide additional nutritional value while contributing to sustainable development.

Keywords: brassica, glucosinolates, isothiocyanates, agro-industrial by-products, antioxidant properties, functional powders.

1. Introduction

The Brassicaceae (or Cruciferae) family comprises about 3200 species, including cruciferous vegetables of commercial interest such as cabbage, cauliflower, broccoli and mustards, as well as oilseed and condiment crops and ornamental plants, among others [1,2]. A characteristic of cruciferous plants is the synthesis of the secondary metabolites named glucosinolates [3,4]. Glucosinolates (GSLs) consist of a generic chemical structure formed by the thiohydroximate-O-sulfonate group linked to glucose and a variable aglycone side chain (R-chain) derived from one of eight amino acids [4,5,6]. These bioactive chemicals play an important role in biotic stress resistance, specifically in the defence against herbivores and microorganisms [6,7]. GSLs role in plant defence is mainly due to their interaction with the enzyme myrosinase [8]. Once plant tissue damage occurs because of chewing, heating, grinding, digestion, or insect or herbivore attack [5,7], myrosinase hydrolyse GSLs producing a variety of bioactive and/or toxic compounds [5] such as isothiocyanates, thiocyanates, nitriles, goitrin and epithionitriles, depending on the precursor molecule, reaction conditions (temperature and pH) or the presence of certain cofactors [4,9]. Interest regarding the human health-promoting effects of GSLs and their hydrolyzation products such as isothiocyanates is increasing, which particularly applies to sulforaphane as one of the most abundant in brassicas [10]. Several in vitro and in vivo studies have reported the protective function of these compounds against diabetes or cardiovascular diseases [5]; moreover, the chemopreventive activity, anti-inflammatory effect and epigenetic mechanism's regulation of brassica-derived phytochemicals in humans has also been investigated [4,6,11,12,13]. These health-promoting effects present an opportunity for the development

of anti-inflammatory and chemopreventive functional foods, dietary supplements and drugs [11].

A large amount of fruit and vegetable wastes are generated along the whole food chain. In the very first stages of processing, these are due to discards because of high commercialization standards, as well as to the removal of non-desired or damaged parts. In primary production, losses in vegetables, fruits, tubers and roots vary from 10 to 30% of the production volume [14]. Brassica varieties such as broccoli or white cabbage generate large quantities of residual organic matter that are not consumed or commercialized but are still rich in bioactive compounds. These varieties are widely produced in Europe: in 2020, more than 4 million tonnes of cabbage and 2 million tonnes of cauliflowers and broccoli were produced, with Spain producing 7% and 34%, respectively [15].

Towards the development of a circular economy system and the reduction in food loss and wastes, there is the need for developing new production systems and products that minimize waste and reintroduce residues into the food chain. These approaches contribute to the development of more sustainable food systems [16] focusing on the four priorities: nutrition and health, climate and sustainability, circularity and resource use, and innovation and communities.

Fruit and vegetable by-products and discards, essentially outer layers and extremities, contain large amounts of bioactive compounds such as dietary fibers, antioxidant compounds and others [17], with beneficial effects on human health [18,19], and a great potential for sustainable and functional food product development. These materials in raw form are susceptible to microbial spoilage, so their transformation into powdered ingredients is an interesting alternative. Transformation of these wastes into powders implies a rather simple processing resulting in versatile products, rich in bioactive compounds, with increased physicochemical and microbiological stability [17,18]. These powdered ingredients offer wide applicability in the food industry, improving the nutritional characteristics of foodstuffs, as food preservatives and additives (colourings, flavourings, etc.), and might be incorporated into meat and bakery products [17,18,20] or

added to prepared foods such as instant soups [21] or infant formulations [20]. Furthermore, and particularly with brassica powders, the products could have potential uses in agriculture as a natural resource for pest control and disease prevention in crops due to their glucosinolates and isothiocyanates content [22,23].

Powder manufacturing requires the use of several stages (cleaning, grinding, drying, powdering), which in turn determine the properties of the products obtained. Thus, an important step before production is the assessment of the impact of processing parameters on the powders' properties. The intensity of disruption can affect the properties of the products as, for instance, excessive damage to plant tissue may negatively affect the content of antioxidant compounds [24]. Moreover, factors such as the size of the particles generated, the contact surface with air or the internal structure of the matrix generated are decisive for the dehydration process and therefore for the characteristics of the powders obtained [25]. Likewise, the effect of dehydration depends on the type of product and its structure, as well as on preprocessing stages and drying conditions, such as air temperature [26,27]. The advantages and disadvantages of different dehydration techniques commonly applied to develop functional ingredients and foods have been previously discussed [28]. In this review, air drying and freeze drying are proposed as appropriate drying methods for the integral valorisation of vegetable wastes and discards.

In this context, the present work was developed under a project in collaboration with the agricultural cooperative Agrícola Villena Coop. V., aiming to transform these brassica wastes into powdered food ingredients. Therefore, the purpose of the present work was to develop the process for obtaining powdered products from broccoli (*Brassica oleracea* var. *italica*) and white cabbage (*Brassica oleracea* var. *capitata*) residues (stems and outer leaves, respectively), and their subsequent physicochemical and functional characterization in order to evaluate the impact of processing on the powder's properties and their applications.

2. Materials and Methods

2.1. Raw Material and Powder Manufacturing

White cabbage (*Brassica oleracea* var. *capitata*) and broccoli (*Brassica oleracea* var. *italica*) residues were used in this work. White cabbage wastes, corresponding to the outer leaves generated in the manufacturing lines for the commercialization of fresh cabbage, were provided by the cooperative Agrícola Villena Coop. V. (Alicante, Spain). Broccoli was purchased from a local supermarket in Valencia (Spain) and residue (stems) was separated manually in the laboratory with a knife. Fresh vegetable discards were disinfected with a 1% (*v*/*v*) sodium hypochlorite solution in water.

The first step in obtaining the powders was to disrupt the tissue with a Thermomix® TM6 food processor (Vorwerk, Madrid, Spain) into pieces of ≤10 mm diameter, called hereinafter chopped (C), or into ground (G) pieces of ≤5 mm diameter. Disruption conditions were set according to preliminary tests, as in Bas-Bellver et al. (2020) [29]. Chopping was carried out at 5000 rpm for 5 s and grinding at 10,000 rpm for 10 s. Wastes were then dehydrated by hot-air drying (HAD) or freeze drying (FD). HAD was carried out in a convective CLW 750 TOP+ transverse flow tray dryer (Pol-Eko-Aparatura SPJ, Katowice, Poland) with an air velocity of 2 m/s and drying temperature of 50, 60 or 70 °C. Drying conditions were decided based on preliminary experiences. Ground or chopped residues were distributed on the dryer trays (~200 g of residue/tray) in 10 mm thick layers. Convective drying was conducted until water activity (a_w) measurements on the samples taken from the trays were reduced to 0.3, to guarantee stability of the final product [26]. FD consisted of deep-freezing ground samples at −40 °C in a CVN-40/105 freezer (Matek, Barcelona, Spain) and further freeze drying in a LyoQuest-55 laboratory freeze drier (Telstar, Terrasa, Spain) at 0.1 mbar and −45 °C (condenser temperature). Once dehydrated, the product obtained, either air-dried or freeze-dried, was ground to obtain a fine powder (10,000 rpm at 30 s intervals for a total 2 min milling) in a Thermomix® TM6 food processor (Vorwerk, Madrid, Spain). The powders obtained were packed into glass jars in a light-free environment.

Hereinafter, powdered products are identified according to the type of residue: white cabbage (WC) or broccoli (B); the pretreatment applied: grinding (G) or chopping (C); and the drying method used: HAD50, HAD60 and HAD70 for HAD at 50, 60 and 70 °C, or FD for freeze drying.

2.2. Matrix Behaviour during Air Drying: Drying and Drying Rate Curves

Both the weight and the water activity of samples were registered at different times throughout the drying process as explained elsewhere [29]. This procedure allowed the drying curves of the Brassica vegetable wastes to be obtained at 50, 60 and 70 °C, as well as to register the time required to reach a water activity value (a_w) lower than 0.3. For white cabbage outer leaves, this took 16 h at 50 °C, 14 h at 60 °C and 12 h at 70 °C, and for broccoli stems, 15 h at 50 °C, 10 h at 60 °C and 8 h at 70 °C.

The drying curves (moisture on dry basis vs. time) and the drying rate curves (drying rate vs. moisture on dry basis) were obtained by stating dry matter balances between the final conditions of drying (M_f: sample weight at the end of drying; x_{wf}: sample moisture content at the end of drying) and each measuring time along the drying process (M_t: sample weight at time t; x_{wt}: sample moisture content at time t) following Equation (1). A dry matter balance states that the amount of dry matter remains constant along the drying process and the only mass transfer taking place corresponds to the water being removed from the product.

$$M_t \cdot (1 - x_{wt}) = M_f \cdot (1 - x_{wf}) \quad (1)$$

In order to compare the different samples and drying conditions applied, the moisture represented in the drying curves was calculated relative to the initial moisture (X_w/X_{w0}), in dry basis, for each experiment.

2.3. Analytical Determinations

Brassica powders were characterized in terms of physicochemical properties including moisture content, water activity, total soluble solids content, particle size distribution, colour, specific volume and solubility. Technological properties of interaction with water (hydration properties)

and oil (emulsifying properties) were also evaluated. Moreover, antioxidant properties and glucosinolate and isothiocyanate contents were assessed.

2.3.1. Physicochemical Properties

Water activity (a_w) was measured with an Aqualab 4TE dew point hygrometer at 25 °C (Decagon devices Inc., Pullman, Washington, DC, USA). Moisture content (x_w) was obtained gravimetrically by measuring weight loss before and after drying in a Vaciotem-T vacuum oven (JP Selecta, Barcelona, Spain) (p = 10 mmHg) at 60 °C until constant weight, according to the official method of the AOAC 934.06 [30]. Total soluble solids contents (x_{ss}) were determined by a thermostatic Abbe refractometer NAR-3T (Atago, Tokyo, Japan) through the measurement of Brix degrees at 20 °C, according to the ISO 1743:1982 method. In dried samples, measurements were obtained from an aqueous extract of soluble solids in a 1:10 (*w/v*) ratio. Particle size distribution was determined by laser diffraction using a Mastersizer 2000 equipment (Malvern Panalytical Ltd., Malvern, UK). The equipment was coupled to a unit Hydro 2000, setting the particle absorption index at 0.1, and the refraction indexes at 1.52 and 1.33 for the sample and the dispersed phase (deionized water), respectively. Particle size results were obtained in terms of equivalent volume mean diameter D[4,3], surface area mean diameter D[3,2] and the distribution percentiles d_{10}, d_{50} and d_{90}. Colour of samples was measured with a CM-1000R spectrocolorimeter (MINOLTA, Tokyo, Japan) using a D65 illuminant and a 10° angle of vision as reference. To quantify colour, the CIE L*a*b* system was used, where L* is the brightness, a* is the red–green component and b* is the yellow–blue component. Hue (h_{ab}) and chrome (C_{ab}) attributes were also calculated.

2.3.2. Water Interaction and Oil Emulsifying Properties

Specific volume of powders was assessed by measuring the volume of 5 g of sample in a 10 mL test tube. Solubility was obtained following the method described by Mimouni et al. [31], as the mass fraction of dissolved solids in the rehydrated sample. Hygroscopicity was determined according to the method proposed by Cai and Corke [32], which consisted of measuring the gain of water of certain amount of sample when placed in a hermetic

chamber next to a saturated solution of sodium sulphate (Na_2SO_4) for one week at room temperature (25 °C). Results were expressed in g of water/100 g of sample. Wettability was obtained from the time in which 2 g of powder became completely wet in a beaker containing 20 mL of distilled water at 25 °C [33]. It refers to the wetting time taken by all powder particles to sink completely, and it is inversely related to wetting time. Swelling capacity (SC) was measured according to Raghavendra et al. [34], as the ratio between the volume of the sample when is immersed in water excess for 18 h at 25 °C and the initial weight of the sample. Results were expressed in mL/g. Water holding capacity (WHC) was calculated as the amount of water retained by the sample without the application of any external force; that is, the ratio between the amount of water contained in 0.2 g of powder hydrated with 10 mL of water for 18 h at 25 °C and the dry weight of the powder after freeze drying [34]. Water retention capacity (WRC) was defined as the amount of water retained by the sample when subjected to an external force such as pressure or centrifugation [34]. Thus, 1 g of powder was added to 10 mL of water allowing hydration for 18 h at 25 °C. After that, the mixture was centrifuged for 30 min at 2000 rpm in a MegafugeTM 16 centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the resulting precipitate was weighed and freeze-dried to obtain the dry weight of the sample. WRC was calculated as the ratio between the water retained by the powder and the dry weight of the residue.

Oil holding capacity (OHC) was determined by mixing 0.2 g of powder with 1.5 g of sunflower oil and kept overnight at room temperature. Mixture was centrifuged at 3400 rpm for 5 min, the supernatant was removed and the weight of the precipitate obtained. Results were expressed in g of absorbed oil per g of powder [35]. Emulsifying activity (EA) was measured following the method described by Yasumatsu et al. [36]. A 2% (*w/v*) aqueous powder solution was mixed with 7 mL of sunflower oil and homogenised at 2400 rpm for 5 min in a Reax Top Vortex mixer (HeidolphTM, Schwabach, Germany) and, after that, the mixture was centrifuged at 10,000 rpm for 5 min in a Megafuge^{TM} 16 centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA). The volume of the emulsion was measured and referred to the total fluid volume. Emulsifying stability (ES) was measured by the same

procedure explained for EA, but in this case the emulsions were heated at 80 °C for 30 min before centrifugation at 2000 rpm for 5 min.

2.3.3. Antioxidant Properties

Antioxidant compounds were extracted by mixing the samples with an 80% (*v/v*) methanol/water solution in a 1:10 (*w/v*) or 2:10 (*w/v*) ratio for powders and raw samples, respectively. The mixture was stirred for 1 h in a horizontal stirrer (Magna Equipments S. L., model ANC10, Barcelona, Spain) and further centrifuged at 10,000 rpm for 5 min in an Eppendorf centrifuge 5804/5804R (Eppendorf SE, Hamburg, Germany).

Total phenolic content was measured using the Folin-Ciocalteu method [36,37]. In total, 0.125 mL of the extract was mixed with 0.5 mL of bidistilled water and 0.125 mL of Folin-Ciocalteu reagent (Scharlab S.L., Barcelona, Spain), and allowed to react for 6 min in darkness. Then, 1.25 mL of 7% (*w/v*) sodium carbonate solution and 1 mL of bidistilled water were added. After 90 min in darkness, absorbance was measured at 760 nm in a Helios Zeta UV/Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Results were expressed in mg of Gallic Acid Equivalents (GAE) per g of dry matter.

Total flavonoid content was measured following the modified method described by Luximon-Ramma et al. [38]. Amounts of 1.5 mL of the extract and 1.5 mL of a 2% (*w/v*) aluminium chloride solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) were mixed and kept in darkness for 10 min. Absorbance was measured at 368 nm in a Helios Zeta UV/Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and results were expressed in mg of Quercetin Equivalents (QE) per g of dry matter.

Antioxidant activity was measured by the DPPH and ABTS radical methods. According to DPPH method [39], 0.1 mL of the extract was mixed with 2.9 mL of a 0.1 mM solution of DPPH (2,2-diphenyl-1-picryl hydrazyl) in methanol (Merck KGaA and affiliates, Darmstadt, Germany). After reaction during 60 min in darkness, the absorbance was measured at 575 nm in a Helios Zeta UV/Vis spectrophotometer (Thermo Fisher Scientific Inc.,

Waltham, MA, USA). Results were expressed in mg of Trolox Equivalent (TE) per gram of dry matter. For the ABTS method [40], 0.1 mL of the extract was added to 2.9 mL of an $ABTS^{+}$ (VWR International LLC, Radnor, PA, USA) solution in phosphate buffer with an absorbance of 0.70 ± 0.02 at 734 nm. Absorbance was measured in a Helios Zeta UV/Vis sp HeidolphTM, Schwabach spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 734 nm after 7 min of reaction. Results were expressed in mg of Trolox Equivalent (TE) per g of dry matter.

2.3.4. Glucosinolate and Isothiocyanate Content by High Pressure Liquid Chromatography (HPLC)

Glucosinolate content of powders and fresh (not dehydrated) samples of both broccoli stems and white cabbage outer leaves were analysed by HPLC, following the protocol described by Campas-Baypoli et al. [41] with some modifications.

Sample preparation involved the conversion of glucoraphanin into sulforaphane by means of hydrolysis. In total, 0.5 g of powder or fresh sample was added with 4 mL of acidic water (pH 6). The mixture was incubated in a PRECISTERM water bath (JP Selecta, Barcelona, Spain) at 45 °C for 2 h, to complete hydrolysis. In the present work, additionally, in order to quantify the amount of glucosinolates that were transformed into isothiocyanates during the waste-to-powder transformation process, the isothiocyanate (sulforaphane) content in samples not subjected to the hydrolysis or conversion step was also assessed.

Sulforaphane was extracted by adding 20 mL of dichloromethane to the samples and vortexing for 1 min. After 1 h at room temperature, the solution was filtered using Whatman no. 41 paper. The extract was then purified using 3 mL 7086-03 BAKERBOND® solid-phase extraction (SPE) silica gel (SiOH) disposable columns (JT Baker®, Phillipsburg, NJ, USA), previously activated with 3 mL of dichloromethane. The organic extract obtained after filtration was passed through the cartridge, washing the column with 3 mL of ethyl acetate and eluting the sulforaphane with 3 mL of methanol. The sample collected was taken to a Hei-VAP Core rotary evaporator (Heidolph Instruments GmbH & Co., Schwabach, Germany) for evaporation of the

organic solvent and subsequently resuspended in 100 µL of acetonitrile. The resulting solution was filtered using 0.45 µm nylon membrane, and 20 µL aliquot of this solution was finally injected onto the column of the HPLC system.

The chromatographic analysis was performed using an Alliance 2995 system with diode array detector (Waters, Milford, MA, USA) and a C18 Luna column (Phenomenex, Torrance, CA, USA). The analyses were carried out under isocratic conditions using as mobile phase a solution of acetonitrile–ultrapure water (30:70, *v/v*) with a flow rate of 0.6 mL/min, and the temperature of the column was set at 36 °C. Sulforaphane was detected at 202 nm and the time between injections was 20 min. All samples were analysed in duplicate.

A calibration line of known concentrations of sulforaphane was prepared using an HPLC standard (Merck KGaA and affiliates, Darmstadt, Germany; >90% purity). Solutions of 1, 3, 5, 7.5, 10, 15, 20, 25, 50 and 75 µg/mL were prepared using acetonitrile as solvent. Chromatogram pick areas were extracted and correlated with the concentrations according to the calibration curve. Sulforaphane recovery percentage was determined by adding a known amount of standard (sulforaphane) to selected samples prior to their SPE. The percentage of recovery was calculated from the ratio between the amount of standard recovered (difference between the amount of standard in the enriched sample and the amount of standard in the non-enriched sample) and the known amount of standard added. The recovery percentage was used to correct the values obtained after identification and quantification of sulforaphane.

2.4. Statistical Analysis

All analytical determinations were determined at least in triplicate. Statistical analysis of the results obtained was carried out with Statgraphics Centurion software (Centurion XVII.I version, StatPoint Technologies, Inc., Warrenton, VA, USA). Analyses of variance (one-way ANOVA and multifactorial ANOVA) were performed, having previously checked the normality of the data, and using a confidence level of 95% (p-value < 0.05).

3. Results and Discussion

3.1. Drying and Drying Rate Curves

Drying curves and drying rate curves obtained at 50, 60 and 70 °C, for a 10 mm thick layer of ground or chopped white cabbage outer leaves or broccoli stems, are shown in Figure 1. Drying curves describe the relationship between the moisture content (expressed on a dry basis) of a sample and the time elapsed, under constant pressure and temperature conditions. Theoretically, hot-air drying of a product takes place in three stages: an initial induction period, followed by the constant drying rate period (CDRP) and a final period of drying at a decreasing rate called the falling drying rate period (FDRP) [42]. However, depending on the characteristics of the sample and the drying conditions, experimental curves may present all or only some of these periods [43].

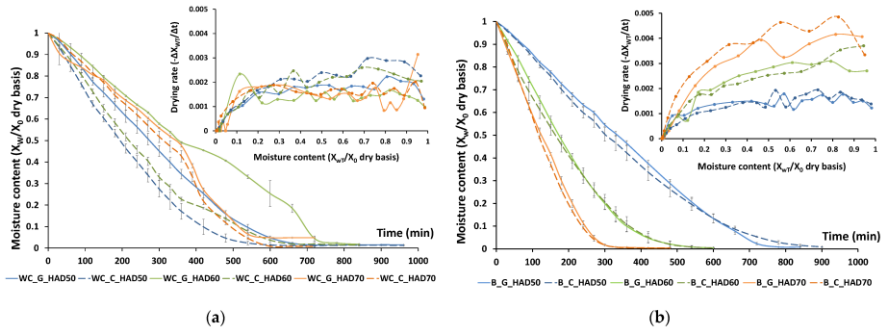


Figure 1. (**a**) Drying curves (moisture content vs. time) and drying rate (drying rate vs. moisture) curves of ground and chopped white cabbage outer leaves (**a**) and broccoli stems (**b**). WC: white cabbage; B: broccoli; G: ground; C: chopped; HAD: hot-air drying at 50, 60 and 70 °C.

Drying periods are typically better identified on the drying rate curves. For the brassica residues analysed, the drying rate curves obtained evidenced that most of the water was removed during a CDRP, for most samples. However, some differences were identified between products and drying temperatures. The cabbage residue showed a clearer and longer CDRP, while the broccoli residue did not exhibit this behaviour at higher drying temperatures (60 and 70 °C), and seemed to enter directly into the FDRP. In addition, the drying rates were initially higher for the broccoli

residue, thus suggesting that water was more easily removed from broccoli waste than from cabbage waste, especially at higher temperatures, coinciding with the curves that enter directly in the FDRP. This could be due to structural differences between both residual matrices, but also to structural modifications during drying. In fact, a more effective removal of surface water from samples, as observed in the broccoli residue, may cause crusting of the product surface increasing the internal resistance to water transport [44]. Crusting phenomena, also known as case-hardening, imply the accumulation of non-volatile compounds that are carried away by water diffusion, compounds formed because of Maillard reactions or oxidation processes, and structural changes (shrinkage, glass transitions) in the tissue layers close to the surface that hinder the diffusion of water through the matrix [45].

The effect of drying temperature on drying kinetics was evident in all cases, although more pronounced in broccoli, suggesting structural differences among matrices, either initial ones or produced during drying (Figure 1). On the other hand, cabbage showed a more homogeneous behaviour regarding the different drying temperatures applied, which allowed certain influences of the disruption pretreatment to be identified. As observed in Figure 1a, the drying rate was higher when the residue was chopped as compared to ground. This may be due to a more packed matrix in the case of ground cabbage, with the particles closer to each other than in the case of chopped cabbage, which would hinder the diffusion of water to the surface [28]. This effect was less significant in broccoli, where both chopped and ground samples presented quite a similar behaviour. This might be attributed to the tissue structure (stems) being harder and more difficult to disrupt than in cabbage leaves. In a previous study [29] on several vegetables' residues (leek, cabbage, carrot and celery), chopping allowed drying times to be reduced in most cases, except for carrot, also with a harder structure.

The study of drying kinetics also made it possible to define the time required to reduce initial moisture to reach the target water activity (a_w < 0.3) for each residue and drying condition applied. Hence, the time required for white cabbage was 16 h for 50 °C, 14 h for 60 °C and 12 h for 70 °C

whereas for broccoli samples, this was 15 h for 50 °C, 10 h for 60 °C and 8 h for 70 °C. Chopped or ground residues required similar drying times at the same drying temperatures.

3.2. Physicochemical Characterization

Table 1 shows the results corresponding to water activity (a_w), moisture content (%) and total soluble solids (x_{ss}) of the raw materials (cabbage outer leaves, broccoli stems) and the different powders obtained.

Table 1. Water activity (a_w), moisture content (g water/100 g) and soluble solids content (x_{ss} in g soluble solids/g dry matter) of white cabbage (WC) and broccoli (B) samples. G: ground, C: chopped; HAD: hot-air drying at 50, 60 and 70 °C, FD: freeze drying. Mean ± standard deviation of six replicates.

Sample	a_w	Moisture content (%)	x_{ss} (g_{ss}/g_{dm})
WC_G_HAD50	0.30 ± 0.04^{bc}	4.3 ± 1.2^{b}	0.62 ± 0.02^{ab}
WC_C_HAD50	0.285 ± 0.009^{abc}	3.2 ± 0.9^{ab}	0.637 ± 0.004^{ab}
WC_G_HAD60	0.256 ± 0.014^{abc}	2.6 ± 1.4^{ab}	0.652 ± 0.013^{bc}
WC_C_HAD60	0.254 ± 0.009^{abc}	2.49 ± 0.12^{ab}	0.61 ± 0.04^{ab}
WC_G_HAD70	0.23 ± 0.09^{a}	3.1 ± 1.1^{ab}	0.61 ± 0.06^{ab}
WC_C_HAD70	0.245 ± 0.08^{ab}	2.6 ± 0.8^{a}	0.58 ± 0.10^{a}
WC_G_FD	0.301 ± 0.033^{c}	3.4 ± 1.4^{ab}	0.64 ± 0.05^{bc}
WC (raw)	0.990 ± 0.003^{d}	91.4 ± 0.2^{c}	0.70 ± 0.02^{c}
B_G_HAD50	0.30 ± 0.04^{ab}	3.7 ± 0.2^{bc}	0.56 ± 0.03^{a}
B_C_HAD50	0.312 ± 0.08^{b}	3.3 ± 0.2^{ab}	0.60 ± 0.03^{abc}
B_G_HAD60	0.253 ± 0.007^{a}	2.3 ± 0.7^{a}	0.58 ± 0.02^{ab}
B_C_HAD60	0.283 ± 0.010^{ab}	2.2 ± 0.64^{a}	0.59 ± 0.02^{b}
B_G_HAD70	0.318 ± 0.045^{ab}	3.8 ± 1.8^{b}	0.59 ± 0.02^{b}
B_C_HAD70	0.30 ± 0.04^{b}	2.6 ± 1.0^{a}	0.61 ± 0.02^{b}
B_G_FD	0.298 ± 0.04^{ab}	2.7 ± 0.4^{b}	0.608 ± 0.018^{b}
B (raw)	0.998 ± 0.004^{c}	92.83 ± 0.09^{c}	0.70 ± 0.02^{c}

$^{a,b,c\ldots}$ Different superscript letters in the same column for the same residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Raw cabbage and broccoli have very high a_w values, which indicates perishability and a high risk of spoilage. The drying methods applied allowed an a_w in the powders to be reached that guarantees product stability [46]. For a similar drying temperature, and for both cabbage and broccoli

powders, those obtained from ground residues showed generally higher moisture values than those obtained from chopped ones. The multifactorial ANOVA analysis confirmed that there were significant differences depending on the milling intensity applied before drying (*p*-value < 0.05). This fact suggests that the structure played a fundamental role in the removal of water. The more packed structure obtained in ground samples could have limited internal water transfer to a higher extent, as compared to chopped ones, in which the presence of interparticle channels might have facilitated the water outflow by diffusion and a capillarity mechanism.

Soluble solid fractions (x_{ss}) of powders, obtained from Brix measurements, are also given in Table 1. Powdered foods rich in low molecular weight sugars, such as fruit powders, are very sensitive to environmental conditions so that caking and stickiness may become a problem during storage [47]. Nevertheless, the soluble solid obtained for vegetable powders is relatively low as compared to fruit ones and in line with fruit skins and bagasse [26]. Raw residues showed slightly higher soluble solid contents than powders. This might be due to the structure resulting from the milling and dehydration operation applied, or either to the success of the extraction step from the raw or dried material. No trend was observed depending on drying conditions or pretreatment intensity.

Particle size distributions, measured by the wet procedure, are shown in Figure 2. In this figure, the volume percentage of particles with a specific particle size is plotted. In addition, characteristic parameters D[4,3], D[3,2], d_{10}, d_{50} and d_{90} of the powders, are summarized in Table 2, which allowed the identification of statistically significant differences among samples.

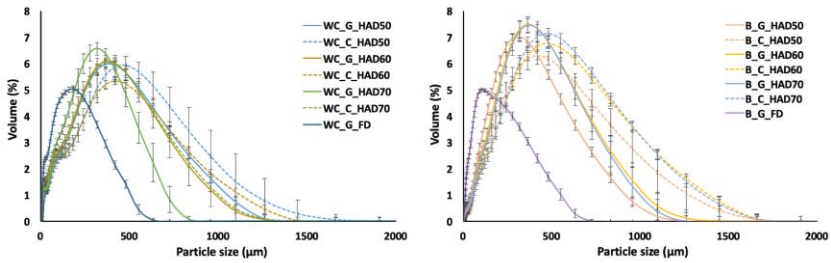


Figure 2. Particle size distribution of powders from white cabbage outer leaves (**a**) and broccoli stems (**b**). The error bars represent the standard deviation of five replicates. WC: white cabbage; B: broccoli; G: ground; C: chopped; HAD: hot-air drying at 50, 60 and 70 °C, FD: freeze drying.

Table 2. Particle size characteristic parameters of white cabbage (WC) and broccoli (B) powders: equivalent volume diameter D[4,3], surface area mean diameter D[3,2], percentiles d_{10}, d_{50} and d_{90}. HAD: hot-air drying at 50, 60 and 70 °C, FD: freeze drying; C: chopped; G: ground. Mean ± standard deviation.

Sample	D[4,3]	D[3,2]	d_{10}	d_{50}	d_{90}
WC_G_HAD50	306 ± 12^{c}	48.7 ± 1.1^{d}	19.2 ± 0.4^{c}	249 ± 7^{d}	688 ± 33^{cd}
WC_C_HAD50	350 ± 49^{d}	58 ± 4^{f}	25 ± 2^{e}	283 ± 38^{e}	792 ± 117^{e}
WC_G_HAD60	292 ± 22^{c}	51.8 ± 1.8^{e}	20.7 ± 0.4^{d}	240 ± 14^{d}	650 ± 59^{c}
WC_C_HAD60	299 ± 24^{c}	42.2 ± 0.7^{b}	16.7 ± 0.2^{b}	218 ± 13^{c}	714 ± 66^{d}
WC_G_HAD70	231 ± 11^{b}	47.0 ± 1.1^{cd}	18.7 ± 0.6^{c}	197 ± 9^{b}	498 ± 29^{a}
WC_C_HAD70	294 ± 20^{c}	45.7 ± 1.6^{c}	16.9 ± 0.7^{b}	241 ± 16^{d}	661 ± 47^{cd}
WC_G_FD	137 ± 3^{a}	34.3 ± 0.6^{a}	13.9 ± 0.2^{a}	101 ± 2^{a}	323 ± 8^{a}
B_G_HAD50	299 ± 13^{b}	83 ± 4^{b}	56 ± 3^{b}	255 ± 8^{b}	609 ± 36^{b}
B_C_HAD50	387 ± 37^{d}	91 ± 5^{c}	56 ± 3^{b}	318 ± 27^{c}	828 ± 94^{d}
B_G_HAD60	352 ± 18^{c}	105 ± 2^{e}	72.2 ± 1.9^{f}	308 ± 10^{c}	698 ± 50^{c}
B_C_HAD60	438 ± 34^{e}	97 ± 3^{d}	63 ± 2^{d}	373 ± 23^{d}	912 ± 83^{e}
B_G_HAD70	341 ± 22^{c}	91 ± 4^{c}	59 ± 3^{c}	303 ± 15^{c}	681 ± 51^{c}
B_C_HAD70	445 ± 38^{e}	104 ± 4^{e}	71 ± 4^{e}	388 ± 28^{d}	906 ± 91^{e}
B_G_FD	145 ± 4^{a}	42.4 ± 0.4^{a}	18.3 ± 0.3^{a}	102 ± 3^{a}	341 ± 11^{a}

$^{a,b,c...}$ Different superscript letters in the same column for the same residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

The results obtained evidence that both the pretreatment intensity and dehydration technique applied determine powders' particle size. For both residues, powders obtained by FD presented smaller particle sizes than HAD powders, which could be attributed to the more porous and fragile structure generated during freeze drying, which facilitates further milling [26,48]. Comparing both residues, broccoli powders were usually thicker than cabbage ones. This could be a consequence of faster initial drying rates promoting crusting phenomena, making it more difficult to reach low moisture contents in the inner part of the samples, thus leading to rubbery behaviour and them being more difficult to mill. In contrast, cabbage powders were more homogeneously dried during a longer period, thus

obtaining a more brittle material, and being easier to mill. Gulati and Datta [49] investigated case-hardening and texture development during the air drying of food materials and evidenced that, for very low average moisture contents, high drying rates lead to case-hardening phenomena and a product core that remains in the rubbery state, while for lower and more homogeneous drying rates, the entire material transitions into the glassy state. On the other hand, regarding the milling ability, the glassy state is related to a crispier character, whereas in the rubbery state, crispness is lost [50].

The drying temperature also determined particle size, its effect being different depending on the residue. In the case of cabbage leaves, increasing the drying temperature implied a reduction in particle size characteristics; in contrast, broccoli powders presented higher particle sizes when higher temperatures were applied (Table 2). This could be related to the differences observed in the drying behaviour between both residues, as previously explained, since the crusting phenomena occurring in broccoli intensified when increasing temperature. On the other hand, grinding prior to drying resulted in powders with a smaller particle size than chopping, this being more remarkable in broccoli as compared to cabbage. This result is in line with previous studies [29].

Figure 3 show the L*a*b* coordinate distribution of powders, where each point represents a sample. As can be observed, luminosity (L*) presented values in the range of 70–80. In general, luminosity was slightly higher in FD than in HAD powders, as also observed by Xu et al. [51] for hot-air dried at 60 °C (49.5 ± 0.4) and freeze-dried (56.5 ± 0.4) cabbage, and by Vargas et al. [52] for hot-air dried at 70 °C (31±2) and freeze-dried broccoli (47±7). Regarding a* (+redness/−greenness) and b* (+yellowness/ −blueness) coordinates, HAD and FD powders were clearly differentiated; however, the drying temperature and intensity of the pretreatment applied did not exhibit a clear trend. a* values were positive for all powders except for the FD ones, indicating a deviation towards the green colour. In the case of the b* coordinate, all values were positive, being generally higher in broccoli powders.

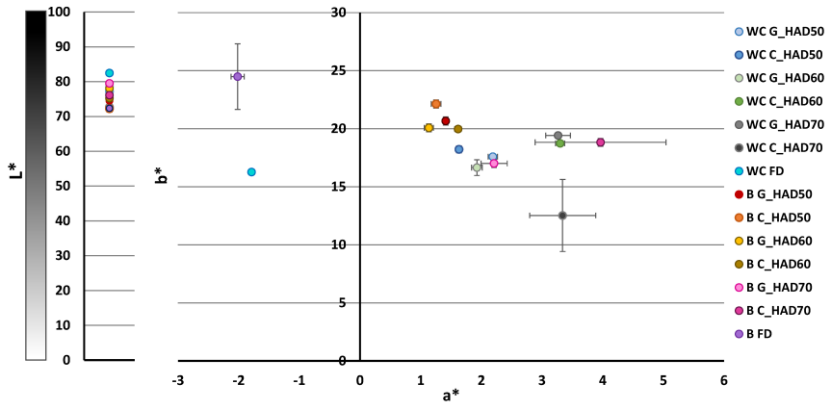


Figure 3. Distribution of L*a*b* coordinates of white cabbage and broccoli waste powders. WC: white cabbage; B: broccoli; G: ground; C: chopped; HAD: hot-air drying at 50, 60 and 70 °C, FD: freeze drying.

Table 3 shows the results of chroma (C_{ab}) and hue (h_{ab}) parameters. There were significant differences (*p*-value < 0.05) among samples for both chroma and hue. The higher chroma values found in broccoli powders indicate a higher intensity of saturation. Hue values were generally higher in broccoli. With respect to the drying technique applied, hue values of FD powders were higher than HAD ones, for both vegetable residues.

Table 3. Colour parameters of white cabbage (WC) and broccoli (B) powders: chroma (C_{ab}) and hue (h_{ab}). HAD: hot-air drying at 50, 60 and 70 °C, FD: freeze drying; C: chopped; G: ground. Mean ± standard deviation of three replicates.

Sample	Chroma (C_{ab})	Hue (h_{ab})	Sample	Chroma (C_{ab})	Hue (h_{ab})
WC_G_HAD50	17.7 ± 0.2^{bc}	82.9 ± 0.2^{c}	B_G_HAD50	20.7 ± 0.4^{bc}	86.08 ± 0.05^{c}
WC_C_HAD50	18.3 ± 0.3^{bc}	84.88 ± 0.05^{d}	B_C_HAD50	22.2 ± 0.4^{c}	86.75 ± 0.16^{c}
WC_G_HAD60	16.7 ± 0.7^{b}	83.39 ± 0.06^{c}	B_G_HAD60	20.1 ± 0.3^{b}	86.76 ± 0.17^{c}
WC_C_HAD60	19.02 ± 0.04^{c}	80.1 ± 0.2^{b}	B_C_HAD60	20.04 ± 0.02^{b}	85.37 ± 0.15^{c}
WC_G_HAD70	19.71 ± 0.16^{c}	80.5 ± 0.5^{b}	B_G_HAD70	17.2 ± 0.4^{a}	82.6 ± 0.6^{b}
WC_C_HAD70	12 ± 3^{a}	74.9 ± 1.1^{a}	B_C_HAD70	19.27 ± 0.16^{b}	78 ± 3^{a}
WC_G_FD	16.37 ± 0.09^{b}	96.26 ± 0.14^{e}	B_G_FD	24 ± 2^{d}	94.7 ± 0.4^{d}

$^{a,b,c...}$ Different superscript letters in the same column for the same residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05).

Table 4 shows the results of the solubility, specific volume, hydration and emulsifying properties of the brassica powders obtained. These are

important properties that determine the functionality of powders when used as food ingredients [53].

Table 4. Results of specific volume, solubility, hydration and water retention properties and emulsifying properties of white cabbage (WC) and broccoli (B) powders. Spec V: specific volume (mL); % solubility expressed as a percentage; HG: hygroscopicity (%) (g_{water}/100 g_{sample}); Wt: wetting time (min); SC: swelling capacity (mL/g); WHC: water holding capacity (g/g); WRC: water retention capacity (g/g); OHC: oil holding capacity (g/g). G: ground; C: chopped; HAD: hot-air drying at 50, 60 and 70 °C; FD: freeze drying. Mean ± standard deviation of three replicates.

Sample	Spec. V (mL)	Solubility (%)	HG (%)	Wt (min)	SC (mL/g)	WHC (g/g)	WRC (g/g)	OHC (g/g)
WC_G_HAD50	1.80±0.04^{c}	52±6^{a}	63.0±0.7^{a}	8.7±0.9^{a}	11.17±0.15^{bc}	18±3^{ab}	9.0±0.3^{a}	3.87±0.11^{b}
WC_C_HAD50	1.807±0.012^{c}	56±4^{a}	61.8±0.7^{a}	9±2^{a}	11.37±0.06^{c}	20±7^{ab}	15±2^{c}	3.1±0.5^{a}
WC_G_HAD60	1.51±0.02^{a}	54±3^{a}	64.8±0.6^{ab}	14±6^{ab}	11.10±0.10^{b}	15±6^{a}	10.1±0.7^{ab}	4.2±0.3^{b}
WC_C_HAD60	1.53±0.02^{a}	55±6^{a}	66.1±1.2^{ab}	18±4^{bc}	10.8±0.3^{a}	20±4^{ab}	9.9±0.9^{ab}	3.0±0.4^{a}
WC_G_HAD70	1.993±0.012^{d}	55±4^{a}	68±12^{ab}	6±2^{a}	11.10±0.11^{b}	28±5^{b}	10±2^{ab}	4.38±0.10^{bc}
WC_C_HAD70	1.67±0.03^{b}	55±3^{a}	67.1±1.3^{ab}	29±4^{c}	11.40±0.10^{c}	21±6^{ab}	11.0±1.5^{ab}	2.9±0.2^{a}
WC_G_FD	2.96±0.03^{e}	58±4^{a}	74±11^{b}	15±6^{ab}	10.8±0.2^{a}	21±7^{b}	13±3^{bc}	4.8±0.5^{c}
B_G_HAD50	1.65±0.04^{c}	54±4^{a}	67.9±0.7^{ab}	2.5±1.5^{a}	10.70±0.11^{c}	33±4^{bc}	13.2±1.6^{b}	4.85±0.14^{c}
B_C_HAD50	1.32±0.02^{a}	45±3^{a}	70.8±0.6^{ab}	4±2^{ab}	11.47±0.06^{f}	35±10^{c}	12.5±1.3^{b}	4.0± 0.5^{b}
B_G_HAD60	1.44±0.04^{b}	53±11^{a}	65.6±0.5^{a}	4.4±1.2^{ab}	10.93±0.06^{de}	24±2^{bc}	11.50±0.14^{b}	4.2± 0.3^{b}
B_C_HAD60	1.307±0.012^{a}	56±6^{a}	65.2±0.6^{a}	7.79±1.16^{bc}	10.91±0.12^{d}	25±4^{bc}	13±2^{b}	3.9±0.3^{b}
B_G_HAD70	1.69±0.06^{c}	55±4^{a}	66±3^{a}	8.9±0.6^{c}	9.47±0.06^{b}	20±4^{ab}	7.7±0.3^{a}	3.3±0.3^{a}
B_C_HAD70	1.27±0.03^{a}	52±7^{a}	77±14^{bc}	10±5^{c}	5.00±0.10^{a}	12±3^{a}	6.7±0.5^{a}	2.81±0.15^{a}
B_G_FD	3.98±0.02^{d}	54±6^{a}	84.3±0.7^{c}	1.9±0.9^{a}	11.03±0.06^{e}	32±4^{bc}	12.7±0.7^{b}	6.5±0.3^{d}

$^{a,b,c...}$ Different superscript letters in the same column for the same residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

As expected, FD powders exhibited higher specific volumes than HAD ones due to the air channels generated during sublimation, which produce a more open and porous structure that determines powder volume [29,54]. Regarding solubility, statistically significant differences were not found among powders obtained from the same residue. Solubility is a physical property describing powder behaviour in an aqueous solution, which is related to several characteristics of the powder such as the structure of the polysaccharides [53], powder microstructure or particle size distribution

[55,56]. The values obtained in the present work were slightly higher than those reported by Shi et al. [57] for freeze-dried broccoli stem powder (42.1 ± 0.2%).

No statistically significant differences were observed in cabbage powders regarding hygroscopicity (HG), although values slightly increased with temperature in HAD samples. This trend was not observed in broccoli powders. HG was higher in FD powders, mainly in broccoli ones, which could be attributed to the larger surface area of these powders. Similar results were observed by Kapoor and Feng [58] for HAD and FD blueberry and cranberry powders. As reported by Si et al. [55], soluble solids content is also positively related to hygroscopicity.

Wettability did not show a clear trend with respect to the dehydration method used. In contrast, an effect of the pretreatment was identified since chopping led to higher wettability (higher wetting time), as compared to ground. As evidenced by several authors [59,60], wettability is higher for larger particle sizes since large particles imply more interparticle spaces and, therefore, more porosity. This was confirmed in the present work when comparing ground vs. chopped air-dried samples.

Swelling capacity (SC), water retention capacity (WRC) and water holding capacity (WHC) exhibited statistically significant differences among powders; however, no trend was observed with respect to the factors evaluated (dehydration technique or previous milling intensity). Hydration properties play an important role in powder quality since a higher water affinity may improve the functionality of the final product [20,53,61]. According to the literature, intense milling and reduced particle size are negatively related to hydration properties [62]. This phenomenon may be due to the damage of the fiber matrix and the collapse of the pore during grinding. A relationship between particle size and hydration properties was also evidenced by Jongaroontaprangsee et al. [63] on white cabbage outer leaf powders; nevertheless, the same authors did not find a relationship between particle size and hydration properties on citrus powders, concluding that not only particle size but also fiber nature and process history contribute to hydration properties. In fact, it has also been reported

that higher drying rates reduce hydration properties [63], a phenomenon that was observed in the present study in the case of broccoli HAD powders obtained at 70 °C. In contrast, and although not significant, FD powders were in the upper range of the values obtained for SC, WHC and WRC. This is in line with other authors who reported that, as compared to FD, HAD reduces the hydration properties and porosity of vegetable and fruit powders [64,65].

In addition to hydration properties, vegetable powders may have the ability to trap fat, which make emulsifying properties interesting parameters for their characterization. Emulsifying properties are affected by the size, shape and superficial area of the particles, as well as by the nature of the fiber present. However, the powders obtained in the present work did not exhibit an interesting behaviour with regard to their emulsifying properties. On the one hand, no results were obtained for the emulsifying activity and stability; on the other hand, small values for oil holding capacity (OHC) were obtained. These values were in the same range as those reported for turnip fibers (between 3.32–6.35 g/g) [66], and slightly lower than those reported for asparagus fibers (between 5.28–8.53 g/g) [67]. Regarding the drying technique applied, FD powders showed better oil absorption capacity than HAD ones, as reported by Que et al. [64].

Indeed, brassica powders exhibited better hydration properties than emulsifying ones. Hydration properties reveal the presence of a greater number of hydrophilic groups and soluble fibers with a high ability to absorb water [20]. On the other hand, both proteins and fibers contribute to emulsion formation due to both their hydrophobic and hydrophilic domains [20,57,65]. The incorporation of ingredients with higher water holding and swelling capacity may improve the viscosity and texture quality [53] of a great variety of foods, especially meat products; hydration properties are also important regarding the consistency of the final product in baking applications [20]. On the other hand, oil absorption properties are important to prevent fat loss during cooking and to enhance food flavour [20,53].

3.3. Antioxidant Properties

Total phenols, total flavonoids and the antioxidant capacity (measured by the DPPH and ABTS methods) of the powders and raw materials are shown in Table 5.

Table 5. Results of antioxidant properties of the raw and powdered residues of white cabbage (WC) and broccoli (B). HAD: hot-air drying at 50, 60 and 70 °C, FD: freeze drying; C: chopped; G: ground. Mean ± standard deviation.

Sample	Total phenols (mg GAE/g_{dm})	Total flavonoids (mg QE/g_{dm})	DPPH (mg TE/g_{dm})	ABTS (mg TE/g_{dm})
WC_G_HAD50	3.3 ± 0.2^{a}	2.5 ± 0.7^{a}	1.5 ± 0.3^{ab}	49 ± 3^{b}
WC_C_HAD50	3.7 ± 0.8^{a}	4.0 ± 1.0^{cd}	3.0 ± 0.6^{d}	51 ± 10^{b}
WC_G_HAD60	3.3 ± 0.5^{a}	2.6 ± 0.7^{ab}	1.3 ± 0.3^{a}	49 ± 6^{b}
WC_C_HAD60	4.1 ± 1.1^{ab}	3.7 ± 0.6^{bc}	2.17 ± 0.14^{c}	59.6 ± 1.2^{b}
WC_G_HAD70	4.8 ± 0.9^{bc}	4.1 ± 1.2^{cd}	1.4 ± 0.7^{a}	48 ± 6^{b}
WC_C_HAD70	5.4 ± 0.4^{c}	4.9 ± 0.6^{d}	2.048 ± 0.016^{abc}	58 ± 15^{b}
WC_G_FD	4.0 ± 1.2^{ab}	1.8 ± 0.4^{a}	1.85 ± 0.14^{abc}	25 ± 6^{a}
WC raw	3.9 ± 0.4^{a}	1.8 ± 0.3^{a}	2.08 ± 0.17^{bc}	30 ± 2^{a}
B_G_HAD50	3.5 ± 0.6^{a}	2.2 ± 0.4^{a}	2.60 ± 1.05^{ab}	69 ± 4^{b}
B_C_HAD50	4.8 ± 0.5^{b}	3.1 ± 0.2^{b}	3.1 ± 0.4^{bcd}	83 ± 11^{c}
B_G_HAD60	3.9 ± 0.6^{ab}	2.6 ± 0.4^{ab}	2.1 ± 0.3^{a}	88 ± 11^{cd}
B_C_HAD60	4.4 ± 0.5^{ab}	3.6 ± 0.8^{c}	2.7 ± 0.5^{abc}	98 ± 14^{de}
B_G_HAD70	6.7 ± 1.2^{c}	5.3 ± 0.4^{e}	2.4 ± 0.4^{ab}	90 ± 10^{cd}
B_C_HAD70	8 ± 2^{d}	8.1 ± 0.4^{f}	3.40 ± 0.17^{cd}	108 ± 8^{e}
B_G_FD	4.3 ± 1.2^{ab}	5.50 ± 0.03^{e}	2.9 ± 0.9^{bcd}	50 ± 5^{a}
B raw	5.0 ± 1.0^{b}	4.6 ± 0.7^{d}	3.7 ± 0.3^{d}	34.0 ± 1.6^{a}

$^{a,b,c\ldots}$ Different superscript letters in the same column for the same residue indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Broccoli stems were richer in total phenols and flavonoids than cabbage outer leaves, in agreement with Jaiswal et al. [68]. Results evidenced a different impact of the dehydration technique used on the phenolic and flavonoid content of powders. On the one hand, FD generally maintained the levels of phenolic and flavonoid compounds that were naturally present in the raw sample, suggesting this is a good technique to preserve the original characteristics of the product. However, results were different when the residues were transformed into powders by means of HAD. In general, but

more markedly in the case of broccoli stems, drying at 50 and 60 °C reduced the total phenolic content by 4–27%, while drying at 70 °C implied an increase by 23–60%. In fact, the use of high temperatures during drying has been reported to increase the formation of new phenolic substances and contribute to the formation of compounds with high antioxidant activity as a result of Maillard reactions [64,69]. On the other hand, the shorter time needed to complete drying when higher temperatures are used might also have reduced the phenolics' degradation.

White cabbage powders exhibited a total flavonoid content 1.4 to 2.7 higher than the fresh residue. In the case of broccoli stems, drying at 50 and 60 °C reduced the total flavonoid content by 33–52% and 22–44%, respectively, while drying at 70 °C increased it by 15–76%. Multifactorial ANOVA revealed statistically significant differences for the dehydration factor (p-value < 0.05), pointing to HAD70 as the best drying treatment for obtaining powders with the highest content of both total phenols and flavonoids. Regarding disruption pretreatments, chopping generally led to higher phenol and flavonoid content than grinding. This fact could be related to a lower cell tissue damage in the case of chopped samples, so that phenols and flavonoids remain trapped by the structure during drying, thus remaining less susceptible to oxidation during this stage of processing [24].

As for the antioxidant capacity, HAD resulted in powders with a noticeably higher ability to scavenge ABTS free radicals and a slightly higher ability to scavenge DPPH radicals compared to FD. This has previously been reported in similar [29] and other powdered products, from pumpkin [64] or lemon pomace [70], for example. The drying temperatures used in the present study may have promoted the formation of Maillard reaction products, which increases the antioxidant activity [71,72]. As reported by Bernaert et al. [69] processing may result in either a depletion or an increase in antioxidant properties in foods. Thermal treatment can also reduce the action of prooxidant elements, thus contributing to better antioxidant properties. In contrast, the loss of antioxidants due to long processing exposure or the formation of compounds with prooxidant action may lower the antioxidant capacity during processing. Powders from chopped broccoli stems dried with air at 70 °C exhibited the highest antioxidant capacity; in

contrast, no statistically significant differences were obtained among temperatures when air drying cabbage leaves. These differences depending on the type of residue could indicate that the antioxidant compounds present in white cabbage were more thermolabile than those present in broccoli stems, or also a reduced generation of new antioxidant compounds during processing. Again, FD kept the antioxidant capacity virtually unchanged, which may be attributed to milder processing conditions, including less exposure to oxygen [51]. Regarding the intensity of tissue disruption prior to drying, chopped samples exhibited higher antioxidant capacity than ground ones. As mentioned before, a higher disruption intensity could imply a loss of antioxidant compounds because of higher tissue de-compartmentation and a larger surface area in contact with drying air, thus boosting oxidative reactions.

3.4. Glucosinolate and Isothiocyanate Content

As described in the Materials and Methods section, the protocol published by Campas-Baypoli et al. [41] includes a hydrolysis step for the conversion of glucoraphanin to sulforaphane, which allows the indirect quantification of glucoraphanin. In this work, however, in order to distinguish between the sulforaphane produced by the transformation process of the residue into powder and that resulting from the hydrolysis conversion included in the experimental protocol, sulforaphane content was analysed in the powders and the raw samples both with and without the hydrolysis step. The results of this analysis are presented in Figure S1 as supplementary material, where it is observed that the sulforaphane content of hydrolysed samples (H) was slightly higher, but not significantly, than non-hydrolysed ones (NH) (Figure S1). Differences between H and NH samples were not statistically significant for any of the treatments applied (p-value ≥ 0.05), indicating that the hydrolysis stage was not necessary to evaluate the effect of processing or to assess which powder presents better characteristics. Therefore, the values presented in Table 6 correspond to powders, and raw residues, obtained without the hydrolysis step.

Table 6. Sulforaphane content (μg/g_{dm}) of powders and raw samples. WC: white cabbage, B: broccoli; G: ground, C: chopped; HAD: hot-air drying at 50, 60 and 70 °C, FD: freeze drying. Mean ± standard deviation.

Sample	White cabbage (WC)	Broccoli (B)
G_HAD50	68.7 ± 0.5^{ab}	470.2 ± 1.1^{a}
C_HAD50	67 ± 9^{a}	479 ± 3^{a}
G_HAD60	71 ± 6^{ab}	476 ± 10^{a}
C_HAD60	70 ± 6^{ab}	472 ± 16^{a}
G_HAD70	69 ± 8^{ab}	493± 52^{a}
C_HAD70	73 ± 3^{ab}	461± 9^{a}
FD	77 ± 4^{ab}	506 ± 11^{a}
Raw	105 ± 1^{c}	787 ± 5^{b}

$^{a,b,c...}$ Different superscript letters for a similar residue indicate statistically significant differences at the 95% confidence level (*p*-value < 0.05).

The sulforaphane content of broccoli stems was about seven times higher than the values obtained for cabbage outer leaves. Values were in the range of those obtained by Thomas et al. [19] for broccoli stems (591 μg/g_{dm}), slightly higher than those reported by Campas-Baypoli et al. [41] (240 μg/g_{dm}) and lower than those given by Domínguez-Perles et al. [73] (around 1800 μg/g_{dm}) for fresh broccoli stems. Regarding cabbage, the results were in the range of those reported by Lekcharoenkul et al. [74] for fresh and powdered white cabbage outer leaves (60–70 μg/g_{dm}). Discrepancies may be due to differences in the extraction and detection methods, as well as differences in the variety of the raw material used or even the sample origin.

In all cases, processing implied a degradation of sulforaphane since the best results were obtained for fresh tissues. On the one hand, drying conditions (temperature, time) could have inhibited the myrosinase action, as described in Vargas et al. [52]. The myrosinase enzyme has an optimal activity at 60 °C but may lose its activity when exposed to higher temperatures or long treatments [74,75]. On the other hand, sulforaphane is a thermosensitive molecule and exposure to temperatures around 60 °C or higher for long periods may adversely affect its stability [8,76]. In this sense, Tanongkankit et al. [75] demonstrated in a previous study the effect of hot-air drying in gradients from 40 to 70 °C on the evolution of sulforaphane, and it was evidenced that sulforaphane is formed at an early

stage of drying, but when the temperature increases at the end of the drying process (more than 60 °C), it is degraded. Consequently, only a fraction of sulforaphane remains in the final powder.

Multifactorial ANOVA analysis of the results revealed significant differences (p-value < 0.05) regarding the dehydration technique applied. For both residues, sulforaphane was better preserved by FD than HAD. It should be noted that FD is carried out under less aggressive conditions for the vegetal material regarding the temperature and low oxygen conditions, which would result in a greater sulforaphane stability during processing. Among HAD powders, the drying temperature or intensity of previous disruption did not have a statistically significant impact on the amount of sulforaphane contained in the powders. As compared to the raw sample, sulforaphane was better preserved in white cabbage powders (64–73%) than in broccoli ones (60–64%).

4. Conclusions

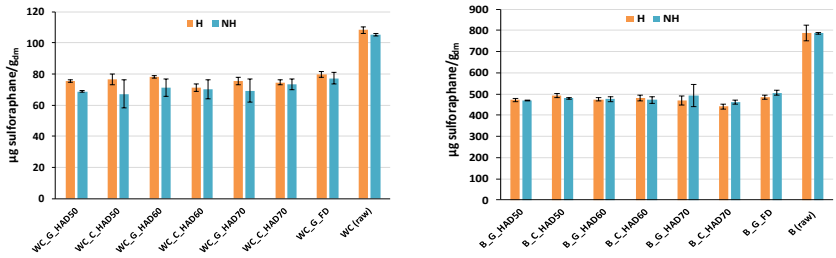
This research made it possible to obtain powdered products from cabbage outer leaves and broccoli stems, and vegetable residues generated during the early stages of processing for fresh and fourth range product commercialization. The results obtained demonstrated the impact of processing on the powders' properties. Both the dehydration technique and conditions applied, as well as the intensity of the disruption pretreatment, were evidenced to have a significant impact on the relevant properties of powders.

On the one hand, freeze drying was revealed as a good technique to preserve the antioxidant properties of raw products; in contrast, hot-air drying promoted the antioxidant properties due to the generation of new compounds with increased antioxidant activity, especially at high temperatures. The relevance of disruption prior to drying was also demonstrated in properties such as the drying rate, particle size or antioxidant properties. In fact, the interdependence of pretreatment intensity and drying, and its impact on particle size was evidenced, particularly for broccoli stems. In general, chopping facilitates subsequent drying and contributes to a product with a smaller particle size and better

antioxidant characteristics than previously ground samples. Other properties, such as water and oil interaction properties mostly depend on the drying technique applied. Regarding potential applications as food ingredients, brassica powders exhibited better hydration than emulsifying properties. Isothiocyanates were identified in all the powders obtained, with broccoli powders being richer than cabbage ones. When transformed into powders, most glucoraphanin was transformed into sulforaphane; nevertheless, processing also had a negative impact on sulforaphane content, mainly attributed to the drying step.

In general terms, it is concluded that white cabbage and broccoli residues produced at the early stages of processing can be successfully transformed into powdered products with interesting properties to be used as functional food ingredients. The reintroduction of these residual organic matter products into the food chain would effectively contribute to the development of more sustainable food systems and is a step towards more sustainable and healthier diets.

Supplementary Materials



(a) (b)

Figure S1: Sulforaphane content (µg/g$_{dm}$) in cabbage (a) and broccoli (b) samples. H: with hydrolysis; NH: without hydrolysis. WC: white cabbage; B: broccoli; G: ground; C: chopped; HAD: hot-air drying; FD: freeze drying.

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ANNEX: *In vitro* digestion effect on antioxidant properties of selected powdered ingredients obtained from brassica vegetable by-products

In vitro digestion was carried out to evaluate its effect on the total phenol content, total flavonoid content and antioxidant activity (DPPH and ABTS methods) of selected cabbage and broccoli powders: freeze-dried powders, which obtained maintained properties more similar to the raw material; and by hot-air dried powders at 70 °C with a previous chopping step, due to their increased antioxidant properties.

The *in vitro* simulation of gastrointestinal digestion (oral, gastric and intestinal phases) was performed by following the standardized INFOGEST method proposed by Minekus et al. (2014). Simulated digestive fluids, i.e., Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF), were reproduced. To simulate the oral phase, samples were mixed with SSF in a 1:1 ratio (w/v) and vortexed (Reax top, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) for 2 min at 37 °C. For the gastric phase, the oral bolus was mixed with SGF in a 1:1 (v/v) ratio and stirred during 2 h at 55 rpm (Intell-Mixer RM-2, Elmi Ltd., Riga, Latvia) and at 37 °C (JP Selecta SA, Barcelona, Spain). For the intestinal phase, the chyme was mixed with the SIF in a 1:1 (v/v) ratio and stirred for 2 h more at 55 rpm (Intell-Mixer RM-2, Elmi Ltd., Riga, Latvia) and 37 °C (JP Selecta SA, Barcelona, Spain).

Sampling for subsequent analysis of total phenols, total flavonoids and antioxidant activity was accomplished at the end of both the gastric and the intestinal phases. Antioxidant properties were analyzed on the soluble and insoluble fractions of the digested samples separated by centrifugation at 10,000 rpm for 5 min (Eppendorf® Centrifuge 5804R, Hamburg, Germany). The bioaccessibility index (BI) and recovery index (RI) were calculated according to equations 1 and 2, respectively. BI is defined as the percentage of compounds which remain solubilized in the chyme after the intestinal phase of digestion, which refers to the proportion of bioactive compounds that could become available for absorption by the intestinal cells. RI is defined as the percentage of compounds present in the total fraction of the

digested sample after the gastric or the intestinal phase of digestion (Ortega et al., 2011).

$$\mathrm{BI}(\%) = \frac{\mathrm{A}}{\mathrm{B}} \cdot 100 \quad (1)$$

$$\mathrm{RI}(\%) = \frac{\mathrm{C}}{\mathrm{B}} \cdot 100 \quad (2)$$

where:

A is the amount (μg) of the compound of interest in the soluble fraction after the intestinal phase of digestion; B is the amount (μg) of the compound of interest in the un-digested powder; and C is the amount (μg) of the compound of interest in the total digest after the gastric or the intestinal phase.

Antioxidant properties were measured on sample extracts obtained from 1 g of non-digested powder or digested precipitate mixed with 10 mL of an 80% (v/v) methanol/water solution. Extraction was performed in dark conditions using a horizontal stirrer (Magna Equipments S. L., model ANC10, Barcelona, Spain) during 1 h (Bas-Bellver et al., 2020a). After that, the mixture was centrifuged at 10,000 rpm for 5 min in an Eppendorf centrifuge (Eppendorf SE, model 5804/5804R, Hamburg, Germany). Measurements were also carried out on the supernatants separated after digestion, with no solvent addition. When necessary, extracts were diluted with an 80% (v/v) methanol/water solution as solvent in a ratio 1:10 (v/v). Bidistilled water replacing the extract was used as a blank.

Results regarding phenols, flavonoids and antioxidant capacity of white cabbage (WC) and broccoli (B) powders, obtained by hot-air drying at 70 °C and prior chopping of the sample (C_HAD70) or by freeze-drying (FD), to the simulated *in vitro* gastrointestinal digestion is summarized in Table 1. All analyses were measured before digestion (BD) and after the gastric (GP) and intestinal phases, both in the supernatant (S) and the precipitate (P).

In line with the previous study (Bas-Bellver et al., 2022), broccoli powders exhibited higher values than cabbage ones before digestion. On the other hand, the dehydration technique used had a statistically significant

effect on antioxidant properties, being higher at 70 °C. As indicated before, this could be the result of new compounds formation, such as Maillard reaction compounds, the inhibition of enzymes which could act as pro-oxidant substances, and a reduced exposure to drying conditions due to shorter treatments (Que et al., 2008; Bernaert et al., 2013; Bas-Bellver et al., 2022).

In vitro digestion had a positive effect on antioxidant properties, as deduced from the values presented in Table 1. A similar trend had been reported in other studies, where the increase of antioxidant compounds after digestion process, comparing with values before digestion, was attributed to structural transformations of polyphenol compounds after the intestinal phase, as well as, to an enhanced extractability and release of matrix-bound antioxidant compounds during digestion process (Chen et al., 2014; Luzardo-Ocampo et al., 2017; Lee et al., 2022; Bas-Bellver et al., 2023).

As for phenolic and flavonoid constituents, similar values were obtained after the gastric and intestinal phases, indicating that values were mostly maintained throughout digestion, with slight positive or negative differences. Bouayed et al. (2011) reported that the release of total phenolics and total flavonoids is mainly accomplished in the gastric phase and its possible increase after duodenal digestion could be due to the additional time of extraction and the effect of intestinal digestive enzymes that facilitate the release of these compounds bound to the matrix. In contrast, in Gunathilake et al. (2018) these compounds decreased after the intestinal phase compared to the gastric on, for several leafy vegetables. This degradation of some phenols and flavonoids could be due to the transition from the acidic gastric conditions to the alkaline intestinal conditions because of the effect of pancreatin and bile acids (Bouayed et al., 2011; Gunathilake et al., 2018).

On the contrary, antioxidant activity after the intestinal phase, for both DPPH and ABTS antioxidant assays, increased significantly as compared to the gastric one. According to literature, radical scavenging activity is strongly pH dependent and at higher pH values might be significantly increased, which is attributed to the hydroxyl moieties deprotonation present on the

aromatic rings of the polyphenolic constituents (Tagliazucchi et al., 2010; Kamiloglu et al., 2014; Gunathilake et al., 2018). In these sense, the small intestine conditions, i.e. alkaline pH and pancreatin, would promote the solubilisation of certain polyphenolic constituents that would had been previously linked to macromolecules or present in a reduced form (Martínez-Las Heras et al., 2017).

In this study, centrifugation has been used to separate the soluble compounds that could be absorbed (supernatant) and the non-soluble or unabsorbed compounds (precipitate). The antioxidant components present in the precipitate after the intestinal phase increased in all cases. However, in the supernatant, antioxidant compounds increased in FD powders but decreased in HAD powders, suggesting an increase or reduction, respectively, of antioxidative compounds solubilisation. The increase in FD powders could be explained because of the porous structure generated during freeze-dying which allows lower particle size, resulting in greater contact surface between the sample and the intestinal fluid, thus facilitating extraction and solubilisation of antioxidant compounds. In both gastric and intestinal stages, the amount of antioxidants collected in the precipitate were significantly higher than in the supernatant, suggesting that most of them remained retained in the structural matrix, albeit a liquid phase is also retained on the pellet being in this case more accessible compounds that the bound ones (Bas-Bellver et al., 2020b; Bas-Bellver et al., 2023). As reported in the literature, antioxidant compounds that are solubilized and liberated to the liquid phase (supernatant) are more available, even though compounds retained in the precipitate could also be continuously and slowly released and then metabolized in the long intestine digestion by the action of colonic microorganisms (Vitaglione et al., 2008; Luzardo-Ocampo et al., 2017; Dong et al., 2021).

Calculated Recovery Indexes (RI) were very high in all cases, with values generally higher in white cabbage than in broccoli powders, although no clear trend was observed regarding the dehydration method used. In most cases, RI obtained after the intestinal phase was higher than after gastric one, reflecting the progressive increase in the extractability of the antioxidant compounds due to the simulated *in vitro* digestion process. As

for the Bioaccessibility Indexes, values obtained were very high indicating a great proportion of antioxidant compounds that could become available for absorption into the systematic circulation. Again, generally higher values were obtained in white cabbage as compared to broccoli powders, and no clear trend was observed with respect to the drying method used.

Table 1. Total phenol content, total flavonoid content and antioxidant capacity (DPPH and ABTS) of white cabbage (WC) and broccoli (B) powders before digestion (BD), after the gastric phase (GP) in the precipitate (P) and supernatant (S), and after the intestinal phase (IP) in the precipitate (P) and supernatant (S) during *in vitro* digestion. Results are given per gram of non-digested sample. Recovery Index (RI) in percentage of the different powders after the gastric and intestinal phase, and Bioaccesibility Index (BI) in percentage. C: chopping; HAD70: hot-air drying at 70 °C, FD: freeze-drying. Mean ± standard deviation of three measurements. $^{a,b,c \text{ or } A,B,C}$ Different superscript letters in the same column for the same residue or for the same property analysed indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

	Sample	BD	Gastric Phase (GP)				Intestinal Phase (IP)				%BI
			S	P	TOTAL	%RI	S	P	TOTAL	%RI	
Total phenol content (mg GAE/g)	**WC_C_HAD70**	4.71 ± 0.04^{bC}	9.65 ± 0.19^{bB}	14.9 ± 0.3^{bB}	24.58 ± 0.18^{bB}	521 ± 4^{bD}	4.25 ± 0.11^{aA}	21.7 ± 0.5^{bD}	25.9 ± 0.5^{bD}	550 ± 10^{bD}	300 ± 8^{aB}
	WC_FD	3.32 ± 0.10^{aA}	5.4 ± 0.3^{aA}	9.1 ± 0.3^{aA}	14.5 ± 0.6^{aA}	437 ± 17^{aB}	6.9 ± 0.8^{bC}	8.93 ± 0.12^{aA}	15.8 ± 0.9^{aA}	476 ± 26^{aC}	338 ± 39^{aB}
	B_C_HAD70	5.73 ± 0.15^{bD}	12.10 ± 0.08^{bC}	15.2 ± 0.9^{bB}	27.3 ± 0.9^{bC}	476 ± 16^{bC}	5.48 ± 0.04^{aB}	17.5 ± 0.2^{bC}	22.99 ± 0.16^{bC}	401 ± 2^{aA}	244.4 ± 1.7^{aA}
	B_FD	4.38 ± 0.13^{aB}	5.8 ± 0.2^{aA}	8.7 ± 0.3^{aA}	14.5 ± 0.4^{aA}	330 ± 8^{aA}	6.1 ± 0.4^{aBC}	13.3 ± 0.7^{aB}	19.4 ± 0.3^{aB}	444 ± 8^{bB}	322 ± 22^{bB}
Total flavonoid content (mg EQ/g)	**WC_C_HAD70**	5.43 ± 0.06^{bB}	4.036 ± 0.011^{bC}	8.62 ± 0.08^{bC}	12.65 ± 0.10^{bC}	232.9 ± 1.8^{aB}	2.042 ± 0.005^{aB}	13.02 ± 0.12^{bC}	15.06 ± 0.12^{bD}	277 ± 2^{bD}	125.3 ± 0.3^{aB}
	WC_FD	2.29 ± 0.05^{aA}	2.87 ± 0.05^{aB}	3.68 ± 0.05^{aB}	6.55 ± 0.02^{aB}	285.7 ± 0.9^{bC}	3.06 ± 0.03^{bD}	3.12 ± 0.04^{aA}	6.18 ± 0.03^{aB}	269.6 ± 1.3^{aC}	219 ± 2^{bD}
	B_C_HAD70	7.48 ± 0.07^{bC}	4.210 ± 0.004^{bD}	10.90 ± 0.09^{bD}	15.11 ± 0.09^{bD}	202.01 ± 1.17^{aA}	2.930 ± 0.007^{bC}	10.45 ± 0.16^{bB}	13.38 ± 0.15^{bC}	179 ± 2^{aA}	100 ± 2^{aA}
	B_FD	2.33 ± 0.09^{aA}	1.665 ± 0.006^{aA}	3.05±0.07^{aA}	4.71±0.07^{aA}	202 ± 3^{aA}	1.319 ± 0.006^{aA}	3.16 ± 0.04^{aA}	4.48 ± 0.04^{aA}	192.3 ± 1.8^{bB}	130.4 ± 0.6^{bC}
DPPH (mg TE/g)	**WC_C_HAD70**	6.7 ± 1.2^{aA}	3.87 ± 0.07^{bC}	32 ± 2^{bC}	36 ± 2^{bB}	541 ± 37^{aB}	2.50 ± 0.09^{aB}	62.7 ± 0.4^{bD}	64.2 ± 1.7^{bD}	961 ± 25^{bB}	125 ± 4^{bD}
	WC_FD	5.9 ± 0.3^{aA}	1.61 ± 0.04^{aB}	27.4 ± 0.6^{aAB}	29.04 ± 0.65^{aA}	489 ± 11^{aB}	2.70 ± 0.08^{bC}	27.9 ± 0.3^{aA}	30.6 ± 0.4^{aA}	515 ± 6^{aA}	74 ± 2^{aB}
	B_C_HAD70	9.3 ± 1.4^{aB}	4.77 ± 0.06^{bD}	31 ± 2^{bBC}	36 ± 2^{bB}	385 ± 24^{aA}	3.4 ± 0.2^{bD}	47 ± 2^{bC}	50 ± 2^{bC}	538 ± 20^{aA}	94 ± 4^{bC}
	B_FD	7.1 ± 1.4^{aA}	0.86 ± 0.16^{aA}	25 ± 3^{aA}	25 ± 3^{aA}	359 ± 47^{aA}	1.47 ± 0.04^{aA}	36.1 ± 0.5^{aB}	37.5 ± 0.4^{aB}	528 ± 6^{aA}	47.6 ± 1.5^{aA}
ABTS (mg TE/g)	**WC_C_HAD70**	56 ± 4^{bC}	49.1 ± 1.8^{bC}	329 ± 33^{bB}	378 ± 34^{bB}	673 ± 61^{aB}	28.4 ± 0.8^{aA}	555 ± 39^{bB}	584 ± 39^{bC}	1040 ± 69^{aC}	169 ± 5^{aB}
	WC_FD	36 ± 2^{aA}	29.6 ± 1.6^{aB}	244 ± 6^{aA}	273 ± 8^{aA}	768 ± 22^{aC}	44.6 ± 1.3^{bC}	295 ± 5^{aA}	340 ± 6^{aA}	954 ± 16^{aB}	204 ± 6^{bC}
	B_C_HAD70	94.71 ± 1.03^{bD}	51.2 ± 1.8^{bC}	414 ± 32^{bC}	465 ± 33^{bC}	491 ± 35^{aA}	38 ± 2^{aB}	550 ± 40^{bB}	588 ± 42^{bC}	621 ± 44^{aA}	103 ± 5^{aA}
	B_FD	45.9 ± 1.8^{aB}	20.8 ± 0.8^{aA}	270 ± 6^{aA}	291 ± 7^{aA}	635 ± 15^{bB}	39.7 ± 0.8^{aB}	474 ± 4^{aB}	512 ± 4^{aB}	1116 ± 9^{bC}	200 ± 4^{bC}

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CONCLUSIÓN

Las propiedades beneficiosas para la salud derivadas de los compuestos bioactivos estudiados sugieren que los productos en polvo obtenidos a partir de residuos de zanahoria, col y brócoli puedan ser utilizados como ingredientes funcionales en la formulación de alimentos.

De nuevo, los estudios llevados a cabo en este capítulo evidenciaron que las técnicas y variables de proces empleadas definierons propiedades de los productos en polvo obtenidos. Mientras la liofilización permitió la obtención de polvos más finos y con mayor contenido en carotenoides y sulforafano, el secado por aire caliente mejoró las propiedades antioxidantes debido a la generación de nuevos compuestos antioxidantes, o a la inactivación de compuestos con capacidad pro-oxidante.

El estudio de la digestión simulada *in vitro*, tanto en los polvos de residuos de zanahoria como en los polvos de residuos de brassicas, reveló que la digestión favorece la liberación de compuestos bioactivos que estarían unidos a la matriz estructural del polvo. En el caso de los polvos de zanahoria, la respuesta a la digestión estuvo muy influida por las condiciones de procesado y por la co-ingestión con grasas, la cual aumentó los índices de recuperación. No obstante, sería necesario llevar a cabo futuras investigaciones con el fin de evaluar mejor el impacto de la co-ingestión de grasas en la bioaccesibilidad de los carotenoides.

Los resultados obtenidos sugieren que los residuos de brassicas y de zanahoria pueden transformarse en ingredientes en polvos estables. Además, debido a su contenido en compuestos bioactivos con propiedades beneficiosas para la salud humana, podrían proponerse como ingredientes funcionales para aumentar el valor nutricional de los alimentos, según se propone en el siguiente capítulo. Por otro lado, en el caso de los polvos de brassicas y en el contexto del proyecto FUNBIOPEST, se colaboró con el Instituto Agroforestal Mediterráneo (IAM-UPV) en la evaluación del efecto de los polvos de brassicas, ricos en isotiocianatos, en la inhibición del del desarrollo de plantas arvenses. Los productos en polvo seleccionados se aplicaron en condiciones controladas en invernadero y campo mediante

diferentes métodos de incorporación (mezcla con suelo y *mulching*), obteniéndose resultados prometedores de estos productos en polvo, como recurso natural para el control integrado de plantas arvenses.

CAPÍTULO 4. Estudio de la aplicación de los polvos obtenidos a partir de residuos de zanahoria y col blanca como ingredientes funcionales en la formulación de alimentos horneados (magdalenas y rosquilletas)

Bas-Bellver, C., Harasym, J., Barrera, C., Betoret, N., Seguí, L. Quality and antioxidant attributes of gluten-free bakery products formulated with vegetables waste flours partially replacing rice flour. *Under preparation*.

RESUMEN DEL CAPÍTULO

Los estudios llevados a cabo anteriormente permitieron seleccionar aquellos productos en polvo con características adecuadas para ser propuestos como ingredientes funcionales. Así pues, se escogieron los ingredientes en polvo obtenidos a partir de zanahoria y col blanca, obtenidos mediante secado por aire caliente a 70 °C, para desarrollar nuevos productos de panadería sin gluten (magdalenas y rosquilletas).

Para la elaboración de los productos de panadería se incorporó polvo de zanahoria a la formulación de magdalenas y polvo de col blanca a la formulación de rosquilletas, en diferentes porcentajes de sustitución de harina de arroz (5, 10, 20 y 30%). El efecto del nivel de sustitución se evaluó en primer lugar sobre las propiedades fisicoquímicas, tecnológicas y antioxidantes de las mezclas de harinas. La capacidad de interacción con agua y con aceite aumentó con la adición de los polvos de residuos, y todos los casos presentaron un predominante carácter hidrófilo. Las propiedades de *pasting* disminuyeron significativamente con niveles más altos de polvos de residuos, mientras que una mayor incorporación de polvos aumentó las propiedades antioxidantes de la harina.

Una vez elaboradas las distintas formulaciones de magdalenas y rosquilletas, la calidad de los productos se evaluó midiendo la reología de la masa cruda y las propiedades fisicoquímicas (actividad del agua (a_w), color, textura y antioxidantes) del producto final tras el horneado y tras una semana de almacenamiento en condiciones controladas. Según los resultados obtenidos, el grado de reemplazo de la harina de arroz por harina vegetal influyó en las características del producto obtenido, tanto en la reología de la masa, como en el color del producto horneado, la textura y las propiedades antioxidantes. Se constató una mejora de las propiedades antioxidantes de los productos elaborados a mayor porcentaje de polvo de zanahoria o col incorporado al producto. Por otro lado, la dureza y la a_w de los productos se vio incrementada tras una semana de almacenamiento en refrigeración. También se observaron cambios en cuanto al color de los mismos. El almacenamiento afectó negativamente a las propiedades

antioxidantes, y se observó un incremento del contenido en azúcares reductores.

Con este trabajo, se consiguió concretar una aplicación de los productos en polvo obtenidos a partir de los residuos de hortalizas para la elaboración de nuevos productos de panadería sin gluten. Al tratarse de productos sin gluten y con propiedades antioxidantes mejoradas, estos productos tendrían un valor nutricional añadido al de otros productos equivalentes disponibles en el mercado, lo que contribuiría a poder confirmar el uso de los ingredientes en polvo obtenidos a partir de residuos de hortalizas como como ingrediente funcional.

Quality and antioxidant attributes of gluten-free bakery products formulated with vegetables waste flours partially replacing rice flour

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Under preparation

Abstract

Fruit and vegetable industrialization generates large amounts of organic residues which are rich in bioactive compounds and might be beneficial for human health. Transforming this waste material into functional powders or flours to be used as functional ingredients is an opportunity for new food products development, contributing to healthier and more sustainable food systems. In the present work, carrot (CP) and white cabbage (WCP) powders were used to partially replace rice flour (5%, 10%, 20% and 30%) in the formulation of gluten-free muffins and breadsticks, respectively. Impact of the percentage of replacement on physicochemical (moisture content, water and oil absorption), pasting and antioxidant properties (reducing sugars, total phenols and antioxidant capacity by DPPH) was evaluated in flour blends. Muffins and breadsticks quality was evaluated by measuring the batter rheology, and physicochemical (aw, colour, texture) and antioxidant properties on the final product just after baking and after one week of storage under controlled conditions. Water and oil absorption capacities increased with powder addition, whereas pasting properties worsen when adding CP and WCP. The incorporation of CP or WCP increased the flour antioxidant value. Dynamic oscillatory tests revealed a predominant elastic behaviour of rice flour and CP and WCP enriched doughs, generally G' and G'' values increased with a higher percentage of CP or WCP. Vegetables waste powder addition had a significant impact on colour attributes. On the other hand, CP and WCP implied a reduced hardness of muffins and breadsticks as compared to control formulations, which could be attributed to an increase in the soluble fibre content. Antioxidant properties were

improved with the addition of both vegetable waste flours, the best results being exhibited by the 30% replacement formulation. Storage had an impact on colour and hardness, which increased in all products after one week of storage, and on antioxidant properties, increasing reducing sugar content, but decreasing total phenol content and antioxidant capacity (DPPH). The present investigation reveals that carrot and cabbage waste flours can be used as functional food ingredients to increase the nutritional properties of new gluten-free bakery products.

Keywords: antioxidant, carrot powder, white cabbage powder, gluten-free, bakery products.

1. Introduction

Food industry annually generates 1.3 billion tons of food wastes and losses and reducing them is an important goal of the 2030 Agenda for Sustainable Development [1]. To minimize problems related to food loss and waste related environmental issues, much research has been focused on food by-product valorisation and alternatives of use [2]. Especially, fruit and vegetable wastes are a rich source of minerals, dietary fibre, antioxidants and bioactive compounds among others, with beneficial effects on human health [3,4]. More particularly, carrot and white cabbage residues contain numerous beneficial nutrients such as antioxidants and specific bioactive constituents such as carotenoids, in carrots, and glucosinolates and isothiocyanates, in cabbage [5,6]. Fruit and vegetable raw by-products are susceptible to microbial spoilage, so their transformation into powdered products results in stable and versatile products rich in bioactive components and with extended applicability in the food industry [6]. Thus, their use as functional ingredients or their incorporation in different food matrices is presented as an alternative for their practical valorisation.

Functional foods or beverages may be obtained by adding ingredients which impart them healthier characteristics, this is the case of antioxidants, fibres, omega-3 or probiotics. Functional ingredients may be added to different foods, including bakery, confectionery, drinks and other non-dairy and dairy food products [7]. Fruit and vegetable derived functional products are key for the functional food sector, due to their health benefits but also

to consumer naturalness perceptions [8]. Consumers are more attracted to natural foods and foods that have additional health benefits other than the usual nutritional properties. Bakery products, for example, muffins, bread, cakes, breadsticks, etc. are consumed worldwide, although they are principally wheat flour processed products [9,10]. It is well known that the presence of gluten in wheat flour causes allergic reactions in celiac patients, that have to consume gluten-free products all their lives to prevent this disease symptoms and alterations [8,11]. In this sense, there is a need for designing new gluten-free products with similar or improved nutritional and organoleptic properties than their gluten-containing counterparts, allowing patients with celiac disease and intolerances to proteins or others cereal components, to consume baking products without changing their dietary pattern [12].

Bakery products such as muffins, bread, biscuits, cakes, breadsticks, etc. are widely consumed worldwide, principally as wheat flour processed products [9,10]. Bakery products possess various attractive characteristics such as relatively long shelf life, high convenience and organoleptic quality [13]. Fortification of bakery products is an increasing current trend [14]. Fruit, vegetables and their by-products or discards have been used in the manufacturing of bakery products to improve their nutritional properties. It has been reported that including vegetable sources in bakery doughs may reduce the glycaemic index, and the incidence of non-transmissible diseases such as cancer, gastrointestinal disorders or coronary disease. On the other hand, it is well-known that gluten content in wheat-based breads cause allergies and intolerance reactions in celiac patients and sensitive individuals. This people have to consume gluten-free products during all their lives to prevent symptoms and alterations [8,11]. In this sense, there is a need for developing new gluten-free products with nutritional and organoleptic properties which are comparable to their gluten-containing counterparts, allowing patients with celiac disease and intolerances to proteins or other cereal components to consume baking products without changing their dietary patterns [12]

Rice is considered a good cereal source as wheat flour substitute for making gluten-free products, although rice flour is rather poor in bioactive

components. In this sense, the addition of fruit or vegetable based functional ingredients might improve the nutritive value of gluten-free baked products [7]. In fact, the development of baked products using fruit and vegetables discards and waste flours is gaining interest [15]; they are considered an economical source of functional components such as antioxidants, fibre or vitamins [11]. Several authors have reported the application of these by-product flours in the development of wheat-based flour cake, bread, muffins or breadsticks using potato peel powder [16], mango peel powder [17], kinnow peel powder [15,18], pomegranate peel powder [19] or grape pomace powders [10]. However, the application of fruit and vegetable residues in gluten-free bakery products is relatively new. Some studies have reported the development of cakes using apple, orange and carrot pomace powders [11]; muffins enriched with mushroom and carrot powders [8]; or the formulation of muffins using amaranth flour [20].

Hence, the aim of the present study was to develop gluten-free bakery products, i.e. muffins and breadsticks, by partially replacing rice flour with carrot waste and white cabbage waste flours; as well as to evaluate the impact of flour replacement on the quality and antioxidant attributes of the products obtained.

2. Material and methods

2.1. Raw materials and ingredients

White cabbage (*Brassica oleracea* var. *capitata*) and carrot (*Daucus carota*) wastes, consisting of outer leaves and discarded sticks of a IV range processing line, respectively, were processed to obtain cabbage waste (WCP) and carrot waste powders (CP) as explained elsewhere [21]. Briefly, the process consisted of chopping the sanitized raw material, disposing it in perforated trays and hot-air drying it at 70 °C, and further milling the dried material to obtain a fine powder. Other ingredients used, i.e. rice flour, eggs, sugar, salt, margarine, sunflower oil, and baking powder, were purchased in a local supermarket in Wroclaw (Poland).

2.2. Muffin preparation

Muffins were prepared following Olawuyi and Lee [8] protocol. Muffins dough was formulated using 200 g of flour, 4.8 g of baking powder, 150 g of margarine, 150 g of sugar and 187 g of whisked eggs. In this formulation, rice flour (RF) was partially replaced with carrot powder (CP) at 5% (CP 5%), 10% (CP 10%), 20% (CP 20%) and 30% (CP 30%) levels, while control muffin was elaborated using RF exclusively. After homogenously mixing all the ingredients, around 100 g of dough was scooped in muffin paper cups and baked in an oven (MPM model MPE-08/T, Milanówek, Poland) for 30 min at 180 °C.

2.3. Breadsticks preparation

Breadsticks were prepared by following Simsek and Süfer [22] protocol. For dough formulation, 100 g of flour, 2.67 g of sugar, 2.67 g of instant yeast, 0.67 g of salt, 1.33 mL of olive oil, and 40 mL of tap water were needed. Rice flour (RF) was replaced by white cabbage powder (WCP) at 5% (WCP 5%), 10% (WCP 10%), 20% (WCP 20%) and 30% (WCP 30%) levels, and the control was prepared using only RF. After hand-kneaded, the dough was cut into strips of 12 cm approximately and baked in an oven (MPM model MPE-08/T, Milanówek, Poland) for 20 min at 200 °C.

2.4. Characterization of rice flour and flour blend

2.4.1. Physicochemical properties

Moisture content of flour blends was obtained according to the Official Method AACC44-19 (AACC, 2000) Moisture-Air-Oven Method, by drying at 135 °C. **Water Absorption Capacity** (WAC) and **Oil Absorption Capacity** (OAC) of the flour mixtures were determined by the centrifugation method described by Abebe et al. [23] with some modifications. Briefly, 1 g of flour was mixed with 10 mL of distilled water or corn oil in centrifuge tubes. The dispersions were vortexed several times during 30 s at high speed (Heidolph Reax, Schwabach, Germany) and let resting for 10 min at room temperature. Mixtures were then centrifuged for 25 min at 3000 × g (Thermo Fisher Scientific, Waltham, USA). The supernatant was removed and weighed, and results were expressed as g of water or oil retained per g of flour.

Hydrophilic/Lipophylic Index (HLI), was calculated as the quotient between WAC and OAC.

2.4.2. Pasting properties of flour blends

Viscometric profile of flour blends was obtained according to Harasym et al. [24] by following ICC Standard method 162 and using Rapid Visco Analyser (RVA-4500, Perkin Elmer, USA). Basically, 3.5 g of sample were transferred to a RVA container with the amount of distilled water (as solvent) adjusted to the total weight of 28.5 g. Each flour suspension was equilibrated at 50 °C for 1 min, then the temperature raised to 95 °C at a rate of 5 °C/min, holding at 95°C for 5 min, then cooled to 50 °C at a rate of 5 °C/min and finally maintained at 50 °C for the last 4 min. Stirring speed was set at 960 rpm for the first 10 s and then maintained at 160 rpm for the rest of the analysis. Each sample was analysed in duplicate. The TCW3 software (Perkin Elmer, UK) was used to calculate the parameters peak viscosity (PV), trough viscosity (TV), breakdown (BD = PV-TV), final viscosity (FV) and setback (ST = FV-TV), pasting temperature and peak time.

2.5. Characterization of muffins and breadsticks batters and products

2.5.1. Rheological measurements

Dynamic oscillatory tests of the muffin and breadstick batters were performed with Anton Paar MC102 rheometer (Anton Paar., Stuttgart, Germany) using a parallel plate geometry (40 mm diameter) of serrated steel surface with 1 mm of working gap, and with a temperature set at 25 °C, controlled by a KNX2002 thermal controller. Dough samples were placed on the plate, removing excess, and the sample was left to equilibrate for 5 min before each test. Viscoelastic behavior was determined in terms of storage or elastic modulus (G') and loss or viscous modulus (G'') with a frequency sweep performed from 10 to 1 Hz in the linear viscoelastic region at a constant stress of 1 Pa. All rheological tests were performed in triplicate.

2.5.2. Evaluation of muffins and breadsticks quality: physicochemical characterisation

Water activity (a_w) of baked products was measured using an AquaLab 3TE analyzer (Decagon Devices, Inc., Pullman, WA, USA). **Weight** of the baked products was determined using an Axis AD1000 (Axis, Gdansk, Poland). **Volume** of the products was measured using a 3D Scanner v2 with Quickscan (MatterandForm, UK) analyser. **Colour** of breadsticks and muffins (crust and crumb) was measured with a Konica Minolta CR-310 chroma meter (Ramsey, NJ, USA) using a D65 standard illuminator and the 2° standard observer. Colour parameters were presented as CIEL*a*b* coordinates. Additionally, the total colour change after one week of storage (ΔE*) was calculated as shown in Equation 1.

$$\Delta E = \sqrt{\left(L_i^* - L_n^*\right)^2 + \left(a_i^* - a_n^*\right)^2 + \left(b_i^* - b_n^*\right)^2} \quad (1)$$

where L_i*, a_i*, and b_i* are the colour parameters of the initial product and L_n*, a_n*, and b_n* are those of the product sored for one week.

Texture of the products was evaluated in two different ways depending on the type of product. For muffins, crumb texture was determined in triplicate with an AXIS texture analyser (Axis, Gdansk, Poland) using the software "Texture Expert" and an aluminium 20 mm diameter cylindrical probe. A Texture Profile Analysis (TPA) double compression test was performed on 2 cm-thickness crumb slices, which were subjected to a 50% deformation at 1 mm/s speed test, with a 30 s delay between the first and second compression. Results of the maximum hardness (N) in the first compression were obtained. Breadsticks texture was evaluated by a flexure and breakage test. The product was placed on two supporting beams kept at 3 cm of distance. Another beam was descended (at 1 mm/s speed) and the peak force required to break the sample was registered as breaking hardness (N).

2.6. Antioxidant properties

Antioxidant properties were measured in flour blends, as well as in the different muffins and breadsticks formulations. Antioxidant compounds were extracted by mixing 0.5 g of sample (flours, muffin crumbs, muffin crusts and breadsticks) with 8 mL of water. The mixture was agitated in a laboratory orbital shaker (WU4, Premed, Marki, Poland) at room temperature for 2 h. After centrifugation at 3500 x g for 10 min (MPW-350,

MPW, Warszawa, Poland), the supernatant was collected. The extraction was repeated, and the total supernatant (extract) was used for antioxidants determination.

Total phenolic content was measured using the Folin–Ciocalteu method [24]. 20 µL of the extract were mixed with 1.58 mL of distilled water and 100 µL of Folin-Ciocalteu reagent. The mixture was incubated for 5-8 min at room temperature, and then 300 µL of a saturated solution of Na_2CO_3 was added. After incubation at 38 °C for 30 min (MLL147, AJLElectronics, Kraków, Poland), the absorbance was measured at 765 nm (UV-VisUltrospec 2000, Pharmacia Biotech, Piscataway, NJ, USA). The results were expressed as mg of Gallic Acid Equivalent (GAE) per g of sample.

Determination of **reducing sugars** was carried out by following the Dinitrosalicylic acid (DNS) method with some modifications [25,26]. For this purpose, 0.5 mL of the extract was mixed with 0.25 mL of DNS reagent (1% of 3,5-dinitrosalicylic acid solution (DNS) in 0.4 M NaOH). The test sample was incubated in a boiling water bath for 5 min, and then cooled to 50-60 °C. After that, 3 mL of distilled water were added. Absorbance was measured at 530 nm, and results given as mg of Glucose Equivalent (GE) per g of sample.

Antioxidant capacity of the samples was determined by **DPPH method** [27,28]. 34.5 µL of the extract were mixed with 1000 µL of a 0.1 mM prepared solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol with an absorbance of 0.9 ± 0.1. After 20 min, absorbance was measured at 517 nm. The antioxidant capacity was expressed as µmol of Trolox Equivalent (TE) per g of sample.

2.7. Storage stability study

Stability of baked products was evaluated after 7 days of storage at 4 °C into hermetic bags. Accordingly, water activity, colour, texture (hardness) and antioxidant properties were analysed. All analyses were evaluated at least in triplicate.

2.8. Statistical analysis

The results were taken at least in triplicate. The analysis of variance (ANOVA) of the results obtained was assessed with Statgraphics Centurion software (Centurion XVII.I version, StatPoint Technologies, Inc., Warrenton, VA, USA). ANOVA was performed with a previous normality of data checked and using a $p < 0.05$ significance level.

3. Results

3.1. Characterization of flour blends

Moisture content and technological properties, water absorption (WAC) and oil absorption capacity (OAC), as well as the hydrophilic/lipophilic index (HLI) of the flour blends analysed are summarized in Table 1. Significant differences were found between rice flour and CP/WCP enriched flours, rice flour showing a higher moisture content, since vegetables powders initially presented lower moisture content [21], which resulted in lower values when mixed with rice flour.

WAC indicates the ability of flour to associate with water, while OAC represents its ability to retain oil, which plays an important role in flavour retention of food products [29]. Statistically significant differences were obtained for WAC, OAC and HLI parameters. Generally, WAC and OAC values increased with rice flour replacement by vegetable waste powders and, consequently, HLI value too. WAC values varied from 2.43 to 3.56 mL/g for CP blends, and from 2.37 to 4 mL/g for WCP blends; in both cases, rice flour replacement with a 30% vegetable residue powder exhibited the highest values. OAC values also increased with vegetable waste powder addition, although not as markedly. An increase in WAC and OAC had been previously reported by Ahmad et al. [29] when replacing wheat flour by carrot pomace powder in cookies formulation, and was attributed to a higher amount of fibre content the higher the proportion of vegetables residue powder, due to an increased competition between the residue powder and the flour for the absorption of water and oil. Another study also reported WAC increase by replacing rice flour with amaranth flour, which was explained by the presence of polar amino acid groups that increased when the amaranth flour was more present [20]. Both CP and WCP based flours had better water

absorption than oil absorption properties, which indicates a major hydrophilic character.

Table 1. Moisture content (x_w), water absorption capacity (WAC), oil absorption capacity (OAC) and hydrophilic/lipophilic index (HLI) of flour blends. CP: carrot powder, WCP: white cabbage powder; 5, 10, 20, 30%: percentage of residue powder in the flour blend. Mean ± standard deviation of three independent measurements.

	x_w (g_w/100 g)	WAC (mL/g)	OAC (mL/g)	HLI
Rice flour	12.282 ± 0.002^{b}	2.25 ± 0.03^{a}	1.57 ± 0.03^{a}	1.43 ± 0.04^{a}
CP 5%	10.16 ± 0.03^{a}	2.43 ± 0.05^{b}	1.66 ± 0.02^{a}	1.47 ± 0.02^{a}
CP 10%	10.13 ± 0.08^{a}	2.62 ± 0.02^{c}	1.64 ± 0.03^{a}	1.60 ± 0.04^{b}
CP 20%	10.63 ± 0.02^{a}	3.07 ± 0.12^{d}	1.66 ± 0.05^{a}	1.85 ± 0.12^{c}
CP 30%	10.5 ± 0.4^{a}	3.56 ± 0.12^{e}	1.680 ± 0.010^{ab}	2.12 ± 0.06^{d}
WCP 5%	11.5 ± 1.7^{ab}	2.37 ± 0.03^{ab}	1.72 ± 0.05^{bc}	1.38 ± 0.03^{a}
WCP 10%	10.5 ± 0.2^{a}	2.64 ± 0.07^{c}	1.68 ± 0.03^{ab}	1.57 ± 0.05^{b}
WCP 20%	10.79 ± 0.06^{a}	3.20 ± 0.02^{d}	1.71 ± 0.03^{bc}	1.87 ± 0.04^{c}
WCP 30%	11.328 ± 0.002^{ab}	4.00 ± 0.16^{f}	1.744 ± 0.013^{bc}	2.29 ± 0.07^{e}

$^{a,b,c,\ldots}$Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Pasting properties of rice flour (control) and vegetable waste flour blends are given in Table 2. As for pasting curves, they are plotted in Figure 1A (CP blends) and Figure 1B (WCP blends). The aim of pasting analysis was to study the mixture viscosity and the behaviour when adding vegetable waste flours (CP, WCP), as well as the ability of the flour blends to form a consistent batter [8]. Generally, the incorporation of CP or WCP increased the viscosity profile as compared with rice flour (table 2, figure 1A and B). These results indicate a greater structural rigidity. As described in previous studies [30,31] a lower amylose content, as well as structural differences in the amylopectin molecules, may be related to higher peak viscosity due to an increased swelling potential when CP or WCP were added.

Textural parameters, i.e. peak, trough, setback and final viscosities values, significantly decreased when CP and WCP content in flours increased. Similar results were reported for other vegetable flour blends in which carrot, mushroom or kinnow peel residues powders [8,18,29] were used. This behaviour could be related to the higher water absorption (Table 1) and binding capacity due to an increased fibre content, consequently reducing

pasting properties [18]. The viscosity decrease with higher levels of CP/WCP could be considered positive, as excessive viscosity could lead to problems during dough handling and industrial manufacturing of the product (e.g., mould filling and machinery cleaning) [32].

The addition of CP and WCP affected the pasting temperature and peak time, parameters that provide information about the minimum time and temperature of flour cooking [33]. A higher pasting temperature indicates larger starch content, which makes flour more resistant to swelling and rupturing [29], as in the case of rice flour. The decrease in pasting temperature as the CP/WCP level increases indicates the decrease in the gelatinization temperature of flour blends [30].

Table 2. Pasting properties of rice flour and powder blends. CP: carrot powder, WCP: white cabbage powder; 5, 10, 20, 30%: percentage of residue powder in the flour blend. Mean ± standard deviation of three independent measurements.

	Peak visc (cP)	Trough visc (cP)	Breakdown (cP)	Final visc (cP)	Setback visc (cP)	Pasting Temp (°C)	Peak time (min)
Rice flour	2874±83^{a}	2413±49^{a}	461±133^{a}	5507±16^{b}	3094±66^{b}	90.55±0.07^{f}	5.97±0.05^{ab}
CP5%	4033±49^{d}	3563±5^{d}	470±54^{ab}	8378±132^{g}	4816±136^{f}	89.3±0.7^{ef}	6.4±0.4^{bc}
CP10%	3665±74^{c}	3130±232^{c}	535±158^{ab}	7160±35^{e}	4030±197^{d}	88.5±0.5^{de}	6.3±0.5^{abc}
CP20%	3308±21^{b}	2806±81^{b}	502±60^{ab}	5803±47^{c}	2997±127^{b}	86.8±0.6^{bc}	5.90±0.14^{ab}
CP30%	2782±84^{a}	2310±56^{a}	472±28^{ab}	4398±61^{a}	2089±5^{a}	85.58±0.04^{ab}	5.89±0.02^{ab}
WCP5%	4481±16^{e}	3849±70^{e}	632±54^{ab}	9112±109^{h}	5264±179^{g}	87.25±0.07^{cd}	6.72±0.03^{c}
WCP10%	4144±62^{d}	3452±88^{d}	642±45^{bc}	7842±244^{f}	4390±156^{e}	85.6±1.2^{ab}	6.3±0.5^{abc}
WCP20%	3768±71^{c}	2956±43^{bc}	813±28^{cd}	6479±144^{d}	3523±100^{c}	86.0±0.6^{bc}	5.74±0.09^{a}
WCP30%	3702±86^{c}	2825±79^{b}	877±6^{d}	5697±40^{bc}	2872±40^{b}	84.4±0.5^{a}	5.69±0.02^{a}

$^{a,b,c...}$Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value<0.05).

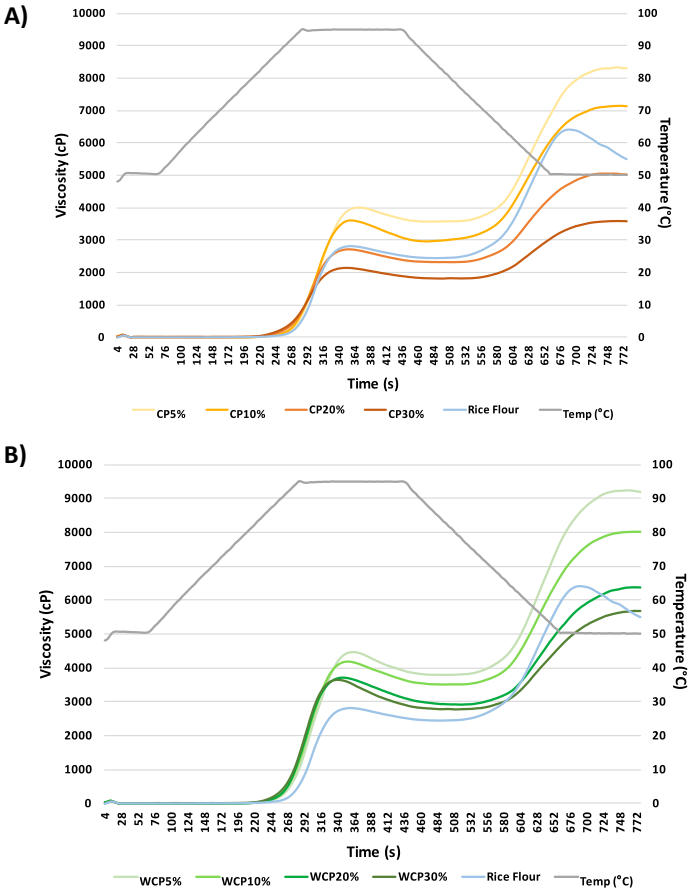

Figure 1. Pasting curves of rice flour (RF) and flours enriched with A) carrot powder (CP) or B) white cabbage powder (WCP). 5, 10, 20, 30% indicate the percentage of residue powder in the flour blend.

Antioxidant properties of rice flour and flour blends are presented in Table 3. The antioxidant potential of CP and WCP has been demonstrated in previous studies [5,6]. As expected, reducing sugars content, total phenols content and antioxidant activity (DPPH) values in both CP and WCP blends were higher than in rice flour. Among flour blends, results were slightly higher for WCP for the different antioxidant properties considered. This is consistent with previous results, where the phenolic content and antioxidant activities of WCP was significantly higher than that of CP [21]. This trend was also observed by Olawuyi and Lee [8] when adding carrot or mushroom

pomace powders to rice flour in different proportions. These results demonstrate that the addition of CP or WCP to rice flour increases the antioxidant and nutritional value of gluten-free mixtures, which could result in better quality and nutritional attributes of the resulting baked products.

Table 3. Antioxidant properties of the rice flour and the flours with partial replacement of rice flour by carrot waste powder (CP) or white cabbage waste powder (WCP). Mean ± standard deviation of three independent measurements.

	Reducing sugars (mg GE/g_{dm})	**Phenols (mg GAE/g_{dm})**	**DPPH (µmol TE/g_{dm})**
Rice flour	1.257 ± 0.019^{a}	0.32 ± 0.07^{a}	4.64 ± 0.05^{a}
CP 5%	8.195 ± 0.018^{b}	0.316 ± 0.011^{a}	4.6 ± 0.2^{a}
CP 10%	11.09 ± 0.05^{c}	0.40 ± 0.04^{ab}	4.54 ± 0.05^{a}
CP 20%	19.14 ± 0.10^{e}	0.42 ± 0.06^{b}	5.67 ± 0.05^{b}
CP 30%	21.82 ± 0.12^{f}	1.03 ± 0.05^{d}	6.25 ± 0.12^{c}
WCP 5%	11.19 ± 0.12^{c}	0.901 ± 0.011^{c}	6.06 ± 0.08^{c}
WCP 10%	17.60 ± 0.04^{d}	1.06 ± 0.02^{d}	7.69 ± 0.05^{d}
WCP 20%	22.8 ± 0.2^{g}	2.01 ± 0.02^{e}	9.30 ± 0.05^{e}
WCP 30%	24.3 ± 0.9^{h}	2.72 ± 0.06^{f}	11.5 ± 0.2^{f}

$^{a,b,c\ldots}$Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

3.2. Muffins characterization

Dynamic oscillatory test was used to study the viscoelastic behaviour of rice flour based and CP enriched doughs. The rheograms of all dough formulations are represented in Figure 2. Storage or elastic (G') and loss or viscous (G'') modulus increased slightly with an increase in frequency, both showing a frequency dependence. In all formulations, G' resulted in higher values than G'' (G' > G'') which means that the elastic component predominates over the viscous component. Likewise, the loss tangent (tan δ = G''/G') values were in all cases in the range of 0.4-0.6 (tan δ < 1) and slightly dependent on frequency. Hence, rheograms depict typical soft gels with more solid-like behaviour [29]. Similar results have been reported in other enriched rice-based batters [11,34].

The incorporation of CP significantly increased the G' and G'' values compared to control indicating a higher viscoelastic behaviour. This increase was observed to be proportional to the amount of CP added, except for 10%

CP, which showed values close to control. The increase in G' and G'' values may be due to the crosslinking degree of the polymer system. Polyphenols and fibre present in the residue powder could contribute to a more viscoelastic dough behaviour, since proteins can form complexes with polyphenols fortifying the batter networks, and fibre can compete for water absorption and serve as a filler for the viscoelastic matrix [35].

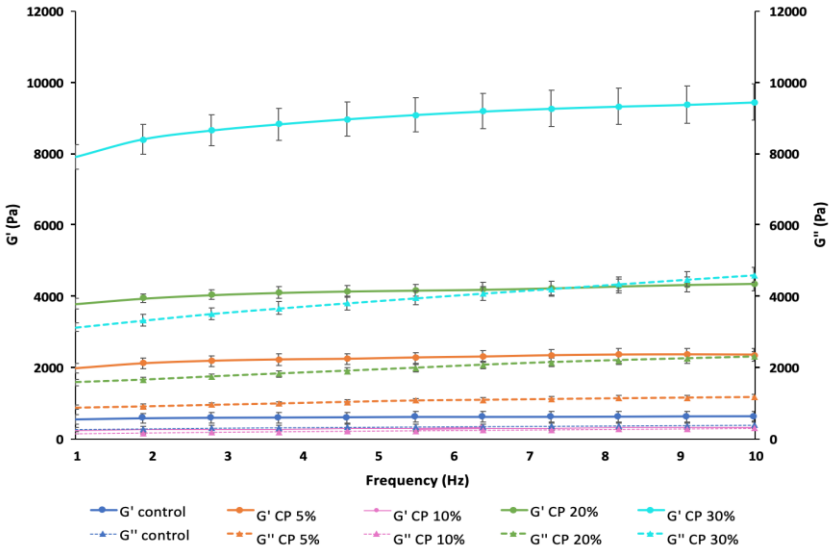

Figure 2. Rheograms of the gluten-free muffin batters obtained by partially replacing rice flour by carrot waste flour. G': storage modulus, G'': loss modulus. 5, 10, 20 and 30% indicate the percentage of carrot waste powder in the flour blend.

Table 4 shows water activity (a_w), weight and volume of muffins immediately after baking, as well as a_w values after one week of storage. The control muffin showed the highest a_w value, whereas M30 formulation exhibited the lowest one. Generally, a_w slightly increased after one week of storage. Weight and volume usually decreased when increasing CP in the formulation, M5 having the highest values and M30 de lowest ones. It has been previously reported that adding mushroom and carrot powder to muffins increases their weight due to their higher fiber content [8]; other studies on flour formulations of refined wheat flour and kinnow peel powder, suggested that higher fibre contents result in less moisture loss during baking, thus increasing muffins weight [15]. Other authors have also

reported a volume decrease when adding pomace powders in cakes [11] and muffins [8], due to the higher fibre content that increase water binding properties affecting muffin volume.

Table 4. Water activity (a_w) and water activity after one week of storage ($a_{w\ storage}$), weight and volume of gluten-free muffins (M) obtained by partially replacing rice flour by carrot waste powder. 0, 5, 10, 20 and 30 indicate the percentage of carrot waste powder in the flour blend. Mean ± standard deviation of three independent measurements.

	a_w	$a_{w\ storage}$	Weight (g)	Volume (mm^3)
M0	0.895 ± 0.007^{c}	0.894 ± 0.008^{c}	42.81 ± 0.07^{b}	84.3 ± 0.3^{a}
M5	0.879 ± 0.004^{bc}	0.89 ± 0.02^{bc}	48.7 ± 0.7^{c}	112 ± 4^{c}
M10	0.84 ± 0.03^{ab}	0.866 ± 0.15^{abc}	41.1 ± 0.7^{a}	89 ± 3^{b}
M20	0.87 ± 0.04^{bc}	0.86 ± 0.03^{ab}	42.1 ± 0.3^{b}	83 ± 2^{a}
M30	0.814 ± 0.007^{a}	0.846 ± 0.013^{a}	40.7 ± 0.3^{a}	81.2 ± 0.4^{a}

$^{a,b,c,...}$Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Colour parameters (L*, a*, b*, and ΔE) of muffins (crust and crumb) are given in Table 5. Statistically significant differences were found among formulations. Crust and crumb lightness (L*) of rice muffins (control) were significantly higher than that of CP enriched muffins. L* of muffin crumb and crust decreased with CP addition. Natural brownish colour of CP decreased brightness of muffins when incorporating to formulation. Similar results were obtained by Olawuyi and Lee [8] and Kirbas et al. [11] in gluten-free carrot powder-based muffins and cakes, respectively. In bakery products, the colour is a result of the ingredients, the process conditions and the interactions between them, such as Maillard or caramelization reactions [12] and enzymatic browning [36].

The a* value for crust and crumb increased with the increase of CP. The darker colour and the increase of redness (positive a*) in muffins with higher CP percentage might be due to the presence of the carotenoid pigment, or either to Maillard reactions caused by sugars, proteins and phenols during baking [37,38]. Greater amounts of CP increased the b* value of crumb, but no clear trend was observed for crust. In both crumb and crust b* values were positive indicating a tendency towards yellow.

Colour change (ΔE) after one week of storage was calculated and results are summarized in table 5. Storage implied colour changes for all formulations being studied. While colour change in crust exhibited significant differences among formulations, colour changes in crumb were not significant among samples. This fact evidences that interactions between ingredients and process parameters determine colour, since crust is the region most exposed to processing and storage conditions.

Table 5. Colour parameters L*, a* and b* of gluten-free muffins (M) crumb and crust, at time 0 and total colour difference after one week of storage (ΔE). 0, 5, 10, 20 and 30 indicate the percentage of carrot waste powder in the flour blend. Mean ± standard deviation of three independent measurements.

		L*	a*	b*	ΔE
CRUMB	M0	63 ± 4^{d}	-3.1 ± 0.2^{a}	25.1 ± 0.8^{a}	8 ± 3^{a}
	M5	57 ± 3^{c}	-0.45 ± 0.10^{b}	32.835 ± 1.006^{b}	8.7 ± 1.9^{a}
	M10	58 ± 3^{c}	1.5 ± 0.5^{c}	39 ± 4^{c}	7.0 ± 3^{a}
	M20	54.2 ± 1.9^{b}	4.02 ± 0.50^{d}	44.6 ± 1.5^{d}	8.3 ± 1.3^{a}
	M30	50.0 ± 1.6^{a}	6.1 ± 0.5^{e}	47.7 ± 1.4^{e}	9 ± 2^{a}
CRUST	M0	60 ± 2^{d}	4.7 ± 1.4^{a}	34.8 ± 0.4^{ab}	7.9 ± 1.2^{a}
	M5	46 ± 3^{b}	15 ± 4^{b}	32 ± 4^{a}	12.1 ± 6^{ab}
	M10	47.7 ± 0.8^{bc}	16.2 ± 1.6^{b}	35.8 ± 0.8^{ab}	13 ± 2^{ab}
	M20	50.52 ± 1.17^{c}	15.5 ± 1.9^{b}	39 ± 2^{b}	16 ± 5^{b}
	M30	40.5 ± 1.5^{a}	16.5 ± 1.4^{b}	32.5 ± 0.5^{a}	11.8 ± 1.3^{ab}

$^{a,b,c,\ldots}$Different superscript letters in the same column for a same muffin zone (crust or crumb) indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Hardness, or the highest force required to compress the muffin crumb [8] is plotted in Figure 3. Addition of CP in different percentages significantly decreased muffins hardness, suggesting a more crumbly and softer texture [32]. This trend was also reported in Bhatt et al. [20] on muffins made by replacing black rice flour with amaranth flour, and was attributed to the increase in the flour porosity. Other studies showed that the addition of peanut milk powder [39] or potato peel flour [16] decrease muffins and cakes hardness; however, opposite results were obtained in a similar study [8] in which the incorporation of mushroom or carrot powder increased the hardness of rice muffins.

Muffins hardness significantly increased after one week of storage. This has been reported in other for gluten-free muffins enriched with pecan nut expeller [40] or orange fibres [41]. This could be the result of crumb water loss and a possible starch retrogradation, i.e. the reassociation of disrupted amylose and amylopectin chains when cooled, creating an ordered structure which increases hardness of the product [40,42].

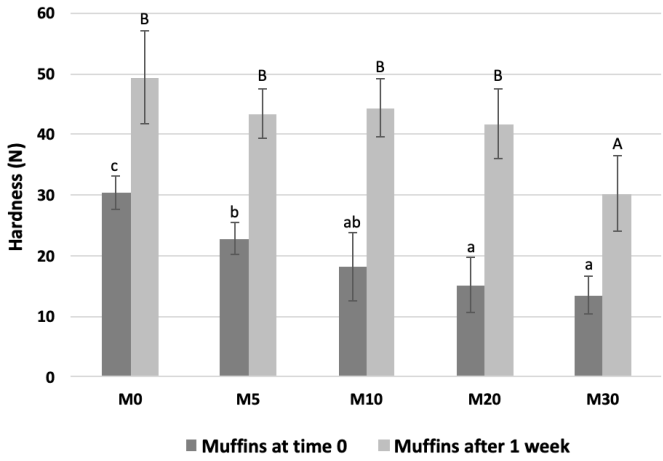

Figure 3. Gluten-free muffins (M) hardness (in N) obtained by replacing rice flour with different percentages of carrot waste powder (0, 5, 10, 20 and 30 indicate the carrot waste powder replacement used in the formulations). $^{a,b,c\ or\ A,B,C}$ different letters in the same series indicate significant differences at the 95% confidence level.

Muffin cuts for all the formulations applied are shown in Figure 4. It can be observed that product appearance is modified as a consequence of rice flour replacement with CP. Colour variation was the most outstanding difference, crust and crumb getting progressively darker and orangish as CP level increases. This has been objectively observed when analysing colour properties. Besides, a decrease in volume as CP proportion increases is also observed.

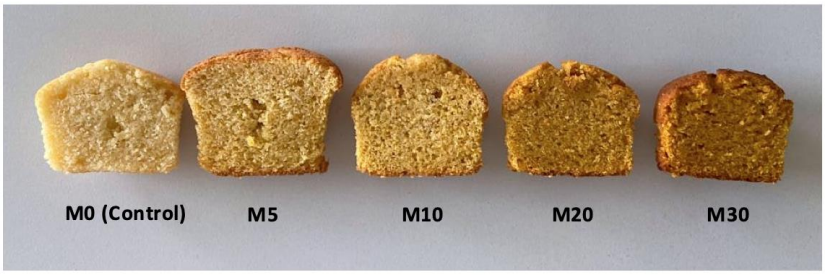

Figure 4. Appearance of gluten-free muffin cuts, obtained by replacing rice flour with different percentages of carrot waste powder (0, 5, 10, 20 and 30 indicate the percentage of carrot waste powder in the flour blend).

Antioxidant properties of muffins (crumb and crust) after baking and after one week of storage, are shown in Figure 5. Sugar reducing content, total phenol content and antioxidant activity (DPPH) significantly increased with a higher proportion of CP in both crumb and crust, as compared to control (rice flour muffin).

Addition of CP to muffins formulation increased the sugar reducing content from 3.17 (M0) to 7.61 mg GAE/g (M30), due to the sugar content that the vegetable powder would provide. However, after one week of storage values obtained were significantly higher than at time 0, which could be explained by the capacity of reducing sugars to accumulate at the temperature (4 °C) at which muffins had been stored. It has been reported that reducing sugar content can increase during storage, when stored at 4 and 8 °C; conversely, reducing sugars decrease when stored at 12 or 16 °C [43].

The increase in total phenol content and antioxidant activity when increasing CP flour in the formulation could be attributed to phenolics and carotenoids content of carrot waste powders [5]. This increase was also observed in other studies, in carrot powder and mushroom powder enriched rice muffins [8], in carrot powder enriched cookies [29] or in mango peel powder enriched biscuits [36]. Phenolic content and free radical scavenging analyses (DPPH) showed slightly higher results in crust than in crumb. This difference could be attributed to the formation of antioxidant compounds due to Maillard browning during baking [29], and this may also be due a

lower moisture content in the crust and therefore the compounds present are more concentrated.

As for the impact of storage on the antioxidant properties measured, phenolic compounds and antioxidant activity (DPPH) reduced during storage for all formulations, and in both muffin crumb and crust. This had been also reported by Croitoru et al. [44] and attributed to possible antioxidant degradation reactions.

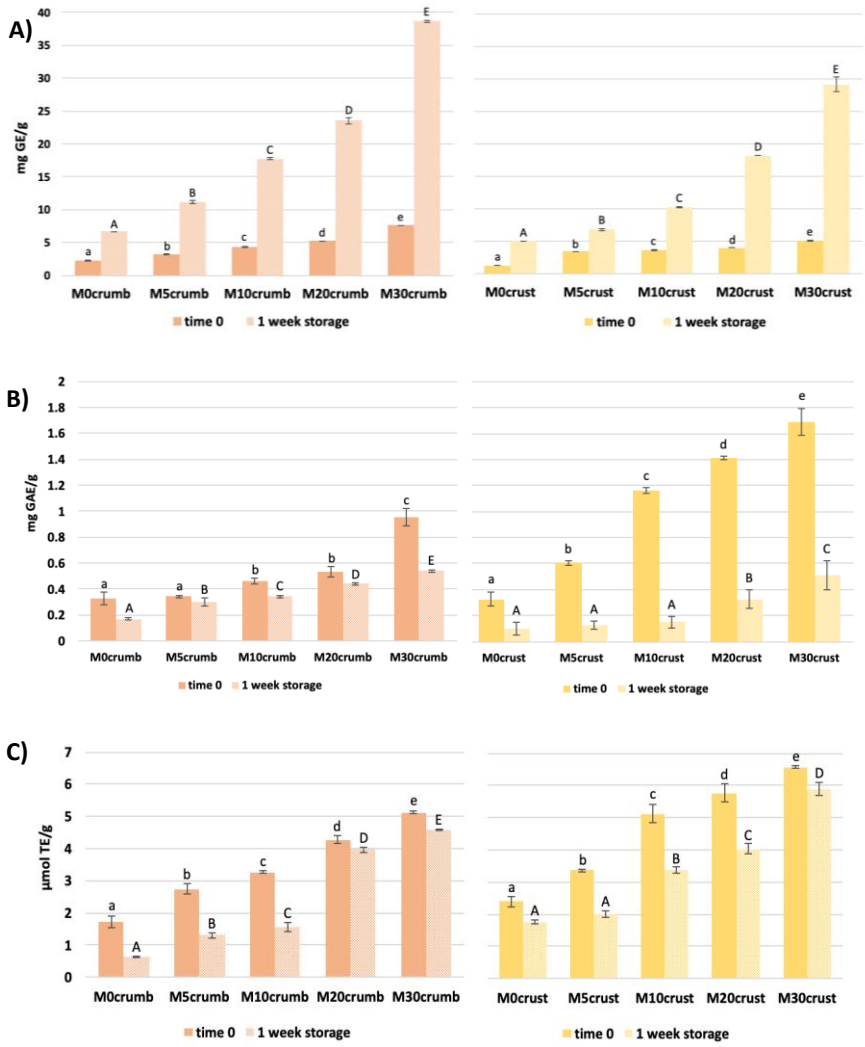

Figure 5. Antioxidant properties of gluten-free muffins crumb and crust obtained by

partially replacing rice flour by carrot waste powder (0, 5, 10, 20 and 30 indicate the percentage of carrot waste powder in the flour blend), after baking (time 0) and after one week of storage. A) Reducing sugar content (Glucose Equivalents, GE); B) Total phenol content (Gallic Acid Equivalents, GAE); C) Antioxidant activity by the DPPH method (Trolox Equivalents, TE). $^{a,b,c \text{ or } A,B,C}$ different letters in the same series indicate significant differences at the 95% confidence level.

3.3. Breadstick products characterization

The viscoelastic behaviour of dough formulations is represented in Figure 6. As occurred with muffins doughs (figure 2), loss modulus (G'') was lower than storage modulus (G') in the whole frequency range. Both parameters increased with the frequency in all samples indicating an elastic behaviour of batters. The loss tangent (tan δ) values were around 0.3 and slightly dependent on frequency; tan $\delta < 1$ indicates a more solid-like behaviour.

When incorporating the vegetables residue powder, the trend was not as clear for breadsticks doughs than for muffins doughs (figure 2). In this case, the addition of WCP in a 20% and 30% increased the G' and G'' values compared to control, as also reported in kinnow peel powder enriched dough (3, 6, 9% of replacement) [18] or in orange peel powder enriched dough (1, 3, 5 and 7% of replacement) [35]. In contrast, values decreased in doughs with a 5% and 10% of WCP replacement.

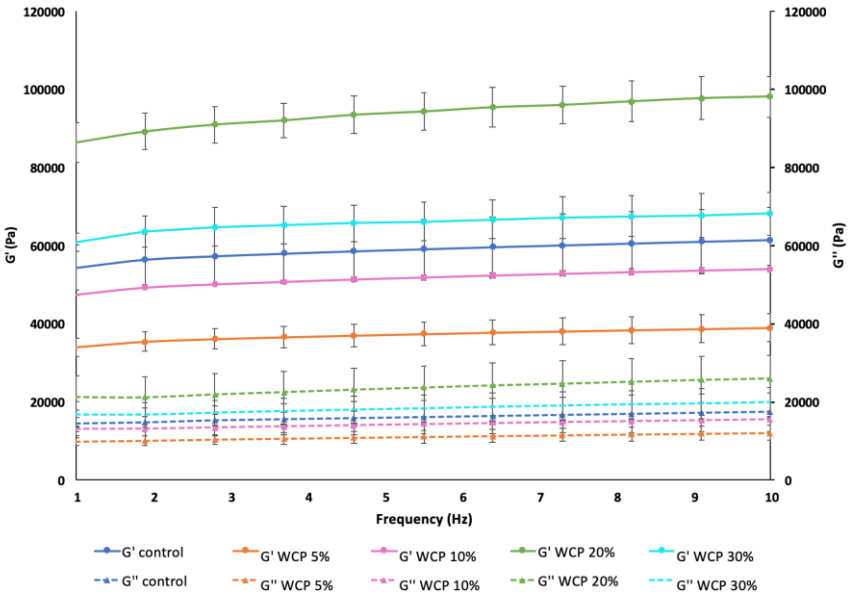

Figure 6. Rheograms of gluten-free breadsticks batters, obtained by partially replacing rice flour with white cabbage waste powders. G': storage modulus, G'': loss modulus. 0, 5, 10, 20 and 30% indicate the percentage of white cabbage waste powder in the flour blend.

Water activity (a_w) results, after baking and after one week of storage, and the weight and the volume of gluten-free breadsticks formulations are shown in Table 6. Although there were significant differences regarding a_w values, no trend was identified as the highest values corresponded to B5 and B10 formulations. Generally, a_w increased after one week of storage, in all cases.

The weight of gluten-free breadsticks increased with WCP replacement, which may be due to the increase in fiber content. Similar results were obtained on soup sticks enriched with kinnow peel powder [18]. No significant differences were obtained regarding volume among formulations.

Table 6. Water activity at time 0 (a_w) and water activity after one week of storage ($a_{w\ storage}$), weight and volume of gluten-free breadstick (B) products obtained by replacing rice flour with white cabbage waste powders. 0, 5, 10, 20 and 30 indicate the percentage of white cabbage waste powder in the flour blend. Mean ± standard deviation of three independent measurements.

	a_w	$a_{w\ storage}$	Weight (g)	Volume (mm^3)

B0	0.82 ± 0.05^{a}	0.837 ± 0.008^{a}	15.7 ± 0.3^{a}	20 ± 3^{a}
B5	0.87 ± 0.03^{b}	0.878 ± 0.005^{c}	16.9 ± 0.5^{bc}	19.0 ± 1.3^{a}
B10	0.879 ± 0.007^{b}	0.867 ± 0.008^{bc}	16.72 ± 0.17^{b}	16.88 ± 1.08^{a}
B20	0.86 ± 0.04^{ab}	0.879 ± 0.007^{c}	16.7 ± 0.8^{b}	16.8 ± 1.6^{a}
B30	0.838 ± 0.012^{ab}	0.852 ± 0.015^{ab}	17.7 ± 0.4^{c}	17.0 ± 0.6^{a}

$^{a,b,c...}$Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Colour parameters (L*, a*, b*, and ΔE) of gluten-free breadsticks are given in Table 7. As occurred with muffin (table 5), L* values decreased significantly with a greater replacement of WCP, whereas a* (redness) values increased with respect to the control sample. Among WCP formulations, b* value (yellowness) decreased with the increase of WCP added but was lower in B0. Likewise, Simsek and Süfer [22] reported a decrease of L* and b* and an increase of a* values when increasing the percentage of olive pomace flour in breadsticks. The one-week storage period did imply a colour change (ΔE) for all formulation; this colour change was more significant for replacements from 10 up to 30 (B10-B30).

Table 7. Colour parameters L*, a* and b* of gluten-free breadsticks (B) obtained by replacing rice flour with white cabbage waste powders, at time 0 and colour difference after one week of storage (ΔE). 0, 5, 10, 20 and 30 indicate the percentage of white cabbage waste powder in the flour blend. Mean ± standard deviation of three independent measurements.

	L*	**a***	**b***	**ΔE**
B0	66 ± 3^{e}	4.9 ± 1.4^{a}	25 ± 2^{a}	5.7 ± 1.8^{a}
B5	55 ± 3^{d}	12.1 ± 1.7^{b}	32.4 ± 0.9^{c}	5.2 ± 1.8^{a}
B10	51 ± 4^{c}	13 ± 2^{bc}	31.3 ± 1.4^{c}	10 ± 2^{b}
B20	45 ± 3^{b}	14.4 ± 1.2^{c}	29 ± 3^{b}	9 ± 4^{ab}
B30	41 ± 5^{a}	13.1 ± 1.4^{bc}	26 ± 3^{a}	7 ± 3^{ab}

$^{a,b,c...}$Different superscript letters in the same column indicate statistically significant differences at the 95% confidence level (p-value < 0.05).

Breadsticks hardness obtained from the maximum force necessary to break the product [45] is presented in Figure 7. Increasing WCP percentages in the formulation implied lower hardness values, which was significantly higher for the control sample than for the enriched ones. This trend was also

reported by Simsek and Süfer [22], where a partial replacement of wheat flour with olive pomace flour reduced hardness values in breadsticks formulations. This was attributed to the higher quantity of soluble fibres, resulting in crispier products. In rice flour crackers enriched with apple pomace powder (3, 6, and 9% of replacement) a decrease in the fracture force was observed, which was again explained in terms of the more soluble fibre content, which reduced hardness of the rice cracker [45]. Storage implied an increase in hardness values in all cases, although more notably in the case of control, and with no significant differences among the rest of formulations. This increase has also been observed and discussed previously, when analysing muffin products (figure 3), which could be attributed to water loss and starch retrogradation when cooled during storage, increasing products hardness.

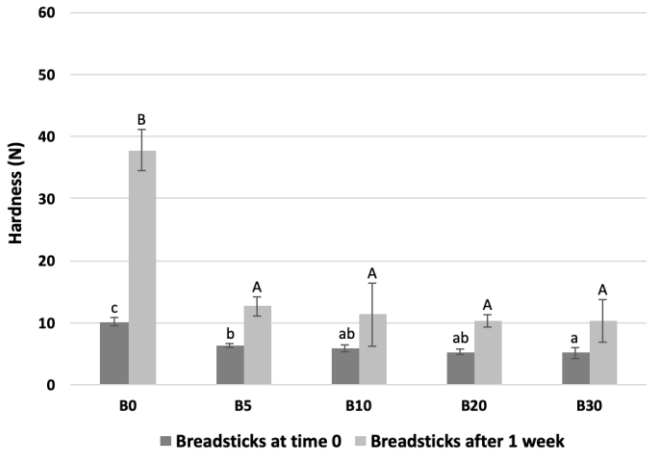

Figure 7. Gluten-free breadsticks hardness (N), obtained by partially replacing rice flour by white cabbage waste powders. 0, 5, 10, 20 and 30 indicate the white cabbage waste powder proportion in formulations. ^{a,b,c} or ^{A,B,C} different letters in the same series indicate significant differences at the 95% confidence level.

Gluten-free breadsticks corresponding to the five formulations used are shown in Figure 8. As observed, the appearance of the products changed as a result of rice flour replacement with WCP. As observed, breadsticks became progressively darker when increasing WCP, since a higher reducing sugars content implies a higher incidence of Maillard reactions, which would

explain the appearance of roasted regions. This is consistent with the objective colour measurements previously discussed.

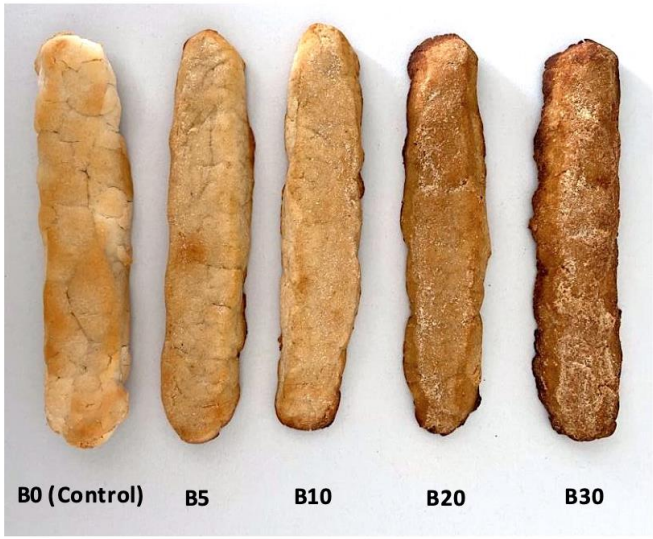

Figure 8. Gluten-free breadsticks products, obtained by partially replacing rice flour by white cabbage waste powders. 0, 5, 10, 20 and 30 indicate the percentage of white cabbage waste powder (WCP) in the flour blend.

Antioxidant properties of gluten-free breadsticks, after baking and after one week of storage, are shown in Figure 9. As in muffins, the sugar reducing content, total phenol content and antioxidant activity (DPPH) of WCP enriched formulations significantly increased as compared to rice breadstick formulation. Ajila et al. [46] also observed an improvement of antioxidant properties in macaroni formulations enriched with mango peel powders. This fact reinforced the idea that adding WCP to breadsticks formulation may have a significant impact on the nutritional quality and functional potential of the food.

Again, the sugar reducing content exhibited after one week of storage increased significantly. However, total phenol content and DPPH values decreased after storage. Reduction in total phenol content and antioxidant activity after 7 days of storage were also reported by Lafarga et al. [47] in

crackers enriched with broccoli stem powders (12.5 and 15% substitution level).

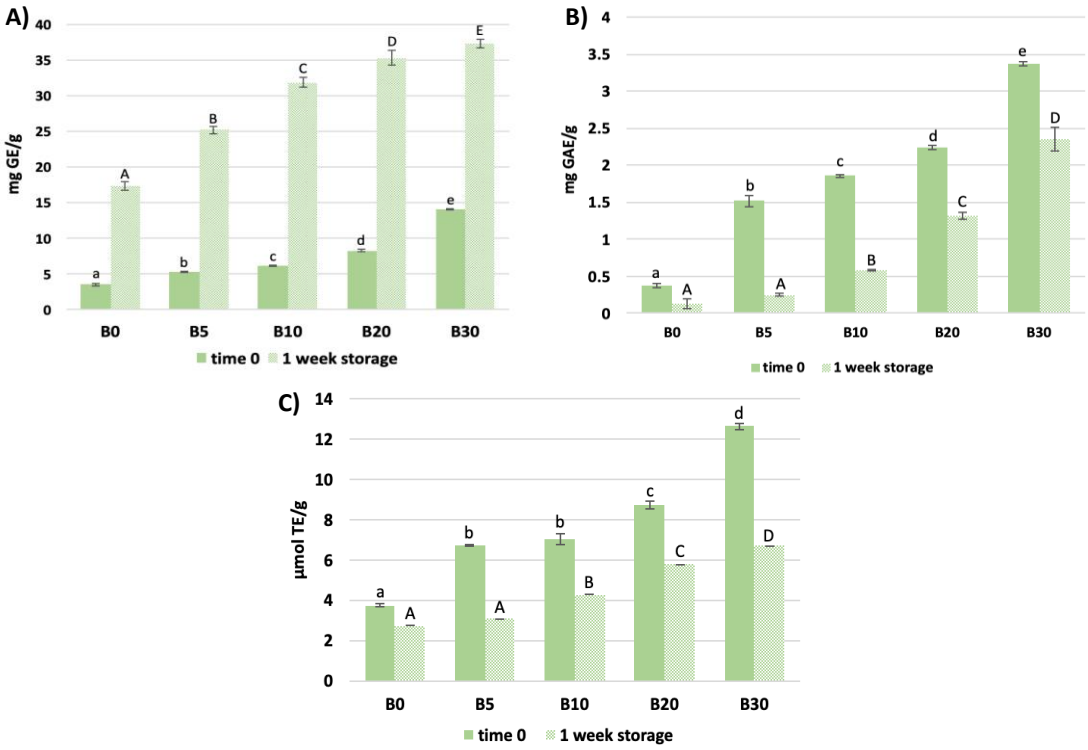

Figure 9. Antioxidant properties of gluten-free breadsticks obtained by partially replacing rice flour with white cabbage waste powders, after baking (time 0) and after one week of storage. 0, 5, 10, 20 and 30 indicate the percentage of white cabbage waste powder (WCP) in the flour blend. A) Reducing sugar content (Glucose Equivalents, GE); B) Total phenol content (Gallic Acid Equivalents, GAE); C) Antioxidant activity by the DPPH method (Trolox Equivalents, TE). [a,b,c or A,B,C] different letters in the same series indicate significant differences at the 95% confidence level.

4. Conclusions

This study showed that replacement of rice flour with carrot waste (CP) or white cabbage waste powder (WCP) is an excellent approach to improve antioxidant and nutritional value of widely consumed bakery products, such as muffins or breadsticks, in gluten-free formulations. In this way, the use vegetable waste powdered ingredients in bakery products formulation

would contribute to the development of more sustainable and healthier diets.

The incorporation of CP or WCP had a significant impact on physicochemical and antioxidant properties of the flour blends and the products obtained. Pasting, rheological and textural parameters revealed CP and WCP incorporation as more adequate in obtaining quality gluten-free muffin or breadstick. Colour of baked products was influenced by the addition of CP or WCP. It has been evidenced that CP and WCP addition may significantly improve antioxidant properties of gluten-free rice formulated products, resulting in fortified foodstuffs with increased health benefits. Storage under fridge conditions, however, had a negative impact on the quality of the products obtained.

Hence, the partial replacement rice flour by CP or WCP in gluten-free bakery products such as muffins or breadsticks, has been proved to have a great potential to obtain functional products with added value. Nevertheless, it should be noted that further analyses are needed to complete this study. On the one hand, a sensory analysis would be necessary to determine final products acceptability; on the other, microbiological safety and stability of the products should be evaluated.

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CONCLUSIÓN

Los resultados obtenidos en esta investigación suponen una aproximación preliminar al desarrollo de productos de panadería con propiedades nutritivas mejoradas y sin gluten, mediante la adición de productos en polvo obtenidos a partir de residuos de hortalizas, contribuyendo de este modo al desarrollo de dietas más saludables y sostenibles.

Este estudio demostró que la sustitución parcial de harina de arroz por polvo de residuo de zanahoria o de col blanca supone una mejora del potencial antioxidante y nutricional de productos de panadería ampliamente consumidos, tales como las magdalenas y las rosquilletas. No obstante, sería necesario ampliar esta investigación mediante análisis de aceptación sensorial y ensayos microbiológicos de seguridad del alimento, para confirmar la aceptabilidad y estabilidad de los productos de panadería elaborados.

5. RESUMEN GENERAL DE RESULTADOS

La revisión del estado del arte sobre la obtención de productos deshidratados en polvo a partir de residuos vegetales evidenció que las condiciones de proceso definen las propiedades de estos productos, y permitió seleccionar las técnicas consideradas más apropiadas para abordar el proyecto. Las consiguientes investigaciones contribuyeron a profundizar en el conocimiento del impacto que tienen las etapas del proceso de transformación (pretratamientos, secado, molienda) sobre las características de los productos en polvo en cuanto a sus propiedades fisicoquímicas, funcionales, tecnológicas y de bioaccesibilidad, pudiendo seleccionarse aquellas que se consideraron más interesantes.

Los métodos de deshidratación empleados (secado por aire caliente y liofilización) permitieron disminuir la humedad y actividad del agua del producto inicial hasta el valor objetivo (< 0,3). Ambos métodos de secado influyeron sobre las propiedades de los polvos de modo que, en términos generales, la liofilización dio lugar a polvos más finos y con mayor contenido en compuestos bioactivos específicos (carotenoides y sulforafano), mientras que el secado por aire caliente dio lugar a productos con mejores propiedades antioxidantes. Teniendo en cuenta el contexto de aplicación en el que se desarrolla el proyecto y los costes asociados a cada operación, la recomendación general fue la de proponer el secado por aire caliente como operación de deshidratación. Dentro de éste, la mayor temperatura ensayada (70 °C) dio lugar a mejores propiedades antioxidantes y permitió reducir los tiempos de secado, con el consiguiente ahorro energético. En cuanto a las propiedades tecnológicas, los polvos mostraron buenas propiedades de interacción con el agua y escasa interacción con el aceite.

Tanto los pretratamientos (congelación y disrupción del material vegetal) como el tipo de matriz vegetal afectaron de forma diferente a las propiedades fisicoquímicas y antioxidantes de los productos, así como al comportamiento de las muestras durante el secado. Se constató una interdependencia de las etapas de pretratamiento, secado y molienda. No se observó un efecto significativamente positivo de la congelación previa lo que, junto con el coste asociado a la misma, llevó a descartarla y a recomendar el procesado de los residuos en fresco. Respecto a la disrupción, el troceado, combinado con SAC a 70 °C, dio lugar a productos con mejores

características antioxidantes. La investigación también puso de manifiesto la importancia de estudiar no sólo el procesado, sino también el impacto del almacenamiento sobre las características de los ingredientes en polvo. Durante el almacenamiento, se observaron cambios importantes en las propiedades fisicoquímicas y antioxidantes de los productos, lo que sugirió la necesidad de almacenarlos en condiciones más controladas.

La fermentación con *Lactobacillus plantarum* supuso una mejora de las propiedades antioxidantes del tallo de brócoli, además de reducir los tiempos de secado por aire caliente, lo que a su vez se tradujo en una menor exposición al oxígeno y a las altas temperaturas. Sin embargo, el potencial efecto probiótico solamente pudo garantizarse para el producto liofilizado, recomendándose de cara al futuro realizar ensayos de secado por aire caliente a menores temperaturas (< 60 °C) o en rampa descendente.

La digestión gastrointestinal simulada *in vitro* favoreció la liberación de compuestos antioxidantes y carotenoides unidos a la estructura del polvo. Generalmente, los polvos con un menor tamaño de partícula exhibieron índices de recuperación más elevados, lo que se atribuyó a una mayor superficie de contacto entre la muestra y los fluidos de la digestión, incrementándose la extracción y solubilización de los compuestos de interés. Los ensayos de digestión de los polvos dispersos en aceite o emulsión sugirieron que la co-ingesta podría contribuir a una mayor bioaccesibilidad de los carotenoides; no obstante, se hace necesario realizar más investigaciones en este sentido.

Por último, la aplicación de los polvos como ingrediente funcional en la formulación de productos de panadería sin gluten confirmó que la adición de harinas obtenidas a partir de residuos vegetales es una buena estrategia para fortificar y enriquecer nutricionalmente estos productos horneados, a tenor de los resultados obtenidos en productos elaborados con reemplazo parcial de harina de arroz por polvo de residuo de zanahoria, en magdalenas, y polvo de residuo de col blanca, en rosquilletas. En ambos casos, los polvos con mayor porcentaje de reemplazo (30%) son los que presentaron mejores propiedades antioxidantes.

6. CONCLUSIONES GENERALES Y PERSPECTIVAS DE FUTURO

Los resultados obtenidos en la presente tesis doctoral han demostrado el potencial de los residuos generados en la confección de bandejas y productos de IV gama de zanahoria, col, apio, puerro y brócoli para su valorización integral, habiéndose desarrollado un proceso de transformación de los mismos en productos deshidratados en polvo.

Estos resultados han contribuido a generar conocimiento acerca del efecto de diversos tratamientos previos (congelación, triturado, troceado, fermentación) y de diferentes métodos de deshidratación (liofilización y secado por aire caliente) sobre las propiedades fisicoquímicas y el contenido en compuestos bioactivos de los polvos, así como sobre las cinéticas de secado con aire a diferentes temperaturas.

Se prevé que este tipo de aproximaciones pueda contribuir de manera efectiva al desarrollo de sistemas alimentarios más saludables y sostenibles. Sin embargo, más investigaciones son necesarias para el adecuado escalado del proceso a nivel industrial.

El potencial funcional de los polvos obtenidos, evaluado mediante la caracterización de los polvos y, más específicamente, mediante ensayos de digestión gastrointestinal simulada *in vitro*, ha evidenciado su potencial como ingredientes funcionales para aumentar el valor nutricional de alimentos formulados. No obstante, futuros ensayos de digestión *in vitro* con el fin de evaluar la bioaccesibilidad de sus componentes cuando los polvos son adicionados a otras matrices alimentarias (carbohidratos, grasas, incorporados a un alimento formulado…), así como la extensión de la digestión a la etapa colónica, permitirán profundizar en el efecto beneficiosos que estos ingredientes podrían tener sobre la salud.

Con respecto a la aplicación de los ingredientes en polvo a productos de panadería, cabe destacar que la creciente demanda del consumidor por productos naturales, con un valor nutricional añadido y que aporten un beneficio para la salud, constituye una oportunidad de mercado para estos productos de consumo habitual. Por otro lado, la incorporación de estas harinas vegetales en la formulación de productos sin gluten permitirá que personas con alergia o intolerancia a estas proteínas puedan consumirlos sin cambiar su patrón dietético. En este trabajo, la incorporación de harinas

obtenidas a partir de residuos de hortalizas se ha confirmado como una buena estrategia para enriquecer nutricionalmente estos productos horneados. No obstante, para que puedan utilizarse como ingredientes funcionales, será necesario abordar futuros estudios de aceptabilidad sensorial, estabilidad y seguridad.

En el caso de los polvos de brassicas, debido a su contenido en glucosinolatos e isotiocianatos, se ha propuesto su uso para prevenir o limitar el crecimiento de plantas arvenses con el fin de reducir el empleo de plaguicidas sintéticos. Estas investigaciones se están llevando a cabo en colaboración con el Instituto Agroforestal Mediterráneo (IAM-UPV), en el contexto del proyecto FUNBIOPEST, habiéndose obtenido resultados muy prometedores.

Por todo ello, se concluye que la transformación de residuos o destríos hortofrutícolas en productos deshidratados en polvo es una oportunidad para los agentes productivos que generan este tipo de residuos, al tiempo que permite reducir sus costes de gestión. Por otro lado, empresas interesadas en producir nuevos ingredientes o aditivos tienen la oportunidad de dar valor a estos residuos, que están ampliamente disponibles y a bajo coste. En ambos casos, el empleo de materiales de desecho de origen natural es una apuesta por la sostenibilidad y la salud, que supondría un grado de diferenciación para la empresa en el mercado actual.