UNIVERSITAT POLITÈCNICA DE VALÈNCIA

MI QU ALI
Grupo de Investigación Microestructura y Química de Alimentos

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

Aprovechamiento de subproductos de zumos de frutos rojos para el diseño de alimentos

TESIS DOCTORAL

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HACEN CONSTAR QUE:

El trabajo de investigación "Aprovechamiento de subproductos de zumos de frutos rojos para el diseño de alimentos", que presenta Dña. Elena Diez por la Universitat Politècnica de València, y que ha sido realizado bajo nuestra dirección en el Grupo de Investigación de Microestructura y Química de Alimentos de la Universitat Politècnica de València, reúne las condiciones para optar al grado de Doctor.

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Fdo. Dra. Isabel Hernando Hernando

Fdo. Dra. Amparo Quiles Chuliá

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Índice

Resumen

La presente tesis plantea distintas estrategias para el diseño de alimentos a partir del aprovechamiento del bagazo que se genera como subproducto en la industria de elaboración de zumos de frutos rojos. De este modo, se puede aportar un valor añadido al subproducto, al mismo tiempo que se desarrollan alimentos capaces de cubrir las necesidades y exigencias nutritivas que demanda el consumidor actual. Uno de los aspectos más interesantes que tiene el uso de estos subproductos, es su alto contenido en polifenoles y fibra, de gran importancia en la industria alimentaria por su efecto en las propiedades de los alimentos y sobre la salud.

Se realizó un primer estudio para conocer el efecto de la sustitución de grasa en bizcochos por diferentes tipos de fibra: soluble, insoluble y una mezcla de ambas. En este trabajo se observó que las características texturales, estructurales y sensoriales se vieron afectadas con la incorporación de fibra, principalmente en el caso de la fibra insoluble. Por todo esto, se establecieron dos estrategias de mejora. Por una parte, se incorporaron diferentes impulsores químicos en combinación con bicarbonato (normal o encapsulado) en bizcochos formulados con bagazo y se caracterizaron desde un punto de vista físico químico, estructural y sensorial. Por otra parte, se estudió cómo afecta a la textura, estructura y a la digestibilidad *in vitro* del almidón la sustitución parcial de harina de trigo por harina extrusionada en magdalenas formuladas con bagazo. En ambos estudios se obtuvieron resultados satisfactorios en cuanto a la mejora de las propiedades del producto.

Además de las estrategias de mejora en productos horneados, se decidió incorporar bagazo en matrices lácteas para desarrollar nuevas formulaciones de batidos. Se estudió el efecto de un tratamiento no térmico, las altas presiones hidrostáticas, sobre el contenido fenólico y la capacidad antioxidante de los batidos, y sobre la capacidad antimicrobiana de los fenoles presentes en el bagazo.

Se observó que la mayor retención de compuestos fenólicos y capacidad antioxidante con la mínima supervivencia microbiológica

se obtuvo para las presiones y tiempos más altos (500 MPa durante 10 min) en los batidos con las concentraciones más altas de bagazo estudiadas (10%).

Por último, se llevó a cabo un estudio con sistemas modelo para conocer las interacciones que tienen los polifenoles, cuando forman parte del bagazo o cuando se encuentran en forma de extracto, con los principales macronutrientes de los alimentos y sus efectos sobre la bioaccesibilidad de los compuestos fenólicos tras la digestión *in vitro*. La bioaccesibilidad de los polifenoles cuando se incorporaron en los sistemas modelo en forma de bagazo aumentó en comparación con cuando se adicionaron como extracto. Por otro lado, los sistemas modelo formulados con bagazo y un solo nutriente, presentaron una mayor bioaccesibilidad de los compuestos fenólicos que cuando se encontraron todos los nutrientes en el sistema modelo.

Resum

La present tesi planteja diferents estratègies per al disseny d'aliments a partir de l'aprofitament del bagàs que es genera com a subproducte en la indústria d'elaboració de sucs de fruits rojos. D'aquesta manera, es pot aportar un valor afegit al subproducte, al mateix temps que es desenvolupen aliments capaços de cobrir les necessitats i exigències nutritives que demanda el consumidor actual. Un dels aspectes més interessants que té l'ús d'aquests subproductes, és el seu alt contingut en polifenols i fibra, de gran importància en la indústria alimentària pel seu efecte en les propietats dels aliments i sobre la salut.

Es va realitzar un primer estudi per a conéixer l'efecte de la substitució de greix en bescuits per diferents tipus de fibra: soluble, insoluble i una mescla d'ambdues. En aquest treball es va observar que les característiques texturals, estructurals i sensorials es van veure afectades amb la incorporació de fibra, principalment en el cas de la fibra insoluble. Per tot això, es van establir dues estratègies de millora. D'una banda, es van incorporar diferents impulsors químics en combinació amb bicarbonat (normal o encapsulat) en bescuits formulats amb bagàs i es van caracteritzar des d'un punt de vista físic químic, estructural i sensorial. D'altra banda, es va estudiar com afecta a la textura, estructura i a la digestibilitat *in vitro* del midó la substitució parcial de farina de blat per farina extrusionada en magdalenes formulades amb bagàs. En tots dos estudis es van obtindre resultats satisfactoris quant a la millora de les propietats del producte.

A més de les estratègies de millora en productes enfornats, es va decidir incorporar bagàs en matrius làcties per a desenvolupar noves formulacions de batuts. Es va estudiar l'efecte d'un tractament no tèrmic, les altes pressions hidroestàtiques, sobre el contingut fenòlic i la capacitat antioxidant dels batuts, i sobre la capacitat antimicrobiana dels fenols presents en el bagàs.

Es va observar que la major retenció de compostos fenòlics i capacitat antioxidant amb la mínima supervivència microbiològica es va obtindre per a les pressions i temps més alts (500 MPa durant 10 min) en els

batuts amb les concentracions més altes de bagàs estudiades (10%).

Finalment, es va dur a terme un estudi amb sistemes model per a conéixer les interaccions que tenen els polifenols, quan formen part del bagàs o quan es troben en forma d'extracte, amb els principals macronutrients dels aliments i els seus efectes sobre la bioaccessibilitat dels compostos fenòlics després de la digestió *in vitro*. La bioaccessibilitat dels polifenols quan es van incorporar en els sistemes model en forma de bagàs va augmentar en comparació amb quan es van addicionar com a extracte. D'altra banda, els sistemes model formulats amb bagàs i un sol nutrient, van presentar una major bioaccessibilitat dels compostos fenòlics que quan es van trobar tots els nutrients en el sistema model.

Abstract

This thesis proposes different strategies for the design of foods based on the use of pomace generated as a by-product in the berry juice processing industry. In this way, it is possible to add value to the by-product while developing foods capable of meeting the needs and nutritional requirements demanded by today's consumers. One of the most interesting aspects of the use of these by-products is their high content of polyphenols and fiber, of great importance in the food industry due to their effect on food properties and health.

A first study was carried out to determine the effect of replacing fat in sponge cakes with different types of fiber: soluble, insoluble and a mixture of both. In this work it was observed that the textural, structural and sensory characteristics were affected by the incorporation of fiber, mainly in the case of insoluble fiber. For all these reasons, two improvement strategies were determined. On the one hand, different chemical leavenings agents were incorporated in combination with bicarbonate (normal or encapsulated) in sponge cakes formulated with pomace and characterized from a physical-chemical, structural and sensory point of view. On the other hand, it was studied how the partial substitution of wheat flour by extruded flour in pomace-formulated muffins affects texture, structure and *in vitro* starch digestibility. In both studies satisfactory results were obtained in terms of improved product properties.

In addition to the improvement strategies in baked products, it was decided to incorporate pomace in dairy matrices to develop new milkshake formulations. The effect of a non-thermal treatment, such as high hydrostatic pressures, on the phenolic content and antioxidant capacity of the milkshakes, and on the antimicrobial capacity of the phenols present in pomace was studied.

It was observed that the highest retention of phenolic compounds and antioxidant capacity with the lowest microbiological survival was obtained for the highest pressures and times (500 MPa for 10 min) in the milkshakes with the highest pomace concentrations studied (10%).

Finally, a study was carried out with model systems to learn about the interactions that polyphenols have when they are part of the pomace or when they are in extract form, with the main macronutrients of food and their effects on the bioaccessibility of phenolic compounds after *in vitro* digestion. The bioaccessibility of polyphenols increased when it is incorporated into the model systems as pomace compared to when it is added as extract. On the other hand, model systems formulated with pomace and a single nutrient presented higher bioaccessibility of phenolic compounds than when all nutrients were found in the model system.

Introducción

En 2015 la Organización de Naciones Unidas (ONU) estableció la agenda 2030 sobre el Desarrollo Sostenible. Dentro de ella se incluyó como objetivo reducir a la mitad el desperdicio de alimentos *per capita*, a nivel consumidores, en la venta al por menor y en las cadenas de producción y suministro, incluidas las pérdidas posteriores a la cosecha. Para poder conseguir este objetivo se propuso a las empresas que plantearan acciones de innovación enfocadas al logro de una producción sostenible (ONU, 2015).

El consumo de zumos y néctares en Europa ascendió a 9.067 millones de litros en el año 2018 (AIJN, 2013). Después del prensado de la fruta para la obtención de zumo, se obtiene un subproducto denominado bagazo, el cual está formado por piel, tallos, semillas y otras partes no usadas del producto principal. Estos componentes son ricos en compuestos bioactivos, los cuales tienen un efecto beneficioso sobre la salud que va más allá del simple efecto nutritivo de su composición (Fraga et al., 2019).

Para evitar el impacto ambiental provocado por el desecho de estos subproductos, y la pérdida de los nutrientes y compuestos beneficiosos que contienen, es interesante el desarrollo de estrategias alternativas que permitan su revalorización y aprovechamiento, tales como el diseño o la obtención de ingredientes para su incorporación en la formulación de diferentes productos alimentarios. El *Upcycling*, es una práctica de la economía circular que consiste en dar una segunda oportunidad a los subproductos de la industria para ser usados nuevamente, con beneficios tangibles para el medio ambiente y la sociedad (Spratt et al., 2020). Su mayor diferencia con otros procesos, como el reciclaje, es que no intenta continuar con modelos lineales como la degradación o descomposición de materiales. Esta práctica tiene por lo tanto un fuerte impacto sobre la sostenibilidad ya que se realiza una gestión eficiente de estos subproductos -que de otra forma serían eliminados- que se utilizan como ingrediente para ser reincorporados de nuevo en la cadena alimentaria (Figura 1).

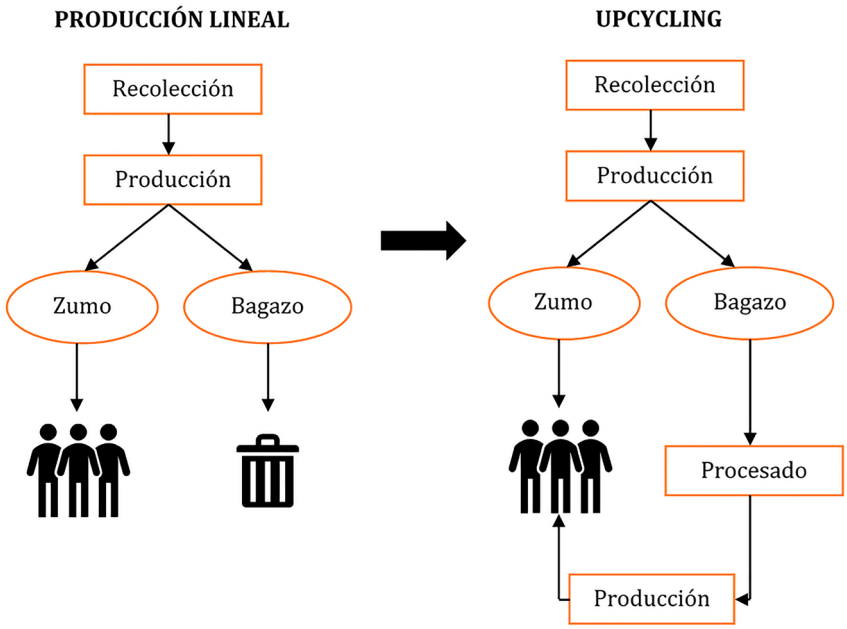


Figura 1. *Upcycling* dentro de la industria de producción de zumos de frutos rojos.

Los subproductos derivados de la industria de los zumos de frutos rojos están compuesto en su mayoría por compuestos fenólicos con una alta capacidad antioxidante y por fibra (Reißner et al., 2019). Entre los beneficios que aporta el consumo de polifenoles se encuentran la mejora de la salud cardiovascular, la reducción de la inflamación, la prevención de cáncer o la modulación de la microbiota intestinal (efecto antimicrobiano y prebiótico) (Del Rio et al., 2013; Nile & Park, 2014).

Los polifenoles son una clase de compuestos fenólicos que consisten en uno o más grupos hidroxilo (—OH) unidos directamente a un anillo aromático. Estos están divididos en dos grandes categorías como son flavonoides y ácidos fenólicos. La estructura básica de los flavonoides consiste en dos anillos aromáticos (A y B) que se están unidos mediante tres átomos de carbono que forman un heterociclo oxigenado (C).

Asimismo, los flavonoides se pueden separar en subclases en función del estado de oxidación del heterociclo: flavonas, flavanonas, flavonoles, flavanonoles, isoflavonas, flavanolas y antocianidinas. Dentro de cada clase, se distinguen diferentes tipos por el patrón de hidroxilación de sus dos anillos fenólicos, y por la naturaleza y posición de los sustituyentes (Figura 2).

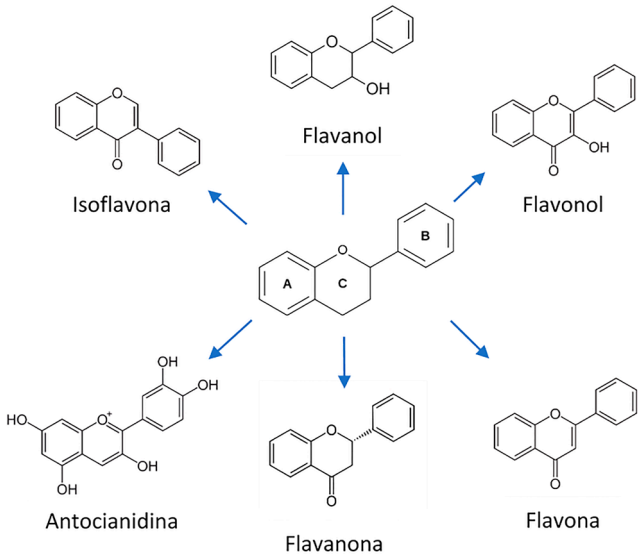
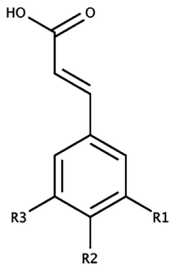


Figura 2. Estructura básica de los diferentes flavonoides.

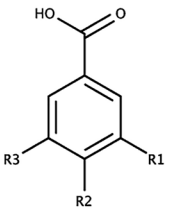
Los flavonoides se encuentran en gran cantidad en los alimentos de origen vegetal. En el caso de los frutos rojos, la mayor parte de polifenoles que se encuentran en su composición pertenecen al grupo de los flavanoles, flavonoles y antocianidinas. Los flavonoles pueden encontrarse en forma monomérica (epicatequina o epigallocatequina) o formando polímeros que reciben el nombre de proantocianidinas o taninos condensados. Además de flavonoides, también hay ácidos fenólicos, los cuales a su vez se dividen en dos subclases como son los ácidos hidroxibenzoicos y los ácidos hidroxicinámicos (Figura 3).

ÁCIDOS HIDROXICINÁMICOS



R1 = R2 = R3 = H: Ácido cinámico
R1 = R2 = OH, R3 = H: Ácido cafeico
R1 = R3 = OH, R2 = OH: Ácido cumárico

ÁCIDOS HIDROXIBENZOICOS



R1 = R2 = R3 = OH: Ácido gálico
R1 = R3 = H, R2 = OH: Ácido hidroxibenzoico

Figura 3. Estructura básica de ácidos fenólicos

Los compuestos fenólicos del bagazo pueden ser utilizados en los alimentos como ingredientes para aumentar la estabilidad oxidativa. Estos compuestos fenólicos poseen capacidad antioxidante por lo que actúan como defensa ante procesos de oxidación. Muchos compuestos antioxidantes reducen la oxidación lipídica al eliminar los radicales libres. Estos compuestos reaccionan con los radicales libres evitando que reaccionen con los ácidos grasos insaturados y por lo tanto inhibiendo la oxidación lipídica. La eficacia de la eliminación de radicales libres va a depender de varios factores como la posición y el grado de hidroxilación, polaridad, solubilidad, potencial reductor, estabilidad de los compuestos fenólicos a operaciones de procesado de alimentos y estabilidad del radical fenólico.

Además de la capacidad antioxidante, se ha observado que estos polifenoles tienen un efecto antimicrobiano. Los extractos fenólicos de los frutos rojos inhiben el crecimiento de los patógenos del tracto gastrointestinal y por lo tanto se les podría atribuir un efecto terapéutico (Lavefve et al., 2020). Esta actividad antimicrobiana puede estar causada por múltiples mecanismos, aunque uno de los más aceptados es que favorecen la desintegración de la membrana externa (Nohynek et al., 2006).

Los compuestos fenólicos pueden verse afectados por las condiciones de procesado del bagazo. Por ejemplo, el uso de altas temperaturas (60-125 °C) provoca la degradación de estos compuestos (Khanal et al., 2010). Es por ello por lo que se están estudiando otros tipos de procesado como la aplicación de tratamientos no térmicos, los cuales parecen favorecer la extractabilidad de los compuestos fenólicos sin degradarlos y sin comprometer la seguridad alimentaria (Corrales et al., 2008; Tadapaneni et al., 2014).

La fibra, el otro componente mayoritario del bagazo de los frutos rojos, es un grupo de sustancias químicamente similar a los carbohidratos, pero que no son hidrolizadas a lo largo del tracto gastro intestinal. En base a sus propiedades químicas, físicas y funcionales, la fibra puede ser clasificada como fibra soluble e insoluble. La fibra soluble incluye pectinas, gomas, fructanos tipo inulina y algunas hemicelulosas, que se disuelven en agua formando geles viscosos. Estos son resistentes a la digestión en el intestino delgado, pero fácilmente fermentables por la microbiota presente en el intestino grueso (Quiles et al., 2016). La fibra posee un efecto prebiótico, ya que estimula el crecimiento de bacterias beneficiosas del intestino (Buttriss & Stokes, 2008). Asimismo, el consumo de fibra se ha relacionado con una reducción del riesgo de padecer cáncer colorrectal. La fibra, además, aporta volumen a las comidas y una menor densidad energética, lo que puede ayudar a controlar la saciedad y por lo tanto ayudar en dietas para la reducción de peso.

A pesar de que la recomendación de consumo de fibra es de 25 g por persona y día (EFSA, 2016), la mayor parte de la población no lo cumple (De Mora & Conde, 2010). Por lo tanto, el bagazo de frutos rojos puede ser una alternativa para ayudar a llegar al consumo diario recomendado de fibra. Sin embargo, si la fibra es añadida a un producto alimentario, es necesario tener en cuenta que puede afectar a las características reológicas, texturales y sensoriales. Por ejemplo, en productos horneados la fibra puede producir efectos negativos sobre su estructura, lo cual repercute en el producto final (Quiles et al., 2016).

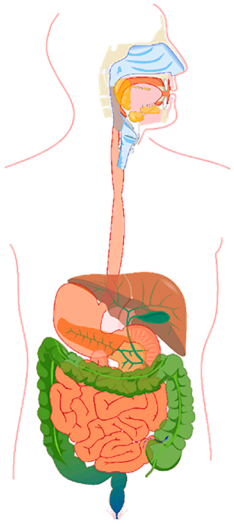
Los consumidores son cada vez más conscientes de la relación entre alimentación y salud, es por ello que se ha aumentado el consumo de ciertos alimentos con efectos beneficiosos sobre la salud. Dado que las declaraciones nutricionales relacionadas con la salud influyen positivamente en las intenciones de compra de los consumidores, la elaboración de productos con un reclamo de salud hace que un producto alimentario sea más atractivo para el consumidor (Curutchet et al., 2019). Por ello la incorporación de bagazo de frutos rojos en diferentes productos alimentarios puede ser interesante para la elaboración de productos que posean un valor añadido más allá de la composición nutricional.

A la hora de formular productos alimentarios es necesario tener en cuenta que, cuando el bagazo es incorporado, puede haber interacciones entre los compuestos bioactivos y otros componentes de la matriz alimentaria. Estas interacciones pueden causar cambios en la absorción de nutrientes a nivel intestinal o incluso evitar la degradación de estos compuestos fenólicos a lo largo de todo el tracto digestivo (Diez-Sánchez et al., 2021). En este sentido es interesante estudiar los efectos que tiene el proceso de la digestión *in vitro* y el comportamiento de estos compuestos bioactivos en la matriz alimentaria.

El proceso de digestión sucede en varias fases: la ingestión, la descomposición tanto mecánica como química de los alimentos, la absorción de nutrientes y la eliminación de alimentos no digeribles. La primera transformación estructural que se produce en los alimentos durante su ingesta sucede en la boca. Por tanto, la masticación se considera el primer paso del proceso de digestión y consiste en triturar el alimento en piezas pequeñas e impregnarlas con saliva para formar un bolo que se pueda tragar fácilmente. La disminución en el tamaño de partícula aumenta la superficie disponible para el ataque de las enzimas digestivas a lo largo de todo el tracto gastrointestinal, favoreciendo la digestión. Estas enzimas se encargan fundamentalmente de romper las moléculas de los principales componentes de los alimentos -hidratos de carbono, lípidos y proteínas- produciendo un importante cambio en la estructura de los componentes y, en consecuencia, en sus interacciones.

En el intestino delgado es donde se lleva a cabo el proceso de absorción de nutrientes en la circulación sistémica, lo que no se ha absorbido pasa al colon, donde es metabolizado y fermentado por las bacterias.

Los ensayos *in vitro* permiten la simulación de las condiciones que se dan a lo largo de todo el proceso de digestión en el organismo. Estos ensayos son ampliamente usados para conocer el comportamiento de un alimento en el tracto gastro-intestinal ya que son más rápidos, baratos y reproducibles que realizar estos mismos ensayos *in vivo*. En los ensayos de digestión *in vitro* normalmente se incluyen la fase oral, gástrica e intestinal (intestino delgado), y en menor medida la fase de fermentación en el intestino grueso. En cada una de las etapas se tratan de imitar las condiciones reales, tales como el pH, tiempo de digestión de cada fase, concentración de sales y la presencia de enzimas y su concentración (Minekus et al., 2014) (Figura 4). Por lo tanto, este



FASE ORAL

- Descomposición mecánica
- Hidrólisis enzimática
  - Amilasa salivar

SALIVA
pH 7
2 min

FASE GÁSTRICA

- Agitación
- Hidrólisis enzimática
  - Pepsina
  - Lipasa gástrica

FLUIDOS GÁSTRICOS
pH 3
1-2h

FASE INTESTINAL

- Agitación
- Hidrólisis enzimática
  - Pancreatina o enzimas individuales (tripsina, quimiotripsina, lipasa pancreática, colipasa y amilasa pancreática)
  - Bilis

FLUIDOS INTESTINALES
pH 7
2h

Figura 4. Esquema de proceso de digestión *in vitro* de alimentos basado en Minekus et al. (2014)

tipo de ensayos puede ser usado para conocer la bioaccesibilidad de los compuestos bioactivos y cómo varía en función del alimento en que se encuentren y las interacciones que se den lugar. La cantidad de compuestos bioactivos con capacidad antioxidante a lo largo del tracto gastro intestinal dependerá de la estabilidad digestiva y su liberación de la matriz alimentaria (Palafox-Carlos et al., 2011).

Según los antecedentes expuestos, diseñar y desarrollar ingredientes, a partir del bagazo de frutos rojos, es una línea de acción importante porque supone la revalorización y el aprovechamiento de estos subproductos, procedentes de la elaboración de zumos de frutos rojos, y da solución a un importante problema de gestión medioambiental. Por otra parte, la incorporación de estos ingredientes, con elevado contenido en polifenoles y fibra, en la formulación de alimentos, es de gran interés porque va a permitir el desarrollo de alimentos con efectos beneficiosos sobre la salud. Para conseguirlo, es necesario profundizar en el estudio de las interacciones entre estos subproductos y sus componentes con los otros componentes de los alimentos, en el impacto que ejercen en las propiedades fisicoquímicas y sensoriales del alimento y en su bioaccesibilidad.

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Use of berry pomace to design functional foods

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A B S T R A C T

The demand for healthy products has led researchers and industry to develop sustainable foods with high nutritional properties. Berry pomace is a by-product from the juice industry, and a valuable source of bioactive compounds. Its composition allows it to be used as a functional ingredient with antioxidant and antimicrobial properties. Moreover, pomace possesses specific techno-functional properties that can lead to changes in the characteristics of the food where it is incorporated. The objective of this work is to collect current knowledge of composition, nutritional, and techno-functional properties of berry pomace, and its use as an ingredient in different foods.

Keywords: by-product; valorization; polyphenols; dietary fiber

1. INTRODUCTION

To date, there is a growing market for natural foods where, beyond the nutritional benefit, a health benefit is obtained. This growth is driven by a growing demand for healthy food from an increasingly health-conscious consumer base. These food products are known as "functional foods," which are defined as:

"*the food comprising at least one ingredient which when consumed brings a beneficial effect exceeding the normal and adequate nutritional effect, whereas this ingredient (or these ingredients) may be naturally present in food, or food may be fortified (enriched) with it*" (Tanska et al., 2019).

Therefore, the food and beverage industries' technology has progressed and explored alternative sources of bioactive ingredients because of its great interest and importance (Granato et al., 2020; Sun-Waterhouse, 2011).

Generation of food waste is a great concern in the social, economic, and environmental points of view. Main surplus in fruit and vegetable processing are by-products and production line waste. The by-products are attracting special attention as functional, novel, and inexpensive ingredients (Déniel et al., 2016), because these by-products contain health-promoting phytochemicals and fibers. It is not a matter of trying to avoid waste generation, but to avoid the loss of the added value these by-products provide as health-promoting phytochemicals and fibers (May & Guenther, 2020). Thus, to change the concept of the by-product as a waste or something disposable (discarded material) toward something usable such as feedstock or raw material in food production, providing an added value is key (Banerjee et al., 2017; Majerska et al., 2019).

The by-products of berry juice processing are promising sources of dietary fiber and may be useful as a value-added ingredient with enhanced benefit for the formulation of functional foods and nutraceuticals (Alba et al., 2019; Mokhtar et al., 2018; Vagiri & Jensen, 2017). Especially berry pomace, which comprises skin, pulp, and seeds, still have high amounts of valuable compounds. The seeds contain oil rich in polyunsaturated fatty acids and other pomace parts are rich in phenolic compounds and dietary fibers, which hold the characteristics required to be considered as functional ingredients due to their effects on human health (Quirós-Sauceda et al., 2014). Polyphenols can vary in their chemical structure and properties, from simple molecules, such as phenolic acids, to highly polymerized molecules, such as proanthocyanidins. Its characteristic structure confers them its antioxidant capacity, which is the property related to health benefits (Fraga et al., 2019). Further, the effects of polyphenols in the prevention of certain diseases such as cancer (Lavefve et al., 2020; Sharma et al., 2017) or type 2 diabetes (Gowd et al., 2018), and the alleviation of symptoms from gut inflammation (Lavefve et al., 2020) have been studied.

Notably, despite the high content of polyphenols in pomace, they are usually trapped or associated with fiber, which can affect their bioavailability and bioaccessibility. Therefore, their antioxidant capacity effect will be also affected (Quirós-Sauceda et al., 2014). During the *in vitro* digestion, the polyphenols may be released, and can undergo changes throughout the gastrointestinal tract due to the different conditions that occur along it (Wojtunik-Kulesza et al., 2020). For example, at low pH (gastric phase) anthocyanins, one of the most found phenolic compounds in berries, are in their most stable form, whereas at higher pH (intestinal phase) they can undergo modifications that make their absorption difficult (Braga et al., 2018; Fleschhut et al., 2006). Moreover, these polyphenols can interact with the different macronutrients in food, causing changes in the absorption of both phenolic compounds and some of the major components such as protein, fat, and carbohydrates (Diez-Sánchez et al., 2021). The primary mechanisms involved are the formation of complexes or the inhibition of certain enzymes.

Giving an added value to these fruit residues by finding applications like food additives and valuable ingredients for functional food is important (Campos et al., 2020). It is also a promising trend for achieving a substantial reduction in waste generation, one of the targets of the sustainable development goals set by FAO for the 2030 agenda (FAO, 2015). Thus, in accordance with these consumer trends, using by-products generated from food industries could be a great opportunity to design healthy and natural food.

Thus, this review summarizes the current information available about berry pomace composition, nutritional value, and techno-functional properties. It is also focused on applying the different berry pomaces as an ingredient in food products.

2. WHY CHOOSE BERRY POMACE?

The pressing of berries in the fruit juice industry generates two materials: juice and pomace. Berry pomace comprises peel, stems, and seeds. As described before, the pomace has an added value and could be used in the formulation of functional foods. However, one must remember that the pomace is a high-moisture product susceptible to microbial growth if storage conditions are not well controlled, thus, before being introduced into a formulation it must be processed (Gouw et al., 2017; Majerska et al., 2019).

2.1. Berry pomace processing: drying

To facilitate its storage and stability, the pomace is subjected to moisture content reducing methods; for this, drying can be a good option to maintain its quality.

The drying process minimizes chemical and biological reactions during storage due to water removal (Majerska et al., 2019). However, drying processes affect the structure of the products giving place to powders with different solubility, bulk density, porosity, and color (Michalska, Wojdyło, Lech, et al., 2017). Moreover, drying can produce degradation of bioactive compounds, such as polyphenols; there is a greater degradation with the increase in the processing temperature (Michalska, Wojdyło, Łysiak, et al., 2017; Zielinska & Michalska, 2018). Thus, it is important to choose the right drying process to obtain the best quality in the final products.

Hot-air convective drying is one of the most common method used for pomace powder obtention, but the main disadvantages are related to the degradation of phenolic compounds, such as anthocyanins, with high processing temperatures (Michalska, Wojdyło, Łysiak, et al., 2017; Zielinska & Michalska, 2018). High temperatures (from 60 to 125 °C) during processing are related to a reduction in the final phenolic content, and thus antioxidant capacity (Khanal et al., 2010; Michalska,

Wojdyło, Łysiak, et al., 2017). To overcome this problem, other types of drying have been developed. An example is the microwave-assisted drying, this does not involve high temperatures and long times and could retain bioactive compounds present in non-processed pomace, but it has high installation and operating costs (Zielinska & Michalska, 2018). However, good results are not always obtained with using microwave-assisted drying; Oliveira et al. (2019) found there were slight differences in phenolic compounds between hot-air drying and microwave-assisted drying. To optimize costs, both techniques could be combined; Zielinska et al. (2018) found that hot-air drying at 60 °C combined with microwave-assisted drying allowed shortening the time of hot-air drying and thus avoiding the chemical changes that occur with longer treatment times, and to lower the cost when using microwave-assisted drying alone.

Kerbstadt et al. (2015) reported on different novel drying techniques such as infrared drying, infrared impingement drying, and microwave-assisted hot-air drying and compared them to freeze drying in bilberry press cake. They found that novel drying techniques could better preserve the anthocyanin content in comparison with freeze drying. In another study, Grimm et al. (2020) compared continuous cyclone drying with the conventional batch fixed-bed convective drying on press cakes from different berries (bilberry, blackcurrant, and cloudberry). The results showed that if both drying processes are conducted at the same temperature, total phenolic contents are very similar regardless of the technique used. Thus, as cyclone drying consumes less energy, it could be a method for quick removal of free water before other drying processes.

There are different drying methods and process conditions, and the choice of their use will depend on various aspects such as cost, time, or phenolic degradation. However, it can be assured that high temperatures and time treatments, regardless of the drying method, negatively affect the phenolic content of the pomace.

2.2. Composition and nutritional properties of berry pomace

Besides the phenolic compounds, the pomace is composed of other compounds of great nutritional value. Table 1 summarizes the proximate composition of several berry pomaces. Notably, each fraction comprising the pomace is rich in different compounds. For example, regarding the lipid composition, the oil obtained from seeds is rich in γ-linolenic acid and stearic acid, whereas the seedless pomace is composed by policosanols and phytosterols (Dobson et al., 2012). Yao et al. (2021) found that raspberry pomace without seeds and the raspberry seeds not only differed in lipid composition but also contained different amount of free, soluble-bound and insoluble-bound phenolics; and the raspberry pomace free phenolics had higher antioxidant capacity. Seeds can be a valuable material for fat extract obtention, whereas the seedless fractions are rich in dietary fiber and phenolic compounds.

The dietary fiber present in pomaces is usually higher than 20% (Table 1). This compound has important functional properties, which will depend on plant source, isolation method, degree of processing, insoluble dietary fiber to soluble dietary fiber (IDF/SDF) ratio, and particle size. From a nutritional viewpoint, the sources of DF used for fortification of foods should have a ratio of IDF/SDF between 1:1 and 2:1 to yield maximum health benefits (Alba et al., 2019). Berry pomace widely exceeds the 2:1 IDF to SDF ratio usually (Table 1). In addition, the fiber pomace retains compounds with interesting characteristics such as polyphenols with antioxidant capacity bound to the cell wall. Thus, according to Saura-Calixto et al. (1998) the pomace fiber can be considered as an antioxidant dietary fiber because of its capacity to act as a carrier of phenolic compounds with a potential antioxidant effect.

The fat content ranges from 0.67 to 20.21% in the different berry pomaces, being the lowest for blueberry and the highest for blackcurrant. The fat content will depend mainly on the seed content, differing between berries and pomace processing conditions. Protein

Table 1. Proxymate composition of berry pomaces

Berry	*Dry weight (% w/w)*	*Moisture (% w/w)*	*Protein (% in DM)*	*Fat (% in DM)*	*Carbohydrates (% in DM)*
Bilberry	94.20	8.40	17.00	-	-
Black chokeberry	93.80-94.7	-	6.44-8.40	4.10-4.49	45.12
Black currant	89.21-96.80	3.43	11.80-17.00	2.0-20.21	2.20-14.90
Blackberry	95.50	-	10.80	10.80	-
Blueberry	53.62-81.45	1.96	4.67-12.03	0.67-5.43	60.94-87.05
Chokeberry	90.21-97.28	2.72	5.97-10.77	3.61-5.15	28.88
Cranberry	68.37-71.53	2.20-4.50	2.20-5.76	2.50-12.00	88.78-91.25
Goldenberry	94.13	5.87	15.89	13.72	61.00
Gooseberry	94.95	5.05	12.40	10.93	16.26
Raspberry	87.80	0.93	10.00	21.84	-
Red currant	94.82	5.18	11.76	14.23	12.65
Rowanberry	97.31	2.69	7.09	3.97	18.71

[k]DM: dry matter; TDF: total dietary fiber; SDF: soluble dietary fiber; IDF: insoluble dietary fiber

Ash (% in DM)	*TDF (% in DM)*	*SDF (% in DM)*	*IDF (% in DM)*	*References*
1.23	58.90	6.90	52.00	(Aura et al., 2015; Hilz et al., 2005; G. Oliveira et al., 2019)
1.80-1.95	42.00-63.50	-	-	(Sójka et al., 2013; Witczak et al., 2021)
1.95-4.10	20.18-90.8	3.97	55.16	(Hilz et al., 2005; Nawirska & Kwaśniewska, 2005; Pieszka et al., 2017; Reißner et al., 2019; Sójka & Król, 2009)
1.70	60.30	2.80	-	(Kosmala et al., 2017)
0.95-2.06	26.15-38.50	0.97-8.20	48.95-53.8	(Calabuig-Jiménez et al., 2018; Gouw et al., 2017; Kosmala et al., 2017; Reque et al., 2015; Ross et al., 2017, 2020; Tagliani et al., 2019)
1.92-6.94	21.79-95.77	7.04	52.46	(Mayer-Miebach et al., 2012; Nawirska & Kwaśniewska, 2005; Pieszka et al., 2017; Reißner et al., 2019; Rodríguez-Werner et al., 2019)
0.70-1.10	-	0.75-5.70	57.7-65.5	(Gouw et al., 2017; Ross et al., 2017, 2020; White et al., 2010)
3.50	16.74	-	-	(Mokhtar et al., 2018)
3.40		7.04	49.56	(Reißner et al., 2019)
4.10	59.50	0.34-2.30	38.13-57.2	(Gouw et al., 2017; N. R. McDougall & Beames, 1994)
3.00		7.00	51.08	(Puganen et al., 2018; Reißner et al., 2019)
2.84		7.68	59.49	(Reißner et al., 2019)

varies between 2.20 and 15.89%, with cranberry having the lowest values and goldenberry the highest.

Therefore, the importance of pomace composition mainly lies in its fiber content (mostly insoluble fiber) and in its phenolic compounds.

2.3. Polyphenols

Polyphenols from berry pomaces engage in a wide range of biological and antioxidant activities. Phenolic composition is different for each class of berry and also may vary within the same species but between cultivars (Bobinaitė et al., 2013; Tumbas Šaponjac et al., 2014) and years of harvest seasons (Sójka & Król, 2009). To determine the phenolic content of polyphenols and their antioxidant capacity, there are different methods usually based in UV/VIS spectrophotometric methodologies. The most widely spectrophotometric method used for phenolic content determination is the Folin Ciocalteau, which measures the total reducing capacity of a sample. Table 2 shows the total phenolic content values from different berries determined using this method.

However, these spectrophotometric methods only give the total phenolic content and do not identify the individual compounds. Therefore, more sensitive and specific procedures are needed. High-performance liquid chromatography (HPLC) is a simple and highly efficient procedure, which is sensitive and specific, but it is very time consuming and requires special expertise and equipment. Rodríguez-Werner et al. (2019) analyzed the chokeberry pomace phenolic composition. They found that the major compounds were polymeric procyanidins and glycosylated anthocyanins such as cyanidin-3-*O*-galactoside and phenolic acids such as neochlorogenic and chlorogenic acid. Likewise, Sojka et al. (2013) obtained similar results for black chokeberry pomace, showing proanthocyanins (polymeric molecules) were the most abundant compounds, followed by the anthocyanins such as cyanindin-3-*O*-galactoside y cyanidin-3-*O*-arabinoside.

These polyphenols also present antioxidant capacity, which can also vary depending on the type of processing. The methods used for measuring the antioxidant capacity are also based in UV/VIS spectrophotometric methodologies, being the most used: ABTS (Trolox equivalent antioxidant capacity) (Calabuig-Jiménez et al., 2018; Jara-Palacios et al., 2019; Mayer-Miebach et al., 2012; Michalska, Wojdyło, Lech, et al., 2017; Reque et al., 2015; Zafra-Rojas et al., 2020), DPPH (Bobinaitė et al., 2013; Calabuig-Jiménez et al., 2018; Četojević-Simin et al., 2015; Metzner Ungureanu et al., 2020; Puganen et al., 2018; Reque et al., 2015; Sójka & Król, 2009; Su & Silva, 2006; Zafra-Rojas et al., 2020), and FRAP (ferric reducing antioxidant power) (Li et al., 2012; Michalska, Wojdyło, Lech, et al., 2017; Ross et al., 2017; Zafra-Rojas et al., 2020). These antioxidant capacity assays are based on single electron transfer reactions.

The antioxidant capacity derived from the phenolic compounds confers them certain characteristics, such as antimicrobial capacity (Aly et al., 2019; Bartkiene et al., 2019; Caillet et al., 2012; Das et al., 2017). However, the antimicrobial effect is not only due to the bioactive composition, the low pH values of pomace also affects the microbiological survival (Yin Lau et al., 2019).

Another important property derived from the antioxidant activity of polyphenols is the ability to reduce the lipid oxidation of food (Ospina et al., 2019). Food oxidation can be caused by oxygen free radicals or reactive oxygen species, and can alter the food texture, flavor, or odor, as well as reduce the shelf life. The antioxidant capacity of polyphenols is related to their ability to scavenge these free radicals. Different authors have studied the effect of berry pomace used as a natural ingredient to elude these oxidation processes (Bialek et al., 2016; Peiretti et al., 2020).

Furthermore, berries can have a prebiotic effect, due to the polyphenolic compounds capacity to improve the gut health favoring the growth of beneficial microorganism (Lavefve et al., 2020; Molan et al., 2009). However, this effect has not been studied in pomaces yet.

Table 2. Total phenolic content of different berry pomaces.

Berry	*Extraction conditions*	*Total phenolic content*	*Unit*	*References*
Bilberry	MetOH 70% + 1% trifluoroacetic acid	4552 ± 116	mg GAE/100 g DM	(G. Oliveira et al., 2019)
Black currant pomace	Acetone & sonication	2004 ± 17	mg GAE/100 g of sample	(Holtung et al., 2011)
	EtOH 92%; solid:solvent 1:2 (w/v); 30 min, room temperature, dark	5530 ± 1080	µmol GAE/100 g FW	(Puganen et al., 2018)
	MetOH:water:formic acid at 50:48:2 v/v/v; solvent:solid 10:1; sonication 5 min and dark 15 min	2077.0 ± 44.4	mg/100 g pomace	(Sójka & Król, 2009)
	MetOH:water:formic acid at 50:48:2 v/v/v; solvent:solid 10:1; sonication 5 min and dark 15 min	2241.6 ± 81.9	mg/100 g pomace	(Sójka & Król, 2009)
Blackberry pomace	EtOH 50%; 1:10 solid:solvent (w/v); 20 °C 48h	3967.7 ± 21.43	mg GAE/100 g DM	(Metzner Ungureanu et al., 2020)
	EtOH:water 1:1; 1:10 solid:solvent (w/v); 20 °C 48h	2828.66 ± 12.1	mg GAE/100 g DM	(Metzner Ungureanu et al., 2020)
	MetOH 75% (1% HCL 1N); solid:solvent 1:5; 12h 25 °C	1699.62 ± 174.50	mg GAE/100 g DM	(Jara-Palacios et al., 2019)
	MetOH/water (50:50, v/v); 1h, room temp + acetone/water (70:30, v/v); 1h, room temp	4016.43 ± 13.44	mg GAE/100 g DM	(Zafra-Rojas et al., 2020)
Blueberry pomace	MetOH 80%; solid:solvent 1:100 w/v	3.02 ± 0.12	mg GAE/100 g DM	(Calabuig-Jiménez et al., 2018)
	MetOH 75% (1% HCL 1N); solid:solvent 1:5; 12h 25 °C	1954.54 ± 177.82	mg GAE/100 g DM	(Jara-Palacios et al., 2019)
	EtOH 70%+ glacial acetic acid 0.5%; 24h 4 °C in dark	2694 ± 29	mg GAE/100 g DM	(Li et al., 2012)
	MetOH 70%; solid:solvent 1:10; 60 min	3113 ± 34	mg GAE/100 g DM	(Ross et al., 2017)
	HCl 1% in metOH; solid:solvent 1:30 (w/v); 60 min, room temperature	2920 ± 58	mg GAE/100 g sample	(Su & Silva, 2006)
	HCl/metOH/water (1:80:10 v/v); solid:solvent 1:20; 2h, room temperature	10234 ± 244	mg GAE/100 g DM	(Tagliani et al., 2019)
Blueberry extract	EtOH 80%; solid:solvent 1:5 (w/v); 1h	7201 ± 193	mg GAE/100 g DM	(Ross et al., 2017)
Chokeberry pomace	MetOH 75% + Formic acid 0.1%	6310 ± 50	mg GAE/ 100 g FW	(Kapci et al., 2013)
	MetOH 60%:water:formic acid (85:12:3)	3100 - 6300	mg / 100 g wb	(Mayer-Miebach et al., 2012)

Cranberry pomace	MetOH 70%; solid:solvent 1:10; 60 min	2487 ± 66	mg GAE/100 g DM	(Ross et al., 2017)
Cranberry extract	EtOH 80%; solid:solvent 1:5 (w/v); 1 h	5435 ± 85	mg GAE/100 g DM	(Ross et al., 2017)
Green currant	EtOH 92%; solid:solvent 1:2 (w/v); 30 min, room temperature, dark	1710 ± 290	µmol GAE/100 g FW	(Puganen et al., 2018)
Raspberry extract	MetOH 80%; 60 min, shaking	14934 ± 401	mg GAE/100 g dry extracts	(Bobinaitė et al., 2013)
	MetOH 80%; 60 min, shaking	9388 ± 445	mg GAE/100 g dry extracts	(Bobinaitė et al., 2013)
	MetOH 80%; 60 min, shaking	13243 ± 457	mg GAE/100 g dry extracts	(Bobinaitė et al., 2013)
	MetOH 80% y acetic acid 0.05%	4370 ± 202	mg GAE/100 g dry extracts	(Četojević-Simin et al., 2015)
	MetOH 80% y acetic acid 0.05%	2630 ± 128	mg GAE/100 g dry extracts	(Četojević-Simin et al., 2015)
Red currant	MetOH 75% (1% HCL 1N); solid:solvent 1:5; 12h 25 °C	3446.59 ± 805.17	mg GAE/100 g DM	(Jara-Palacios et al., 2019)
	EtOH 92%; solid:solvent 1:2 (w/v); 30 min, room temperature, dark	2050 ± 710	µmol GAE/100 g DM	(Puganen et al., 2018)
Red raspberry pomace	MetOH 75% (1% HCL 1N); solid:solvent 1:5; 12h 25 °C	2014.66 ± 100.91	mg GAE/100 g DM	(Jara-Palacios et al., 2019)
Sea buckthorn north pomace	EtOH 92%; solid:solvent 1:2 (w/v); 30 min, room temperature, dark	1360 ± 110	µmol GAE/100 g FW	(Puganen et al., 2018)
Sea buckthorn south pomace	EtOH 92%; solid:solvent 1:2 (w/v); 30 min, room temperature, dark	880 ± 60	µmol GAE/100 g FW	(Puganen et al., 2018)
White currant pomace	EtOH 92%; solid:solvent 1:2 (w/v); 30 min, room temperature, dark	2470 ± 440	µmol GAE/100 g FW	(Puganen et al., 2018)
Yellow raspberry extract	MetOH 80%; 60 min, shaking	10124 ± 323	mg GAE/100 g dry extracts	(Bobinaitė et al., 2013)

*GAE: galli acid equivalents; DM:dry matter; FW:fresh weight; wb:water basis

2.4. Techno-functional characteristics of berry pomace

Berry pomace has specific techno-functional properties, which must be known before incorporating pomace into food products. Several authors have studied different techno-functional properties of berries such as water and oil absorption, swelling and foaming capacity, and bulk density. These properties influence other important characteristics of the final product, e.g.: water absorption has a key role on texture quality, and oil absorption is significant in terms of consistency and bulking.

Reißner et al. (2019) observed that low water binding capacity was related to small particle sizes, whereas larger surface areas favored water adsorption; however, the oil absorption capacity was related to particle porosity rather than chemical composition or molecular affinity to oil. Mokhtar et al. (2018) obtained similar values to Reißner et al. (2019) for water and oil absorption in goldenberry pomace, being the oil absorption lower than water absorption, which they attributed to the higher number of hydrophilic groups capable of binding water, and to the soluble dietary fiber content that possess high water absorption capacity. In addition, Gouw et al. (2017) explained that the property of oil absorption could be due to the fiber-fiber interactions that would be expose the hydrophobic surface to adsorb oil.

Swelling capacity is highly related to water absorption, thus lower water absorption will be in accordance with low swelling capacity. Reißner et al. (2019) and Moktar et al. (2018) obtained similar results for this parameter. Mokhtar et al. (2018) also observed good foaming capacity and stability due to the berry pomace protein content, which formed a continuous cohesive film around the air bubbles in the film; this property mainly depended on pH, viscosity, and processing methods. Berry pomace also has low bulk density, which evaluates the suitability for its incorporation in food formulations (Mokhtar et al., 2018).

Thus, berry pomace can be used in the formulation of a wide range of food products; however, it is necessary to consider its techno-functional characteristics, which will affect rheological, textural, and sensory properties, as well as the nutritional value.

3. USES OF BERRY POMACE IN FOOD PRODUCTS

Functional foods can be classified as: fortified products (increased content of existing nutrients), enriched products (incorporation of new nutrients), altered products (replacement of existing nutrients by others with beneficial functions), and enhanced commodities (alteration of nutrient composition by changes in the raw commodities) (Domínguez Díaz et al., 2020). In all the cases using pomace in the formulation influences their nutritional, physicochemical, or organoleptic characteristics. There are different ways of incorporating the pomace in food. It can be incorporated as a fresh or dried ingredient, and sometimes, an extract of the phenolic compounds is obtained from the pomace and then added to the food.

As phenolic compounds are bound to the cell wall, using a polyphenolic extract from the berry pomaces for specific purposes is interesting. Thus, a high concentration of bioactive compounds excluding other compounds such as dietary fiber and proteins, is obtained. Polyphenols are soluble in water and in alcohol due to its conformation and chemical structure; consequently, most extraction methods use water, ethanol, or their mixtures as solvents. The main extraction methods are mentioned in Table 3.

Traditional methods of extraction are mostly based on using high amounts of solvents that generates large amounts of waste that can be hazardous. These methods use heat to improve mass transfer and usually long extraction times that could have a risk on bioactive thermal degradation. To overcome this problem and mitigate the limitations associated to these extraction methods, innovative technologies

Table 3. Extraction methods for obtaining berry pomace extracts

Extraction methods		***References***
Traditional methods		(L. Laroze et al., 2010; Seabra et al., 2010; Wajs-Bonikowska et al., 2017; Woźniak et al., 2017)
Novel extraction processes	Supercritical fluid extraction	(Kraujalis et al., 2017; L. E. Laroze et al., 2010; Wajs-Bonikowska et al., 2017; Wenzel et al., 2020; Woźniak et al., 2017)
	Microwave-assisted extraction	(Ferreira et al., 2020)
	Ultrasound-assisted extraction	(Galván D'Alessandro et al., 2014; He et al., 2016; Klavins et al., 2018; Sady et al., 2019; Xu et al., 2017; Zafra-Rojas et al., 2020)
	Pulse electric field extraction	(Bobinaitė et al., 2015)
	High pressure extraction	(Grunovaite et al., 2016; Kitrytė et al., 2017; Tokuşoğlu, 2016)
Green chemistry-based extraction techniques	Enzyme-assisted extraction	(Karaś et al., 2017; Kitrytė et al., 2017; Saad et al., 2019; Szymanowska & Baraniak, 2019)

are being investigated to meet the ongoing consumer demands for minimally processed products, and to meet the requirements of a green extraction concept. The main extraction methods used for berry pomace extraction are summarized in Table 3.

3.1. Berry pomace in bakery products

In the last years, the bakery industry has been developing products toward the functional food market; these products provide an ideal matrix to introduce functional ingredients with potential health benefits, supplying a product capable of satisfying consumer demands in terms of appearance, taste, and texture (Alba et al., 2019; Granato et al., 2020; Siró et al., 2008). Pomace incorporation into bakery products is simple and quick to manage, which is why it has aroused great interest, especially in products made with white flour, such as white bread, cookies, cakes, or muffins. However, berry pomace incorporation leads to certain changes in the food, thus it is useful to know its impact on dough technological properties to obtain a good integration of the by-product into bakery products; achieving greater acceptance of the product by the consumer (Gómez et al., 2003; Laurikainen et al., 1998; Struck et al., 2018).

From a technological viewpoint, the composition of the pomace, as well as its ratio of insoluble and soluble fiber (IDF/ SDF) influences the rheological behavior of the dough of bakery products, the development time, and the rate of water absorption in the mixture (Alba et al., 2019). Specifically, berry pomaces contain a higher insoluble fraction (cellulose, hemicellulose, and lignin) than soluble, which causes alterations, such as the reduction in the hydration rate of wheat proteins due to the porosity of insoluble fiber that facilitate the water

absorption. This effect leads to a weaker gluten network, which causes detrimental effects for the creation of a well aerated structure, partly due to a decrease in the air retained by the dough, which results in a firm, less fluffy texture and a reduction in volume in the product (Alba et al., 2019; Diez-Sánchez et al., 2020; Foschia et al., 2013).

3.1.1. Bread

Bread is a product consumed daily, and it has seen a growing interest in incorporating functional ingredients, since it is a key vehicle for the contribution of fiber and bioactive compounds to the population. However, good acceptance by the consumer must be guaranteed to compete with conventional or traditional wheat bread (Gallagher et al., 2004; Martins et al., 2017).

Struck et al. (2018) partially replaced wheat flour (10, 20, or 30%) with dried blackcurrant pomace to analyze the behavior of model bread doughs. They observed that increasing quantities of the by-product caused an increase in dough water absorption, dough development time, and structural strength when the model doughs were baked, leading to the disruption of the gluten matrix. They concluded that lower substitutions (10%) give doughs with desirable characteristics, whereas higher substitutions would need the use of additives to increase the gluten strength. Likewise, Alba et al. (2020) not only observed the effects of flour replacement with blackcurrant pomace (5, 10, 15, or 20% w/w) in bread dough, but also in the final baked bread. Incorporating berry pomace decreased the extent of gluten hydration resulting in stiffer doughs, modifying the morphology of gas bubbles, and resulting in breads with lower specific volume and harder crumb structures.

Furthermore, Martins et al. (2017) used the fiber rich fraction from elderberry pomace as fortification in bread formulations (5, 7, and 10%, added in % wheat flour). The pomace addition caused a higher

percentage of small cells, but lower cells distributed in the medium. The authors observed that higher acceptability was obtained for breads with a 7% pomace addition when compared to the control. Thus, they concluded that bread sensory characteristics were influenced by adding a fiber rich fraction from elderberry pomace.

Reiβner et al. (2020) studied blackcurrant pomace addition into bread, and highlighted the importance of considering the hydration of dietary fiber, which is often neglected in bakery products because the degree of hydration could substantially affect the bread-making process. The pre-hydration of pomace affected the water absorption of dried pomaces; the hydrated fiber had less competition for water, allowing the correct gluten network development enhancing the final bread properties.

In terms of physical properties, the pomace incorporation into breads mainly lead to textural changes and these changes were also appreciable in sensory analysis. The physical and sensory properties of doughs and breads have been studied by different authors, but more studies are needed to understand how the pomace incorporation affects the nutritional quality in terms of bioactive compounds such as fiber and phenolic compounds.

3.1.2. Other bakery products

Incorporating berry pomace in different bakery products such as cookies, muffins, or cakes has also been studied.

Cookies are widely consumed baked foods; they usually contain flour, sugar, and some oil or fat. They may include other ingredients such as raisins, oats, chocolate chips, nuts, fiber, etc. A way of incorporating fiber is using berry pomace. For example, Górecka et al. (2008) incorporated raspberry pomace in short crust cookies as a partial replacement (25 and 50%) of flour to obtain cookies with an added health claim for fiber content to improve the nutritional profile. They obtained cookies with

high contents of fiber without a negative influence on organoleptic properties.

The pomace has not only been used for improving the nutritional profile but also for the different physicochemical characteristics it can provide to the cookies. In the study carried out by Tańska et al. (2016), the pomace of different fruits such as blackcurrant and elderberry, among others, was used in cookies formulations. These pomaces were used at different concentrations (5, 10, 15, 20, 25, 30, and 50% w/w) as a flour replacement. Specifically, 20% blackcurrant pomace stood out for its high anthocyanin content, contributing significantly to the total phenol content in cookies. Regarding the characteristics of the cookies and compared to controls, adding pomace maintained their round shape, diameter, and thickness. They showed a greater hardness, as the percentage of replacement increased, although the organoleptic evaluation showed that a more crunchy and hard texture was still desirable by the panelists. Furthermore, the inhibition efficiency of the formation of free radicals, which indicates the oxidative stability of the lipid fraction, was evaluated, showing the oxidation of lipids was negligible when pomace was used. The antioxidant effect of berry pomace extracts has been also studied. Bialek et al. (2016) used a chokeberry polyphenolic extract instead of the pomace in cookies, and observed that it had a significant impact decreasing oxidation levels even during the storage period in which oxidative changes can occur. Thus, chokeberry polyphenolic extract can extend the shelf life of cookies in terms of lipid oxidation. However, high contents of this extract can give undesirable sensory characteristic, such as taste, decreasing the consumer acceptance.

Therefore, besides the changes in physicochemical properties, it is important to assess if incorporating berry pomaces has a detrimental effect on sensory characteristics. Curuchet et al. (2019) evaluated the sensory attributes of cookies enriched with antioxidant fiber from blueberry pomace. They found that even though the consumers were positively influenced by the appearance, most consumers negatively

ranked the acceptability when the cookies were tasted. Thus, if pomace fiber is used as an ingredient, the formulation needs to be optimized considering the consumers' appeal.

A similar cookie product is crackers, which are savory or salty flat biscuits usually formulated with wheat flour and different gluten-free flours. Schmidt et al. (2017) incorporated dried blueberry pomace to a formulation as a total or partial substitution of gluten-free flours. This incorporation restricted the development of the protein network due to the alterations caused by fiber interactions, which with the low starch content resulted in low extensible doughs and soft crackers (Schmidt et al., 2017). However, the general acceptance was not affected by the pomace incorporation.

Other widely consumed sweet bakery products are cakes and muffins, which are mainly made with eggs, flour, oil, and sugar, sometimes leavened with baking powder. Quiles et al. (2018) showed the effect of using dried pomace of black currant and chokeberry berries as a substitution of flour, fat, or sugar in cakes. The substitution of the three components had an influence on dough and cake properties, because they all play a key role in developing the product. Sugar replacement caused a premature gelatinization of the starch, reducing the competition for water, causing a higher cake dough viscosity, giving rise to a harder final cake, and with a greater formation of small alveoli in the crumb. Flour replacement led to softer cakes with an increase in the size of crumb alveoli, which could be related to the decrease in the capacity to retain gas. Fat replacement gave doughs and cakes with intermediate values for cake texture, and number and size of alveoli. The *in vitro* starch digestibility was also studied and cakes with flour replacement showed the lowest hydrolysis and glycemic indices, whereas fat replaced cakes showed the highest values.

Thus, incorporating pomace in cakes and muffins possesses some aeration problems in the dough that affects the final characteristics of the product. Some alternatives that have been studied to improve this

problem are the use of different leavening agents, such as encapsulating bicarbonate with citric acid or sodium acid pyrophosphate. The combination of pyrophosphate with bicarbonate improved the incorporation of air into the product formulated with pomace, leading to larger gas alveoli and a smoother texture (Diez-Sánchez et al., 2020).

Another alternative to aeration problems is the use of pregelatinized flours. Diez-Sánchez et al. (2019) substituted 50% of wheat flour for extruded wheat flour in muffins formulated with dry blackcurrant pomace at 20% w/w. This allowed the formation of a more compact and consistent network with a more homogeneous fat distribution, and the muffins did not present differences in texture properties or consumer acceptance compared to a standard formulation. Despite the benefits of pregelatinized flour on texture, it can increase the glycemic index, which can be a negative effect; however, this increase is counteracted by the inhibitory effect of berry pomace on digestive enzymes such as α-amylase (G. J. McDougall et al., 2005).

Other studies are related to the optimization of the production parameters to obtain a product with optimal characteristics. Mildner-Szkudlarz et al. (2016) used European raspberry and American cranberries dried pomaces as wheat flour replacers (10 and 20% (w/w)) in muffins and studied different baking conditions and their effect on microstructure, texture, and phenolic content. The baking conditions affected the texture and the polyphenol content. Moreover, the optimal baking conditions were 180 °C for 20 min, when the best texture properties were obtained without excessively affecting the phenolic content. Gornas et al. (2016) also studied how baking temperature (140, 180, and 220 °C) and type of oven (conventional or halogen) affected the stability of muffins enriched with blackcurrant and raspberry pomaces, among other fruit pomaces. The muffins remained in the oven until the temperature inside the muffins reached 107 °C. An increase of free ellagic acid content was positively correlated with the baking time, which resulted from thermal hydrolysis of ellagitannins and ellagic acid glycosides. Short baking time (14 min) combined with

the highest temperature (220 °C) in the conventional oven had the best effect on polyphenol preservation. Finally, the reformulated muffin did not differ from the control in terms of overall acceptability.

3.1.3. Gluten-free products

Gluten-free bakery products on the market entail a higher cost for the consumer compared to their gluten-containing counterparts. These products are usually reformulated with refined flours and starches, thus sacrificing its nutritional profile in terms of fiber content (Šarić et al., 2019). Despite being numerous investigations on new alternatives to replace refined flours and starches, investigation is still necessary to obtain products with physicochemical, nutritional, and sensory characteristics acceptable to the consumer. A good alternative is the reuse of low-cost ingredients, such as fruit by-products, which will contribute to improve nutritional and functional properties of gluten-free products (Šarić et al., 2019).

Pomace from blueberry and raspberry were incorporated in substitution of 30% of flour in gluten-free cookies formulated by Šarić et al. (2019). Even though the addition of pomace caused changes in the properties of the dough and appreciable sensory changes for smell, taste, and appearance, the overall acceptability was positively ranked. Regarding the nutritional profile of the cookies, notably, the enrichment with berry pomaces provided between 6.92 and 7.45 g / 100 g of fiber, depending on the berry used, but the fiber content was higher than 6 g per 100 g, which makes it possible to label the product as "high in fiber," thus satisfying around 30% of the dietary reference intake for dietary fiber, which is 25 g / day.

In another study carried out by Gagneteen et al. (2020), blackcurrant pomace was incorporated in a gluten-free chocolate cookie formulation. Their results showed that the blackcurrant pomace gave gluten-free cookies with higher fiber content and with interesting amounts of

polyphenolic compounds without affecting the acceptability of the final product. In addition, they suggested that if consumed, part of the phenolic compounds reach the large intestine where they can exert their antioxidant activity.

Therefore, although the pomace provides an extra fiber content in products with a deficiency in this component, it must be considered that it will affect the characteristics of the final dough, giving less extensible and weaker doughs. However, these products still had good acceptability by the consumers, besides an increase in their nutritional quality compared to gluten-free cookies present in the market. Consequently, it could be said that the pomace of berries can be incorporated in gluten-free doughs to provide an ingredient of greater value in both quality and health, improving the nutritional quality of common gluten-free bakery products.

3.2. Berry pomace in meat products

One of the greatest factors for deterioration in meat products is the oxidation of lipids, limiting their quality and acceptability because it causes rancidity, modification of the texture, undesirable odors, nutritional losses, and formation of detrimental compounds (Peiretti et al., 2020). In addition, if the meat is heat-treated (e.g. cooked), physicochemical changes are accelerated, causing a greater susceptibility of the meat to undergo oxidative reactions, with the consequent changes in the flavor that could eventually pose problems on the consumers acceptability (Ganhão et al., 2013; Peiretti et al., 2020; Yin Lau et al., 2019).

Specifically, the minced meat used to make hamburgers is often more prone to oxidative changes than the entire piece, since it has a greater contact surface exposed to the oxygen in the air, which causes its deterioration. These reactions modify the texture, causing undesirable odors and nutritional losses that can form toxic compounds, reducing the nutritional quality.

Synthetic antioxidants easily decompose at high temperatures and there is a potentially toxic effect in their use, thus the meat industry has looked for new alternatives to synthetic antioxidants (Ahmad et al., 2015), such as natural antioxidants derived from fruits or by-products like pomace (Lourenço et al., 2019; Muzolf-Panek et al., 2016).

Peiretti et al. (2020) analyzed the effect of blueberry pomace at a concentration of 1 and 2% w/w on oxidative stability of pork patties stored in refrigeration for 7 days. The pomace-pork patties showed a decrease in some volatile compounds, such as hexanal, indicating that lipid oxidation was lower than the control without pomace. This could be due to the high content of phenolic compounds of berry pomaces and their intense antioxidant activity, capable of stabilizing lipid oxidation, prolonging storage for 7 days. Other studies with pork meat, used frozen blackberry extract to reduce meat oxidation caused by light, heat, enzymes, metals, and microorganisms (Jia et al., 2012); using the extracts inhibited lipid oxidation, blocking the formation of free radicals. Furthermore, the formation of undesirable flavors and colors (metmyoglobin), which could damage the organoleptic characteristics of the meat, was avoided in the final product.

These studies show that using pomace can be a potential natural alternative for the substitution of synthetic phenolic antioxidants, allowing preservation of the quality of meat products for a longer time.

The antimicrobial properties of pomaces have been also widely explored. Thus, these by-products could be used as preservatives against pathogens in meat products. There are some preliminary *in vitro* studies related to using pomace to reduce the microbial growth of typical meat pathogens like *Campylobacter jejuni* (Salaheen et al., 2014) or *Escherichia coli* O157: H7, *Salmonella enterica*, and *Listeria monocytogenes* (Yin Lau et al., 2019). Salaheen et al. (2014), showed the effect of extracts from blackberry and bilberry pomaces on the growth and pathogenicity of *Campylobacter jejuni*. The results

showed that both extracts had a potential bactericidal effect; the blackberry extract had a long-term bactericidal effect extended until after 72 h, whereas the blueberry extract lost effect after 24 h. Consequently, it was shown that blackberry and blueberry pomace extracts have a high potential in the control of pathogens in meat and meat products, acting as natural and organic preservatives. Yin Lau et al. (2019) investigated the use of the ethanolic extract of lingonberry pomace for the inhibition of pathogens present in meat such as *Escherichia coli* O157: H7, *Salmonella enterica*, and *Listeria monocytogenes*. At the highest concentrations in the study, total inhibition was achieved, which the authors attributed to a combined effect of the acidic pH of the pomace and the high content of bioactive phenolic compounds.

Tamkutė et al. (2019, 2021) studied the effect of cranberry water and ethanol extracts and defatted chokeberry on bacterial cultures—*Listeria monocytogenes*, *Brochothrix thermospacta*, *Pseudomonas putida*, lactic acid bacteria, aerobic mesophilic bacteria— and on pork meat products—pork slurry, burgers, and cooked ham. Both cranberry water and ethanol extracts effectively inhibited the growth of tested bacteria. Using the ethanol extract was also effective in the meat products studied. It was also effective for the inhibition of malondialdehyde, which is a product from the lipid oxidation, and did not have a severe impact on some of the physicochemical properties of the meat products studied, although it provoked color changes that did not affect the overall acceptability.

In the study by Kryževičūtė et al. (2017) extracts of raspberry pomace were obtained using supercritical CO_2 and pressure liquid extraction (PLE) and added to beef burgers. They observed that using extracts isolated with PLE can be a way to inhibit lipid oxidation during storage and to reduce the spoilage microorganism load in meat. Moreover, its incorporation in burgers did not have a negative effect on sensory attributes and thus could be a promising natural additive for increasing the stability of meat products. Regarding defatted chokeberry extract,

the ethanolic extract (2%) inhibited bacteria in all the meat products studied; thus, it could be a potential natural antimicrobial ingredient extending the shelf life of meat products.

In the described studies, an antimicrobial effect both in *in vitro* studies and in meat products has been proven. Therefore, and due to the growing interest that the consumer has toward natural, organic food products without added chemicals, all this information is of special importance, since the berry pomace or its extract could be used as promising antimicrobial agents.

Berry pomace has also been investigated regarding its potential use as a promoter of starter cultures in meat. The fermentation of meat is an ancient form of preservation; during fermentation, certain autochthonous microorganisms grow, allowing the meat to acidify, inhibiting the growth of spoilage/pathogenic microorganisms. However, exogenous microorganisms are often added to achieve certain biochemical characteristics that the native flora cannot achieve; they are the so-called starter cultures. Its function usually is to cause an accelerated drop in pH, through the production of lactic acid, favoring the development of flavor, texture, and microbiological stability of the final product. In the study carried out by Yin Lau et al. (2019), the extract of cranberry pomace improved the yield and growth rate of some microorganisms used as meat ferments, such as *Lactobacillus* and *Pediococcus* spp. Thus, the phenolic compounds present in berry pomaces can stimulate the growth of meat starter cultures.

In conclusion, the addition of pomace or its extract in different meat products has shown an antioxidant effect, sequestering the oxidants species, and delaying the formation of toxic products, due to the polyphenols in their composition. It also has an antibacterial effect against spoilage and pathogenic microorganisms and can be a substrate for the growth of starter cultures. Likewise, the high dietary fiber content provides the meat with greater retention of moisture and fat, which is a positive influence on the cooking properties. This

makes it possible to extend the useful life of the product and reduce the environmental impact produced by meat waste.

3.3. Berry pomace in dairy products

Numerous studies indicate the fundamental role of dairy products in the world's diet's population. Yogurt is one of the most consumed dairy products, both for its organoleptic and nutritional value. Yogurt is easily digested, providing a bioaccessible matrix to incorporate bioactive compounds, thus obtaining a positive effect on health (Cutrim & Cortez, 2018). Using pomace in its formulation made it possible to fortify the product and provide it with suitable characteristics to consider it an excellent functional food (Raikos et al., 2018).

Several authors have studied the antioxidant effect of berry pomace when incorporated into yogurt. In the study carried out by Raikos et al. (2018) the incorporation of blackcurrant pomace extract was compared with a Gaultheria shallon berry extract in yogurt formulations. Incorporating blackcurrant pomace at 20% w/w caused an increase in total phenols in all yogurts stored for 4 weeks, although during storage there were fluctuations in the phenolic content. These fluctuations could be due to the release of amino acids with phenolic side chains, such as tyrosine, which could increase the phenol content when the proteolysis of milk proteins occurred (Raikos et al., 2018) or to the interactions of polyphenols with milk proteins forming insoluble complexes that could decrease the phenolic content (A. Oliveira et al., 2015). Regarding the antioxidant capacity, a decrease was observed when Gaultheria shallon extract was used, whereas using blackcurrant pomace increased antioxidant capacity was maintained during storage of 4 to 5 weeks (Raikos et al., 2018).

Beyond the antioxidant capacity, an antidiabetic effect has been observed in berry pomace by Ni et al. (2018). These authors used 20% w/w extracts of blackcurrant pomace to reformulate the original composition of yogurt and study its possible antidiabetic effect. The

extract promoted the gradual release of bioactive peptides during the storage of the yogurt, which inhibited the α-amylase and α-glucosidase enzymes, delaying the release of monosaccharides from complex carbohydrates and giving place to a reduction in plasma glucose levels. The possible inhibition mechanism could be because the polyphenols present in pomace bind to the active site of the enzyme, preventing its action (G. J. McDougall et al., 2005). These findings suggest that yogurt with extracts can help blood glucose regulation, although further research is needed to evaluate the bioactivity of peptides, and their potential as α-amylase and α-glucosidase enzymes inhibitors (Ni et al., 2018).

Besides using pomace or its extract as antioxidant or antidiabetic, it has been used for other purposes. For example, Diez-Sánchez et al. (2020) studied the effect of different processing conditions with high hydrostatic pressures (200-500 MPa; 1-10 min) in milkshakes with chokeberry pomace, to obtain the treatment that provided a higher phenolic content and a higher antioxidant capacity with the lowest survival of the pathogenic microorganism *Listeria monocytogenes*. The treatment that obtained the best results was at 500 MPa for 10 min and with a pomace concentration of 10%.

Thus, berry pomace can be a great option in dairy products as an ingredient that could improve its nutritional value and provide beneficial properties (antioxidant, antidiabetic…).

3.4. Berry pomace in snack foods

Snack products are widely consumed but have low nutritional value. Thus, Drożdż et al. (2019) formulated extruded puffed corn snacks with chokeberry and blackcurrant pomace to obtain snacks with an enhanced nutritional value, increasing the total phenolic content. The pomace incorporation affected the expansion rate, the water absorption, and solubility index, giving lower values by increasing proportions of pomace. They concluded that the puffed snacks with berry pomace

addition can be an interesting functional food due to its high content of phenolic compounds and antioxidant capacity. In another study carried out by Mäkilä et al. (2014) snacks were formulated with blackcurrant pomace (30%), cereal materials (40%- oat flour, barley flour, or oat bran), potato starch (30%), sugar, and salt. The results showed that the snacks had desirable physicochemical characteristics, but with the need to improve sensory characteristics. The authors observed that maintaining the berry-like characteristics was important for consumers.

Wang et al. (2019) studied the effect of cranberry and blueberry pomaces incorporation on the expansion characteristics of corn starch extrudates. The expansion was influenced by several factors such as the pomace level incorporation, and specifically, the authors observed that pomaces with high soluble dietary fiber led to higher expanded extrudates. They concluded that the different results were due to the interactions between the pomace components and the starch.

Therefore, incorporation of berry pomace in extruded snacks can be a good alternative for developing new functional foods. Its incorporation did not affect the properties negatively, but the sensory aspects should be improved.

3.5. Other applications of pomace

In recent years there has been a trend toward natural polymers based on food products to replace non-biodegradable plastics. Compounds are incorporated into these polymers to turn them into active packaging material; besides the primary effect as a preservative, they include antioxidant or antimicrobial activities. Staroszczyk et al. (2020) used the aqueous extracts from rowanberry, blueberry honeysuckle, and chokeberry pomaces to formulate fish gelatin films. They observed an antioxidant, and an antimicrobial effect of berry pomace extracts due to its content in polyphenols, as well as improved mechanical and water barrier properties.

Other authors also studied the incorporation of a pomace extract as a new film-forming material. Park and Zhao (2006) developed films by adding cranberry pomace extract to provide a unique flavor and color. Singh et al. (2020) developed starch-based edible packaging films incorporating blueberry pomace powder and investigated the feasibility of using these films for food packaging.

Pomace has not been only used as antimicrobial or antioxidant material in film preparations, but also as a natural indicator in bio-based polymer packaging. Kurek et al. (2019) used blueberry and red grape skin pomace extracts as indicators of chicken meat quality in a novel bio-based polymer packaging. The extracts worked changing its color; the results showed there was a change in color film in correlation with pH changes of spoiled meat samples. Thus, the authors developed a colorimetric indicator of changes in perishable food products were changes in pH determine the spoilage of the product.

Therefore, berry pomaces and their extracts can be used as carriers of bioactive compounds not only for functional food production but also for developing active packaging or even a colorimetric bio-based sensor.

4. CONCLUSIONS

Using by-products rich in bioactive compounds to formulate foods is an emerging topic in food science research. The current review surveys the usage of berry pomace in bakery, dairy, and meat products, snacks, and some alternative uses like packaging films. From a nutritional viewpoint, the pomace provides food with important benefits, because it improves antioxidant capacity, microbiological stability, and avoids lipid oxidation; however, its techno-functional properties lead to changes in some final characteristics of the food texture and flavor. Berry pomace can be included in foods at significant levels while maintaining consumer acceptability and offering an added value. The valorization of berry by-products contributes to a sustainable food chain from an environmental viewpoint.

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Objetivos

OBJETIVO GENERAL

El objetivo general de este proyecto es valorizar el bagazo obtenido como subproducto en el proceso de elaboración de zumos de frutos rojos, mediante su uso como ingrediente en la elaboración de diferentes productos alimentarios y estudiar las interacciones que pueden ocurrir entre dicho ingrediente y las matrices alimentarias en las que se incorpora.

OBJETIVOS ESPECÍFICOS

- Estudiar el efecto de la incorporación de distintos tipos de fibra y de bagazo de frutos rojos en la formulación de productos horneados, sobre sus propiedades físicas, químicas, estructurales y sensoriales y sobre la digestibilidad *in vitro* del almidón.
- Estudiar el uso de diferentes impulsores químicos y de harinas pregelatinizadas como estrategias para la mejora de las propiedades estructurales, texturales y sensoriales de productos horneados formulados con bagazo.
- Estudiar el efecto de un tratamiento no térmico, las altas presiones hidrostáticas, sobre la estructura, el contenido fenólico y la capacidad antioxidante de los batidos formulados con bagazo y sobre la capacidad antimicrobiana de los compuestos bioactivos del bagazo.
- Estudiar el impacto de las interacciones entre los compuestos bioactivos del bagazo y los principales macronutrientes de los alimentos sobre el contenido fenólico, la capacidad antioxidante y la bioaccesibilidad de los compuestos fenólicos en diferentes sistemas modelo.

Estructura de la tesis

El trabajo de investigación realizado ha dado lugar a seis publicaciones científicas, las cuales se han estructurado en la introducción y en cuatro capítulos.

Todo el trabajo de esta tesis ha sido desarrollado dentro del Proyecto europeo BERRYPOM, establecido en la segunda convocatoria de la red de producción y consumo sostenible de alimentos (SUStainable FOOD Production and Consumption - SUSFOOD). En el proyecto se propone el análisis del valor del bagazo de frutos rojos con el objetivo de recuperar los compuestos bioactivos e incorporar el subproducto en alimentos para aprovechar los beneficios nutricionales derivados de la fibra y su contenido en polifenoles.

Con el fin de aunar y discutir la información disponible sobre la composición y propiedades del bagazo y su uso en diferentes alimentos, se elaboró un trabajo de revisión bibliográfica, que forma parte de la introducción, ya que contextualiza el resto de los trabajos elaborados en la tesis.

Para conocer, de forma general, la influencia de la incorporación de fibra sobre las propiedades estructurales y sensoriales de los alimentos se formularon bizcochos en los que se reemplazó la grasa por diferentes tipos de fibras (soluble, insoluble y una mezcla de ambas) y se estudió su efecto sobre la textura, estructura, aceptabilidad y digestibilidad *in vitro* del almidón de los bizcochos. La publicación resultante se incluye en el capítulo 1 de Resultados de la Tesis. Esta publicación ha permitido avanzar en el conocimiento sobre el impacto que puede tener la incorporación de bagazo, con alto contenido en fibra insoluble, en matrices alimentarias.

Adicionalmente, dentro del proyecto donde se enmarca esta tesis y antes de comenzar la misma se realizó un trabajo sobre el efecto del uso del bagazo de frutos rojos para reemplazar parcialmente harina, grasa o azúcar en bizcochos. La referencia del trabajo es la siguiente:

Quiles, A., Llorca, E., Schmidt, C., Reißner, A. M., Struck, S., Rohm, H., & Hernando, I. (2018). Use of berry pomace to replace flour, fat or sugar in

cakes. *International Journal of Food Science & Technology*, *53*(6), 1579-1587 (DOI: 10.1111/ijfs.13765).

En este trabajo se observó que independientemente del componente que se reemplace, y aunque los bizcochos son bien aceptados por los consumidores, se producen cambios importantes en la textura y en la estructura de los productos obtenidos. Por este motivo, en el capítulo 2 de Resultados de la tesis se plantean estrategias de mejora necesarias para contrarrestar el efecto que tiene el uso de bagazo en productos horneados (bizcochos y magdalenas). Dentro de este capítulo, se engloban dos trabajos.

En el primer trabajo se estudió el efecto de diferentes agentes leudantes y su combinación con bicarbonato encapsulado y no encapsulado en la mejora de las propiedades texturales, estructurales y sensoriales de bizcochos formulados con bagazo de frutos rojos.

En el segundo trabajo se estudió el efecto de la harina pregelatinizada como sustituta de la harina de trigo en magdalenas formuladas con bagazo para mejorar las características texturales y estructurales. También, se estudió el efecto de la incorporación de bagazo sobre la liberación de glucosa durante la digestión *in vitro.*

En el capítulo 3 se indaga sobre el uso de bagazo en la formulación de batidos y el efecto de las altas presiones hidrostáticas, como tratamiento no térmico, sobre los compuestos fenólicos y sus propiedades. Este capítulo engloba un solo trabajo en el que se estudiaron las condiciones óptimas de tratamiento por altas presiones para maximizar el contenido fenólico y la capacidad antioxidante de los batidos con bagazo y a la vez minimizar el crecimiento microbiano.

Por último, en el capítulo 4, se profundiza en el conocimiento sobre el proceso de digestión *in vitro* de alimentos con bagazo. Este capítulo está formado por un trabajo en el que se estudiaron las interacciones de los principales macronutrientes de los alimentos y los polifenoles del bagazo, así como su efecto sobre la bioaccesibilidad de los fenoles tras el proceso de digestión *in vitro.*

Las referencias de las publicaciones científicas derivadas de esta Tesis se presentan a lo largo de los capítulos en el siguiente orden:

INTRODUCCIÓN

Diez-Sánchez, E., Quiles, A., Hernando, I. (2021). Use of Berry Pomace to Design Functional Foods. Enviado a *Food Reviews International*

CAPÍTULO 1. ESTUDIO DEL EFECTO DE LA FIBRA SOBRE PROPIEDADES ESTRUCTURALES Y TEXTURALES EN PRODUCTOS HORNEADOS

Diez-Sánchez, E., Llorca, E., Quiles, A., & Hernando, I. (2018). Using different fibers to replace fat in sponge cakes: In vitro starch digestion and physico-structural studies. *Food Science and Technology International*, *24*(6), 533-543 (DOI: 10.1177/1082013218771412).

CAPÍTULO 2. USO DE BAGAZO DE FRUTOS ROJOS EN PRODUCTOS HORNEADOS. ESTRATEGIAS DE MEJORA

Diez-Sánchez, E., Llorca, E., Tárrega, A., Fiszman, S., & Hernando, I. (2020). Changing chemical leavening to improve the structural, textural and sensory properties of functional cakes with blackcurrant pomace. *LWT*, *127*, 109378 (DOI: 10.1016/j.lwt.2020.109378).

Diez-Sanchez, E., Quiles, A., Llorca, E., Reiβner, A. M., Struck, S., Rohm, H., & Hernando, I. (2019). Extruded flour as techno-functional ingredient in muffins with berry pomace. *LWT*, *113*, 108300 (DOI: 10.1016/j.lwt.2019.108300).

CAPÍTULO 3. USO DE BAGAZO DE FRUTOS ROJOS EN BATIDOS. SINERGIA ENTRE TRATAMIENTO NO TÉRMICO Y POLIFENOLES DEL BAGAZO

Diez-Sánchez, E., Martínez, A., Rodrigo, D., Quiles, A., & Hernando, I. (2020). Optimizing high pressure processing parameters to produce milkshakes using chokeberry pomace. *Foods*, *9*(4), 405 (DOI: 10.3390/foods9040405).

CAPÍTULO 4. DIGESTIÓN *IN VITRO.* INTERACCIONES ENTRE POLIFENOLES DEL BAGAZO DE FRUTOS ROJOS Y PRINCIPALES MACRONUTRIENTES DE LOS ALIMENTOS

Diez-Sánchez, E., Quiles, A., & Hernando, I. (2021). Interactions between blackcurrant polyphenols and food macronutrients in model systems: *in vitro* digestion studies. *Foods*, *10*(4), 847 (DOI: 10.3390/foods10040847).

Resultados y discusión

Capítulo 1

Estudio del efecto de la fibra sobre propiedades estructurales y texturales en productos horneados.

Using different fibers to replace fat in sponge cakes: *In vitro* starch digestion and physico-structural studies

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Food Science and Technology International, (2018), 24(6)

A B S T R A C T

This study assessed the effect of substituting 30% of fat by soluble, insoluble fiber, or a mix of both fibers in sponge cake quality, structure, acceptability, and starch digestibility. The apparent viscosity of the different formulations was measured and micro-baking was simulated. Texture profile tests were carried out and the crumb structure was examined. In vitro digestion was performed to study the digestibility of starch and a sensory test was carried out to know consumer acceptance. The soluble fiber (maltodextrin) affected the structure and quality of the cakes less than the insoluble fiber (potato fiber) and the use of soluble fiber in the formulation resulted in lower glucose release under in vitro conditions. Moreover, the consumer did not find differences among the control cake and the cakes prepared with soluble fiber. Considering the results as a whole, soluble fiber may be used for partial replacement of fat in sponge cake formulations and may constitute an appropriate strategy for obtaining healthy sponge cakes.

Keywords*:* Sponge cake; fiber; quality; structure, starch digestion

1. INTRODUCTION

Sponge cakes are a well-known product worldwide and are deeply rooted in the culture of each country. They are popular with consumers, who consider them delicious products with particular organoleptic characteristics (Matsakidou et al., 2010).

The major ingredients that give sponge cakes their specific properties include not only eggs, flour, and sugar but also fat, which comprises approximately 15–25% of the batter (Rodríguez-García et al., 2012). Fat contributes to air incorporation into the batter in the form of small bubbles, which will improve the stability of the batter minimizing the coalescence phenomena, thus increasing the volume of the cakes; fat also interferes with the continuity of the gluten, favoring

the formation of a final product with a smoother, softer texture (Román et al., 2015). Nevertheless, it is the food component with the highest energy value and the high percentage of fat in sponge cakes gives them a high calorie content (Rodríguez-García, Salvador, et al., 2014; Zahn et al., 2010). Many studies have demonstrated the close connection between excessive fat consumption and the development of excess weight, obesity, and certain cardiovascular diseases (Kratz et al., 2013; Mente et al., 2009). The World Health Organization (WHO, 2014) has warned that excess weight and obesity, considered as a typical problem of high-income countries, are becoming major public health problems in many parts of the world. The United Nations Food and Agriculture Organization (FAO, 2017) also states that good nutrition is the first line of defense against disease and requires special attention on the part of the food industry, starting with food design.

Nowadays, the nutritional value of food is becoming increasingly important, as well as the fact that the nutrients contained in them meet the specific needs of the individual. This is in agreement with the call of the WHO and the US Senate Commission on Nutrition's general dietary recommendations to limit the energy intake from total fat and raise the quantity of dietary fiber to a minimum of 22 g per day. In fact, there is increasing demand from consumers for low-fat, low-calorie, dietary fiber-rich products (Martínez-Cervera et al., 2012).

Dietary fiber is increasing nutritional and clinical interest owing to its beneficial effects on health and is being used as an ingredient in a large variety of foods (Oh et al., 2014). Fiber can regulate intestinal function, protect the intestinal walls from contact with certain harmful substances, reduce cholesterol absorption, and regulate blood glucose levels (Hardacre et al., 2015; Oh et al., 2014). The agreed definition of dietary fiber refers to carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed in the small intestine of humans (Viebke et al., 2014). Depending on the chemical, physical, and functional properties, dietary fiber can be classified into soluble and insoluble fiber. Soluble dietary fiber (SDF) includes pectins, gums,

inulin-type fructans, and some hemicelluloses whereas insoluble dietary fiber (IDF) includes lignin, cellulose, and some hemicelluloses. SDF is considered to have benefits on serum lipids, lowering the level of serum total cholesterol, while IDF is linked to laxation benefits (Quiles et al., 2016).

Several previous studies have investigated reducing the fat in sponge cakes or other bakery products by replacing it with different types of fiber, such as inulin (Rodríguez-García, Salvador, et al., 2014; Zahn et al., 2010), citrus pectin (Lim et al., 2014; Psimouli & Oreopoulou, 2013), peach fiber (Grigelmo-Miguel et al., 2001), cocoa fiber (Martínez-Cervera et al., 2011) or maltodextrin (Psimouli & Oreopoulou, 2013). These studies have found that fat replacement is feasible but affects the batter and cake properties depending on the type of fiber used for the replacement. Moreover, dietary fiber can influence the digestion of starch by reducing the starch breakdown and thus, reducing glucose release and absorption (Brennan, 2005).

Dietary fiber incorporated into starch-based foods can entrap starch granules and restrict the availability of water during gelatinization. As a result, the accessibility of starch granules to digestive enzymes is limited under human digestion, which results in the lowering of the glycemic index (Angioloni & Collar, 2011). However, *in vitro* studies show different results depending on the kind of dietary fiber used.

The aim of the present study was to investigate the functionality of soluble and insoluble fibers as replacers for 30% fat on the formulation of low fat cakes. Maltodextrin was used as SDF and potato fiber was used as insoluble fiber. The batter viscosity was measured and micro-baking was simulated to assess the evolution of air bubble growth. Texture profile analyses (TPAs) were also performed and the crumb structure was examined. Lastly, the digestibility of the starch was measured through *in vitro* digestion tests and consumer acceptance was assessed.

2. MATERIALS AND METHODS

2.1. Ingredients

Sponge cakes were prepared with the following ingredients: wheat flour (Harinas Segura S.L, Torrente, Valencia, Spain; composition provided by the supplier: 13.5–15.5% moisture, 9–11% proteins); white sugar (AB Azucarera Ibérica S.L.U., Madrid, Spain); egg yolk and white, both as pasteurized liquids (Ovocity, Llombay, Valencia, Spain); skimmed milk powder (Corporación Alimentaria Peñasanta, S.A., Siero, Asturias, Spain); refined sunflower oil (Aceites del Sur-Coosur, S.A., Vilches, Jaén, Spain); sodium bicarbonate E-500ii and citric acid E-300 (Sodas y Gaseosas A. Martínez, S.L., Cheste, Valencia, Spain); salt; Fibersol-2, composed of 90% resistant maltodextrin (Matsutani Chemical Industry Co. Ltd, Hyogo, Japan, total dietary soluble fiber 90%); Vitacel KF200, a potato fiber-rich food ingredient (J. Rettenmaier and Söhne Gmbh + Co Kg, rich in insoluble fiber (55%), total dietary fiber 65%); and distilled water.

2.2. Batter and cake preparation

The four formulations studied (Table 1) were the control formulation (C) and three further formulations in which 30% of the sunflower oil was replaced by a soluble fiber (SF), an insoluble fiber (IF), or a 50/50 mixture of the two fiber ingredients (M). Extra distilled water was added at ratios of 1:1 Fibersol-2 to water and 1:4 Vitacel KF200 to water as recommended by the suppliers of the fiber ingredients.

The batters were prepared using the 'all in' mixing procedure of Rodríguez-García et al. (2014a), with a few modifications. Firstly, all the liquid ingredients – egg white, yolk, milk, and water – were placed in a Kenwood Major Classic mixer (Kenwood, Havant, UK). The solid ingredients – flour, sugar, Fibersol-2 and/or Vitacel KF200, bicarbonate of soda, citrus acid, and salt – were then added to the same bowl. The

last ingredient added was the sunflower oil. To achieve homogeneous batters, all the ingredients were mixed for 30 s at 202 r/min, followed by 1 min at 260 r/min and 3 min at 320 r/min.

To bake each cake, 700 g of batter were poured into a 20 cm diameter Pyrex® mold and placed in a conventional oven (Electrolux, model EOC3430DOX, Stockholm, Sweden) that had been preheated to 180 °C for 30 min. They were baked at 180 °C for 47 min. After removing the cakes from the oven, they were left to cool for at least 1 h and 30 min before they were examined. All the batters and cakes were prepared in triplicate and the tests were performed within 24 h of their preparation.

Table 1. Composition of the formulations studied (% flour base)

Ingredient	C	SF	M	IF
Flour	100	100	100	100
Sugar	100	100	100	100
Egg yolk	27	27	27	27
Egg white	54	54	54	54
Milk	50	50	50	50
Oil	46	32.2	32.2	32.2
Soluble fibre	0	4	2	0
Insoluble fibre	0	0	2	4
Water	0	4	10	16
Sodium Bicarbonate	4	4	4	4
Citric acid	3	3	3	3
Salt	1.5	1.5	1.5	1.5

C: control cake; SF: cake with soluble fiber; M:cake with a mixture of soluble and insoluble fiber; IF: cake with insoluble fiber.

2.3. Apparent viscosity

Batter viscosity was measured with a Haake Viscotester 6 R Plus viscometer (Thermo Scientific, Walthman, MA), using an R3 spindle at 6 r/min at room temperature. The samples were placed in a thermostatic bath to maintain a temperature of 25 °C. The measurements were made in duplicate for each batter and in triplicate for each formulation.

2.4. Batter image analysis (micro-baking simulation)

Microscope observation was performed during the micro-baking simulation using a temperature-controlled stage (Analysa-LTS350, Linkam Scientific Instruments Ltd, Surrey, UK) under the lens of a light microscope (Nikon ECLIPSE 80i, Nikon Co. Ltd, Tokyo, Japan). A drop of the sample was placed in the concavity of the glass slide, which was placed on the temperature-controlled stage. During heating, the temperature ramp was controlled by a refrigeration system with a liquid nitrogen pump (Linkam). The temperature profile employed was 1.5 °C/min from room temperature (25 °C) to 105 °C. The batter samples were observed at 4x magnification (x4/0.13∞/- WD 17.1 objective lens, Nikon). Photographs were taken with a camera (ExWaveHAD, model DXC-190) fitted to the microscope and connected to a computer. During the micro-baking simulation, a video was recorded with photographs taken every 10 s in a 640 x 540 pixel format, using the microscope software (Linksys 32, Linkam). Three samples of each formulation were examined. The images were analyzed with ImageJ software (National Institute of Health, Bethesda, MD).

2.5. Macroscopic structure of the crumb

The cakes were cut in half through the center and scanned with an HP Scanjet G2710 (Hewlett-Packard, Palo Alto, CA) at a resolution of 300 dpi. Central sections of cake with a field size of 10 x 4 cm were analyzed. The cropped image was split into color channels, the contrast

was enhanced and the image was thresholded and binarized with the aid of the ImageJ software program (National Institutes of Health, Bethesda, MD). Total cell area within the crumb (%) and cell size (mm^2) were calculated. Four images of each formulation were analyzed.

2.6. Sponge cake texture

The textural properties were assessed with a TATXTplus texture analyzer (Stable Microsystems, Ltd, Godalming, UK), using the Texture Exponent Lite 32 program (version 6.1.4.0, Stable Microsystems). A TPA was performed on four cubes (3 x 3 x 3 cm) cut from the center of the cake after removing the crust. The cubes were compressed to 40% of their original height at a test speed of 1 mm/s with a 5 s resting time between the two compression cycles. The trigger force was 5 *g*. The cubes were compressed with a 5 cm diameter cylindrical aluminum probe. After the two compression cycles, the following measurements were recorded: hardness, springiness, cohesiveness, chewiness, and adhesiveness. The measurements were carried out in duplicate.

2.7. Field emission scanning electron microscopy (FESEM)

For each formulation studied, 0.5 cm sided cubes were cut, frozen at -80 °C and lyophilized (Lyoquest 55, Telstar, Terrassa, Barcelona, Spain). The samples were then vacuum coated with platinum and observed under FESEM (model Ultra 55 FESEM, Zeiss, Oberkochen, Germany). Each formulation was analyzed in duplicate.

2.8. *In vitro* digestion

Digestion of the sponge cakes covered three stages: oral, gastric, and intestinal. For oral digestion, the protocol described by Smith et al. (2015) was followed, with a few modifications. Consequently, 10 g of cake were crumbled by hand and 3.5mL of saliva solution, previously incubated at 37 °C, were added. This mixture was ground with a blender

(Ufesa, U1EBB40001; BP-4500) for 15 s, then 70 mL of bidistilled water was added and mixed by hand for 1 min to simulate mastication. The saliva solution was prepared as described by Mishellany-Dutour et al. (2011). The following were dissolved in 1L of bidistilled water: 5.208 g of $NaHCO_3$, 1.369 g of $K_2HPO_4.3H_2O$, 0.877 g of NaCl, 0.477 g of KCl, 0.441 g of $CaCl_2.2H_2O$, 2.16 g of mucin from porcine stomach type II (PGM Sigma M2378) and 8.70 g of α-amylase from porcine pancreas type VI-B (Sigma A3176).

In the gastric stage, to digest 25 g of sponge cake that had already been orally digested, 25 g of gastric fluid was placed in the digester, composed of a glass reactor with a thermostat-controlled jacket and continuous magnetic stirring fitted to a controlled temperature water circulator. The gastric fluid was preincubated at 37 °C for 5 min at pH 2. The sample was added to the reactor, the mixture was adjusted to pH 2 with 2M HCl, 0.006 g of pepsin (Sigma P7000) was added, and the mixture was incubated for 1 h at 37 °C with stirring. The electrolyte solution that constituted the gastric fluid was prepared by dissolving the following in 1 L of distilled water: 3.1 g of NaCl, 0.11 g of $CaCl_2$, 1.1 g of KCl, 5.68mL of 1 M $NaCO_3$. The pH was adjusted to 2 with 2 M HCl.

For the intestinal stage, the pH of the sample was raised to 6 with 1 M $NaCO_3$ to which pancreatin (Sigma P1750, 4xUSP) and bile salts (Sigma B8631) had been added. The pancreatin and bile salt solution was prepared with 0.1 g of pancreatin and 0.625 g of bile salts to 25mL of 0.1 M $NaHCO_3$ (Rufián-Henares & Delgado-Andrade, 2009). Amyloglucosidase (A7095 ≥ 300 U/mL, Sigma) was then added at 0.2 mL/g of starch in accordance with Oh et al. (2014) and Soong et al. (2014). The pH was raised to 7.5 with 0.1 M $NaHCO_3$ and the mixture was incubated at 37 °C for 3 h with stirring. Aliquots were removed at 0, 20, 60, 90, 120 and 180 min of digestion, immediately adding 1.4 mL of ethanol to stop the reaction (Bae et al., 2013), and centrifuged at 3000 r/min for 3 min. The glucose concentration was then measured with the glucose oxidase/peroxidase (GOPOD) assay kit at 510 nm. For this measurement, 0.1 mL aliquots of the supernatant were taken, 3mL of

the GOPOD reagent were added, the sample was incubated at 40–50 °C for 20 min and the absorbance was read at 510 nm.

The experimental data were fitted to the first-order equation proposed by Goñi et al. (1997) [$C = C_{\infty} (1 - e^{-kt})$], where C is the concentration at t time, C_{∞} is the equilibrium concentration, k is the kinetic constant, and t is the chosen time.

2.9. Sensory analysis

Consumers were recruited among students and employees of the Universitat Politècnica de València. A total of 82 untrained panelists (consumers) aged 22–63, were used for the study. Of the participants, 49% were women and 51% men.

The samples were assessed in a standardized tasting room equipped with individual booths. Each consumer received four pieces of cakes (C, SF, M, and IF) coded by three-digit random numbers. The pieces of cakes were served at room temperature in random order. Water was supplied to clean the consumers' mouths between each sample.

Consumer acceptance testing was done using a nine-box structured hedonic scale to score the ''appearance'', ''texture'', ''taste'' acceptability, and ''overall acceptance'' of the product (from 1 = ''I dislike it extremely'' to 9 = ''I like it extremely'').

2.10. Statistical analysis

Analysis of variance (ANOVA) was used for statistical analysis of the results. The least significant differences (LSD) were calculated with a significance level of $p < 0.05$. The Statgraphics Centurion XVI.II statistical program (StatPoint Technologies, Inc., Warrenton, VA) was used for this purpose.

3. RESULTS AND DISCUSSION

3.1. Apparent viscosity

The batter viscosity results for the sponge cake formulations studied are expressed in mPa s. On reducing the fat content by 30%, a significant ($p < 0.05$) reduction was observed in the viscosity of the IF (7675.27 ± 53.53) and M (9032.95 ± 233.36) batters in comparison to the control (10,732.04 ± 348.42), however no significant ($p > 0.05$) difference was observed between SF (10,724.73 ± 470.06) and control batters. This tendency has previously been reported by other authors (Rodríguez-García et al., 2014b; Román et al., 2015; Zahn et al., 2010) who also obtained low viscosity values on replacing fat with soluble fibers and functional ingredients and lower viscosity values at higher rates of replacement.

Bearing in mind that the fat reduction level was constant in the present study, the ingredient with insoluble fiber led to the greatest reduction in viscosity. This rheological behavior is largely due to the greater quantity of water added to the insoluble fiber formulation (1:4) compared to the batter with soluble fiber (1:1), giving IF a higher ratio of liquid to solid ingredients, which led to lower viscosity values.

3.2. Light microscopy and image analysis of the batters

Figure 1(a) shows images of the batters of the different formulations (C, SF, M, and IF) at different temperatures (30, 60, 90, and 100 °C) during microbaking simulation.

Visual examination of the batter images showed a clear air bubble expansion effect due to the lower fat content and the addition of soluble and insoluble fibers together with water. In batter C, the size of the bubbles increased in a uniform, controlled way, distributing the bubbles evenly as the temperature rose.

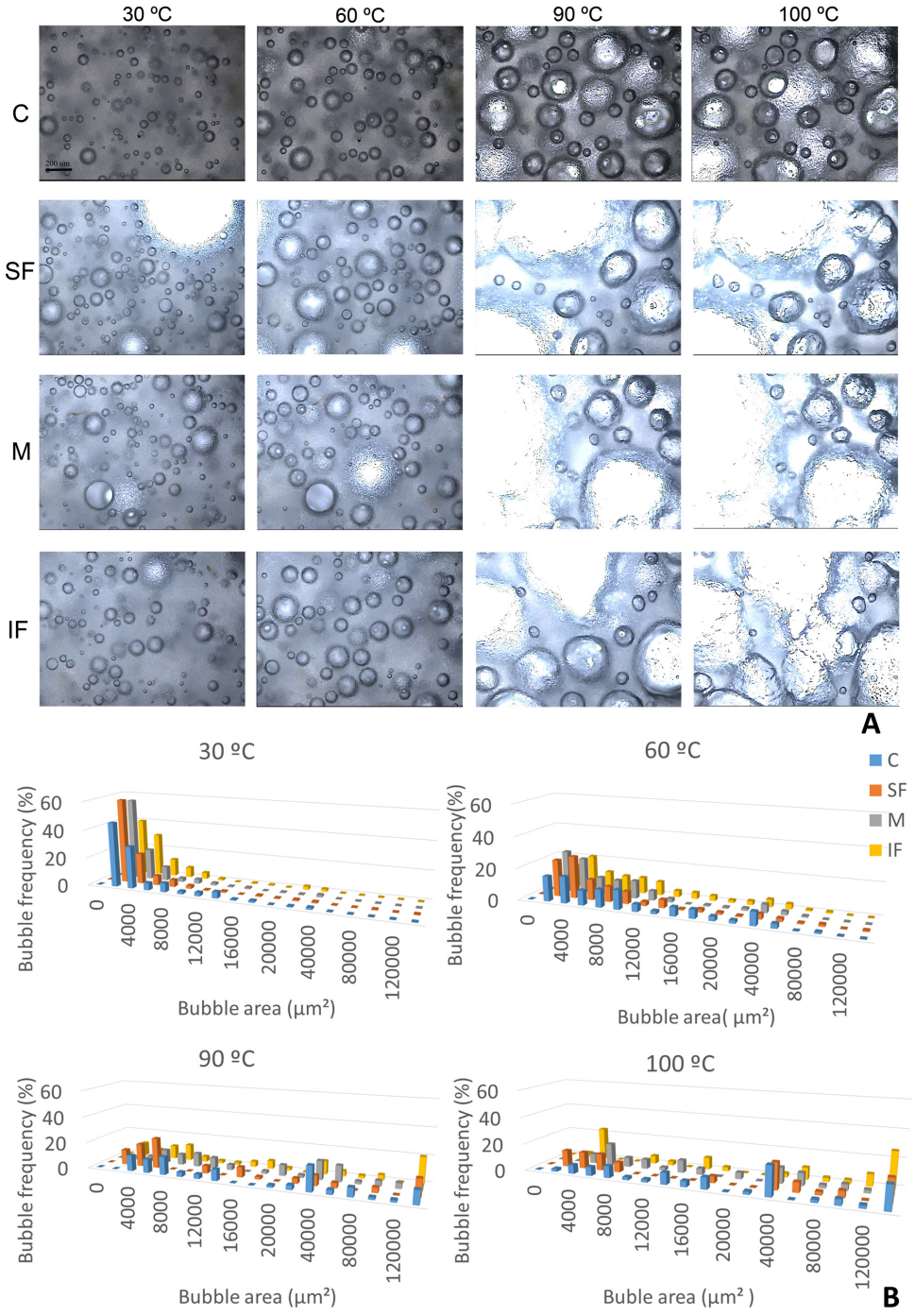

Figure 1. (a) Light microscopy images of bubble expansion at different temperatures during micro-baking. C: control cake; SF: sponge cake with soluble fiber; M: sponge cake with a mixture of soluble and insoluble fiber; IF: sponge cake with insoluble fiber. (b) Histograms of bubble size distribution. C: blue; SF: orange; M: grey; IF: yellow.

In general, the reduction in fat and the addition of soluble and insoluble fibers allowed more bubbles to be incorporated during the mixing process (Figure 1(a) and (b)). As the temperature rose, the bubbles naturally expanded. Bubble expansion was higher in the IF batter, which were the least stable at rising temperatures, with some of the bubbles losing their identity at 100 °C as they coalesced with neighboring bubbles.

The images were analyzed to quantify the bubble size distribution during micro-baking. Figure 1(b) presents histograms of the bubble size distributions at different temperatures. The C formulation batter incorporated fewer bubbles (Figure 1(b)) and showed a tendency to regular distribution of bubble sizes during heating, compared to the other batters. This behavior could be due to the greater apparent viscosity of this batter (C), which would help to make the air bubbles more stable, delaying their movement through the batter and slowing down their disproportionate growth and coalescence as observed previously by Rodríguez-García et al. (2014b).

In general, a lower apparent viscosity of the replaced batters (SF, M, and IF) may have allowed occluding more air during mixing; so, a greater number of bubbles per field is observed at the beginning of the microbaking process, particularly in the case of batters SF and M. During the micro-baking process the air bubble sizes acquired an irregular distribution but towards the end of the heating scale (at 90 °C and 100 °C), the IF batter was found to have a higher percentage of larger bubbles.

The considerable reduction in the apparent viscosity of the IF batters and resulting reduction of air bubble stability in these samples increased the mobility, disproportion ratio, coalescence, and size of the bubbles.

3.3. Macroscopic structure of the crumb

Figure 2 shows scanned, contrasted and binarized images of the different cakes (C, SF, M, and IF).

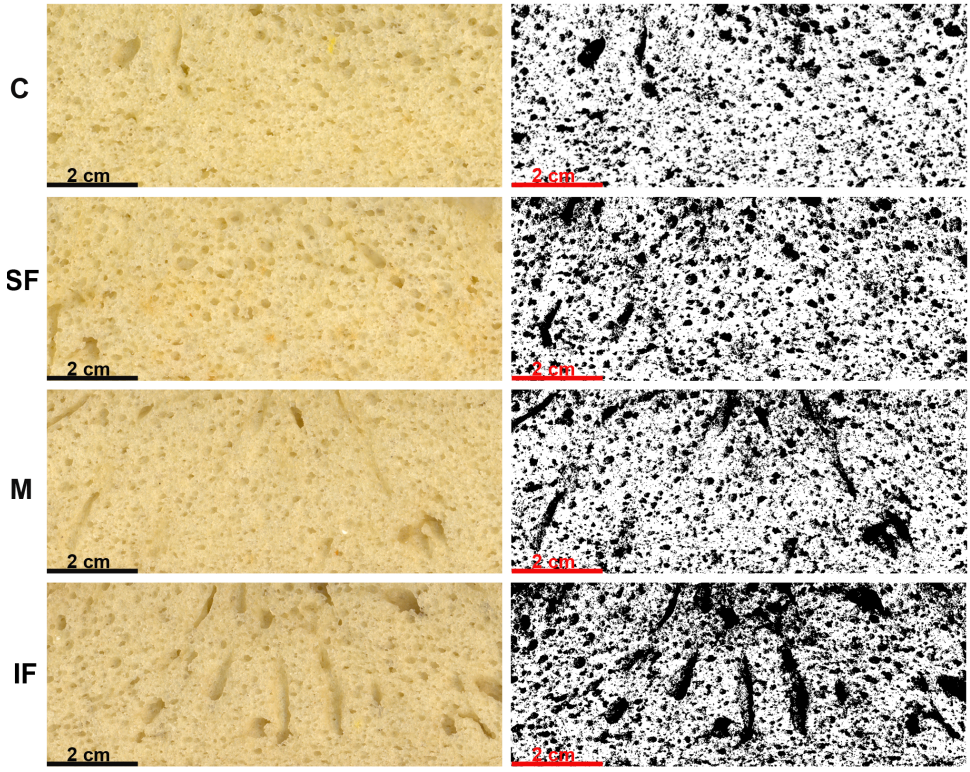

Figure 2. (a) Scanned images of C, SF, M and IF sponge cakes, field size 4 x 10 cm and corresponding binarized images (118 pixels/cm). C: control cake; SF: sponge cake with soluble fiber; M: sponge cake with a mixture of soluble and insoluble fiber; IF: sponge cake with insoluble fiber.

Visual analysis of these cake images shows a practically uniform crumb macrostructure in the control cake (C). In contrast, a series of diffusion pathways appeared in the crumb of the reduced-fat sponge cakes. These pathways were less noticeable in the SF cake and more noticeable and numerous in the IF cake.

The images of the cakes were also analyzed to quantify the crumb macrostructure results (Table 2). IF presented a significantly ($p < 0.05$) higher cell size and a higher total cell area values compared to the other cakes. Consequently, IF presented a more aerated structure with bigger cells. These results agree with the tendency observed in the sponge cake batters during micro-baking, as described in the previous section – IF batter was found to have a higher amount of larger bubbles at the end of the micro-baking. In turn, this is intrinsically affected by viscosity; thus, in IF crumb cake the rising percentage of air would be directly related to the low viscosity found in IF batter. Changes in the thermosetting mechanism, as a consequence of the extra water added, could also be responsible for the presence of the diffusion pathways in replaced cakes.

Table 2. Macroscopic structure of the cumb and textural properties of the cakes.

	Crumb Structure		Cake Texture				
Sample	Cell Size (mm^2)	Total Cell Area (%)	Hardness (N)	Chewiness (N)	Cohesiveness	Springiness	Adhesiveness (g·s)
C	1.0^{a} (0.1)	28.13^{a} (2.37)	4.98^{a} (0.46)	3.15^{a} (0.28)	0.71^{a} (0.01)	0.88^{a} (0.01)	2.55^{a} (1.55)
SF	1.0^{a} (0.2)	30.27^{a} (4.65)	5.62^{b} (0.41)	3.57^{b} (0.22)	0.72^{a} (0.01)	0.88^{a} (0.01)	3.17^{a} (1.01)
M	1.1^{a} (0.1)	32.45^{a} (2.11)	6.22^{c} (0.82)	3.98^{c} (0.49)	0.72^{a} (0.00)	0.89^{b} (0.01)	2.82^{a} (2.01)
IF	1.4^{b} (0.2)	38.52^{b} (3.69)	6.95^{d} (0.69)	4.46^{d} (0.43)	0.72^{a} (0.00)	0.90^{b} (0.01)	2.73^{a} (1.12)

Figures in brackets are standard deviations. $^{a, b, c, d}$. Means with different letters in the same column differ significantly ($p < 0.05$). C: Control cake, FS: cake with soluble fiber; M: cake with a mixture of soluble and insoluble fiber; FI: cake with insoluble fiber.

3.4. Cake texture

The results of the parameters obtained from the TPA curves for the sponge cakes under study are presented in Table 2.

The 30% fat reduction with fiber generated significantly ($p < 0.05$) higher hardness values in SF, M, and IF samples. IF was the hardest of the samples. Hardness values followed the trend IF > M > SF, and significant differences were observed among all of them. This means that more force was required to compress the IF cake than the other formulations. Eslava-Zomeño et al. (2016) obtained significantly higher

hardness values for sponge cakes made with Optisol™ 5300 at different fat replacement ratios. Psimouli & Oreopoulou (2013) also found significantly higher hardness values in sponge cakes prepared with different carbohydrates as fat replacers.

The chewiness values showed a similar trend. The chewiness values of the control cake were significantly lower ($p < 0.05$) than those of the other sponge cakes. When 30% of the fat was replaced by adding soluble and/or insoluble fibers to the formulations, chewiness increased significantly ($p < 0.05$). IF was the chewiest cake. This means that greater energy was needed to chew the IF cake enough to be swallowable.

In general, the tendency for these parameters to increase could be related to the reduction in the batter viscosity of the respective formulations. In the control formulation batter, both the low number of bubbles and the distribution and homogeneous expansion of small bubbles influenced its low hardness values.

In contrast, the greater variation in bubble size observed on adding the soluble and insoluble fibers, particularly the latter, increased the cake hardness considerably. Also, bearing in mind that one of the functions of fat is to make cakes smooth and soft, reducing the fat and adding soluble and insoluble fibers could be expected to increase the hardness of the cakes and, consequently, their chewiness.

The cohesiveness and adhesiveness values showed no significant ($p > 0.05$) differences between the sponge cakes studied. The lower fat content and addition of soluble and insoluble fibers did not influence the work needed to compress the samples a second time compared to the first, nor did they alter the work needed to detach the compression probe from the sample.

The springiness values showed no significant ($p > 0.05$) differences between C and SF or between M and IF, though the latter pair presented significantly ($p > 0.05$) higher springiness.

The use of insoluble fiber in the formulation of the cakes seems to influence in the texture parameters as IF cake is the one with highest hardness, chewiness, and springiness values.

3.5. Field emission scanning electron microscopy

The microstructure of the soluble fibers (Fibersol-2), insoluble fibers (Vitacel K200) and C, SF and IF cakes can be seen in the images obtained through FESEM, shown in Figure 3.

The fibers showed considerable differences in structure. The soluble fiber was made up of numerous particles of varying sizes and shapes, although most were granular and presented a smooth appearance. The insoluble fiber had the typical rough appearance of plant cells, with visible cell walls (labeled pc) and transport tissues (labeled vc).

The structure of the control cake (C) can be seen to be composed of a gluten network, formed by the flour, which contained the other ingredients. The partially gelled starch granules were embedded in the gluten network and the oil acted as a lubricant, creating a continuous

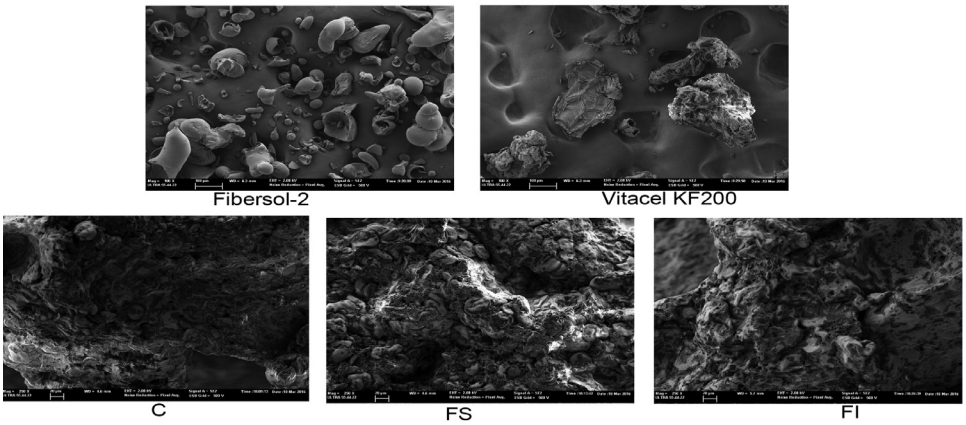

Figure 3. Field emission scanning electron microscopy (FESEM). Images of soluble fiber (Fibersol-2) and insoluble fiber (Vitacel KF200), magnification 100 x, bar = 100 mm. Images of cakes C, SF and IF, magnification 250 x, bar = 20 mm. pc: cell walls; vc: transport tissues. C: control cake; SF: sponge cake with soluble fiber; IF: sponge cake with insoluble fiber.

structure. The structures of the SF and IF cakes, with a 30% reduction in fat, were influenced by the characteristics of the respective fibers and presented a more irregular microstructure, since there was a smaller coating of oil. In the SF cakes, the partially gelled starch granules distributed irregularly through the cake matrix were very evident, as they retained their identity. In the IF cakes, the starch granules were deeply embedded in the matrix, giving rise to a more compact structure that can be related to their harder texture.

3.6. *In vitro* digestion

Figure 4 shows the digestibility curves of cakes C, SF, and IF after *in vitro* digestion. No significant ($p > 0.05$) differences were observed between the samples at 20, 60, 90, and 120 min of digestion. However, SF presented significantly ($p < 0.05$) lower values than the other cakes after 180 min. The parameters obtained after fitting the curves using the first-order model described by Goñi et al. (1997) are shown in Table 3. Although the kinetic constant (k), which indicates the rate of starch hydrolysis is augmented, it can be observed that the area under the hydrolysis curve after 180 min (AUC 180) and the equilibrium concentration (C_∞) values were the lowest for SF, being significant ($p < 0.05$) for C_∞ values. AUC 180 is a comprehensive parameter for the starch hydrolysis, relating the glucose release over a hydrolysis period of 180 min (Gularte et al., 2012) and C_∞ indicates the concentration at the equilibrium point, and a higher concentration of final product reflects increased digestibility of starch (Dura et al., 2014). Taking into account the results obtained for AUC 180 and C_∞ values, the use of soluble fiber in the formulation of the cake would result in lower glucose release under *in vitro* conditions. This could be related to the FESEM images, where the starch granules in the SF cake matrix were observed to be less gelled than those of the IF cake. Moreover, the soluble fiber with greater water absorption capacity would compete with the starch for the available water during sponge cake processing, leading to low starch gelatinization and consequently reducing the release of glucose during in vitro digestion in the case of SF cake.

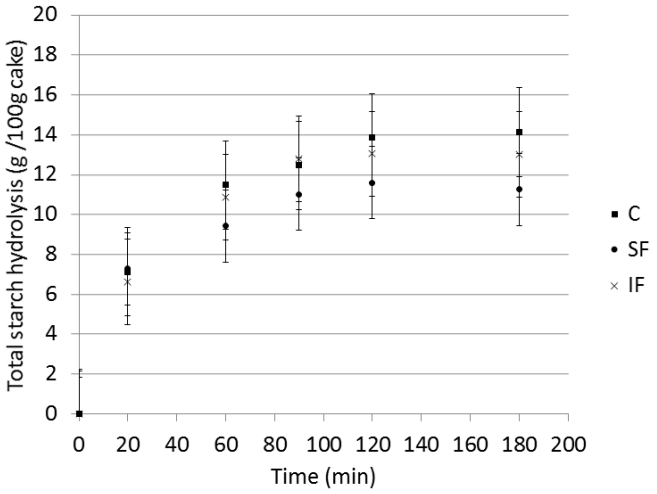

Figure 4. *In vitro* digestibility of starch of the cakes C, SF, M, and IF. C: control cake; SF: sponge cake with soluble fiber; M: sponge cake with a mixture of soluble and insoluble fiber; IF: sponge cake with insoluble fiber.

Table 3. Kinetics of the *in vitro* starch digestibility.

Sample	AUC 180	$C\infty$ (g/100)	k (min^{-1})
C	2075.7[a] (15.6)	14.1[a] (0.4)	0.030[a] (0.003)
SF	1794.1[a] (279.3)	11.6[b] (1.5)	0.039[a] (0.007)
IF	1971.6[a] (64.6)	13.1[a] (0.3)	0.033[a] (0.001)

Values in brackets are standard deviations. [a, b, c, d] Means with different letters in the same column differ significantly ($p < 0.05$). C: Control cake, SF: cake with soluble fiber; IF: cake with insoluble fiber.

3.7. Sensory acceptance

Table 4 presents the mean liking scores for the ‘‘appearance’’, ‘‘texture’’, ‘‘taste’’, and ‘‘overall acceptance’’ of the control cake and the cakes with the different type of fibers.

Table 4. Liking for appearance, texture, taste and overall acceptance of cakes.

Sample	Appearance	Texture	Taste	Overall acceptance
C	7.00[a]	6.90[a]	7.00[a]	7.15[a]
SF	6.93[a,b]	6.78[a]	6.91[a,b]	7.04[a]
M	6.80[a,b]	6.73[a]	6.90[a,b]	7.03[a]
IF	7.03[b]	6.18[b]	6.46[b]	6.53[b]

Values are mean (n=82). a, b, c, d Means with different letters in the same column differ significantly ($p < 0.05$). C=Control cake, SF: cake with soluble fiber; M: cake with a mixture of soluble and insoluble fiber; IF: cake with insoluble fiber.

Statistical analysis showed that the control cake (C) and the cakes where fat was replaced by soluble fiber (SF) and the mix of fibers (M) did not differ significantly ($p < 0.05$) in all the attributes. However, IF cake obtained the lowest value when all the attributes were scored; being significantly ($p < 0.05$) lower than the other three samples for ''texture'' and ''overall acceptance'' attributes.

These results revealed that quality differences due to fat replacement by soluble fiber or by the mix of fibers were not perceived by consumers. However, the replacement by insoluble fiber gave place to significantly ($p < 0.05$) lower scores for ''texture'' and ''overall acceptance'' attributes. If the texture hedonic results are compared with the instrumental measurements it can be observed that the highest hardness and chewiness may have had an important negative influence in hedonic acceptability. In this context, IF was the hardest of the samples but its texture was the less liked by consumers.

4. CONCLUSIONS

Replacing 30% of the fat in a sponge cake formulation with ingredients that are rich in insoluble fiber and extra water caused a reduction in viscosity. As a result of their low viscosity, the batters with

insoluble fiber incorporated a greater quantity of air bubbles during mixing, as observed in micro-baking, and IF batter presented larger bubble size than the other formulations at the same temperatures. In the macroscopic analysis of the crumb, the IF cake also showed a larger quantity of diffusion pathways and a greater percentage of air. Replacing 30% of the fat in the sponge cake formulation with ingredients that are rich in soluble and/or insoluble fiber also caused increased hardness and chewiness. The IF cake was spongier because it contained a greater quantity of air but was also harder, which is related to the more compact matrix observed by FESEM. During *in vitro* digestion, SF showed a lower glucose release at 180 min. Regarding the sensory acceptance, the consumers did not find differences among C, SF, and M cakes, however, IF cake was the less liked by consumers. Overall, considering the physicochemical, sensory, and nutritional quality, soluble fiber may be used for partial replacement of fat in sponge cake formulations and constitutes an appropriate strategy for obtaining healthy sponge cakes.

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Capítulo 2

Uso del bagazo de frutos rojos en productos horneados.

Estrategias de mejora.

Changing chemical leavening to improve the structural, textural, and sensory properties of functional cakes with blackcurrant pomace

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A B S T R A C T

Blackcurrant pomace is a by-product with bioactive compounds and dietary fibre, which can be used as ingredient to elaborate bakery products. However, its high content of fibre results in techno-functional problems affecting texture and sensory properties. We hypothesised that the use of different chemical leavening agents can counteract the negative effects of pomace addition improving the quality of the final product. Citric acid, sodium acid pyrophosphate, and glucono-δ-lactone were used as leavening agents in combination with sodium bicarbonate (encapsulated and free). A micro-baking simulation showed the expansion of the bubbles in the batter. In the cakes, the structure, texture, colour and sensory profile were studied. Cakes prepared with pyrophosphate (regardless bicarbonate type) and glucono-δ-lactone (plus free bicarbonate) incorporated more air, which led to bigger gas cells and a softer instrumental texture. These cakes were perceived as brittle and spongy. All the formulations were acceptable according to an untrained sensory panel.

Keywords: Leavening agent; Structure; By-product; Flash profile; Bakery products

1. INTRODUCTION

By-products from the food industry can have a high nutritional value. This is the case of the by-products generated in the production of blackcurrant juice, composed of peel and seeds; rich in polyphenols and dietary fibre (DF) (Borges et al., 2010; Paunović et al., 2017). These components have beneficial effects on health; polyphenols have a positive impact on cardio-vascular health, reduce inflammation, and modify intestinal microbiota, among other effects; these effects are because of their inner antioxidant capacity (Del Rio et al., 2013;

Shahidi & Ambigaipalan, 2015). DF is involved in disease prevention and health improvement, because of its contribution to physiological attenuations, such as cholesterol and fat binding, reduction of blood glucose levels, prevention of constipation, and facilitating good colonic health (Foschia et al., 2013). To appreciate these value-added ingredients, its incorporation in a food matrix could be interesting (Foschia et al., 2013; Zhao, 2007). Fruit pomace has been previously used in preparing bakery products like muffins and sponge cakes; Quiles et al. (2018) used blackcurrant and Aronia pomace to replace flour, fat, and sugar in cakes. Sudha et al. (2007) and Masoodi et al. (2002) incorporated apple pomace in cakes, and Diez-Sánchez et al. (2019) incorporated blackcurrant pomace in muffins. Finally, Walker et al. (2014) studied the substitution of flour with wine grape pomace in muffins, breads, and brownies. One of the major drawbacks arising from these studies, on inclusion of ingredients high in fibre, come from detrimental effects on the creation of a well-aerated structure (Lebesi & Tzia, 2011; Quiles et al., 2018). This lack of aeration in cakes, which is determined by the amount of gas occluded, produced, and retained by the batter, produces a firm texture and a reduction in cake volume. As expected, such deficiencies increase with higher levels of wheat flour substitution with fibre-rich ingredients.

In sponge cakes, the batter expansion during baking results from carbon dioxide release when an acid (or an acidic salt) reacts with sodium bicarbonate in the presence of moisture and heat to form a salt, water, and carbon dioxide (De Leyn, 2014; Narsimhan, 2014). This chemical reaction influences the expansion of the initial bubbles incorporated during the mixing process that function as nuclei for larger bubbles. Presence and good distribution of bubbles in cakes favour good final product characteristics like colour, texture, and volume (Book & Brill, 2015).

Sodium bicarbonate has been the most used leavening agent in general domestic baking, which reacts with the lactic acid of other ingredients like sour milk (Bennion et al., 1997). Now, there are chemical leavening agents with different characteristics that make them suitable

for application in different conditions (De Leyn, 2014). When free bicarbonate (B) is used, the bubble formation in the first stages of baking results not only from the incorporated air due to the batter mixing but also from the CO_2 released by early leavening chemical reaction (Germain & Aguilera, 2008) and the CO_2 loss by diffusion through the batter (Godefroidt et al., 2019). However, as the bicarbonate dissociates in water almost immediately, the rate of carbon dioxide production is determined by the acid's rate of dissociation (Bellido et al., 2008). The use of encapsulated bicarbonate (EB) avoids a rapid release of gas, retarding the chemical reaction until the capsule's external wall melts during baking (Gibbs et al., 1999; Lakkis, 2016; Meiners, 2012). Hence, the type of acid and bicarbonate form (encapsulated or not) used in the formulation would have considerable influence in bubbles creation and growth. Dorko & Penfield (1993) studied that bicarbonate encapsulation resulted in lower initial CO_2 release in muffins, changing their final characteristics. Though different leavening agents have been used over the years, there are no recent studies comparing the effect of traditional leavening agents with newer ones.

Our hypothesis is that certain combinations of leavening agents including encapsulated sodium bicarbonate could improve aeration of high-fibre bakery products. Thus, the aim of the present study was to evaluate the effects of different leavening agents and their combinations, with encapsulated and free bicarbonate, on the improvement of the structural and sensory characteristics of sponge cakes, prepared with blackcurrant pomace.

2. MATERIALS AND METHODS

2.1. Cake ingredients

The ingredients used in the cake's batter preparation were: wheat flour (Harinas Segura S.L., Torrente, Valencia, Spain; composition

provided by the supplier: 13.5–15.5 g/100 g moisture, 9–11 g/100 g protein), white sugar (AB Azucarera Ibérica S.L.U., Madrid, Spain), pasteurised egg yolk and white (Ovocity, Llombay, Spain), skimmed milk powder (Corporación Alimentaria Peñasanta, S.A., Siero, Asturias, Spain), refined sunflower oil (Aceites del Sur-Coosur, S. A., Vilches, Spain), sodium bicarbonate (E−500ii, Sodas y Gaseosas A. Martínez, S. L., Cheste, Spain), micro-encapsulated bicarbonate (Grupo Indukern, S. L., Barcelona, Spain, melting point of the encapsulation provided by the supplier: 69–73 °C), citric acid (E−300, Sodas y Gaseosas A. Martínez, S. L., Cheste, Spain), sodium acid pyrophosphate (E−450i, Chemische Fabrik Budenheim KG, Budenheim, Germany), glucono-δ-lactone (E−575, Emilio Peña, S. A., Torrente, Spain), mineral water (Pascual S.A.U., Aranda del Duero, Spain), and salt (Sal Bueno S.L., Xirivella, Spain). Blackcurrant pomace was kindly supplied by the Institute of Natural Materials Technology (Technische Universität Dresden, Germany). It was prepared by drying the fresh pomace at 70 °C for 2 h and milling it in a ZM 100 ultracentrifuge mill (Retsch GmbH, Haan, Germany) at 14000 rpm using a 1 mm sieve (Reißner et al., 2019).

2.2. Cake preparation

Six different formulations contained 100 g wheat flour, 100 g sugar, 50 g reconstituted skim milk, 27 g egg yolk, 54 g egg white, 5 g water, 20 g blackcurrant pomace powder, 46 g sunflower oil, and 1.5 g salt; different leavening agents were added to each formulation (Table 1). Encapsulated (EB) and free bicarbonate (B) were used in combination with three different acidic ingredients: citric acid (CA), sodium acid pyrophosphate (SAPP), and glucono-δ-lactone (GDL). These leavening agents were chosen for their different rates of carbon dioxide production, which is a function of the solubility of the acidic constituents. CA is a fast-acting acid, SAPP is a slow-acting acid and GDL is a continuous-releasing leavening agent by a two-step reaction mechanism. The ratios of acid and bicarbonate were selected according to Brose et al. (2001).

Table 1. Amount of leavening agents in the six cake formulations.

Ingredient (in % flour basis)	Sample					
	CA-B	CA-EB	SAPP-B	SAPP-EB	GDL-B	GDL-EB
Free sodium bicarbonate (B)	4	-	4	-	4	-
Encapsulated sodium bicarbonate (EB)	-	4	-	4	-	4
Citric acid (CA)	3	3	-	-	-	-
Sodium acid pyrophosphate (SAPP)	-	-	5.6	5.6	-	-
Glucono-δ-lactone (GDL)	-	-	-	-	9	9

The batters were prepared using the "all in" mixing procedure (Rodríguez-García et al., 2012), with some modifications. Egg white, egg yolk, milk, and water were placed in a planetary mixer Kenwood KM800 Major Classic mixer (Kenwood, Havant, UK), then the solid ingredients were added to the bowl, with the oil added last. All the ingredients were mixed for 30 s at 202 rpm, followed by 1 min at 260 rpm and 3 min at 320 rpm to achieve a homogeneous batter. An oven (Electrolux, model EOC3430DOX, Stockholm, Sweden) was preheated (20 min, 180 °C). The batter was placed in a 20 cm diameter Pyrex baking pan and baked in the pre- heated oven at 180 °C for 43 min. Cakes were kept covered at room temperature for 24 h and then analysed. All the batters and cakes were prepared in triplicate on three different days.

2.3. Light microscopy and image analysis of the cake batters at different baking temperatures

A microscopic examination during simulated micro-baking was carried out to record bubble changes of batter as previously described (Rodríguez-García, Salvador, & Hernando, 2014) using a temperature-controlled stage (Analysa-LTS350, Linkam, Surrey, UK), mounted under the lens of a light microscope (Nikon ECLIPSE 80i, Nikon Co., Ltd., Tokyo, Japan). Images were captured and stored every 10 s while

three samples from each batter were examined. The images were analysed using the software ImageJ (National Institutes of Health, Bethesda, MD).

2.4. Crumb cellular structure

The baked product was cut into vertical slices of 1.5 cm thickness and scanned using a computer scanner (Epson Perfection 1250, Epson America Inc., Long Beach, CA). The images were acquired with a resolution of 300 dpi and were analysed using the software ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The image was cropped to a 10 × 4 cm section, on which the analysis was performed. The image was split into colour channels and the contrast was enhanced; the image was then binarized after a grayscale threshold. The parameters calculated were air cell density (number of cells per field), air cell area (mm^2), and total air cell area within the crumb (%). Measurements were performed on three different slices of each sample.

2.5. Instrumental texture

Texture profile analysis (TPA) was conducted using a TA-TX plus Texture Analyser (Stable Micro Systems, Ltd., Godalming, UK) with the Texture Exponent Lite 32 software (version 6.1.4.0, Stable Micro Systems). TPA was performed on cubes (15 × 15 × 15 mm) taken from the central crumb of each cake. The test speed was 1 mm/s with a strain of 40% of the original cube height and a 5 s interval between the two compression cycles; the trigger force was 0.049 N. The double compression test was performed with a 35 mm diameter aluminium plate (P/35). The parameters obtained from the curves were hardness, chewiness, cohesiveness, and springiness. Eight cubes of each cake were measured.

2.6. Colour measurements

The colour of the cake crumb was measured using a Chroma meter CR-400 (Minolta Co., Ltd., Osaka, Japan). The results were expressed per the CIE $L^*a^*b^*$ system, with reference to illuminant C and a visual angle of 2°. The parameters were L^* ($L^* = 0$ [black]; $L^* = 100$ [white]), a^* ($-a^*$ = greenness; $+a^*$ = redness), b^* ($-b^*$ = blueness; $+b^*$ = yellowness), C^* (chroma $C^* = [(a^{*2} + b^{*2})^{1/2}]$), and h_{ab^*} (hue [h_{ab^*} = arctan (b^*/a^*)]). For each cake, three measurements were done.

2.7. Sensory analysis

2.7.1. Sensory cake characterisation

Cake sensory characterisation using consumers was conducted through a Flash Profile test. This method, combines the free choice of the terms that characterise each of the samples, and the ratings of these terms by the panellist (Dairou & Sieffermann, 2002). Twenty-one untrained participants completed the test in two different sessions. In the first session, the six samples (CA-B, CA-EB, SAPP-B, SAPP-EB, GDL-B, and GDL-EB) were presented in triads and the participants created a list of attributes to describe the similarities and differences between them. The participants were told to focus on descriptive parameters such as flavour, texture, and appearance; and to avoid hedonic terms (Tárrega & Tarancón, 2014). In the second session each panellist ranked the cakes according to her/his own list of attributes created in the first session.

2.7.2. Sample liking

A consumer liking test of the six samples was conducted with a total of 89 consumers aged 17–45 years. Each consumer received the six samples coded with three-digit random numbers monadically and

randomly served at ambient temperature. The test was done using a 9-point hedonic scale (1 = "dislike extremely"; 9 = "like extremely") to score the liking of the 'appearance', 'texture', 'taste', and 'overall liking' of the cakes.

2.8. Data analysis

A categorical multifactorial experimental design with two factors: type of sodium bicarbonate (B or EB) and type of leavening acid (CA, SAPP, and GDL) was performed on the values for texture, cellular structure, and colour parameters. Analysis of variance (ANOVA) was performed on the data. The least significant differences (LSD) were calculated at the $p < 0.05$ significance level to compare the test means. A principal component analysis (PCA) was also performed to study the correlation between crumb structure parameters and texture parameters.

For the sensory analysis, a multi factorial analysis (MFA) was used for the Flash Profile data to identify the samples and terms most closely related to the characteristics of each cake formulation. A factorial map was generated to evaluate the general sensory positioning of the samples according to the participants' perception (Tarrega et al., 2017). To facilitate the interpretation of Flash Profile results, a hieratical cluster analysis (HCA) was subsequently performed. Beside this, the data obtained for consumer liking test were analysed using the analysis of variance (ANOVA), and the least significant differences (LSD) were calculated at the $p < 0.05$ significance level.

All the statistical analysis was performed with software XLSTAT version 2018.1 (Addinsoft España, Barcelona, Spain).

3. RESULTS AND DISCUSSION

3.1. Analysis of the crumb structure

3.1.1. Light microscope and image analysis of the cake batters at different baking temperatures

The images corresponding to the simulated micro-baking process are shown in Fig. 1A. They were taken at different temperatures to observe the evolution of the batter bubbles' expansion. The bubbles expand due to moisture evaporation and CO_2 produced by the reaction of the leavening agent (Narsimhan, 2014). The images obtained were analysed to quantify the size distribution of the bubbles for each formulation at different temperatures during micro-baking (Fig. 1B).

Batters formulated with encapsulated bicarbonate (EB) below temperatures of 70–80 °C showed a higher frequency of small bubble sizes (0–10,000 μm^2) in comparison with batters formulated with free bicarbonate (B). The melting temperature of the bicarbonate encapsulation is 69–73 °C; from that temperature on it can be observed that there is a sudden change in bubble size in formulations with EB, being the frequency of big bubbles (> 100,000 μm^2) lower than B batters. At that time, the encapsulated bicarbonate is released thus allowing it to react easily with the acid. In addition, at temperatures ranging from 80 to 95 °C the simultaneous occurrence of both starch gelatinisation and protein denaturation results in a large increase in batter viscosity leading to the matrix thermal setting (solid cake structure) (Germain & Aguilera, 2008; Godefroidt et al., 2019; Wilderjans et al., 2013). Therefore, at the end of the baking process, the bubble size distribution is mainly affected by the leavening agent rate of reaction and the matrix thermal setting. As a result, when reaching 90 °C formulations with EB have less time for the CO_2 release due to the encapsulation; this fact

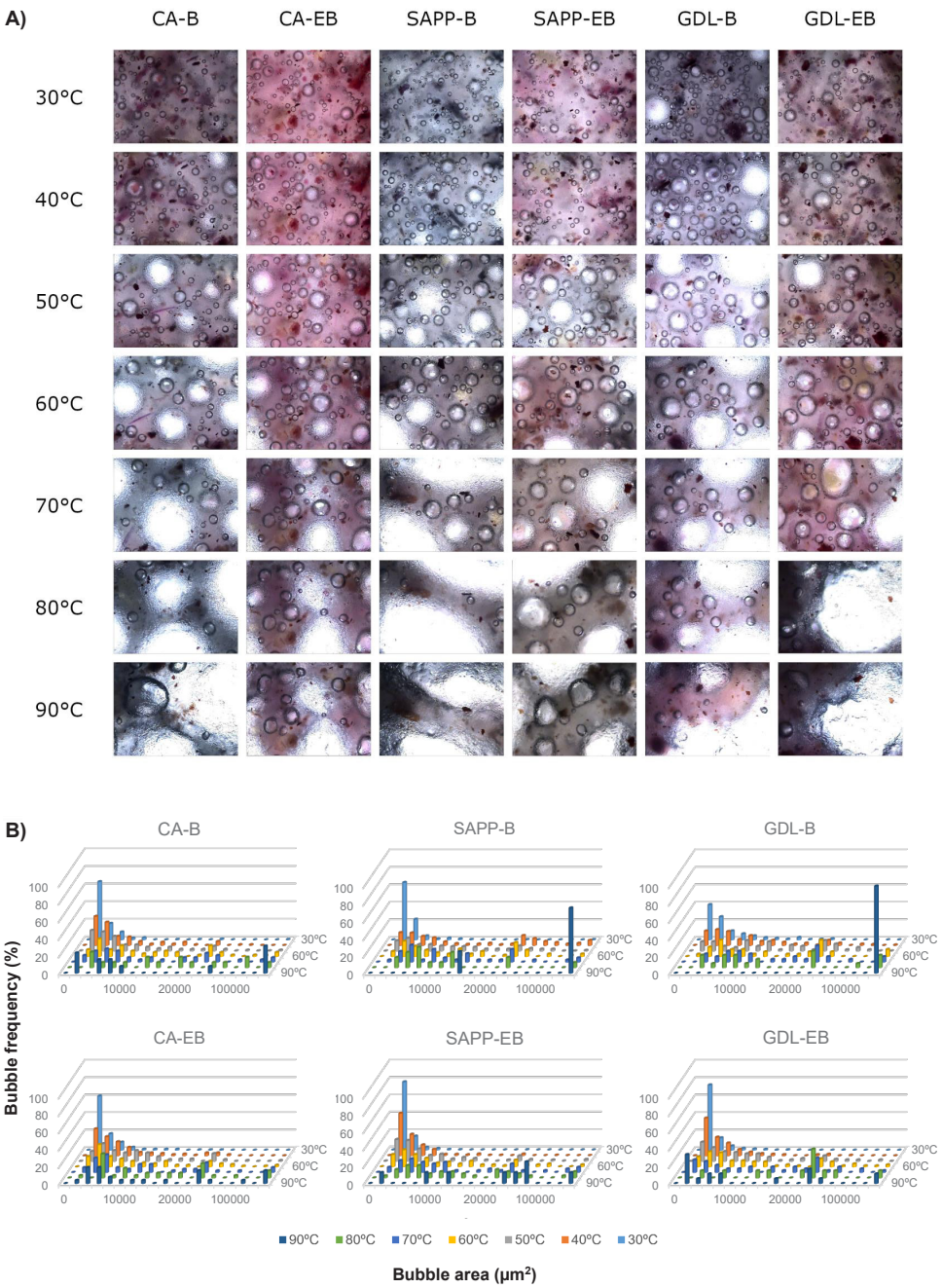

Figure 1. (A) LM images (4x) of bubble expansion in the six batters at different temperatures during micro-baking. (B) Bubble size distribution histograms on the six batters at the different temperatures during micro-baking. CA: citric acid; SAPP: sodium acid pyrophosphate; GDL: glucono-δ-lactone; B: free bicarbonate; EB: encapsulated bicarbonate.

together with the viscosity increase lead to the formation of smaller bubbles than formulations with B.

Citric acid (CA) has a fast reaction with sodium bicarbonate, thus during batter preparation at ambient temperature most of the CO_2 is released (Brose et al., 2001). For batters made with this acid, the images show that the bubbles have a constant growth. In the initial stages, though the fast reaction with the sodium bicarbonate, the bubbles are small for the initial release of CO_2. In addition, bubbles at higher temperatures do not have a large size compared to the rest of the batters, even in batters with free bicarbonate. This behaviour can be seen in the histograms corresponding to the cakes prepared with CA (with both, free and encapsulated bicarbonate) (Fig. 1B, CA-B and CA-EB). The lower frequency of big bubbles (> 100,000 μm^2) at high temperatures could be due to its fast reaction from low temperatures, leading to a smaller amount of CA or bicarbonate available at the end of the baking process.

In contrast to CA, sodium acid pyrophosphate (SAPP) is a slow-acting acid readily soluble in hot water; therefore it reacts with the bicarbonate later than CA in the baking process (De Leyn, 2014). In batters with sodium acid pyrophosphate (SAPP) the bubbles grew when the temperature increased due to the need of heat for SAPP to react with bicarbonate (Fig. 1A). The histograms corresponding to the batters prepared with SAPP show a high frequency of big bubbles at high temperatures (Fig. 1B, SAPP-B and SAPP-EB). In SAPP-EB sample, the bubble size distribution is broader and shows a homogeneous distribution of bubble sizes which are smaller than is SAPP-B. This is because the encapsulation of bicarbonate melts near 70 °C, which together with the need for solubilisation of the acid that occurs at temperatures close to the batter thermal setting and the end of the baking process, led to the reaction taking place at a higher temperature; therefore, the release of CO_2 is lower compared to SAPP-B.

Glucono-δ-lactone (GDL) is an acidic agent that needs to hydrolysate into gluconic acid to react with sodium bicarbonate to form CO_2. In both reactions carbon dioxide is released continuously being the reaction with bicarbonate much faster than the hydrolysation (Bellido et al., 2008; Brose et al., 2001). Bubbles in the GDL-B sample at low temperatures show a broader size distribution, *i.e.*, compared with the other formulations (Fig. 1B, GDL-B and GDL-EB), the frequency of small bubbles (< 10,000 μm^2) is lower but there is a higher frequency of bigger bubbles (10,000–20,000 μm^2). This phenomenon is because at the first stages there is a slow release of CO_2 from the hydrolysation of GDL into gluconic acid, which has a faster reaction with the bicarbonate in the baking stage with the subsequent CO_2 release (Bellido et al., 2008). Moreover, the sample GDL-EB has smaller bubbles in the first baking stages because the encapsulation of the base has not melted.

3.1.2. Cellular structure of the cake crumb

The images were analysed to quantify and compare the macrostructure of the crumb between samples. Two factors were considered: type of acid (CA, SAPP, and GDL) and type of bicarbonate (encapsulated or free). Fig. 2 shows the numerical data corresponding to the image analysis of the crumb. The results are presented only when there is a significant effect or an interaction between factors ($p > 0.05$).

Significant interactions ($p < 0.05$) between factors were only found for cell density, thus both the acid and the base had an influence on the number of cells In Fig. 2A, results corresponding to B-samples did not show significant differences among them regardless the type of acid used. In EB-samples, cakes with SAPP had a significantly lower cell density ($p < 0.05$) compared to CA and GDL samples. This was due to the slow-acting effect of SAPP explained earlier. In addition, there were no significant differences among samples with B or EB with the same acid. Hence, despite the interaction between both factors, there were only slightly differences between samples that could be negligible.

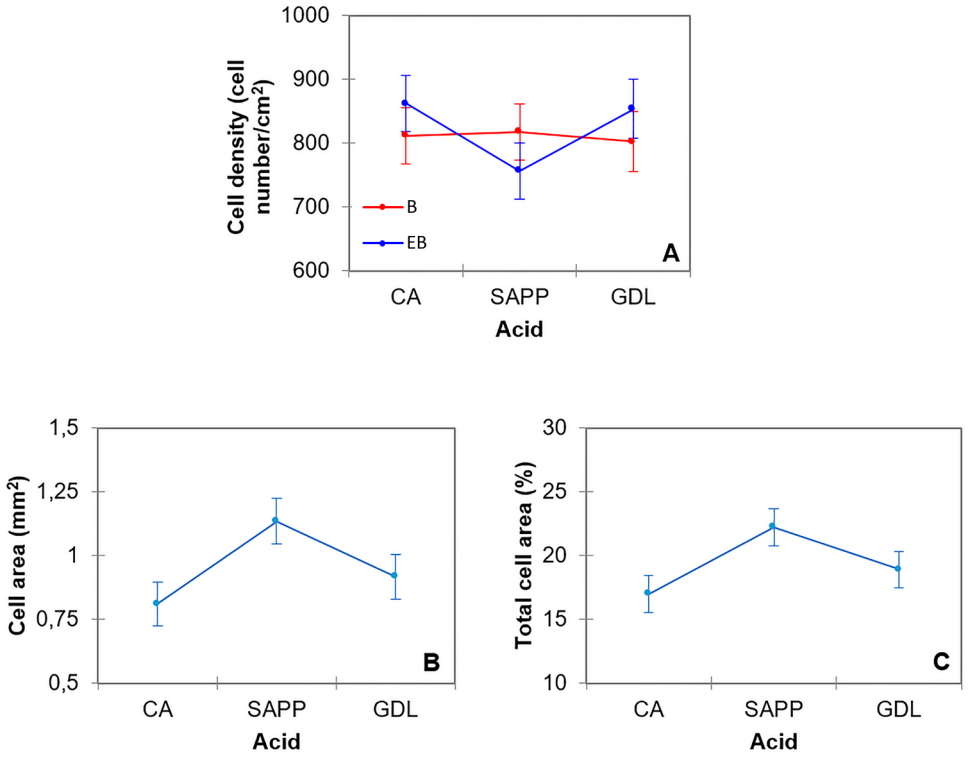

Figure 2. Mean values for cell structure with LSD intervals. (A) Interaction between the acid agent and bicarbonate (free bicarbonate in red and encapsulated bicarbonate in blue) for cell density. (B) Mean values for cell area; effect of acid agent. (C) Mean values for total cell area; effect of acid agent acid used. CA: citric acid; SAPP: sodium acid pyrophosphate; GDL: glucono-δ-lactone.

The type of acid used significantly affected ($p < 0.05$) the values for cell area and total cell area (Fig. 2B and C), but the effect of bicarbonate type (free or encapsulated) was not significant. In cakes with SAPP, the values for cell area and total cell area were significantly higher ($p < 0.05$) in comparison with CA and GDL. These results are consistent with those obtained for bubble size distribution during micro-baking, where a high frequency of big bubbles was observed at high temperatures. The late gas release from SAPP, when the batter viscosity has increased (temperatures near to the thermal setting point), lead to bigger bubbles due to a better retention of the CO_2 into de matrix. On the contrary, the

rapid gas release of CA could lead to an excessive loss of leavening gas thus forming smaller bubbles at the end of the baking process (Penfield & Campbell, 1990).

The different macroscopic structures seen in the crumb may be explained by the uneven expansion of the bubbles during baking, as observed in the micro-baking experiment (Fig. 1). Therefore, the formulations that achieved a lower bubble size at high temperatures (CA and GDL), presented a greater number of smaller cells, giving a lower percentage of total cell area, which implies less air in the crumb. Sodium acid pyrophosphate, regardless of the bicarbonate used, produced cakes with a higher amount of air incorporated in the crumb, since they presented a total cell area significantly higher ($p < 0.05$) than CA and GDL formulations.

3.2. Instrumental cake texture

The instrumental texture results for hardness, chewiness, cohesiveness, and springiness are shown in Fig. 3A–E. The results are only presented when a significant effect or an interaction between acid and base factors ($p > 0.05$) were found.

A significant interaction ($p < 0.05$) between acid and base for hardness values was detected (Fig. 3A). Hardness values of B-cakes (prepared with free bicarbonate) were significantly higher ($p < 0.05$) for cakes with CA, followed by GDL and SAPP (Fig. 3A). The same order was observed for the hardness values in EB-cakes (prepared with encapsulated bicarbonate) but significant difference ($p > 0.05$) was not found between CA and GDL samples. As expected, these results are in line with the macroscopic crumb structure features already analysed. SAPP samples had the highest total cell area values, which implies a more aerated structure that offers less resistance to compression. This relationship has been previously observed in sponge cakes prepared with oil substituted by inulin (Rodríguez-García et al., 2013). Chewiness presented a similar trend as hardness values (Fig. 3B).

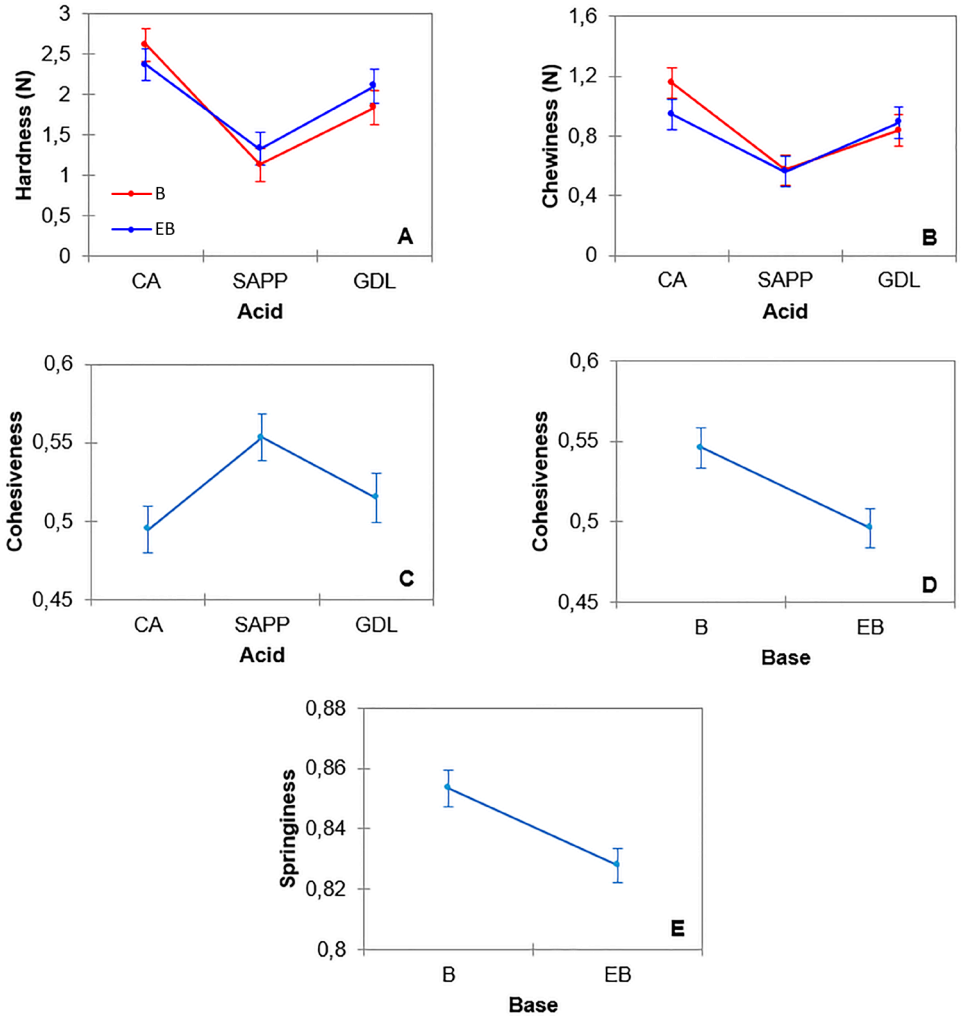

Figure 3. Mean value of instrumental texture parameters of the six cakes with LSD intervals. (A, B) Interaction effect between the acid and bicarbonate form for hardness and chewiness, respectively (free bicarbonate in red and encapsulated bicarbonate in blue). (C, D) Mean values for cohesiveness; effect of the acid agent and bicarbonate form, respectively. (E) Mean values for springiness; effect of the bicarbonate form. CA: citric acid; SAPP: sodium acid pyrophosphate; GDL: glucono-δ-lactone; B: free bicarbonate; EB: encapsulated bicarbonate.

No significant interactions ($p > 0.05$) between acid and base factors were found for cohesiveness and springiness parameters. Fig. 3C and D shows the means plots with the least significant difference (LSD) intervals for cohesiveness. Both the acids and bases had a significant influence on the results. The use of CA and GDL produced significantly ($p < 0.05$) less cohesive cakes compared to those prepared with SAPP. In addition, the use of EB decreased cohesiveness values significantly ($p < 0.05$) in comparison with B. Rodríguez-García el al. (2013) described that higher cohesivity in cakes was related to large air cells and compact crumb structures. Therefore, when the cells are bigger (Fig. 2B), a more cohesive final structure is created, *i.e.*, big bubbles give place a more compact structure.

Springiness values showed no significant interaction ($p > 0.05$) between the type of acid and base used (Fig. 3E). The type of bicarbonate was the factor with higher influence on the results, with EB-cakes having significantly lower springiness values ($p < 0.05$). Here, the generation of CO_2 is retarded until the encapsulation melted, which could lead to a more compact structure. Other authors (Rodríguez-García et al., 2014) related the lower springiness values to a decrease in the number of crumb cells and the existence of a denser matrix. Interestingly, while no differences were detected in the crumb structure when different bicarbonates were used, springiness values showed that the leavening agent reaction rate had an impact on the height recovery of the crumb after the first compression.

As Dewaest et al. (2018) described in their work, a principal component analysis (PCA) was carried out to understand the correlation between crumb structure and texture parameters. The main conclusions obtained were that hardness and chewiness parameters were strongly inversely correlated with bubble size. Thus, higher values of hardness and chewiness are related to smaller bubble size. The springiness parameter was not correlated with crumb cell structure and on the contrary, cohesiveness was negatively correlated with the number of cells and positively correlated with the cell size, being the latter factor the one with higher correlation.

3.3.Colour measurements

Table 2 shows the colour parameters (L^*, C^*, and h_{ab}^*) for the crumb of the different cakes. For crumb colour parameters, no interactions were detected between factors. L^* values did not present significant differences ($p > 0.05$) between samples, and the values indicated that all samples were dark.

C^* values presented significant differences ($p < 0.05$) between formulations depending on the acid used in the formulation, being significantly higher ($p < 0.05$) for SAPP, followed by GDL and CA. Higher values of C^* indicate that the red colour is less saturated and more vivid. The bicarbonate form used did not present differences in C^* values. GDL samples had a hue angle value (h_{ab}^*) significantly closer to reddish tones ($p < 0.05$) than the crumb from cakes CA and SAPP. Considering the bicarbonate type, the formulations for each acid made with EB had greater reddish hue compared to cakes with B.

Table 2. Mean values of sponge cake colour parameters (L^*,C^* and h_{ab}^*), by formulation. CA: citric acid; SAPP: sodium acid pyrophosphate; GDL: glucono-δ-lactone; B: free bicarbonate; EB: encapsulated bicarbonate.

Sample	L^*	C^*	h_{ab}^*
		CRUMB	
CA-B	$25.37^{a} \pm 2.43$	$8.46^{a} \pm 0.64$	$60.19^{d} \pm 2.29$
CA-EB	$26.48^{a} \pm 0.97$	$8.81^{ab} \pm 0.18$	$55.86^{c} \pm 2.02$
SAPP-B	$25.23^{a} \pm 3.88$	$11.79^{c} \pm 2.44$	$57.51^{c} \pm 0.82$
SAPP-EB	$25.47^{a} \pm 1.24$	$11.74^{c} \pm 0.95$	$56.85^{c} \pm 1.78$
GDL-B	$27.87^{a} \pm 0.50$	$10.25^{b} \pm 0.69$	$50.48^{b} \pm 1.60$

Values for colour parameters are mean ± standard deviation of (n = 9) determinations. Means in the same row without a common letter are significantly different ($p < 0.05$) according to the LSD multiple range test.

3.4. Sensory analysis

3.4.1. Flash profile

All the terms generated by the participants are shown in Table 3. A total of 38 different terms were collected (13 of texture, 6 of appearance, 18 of flavour, and 1 of odour). The participants gave high importance to the attributes of texture, since texture-related terms had 66 mentions, followed by flavour (n = 42), appearance (n = 33), and odour (n = 2).

Fig. 4A shows a two-dimensional multi factorial analysis (MFA) plot of the sample configuration. The two first factors of the plot explain 62.76% of the experimental data's variability. The first factor explains 37.94% of the variability; it separates the samples with CA and SAPP-B (positive values of the X-axis) from the rest although the latter one is placed towards the zero value of this axis. The second factor (27.82% of the variability) separates principally the sample GDL-EB (very positive values of Y-axis) from sample SAPP-B (very negative values of Y-axis); also, in negative values of Y-axis are placed the samples SAPP-EB and GDL-B.

The sensory terms used to describe the samples are shown in Fig. 4B. Many of the attributes were spread all over the map, especially those corresponding to aspect and flavour such as berry flavour, and dark, indicating that they are not distinctive for any cake. Conversely, texture attributes do make the difference. Thus, as described by Lassoued et al. (2008), a hieratical cluster analysis (HCA) was carried out in order to more easily identify the attributes that describe each sample. The HCA revealed three cluster groups of sensory attributes (Fig. 4B). The attributes that are most differentiating between groups are for Cluster 1: dry, greyish and sweet (samples CA-B and CA-EB); for Cluster 2: brittle, spongy, brownish/ reddish and sweet (samples SAPP-B, SAPP-EB and GDL-B); and for Cluster 3: hard, compact, gummy, greyish, oily and rancid (sample GDL-B). These

Table 3. List of the terms generated in the first session of the Flash Profile.

Texture	Global appearance	Taste	Odour
Spongy (n=15) *	Dark (n=9)	Sweet (n=11)	Intense odour (n=2)
Compact (n=11)	Greyish (n=8)	Acid (n=7)	
Brittle (n=7)	Brownish (n=7)	Berry taste (n=4)	
Grainy (n=7)	Reddish (n=7)	Oily (n=3)	
Dry (n=5)	Alveoli size	Bitter (n=2)	
Juicy (n=5)	Intense colour	Fruity (n=2)	
Hard (n=4)		Salty (n=2)	
Gummy (n=3)		Cake	
Soft (n=3)		Carrot	
Sticky (n=3)		Intense taste	
Chewable		Mint	
Pasty		Neutral	
Rough		No identified	
		Rancid	
		Strange	
		Spicy	
		Sweetened	
		Tasty	
TOTAL:66	**TOTAL:33**	**TOTAL:42**	**TOTAL:2**

*in brackets appears the frequency of mention of the terms named in more than one occasion.

characteristics of texture perceived by the panellists could be related to the values obtained from the instrumental texture analysis (TPA) and cellular structure analysis of the crumb. SAPP-B and SAPP-EB from Cluster 2 described above as spongy and brittle cakes, were characterized as softer cakes with higher cohesiveness values and bigger cell area compared with cakes in Cluster 1 (CA-B and CA-EB) which were characterized as dry cakes. On one hand the attributes

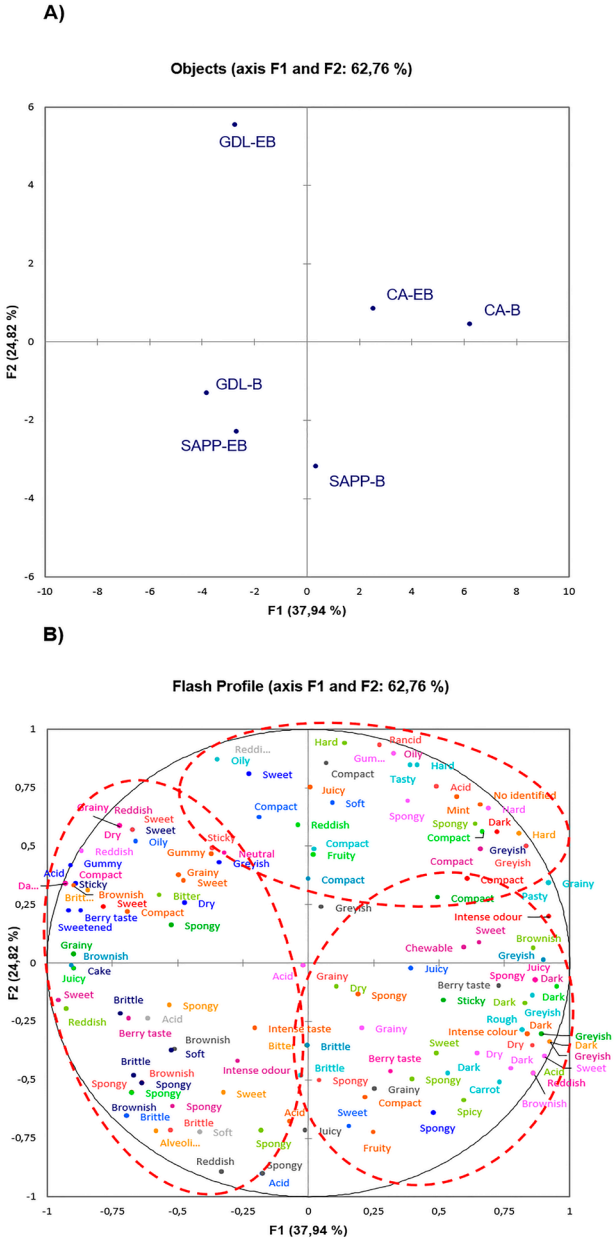

Figure 4. (A) Representation of the six cakes formulations along the first two dimension of the Multi Factorial Analysis (MFA). (B) Representation of the terms from the flash profiling data with the Clusters defined by HCA represented with doted lines. CA: citric acid; SAPP: sodium acid pyrophosphate; GDL: glucono-δ-lactone; B: free bicarbonate; EB: encapsulated bicarbonate.

that principally defined the second cluster confirm the differences in texture and related air cell distribution of the cakes perceived by the panellists, and on the other highlight the importance of the different colours as a discriminant factor between samples. Thus, the flash profile could be considered as a complementary analysis for texture and colour characterisation.

3.4.2. Consumer liking testing

The scores for liking of 'appearance', 'texture', 'taste', and 'overall liking' of the different formulations are shown in Table 4.

The statistical analysis showed that the mean values for liking of cake appearance only had significant difference ($p < 0.05$) between formulations SAPP-EB and GDL-B. Regarding the texture liking, the consumers did not score significant different mean values ($p > 0.05$) between any of the tasted samples. The cakes made with SAPP were the rated the significantly more liked ($p < 0.05$) in terms of taste while the

Table 4. Consumer acceptance test results. CA: citric acid; SAPP: sodium acid pyrophosphate; GDL: glucono-δ-lactone; B: free bicarbonate; EB: encapsulates bicarbonate.

Sample	Appearance	Texture	Taste	Overall acceptability
CA-B	5.6^{ab}	5.8^{a}	6.0^{bc}	6.3^{cd}
CA-EB	5.9^{ab}	6.0^{a}	5.6^{b}	5.7^{bc}
SAPP-B	6.2^{b}	6.3^{a}	6.3^{c}	6.3^{cd}
SAPP-EB	6.0^{b}	6.0^{a}	6.3^{c}	6.4^{d}
GDL-B	5.4^{a}	5.7^{a}	5.6^{b}	5.6^{ab}
GDL-EB	6.0^{ab}	5.9^{a}	4.9^{a}	5.1^{a}

Means in the same row without a common letter are significantly different ($p < 0.05$) according to the LSD multiple range test.

cake GDL-EB obtained the lowest score. Taste liking results are related with the flash profile results, as GDL-EB cakes were characterised as oily and rancid, that could be considered as negative attributes. Finally, the cakes CA-B, SAPP-B, and SAPP-EB had an overall liking mean values significantly ($p < 0.05$) higher than the rest of formulations, which could be attributed to a softer, spongy texture.

4. CONCLUSION

The strategy of changing and combining leavening agents with different rates of CO_2 release produced modifications in the size distribution of the bubbles into the batter, giving place to differences in cake crumb structures that were correlated to texture parameters. These differences were perceived by consumers, which were able to identify attributes describing each sample. Cakes prepared with pyrophosphate (regardless bicarbonate type) were described as brittle, spongy and sweet, and obtained high scores in global acceptability. Therefore, pyrophosphate could be considered as a good option in bakery to facilitate the use of functional high-fibre ingredients, such as by-products of the fruit and vegetable industry. We believe these practices surely would contribute to greater sustainability in the food industry.

5. CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Elena Diez-Sánchez: Investigation, Validation, Formal analysis, Writing - original draft. Empar Llorca: Methodology, Investigation. Amparo Tárrega: Methodology, Formal analysis. Susana Fiszman: Conceptualization, Writing - review & editing. Isabel Hernando:

Conceptualization, Supervision, Resources, Funding acquisition, Writing - review & editing.

6. DECLARATION OF COMPETING INTEREST

Declarations of interest:none.

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Extruded flour as techno-functional ingredient in muffins with berry pomace

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A B S T R A C T

Blackcurrant pomace is a by-product from fruit juice production, which is rich in polyphenols and dietary fibre. When added to bakery products, its high fibre content has detrimental effects on dough/batter viscosity and on the texture and the structure of the final product. In this study, wheat flour extruded at different temperatures was used to replace 50% of wheat flour in a muffin formulation to counteract these detrimental effects. In addition, the effect of blackcurrant on the glucose release during *in vitro* digestion was analysed. The extrusion treatment of the flour caused a disruption of the starch granules, and affected starch characteristics such as water binding capacity and swelling. Its incorporation in muffins did not produce important differences in texture, nor in consumer acceptance, compared to a standard formulation. Moreover, the addition of blackcurrant counteracted the hyperglycaemic effect of pre-gelatinised starch.

Keywords: *In vitro* digestion; pregelatinized flour; bakery products; blackcurrant pomace.

1. INTRODUCTION

Blackcurrant (*Ribes nigrum* L.) is a berry variety rich in phytochemicals such as polyphenols. Beyond the simple nutritional effect of their ingredients, polyphenols have beneficial effects on health. These effects are related to their inner antioxidant capacity which may, among others, have a positive impact on cardio-vascular health, on reducing inflammation and on the intestinal microbiota (Shahidi & Ambigaipalan, 2015).

The production of berry-based juice generates by-products, mainly composed of peel and seeds, which contain a high amount of beneficial compounds, such as polyphenols and fibre, and which may be used for production and recovery of value-added ingredients and other

products (Struck, Plaza, et al., 2016). Górnaś et al. (2016) proposed the enrichment of baked products with fruit pomace, specifically the use of pomace in muffins due to their wide consumption in the population.

The main challenges generated by incorporating ingredients with high fibre content such as pomace in the formulation of muffins come from detrimental effects on the creation of an aerated structure as the fibre modifies batter properties, quality characteristics of the baked product (volume, texture) and nutritional composition (Gularte et al., 2012; Quiles et al., 2016). To improve product quality, the formulation may be modified by adding ingredients that improve related properties, e.g. pre-gelatinised starch. In this regards, Hesso et al. (2014, 2015) and Karaoğlu et al. (2001) studied the impact of pre-gelatinised starches on the texture of cakes, and Miller et al. (2008) evaluated the effect of adding pre-gelatinised modified wheat starch to bread.

Pre-gelatinised starch can be obtained by flour extrusion. Extrusion is a processing technique classified as HTST (high temperature/short-time), where starch granules undergo gelatinisation and melting by the action of heat, pressure, shearing and moisture. Besides starch gelatinisation, there are changes in the flour such as Maillard reactions, improved water absorption, partial or complete destruction of the crystalline structure, protein denaturation and enzyme inactivation (Altan et al., 2009). The degree of these changes depends mainly on extrusion conditions, such as temperature and moisture. Due to the fracture of starch granules, these are more accessible to enzymatic hydrolysis, which leads to a fast conversion of starch to glucose, giving place to an increase in glucose release (Román et al., 2017; Singh et al., 2007). However, dietary fibre and polyphenols can potentially decrease this effect through the inhibition of digestive enzymes (McDougall et al., 2005; Mofasser Hossain et al., 2017).

There are only a few examples where extruded flour is used in bakery products: Gill et al. (2002) substituted wheat flour with extruded barley flour in bread, Jisha et al. (2010) substituted whole wheat flour by pre-

gelatinised cassava flour in muffins and cookies, and Martínez et al. (2013) added extruded wheat flour to bread. The aim of the present study is to use extruded flours in muffins prepared with blackcurrant pomace to counteract the undesirable effects on the batter rheological behaviour due to the high fibre content.

2. MATERIAL AND METHODS

2.1. Muffin ingredients

Muffins were prepared with wheat flour (Harinas Segura S.L, Torrente, Valencia, Spain; composition provided by the supplier: 13.5–15.5 g/100 g moisture, 9–11 g/100 g proteins); white sugar (AB Azucarera Ibérica S.L.U., Madrid, Spain); egg yolk and white, both as pasteurized liquids (Ovocity, Llombay, Valencia, Spain); skim milk powder (Corporación Alimentaria Peñasanta, S.A., Siero, Asturias, Spain); refined sunflower oil (Aceites del Sur-Coosur, S.A., Vilches, Jaén, Spain); sodium bicarbonate E-500ii and citric acid E-300 (Sodas y Gaseosas A. Martínez, S.L., Cheste, Valencia, Spain) and salt. Blackcurrant pomace was provided by Döhler GmbH (Darmstadt, Germany). The pomace was dried at 70 °C for 2 h and milled in a ZM 100 ultracentrifugal mill (Retsch GmbH, Haan, Germany) at 14 000 rpm using a 1 mm sieve. The blackcurrant pomace fibre content was 37.7 ± 2.9 g/kg of soluble dietary fibre (SDF) and 551.6 ± 16.5 g/kg of insoluble dietary fibre (IDF) (Reißner et al., 2019).

2.2. Total phenolic content of blackcurrant pomace

Samples (2.5 g) were extracted with 20 mL methanol/water (50:50, v/v) for 60 min and centrifuged for 15 min at 25400x *g* and 22 °C. The sediment was again extracted with 20 mL acetone/water (70:30, v/v) for another 60 min and centrifuged. The supernatants were combined and

used for further analysis. The total phenolic content was determined by the Folin–Ciocalteu colorimetric method first described by Singleton & Rossi (1965). 0.20 mL sample extract or methanol (blank) were mixed with 2.50 mL 10 mL/100 mL Folin-Ciocalteu reagent (v/v) and 2.00 mL 4 g/100 g sodium carbonate (w/v). The mixture was stirred in sealed containers for 2 h in the dark, decanted and the resulting absorbance was measured at 750 nm. Total phenolic content was calculated using a previously determined gallic acid (GAE) calibration curve and the results were expressed as gallic acid equivalents per mg dry matter sample (mg GAE/g DM).

2.3. Flour extrusion

The wheat flour was extruded using a Kompaktextruder 19/25 DN (Brabender GmbH & Co. KG, Duisburg, Germany), with a 19 mm diameter barrel and a barrel length/diameter ratio of 25/1. A 1:1 screw was used at a speed of 95 rpm; the die diameter was 3 mm. The flour was extruded with the addition of 20% moisture and with a maximum temperature at the die zone of the extruder of 50, 80, 110 and 150 °C (F50, F80, F110 and F150), respectively. The products of two separate batches were dried in a convection oven at 50 °C for 30 min and then ground in a food processor (Thermomix TM 31, Wuppertal, Germany) at 700 rpm for 10 s. The four types of extruded flour were packed in twist-off jars and stored at room temperature until use.

2.4. Analysis of extruded flour

Moisture content of wheat flour, extruded flours and berry pomace was determined by drying to mass constancy (MA30 moisture analyser at 105 °C; Sartorius AG, Göttingen, Germany). The water binding capacity (WBC), defined as the amount of water that is bound per mass unit of flour, dry matter related, was determined using the centrifugation

method described by (Reißner et al., 2019).

Pasting properties were measured with a Physica MCR300 rheometer (Anton Paar GmbH, Ostfildern, Germany) equipped with a ST-24-2D/CC-27 stirrer/cylinder geometry. Suspensions of flour in water (10 g dry matter/100 g) were continuously stirred at 150 rpm, starting at 50 °C for 15 min. With a heating rate of 2 K/min temperature was raised to 92 °C, kept constant for 10 min, decreased to 50 °C at 1 K/min, followed by another 10 min isothermal section. Parameters used for interpretation are maximum torque (M_{max}), temperature at maximum ($T(M_{max})$), and the torque at the end of the last holding phase (M_{end}).

For light microscopy analysis of the flours, solutions of iodine (10 g/L) or toluidine blue (1 g/L) in distilled water were used to stain starch or proteins, respectively. The flours were also studied with polarized light. The micrographs were stored at 1280 x 1024 pixel resolution using the microscope software (NIS-Elements F, Version 4.0, Nikon, Tokyo, Japan).

2.5. Batter and muffin preparation

The control formulation contained 140 g wheat flour, 200 g sugar, 100 g reconstituted skim milk, 54 g egg yolk, 108 g egg white, 10 g water, 40 g blackcurrant pomace powder, 92 g sunflower oil, 6 g citric acid, 8 g sodium bicarbonate and 3 g salt. The amount of pomace added results in 3,1g of fibre by 100g of muffin, so it is possible to claim these muffins as a "source of fibre" (European Commission, 2006). In the test formulations, 50% of the wheat flour was replaced by wheat flour extruded at 50, 80, 110 or 150 °C. Moisture contents of wheat flour and extruded flour were corrected to 14 g/100 g. The batters were prepared using the 'all in' mixing procedure of Rodríguez-García et al. (2014). Ingredients were mixed in a Major Classic mixer (Kenwood Ltd., Havant, UK) for 30 s at 202 rpm, followed by 1 min at 260 rpm and 3 min at 320 rpm. 45 g of batter were poured into 6 cm diameter and 4 cm height moulds and placed in an EOC3430DOX convection oven

(Electrolux AB, Stockholm, Sweden) preheated to 180 °C for 30 min. The muffins were baked at 180 °C for 33 min. After removing from the oven, they were left to cool for 24 h. Half of the muffins were used for muffin characterisation (texture and sensory analysis) and the other half were vacuum packed and stored at -20 °C for the *in vitro* digestion analysis. All the batters and muffins were prepared in triplicate.

2.6. Muffin batter analysis

Batters for rheological experiments were prepared in duplicate without sodium bicarbonate and citric acid to avoid gas formation and measurement interference. A Peltier controlled plate/plate geometry of the MCR300 was loaded with batter, a gap of 2 mm was adjusted, and excess material trimmed with a spatula. Flow curves were measured as a logarithmic function of shear rate (0.1 – 100/s).

Simulated baking was performed in small strain oscillation at 1 Hz and a strain of 0.05%. After a resting period of 10 min at 25 °C, temperature was increased to 100 °C at 5 K/min, reflecting the heating rate during oven baking of muffins. During oscillation, the gap was covered with vaseline oil and a solvent trap was applied to prevent moisture loss.

Microstructure of the batters was analysed using Confocal Laser Scanning Microscopy (CLSM), following the methodology used by Rodriguez Garcia et al. (2012).

2.7. Characterisation of muffins

Muffin texture was assessed with a TAXTplus texture analyzer (Stable Microsystems, Ltd, Godalming, UK), using the Texture Exponent Lite 32 program (version 6.1.4.0, Stable Microsystems). Cubes (1.5x1.5x1.5 cm) cut from the centre of the muffins were compressed to 40% of their original height at 1 mm/s using a 35-mm diameter aluminium plate. After decompression and a 5 s resting time, a second compression was performed. The trigger force was set to 0.05 N. After the two

compression cycles, the following parameters were recorded: hardness (force maximum in the first compression), cohesiveness (ratio of the areas under second and first compression), and springiness (remaining relative sample height at the start of the second compression; Zahn et al., 2013). The measurements were carried out in triplicate.

Consumers were recruited among students and employees of the Universitat Politècnica de València (Spain). A total of 80 untrained panellists (consumers) aged 18-65 were asked to contribute to the study.

The samples were assessed in a standardized tasting room equipped with individual booths. Muffins were cut in four pieces, and one piece of each formulation (CM, M50, M80 and M150) coded by three-digit random numbers, was given to the consumers. The pieces of muffins were served at room temperature in random order. Water was supplied for mouth cleansing. Consumer acceptance testing was done using a nine-box structured hedonic scale to evaluate 'appearance', 'texture', 'taste' acceptability, and 'overall acceptance' of the product (from 1 = 'I dislike it extremely' to 9 = 'I like it extremely').

2.8. *In vitro* digestion

In vitro digestion was carried out for the flours (CF, F50 and F150) and for the baked products (CM, M50 and M150). Digestion of the muffins covered three stages: oral, gastric, and intestinal. The protocol described by Quiles et al. (2018) was used to simulate the biological fate of ingested samples.

The experimental data were fitted to the first-order equation proposed by Goñi et al. (1997):

$$C = C_{\infty} (1 - e^{-kt})$$

where C is the concentration at t time, C_{∞} is the equilibrium concentration, k is the kinetic constant, and t is the chosen time.

The areas under hydrolysis curves (AUC, 0–180 min) were calculated as the integral of the kinetic equation and were used to obtain the starch hydrolysis index (HI). The HI was calculated as the ratio of sample AUC and the AUC for a reference food, white bread, expressed as a percentage (Goñi et al., 1997). The predicted glycaemic index pGI was calculated according to Goñi et al. (1997):

$$pGI = 39.71 + 0.549HI$$

For each formulation, this procedure was performed in triplicate.

2.9. Statistical analysis

Statistical analysis of variance (ANOVA) was performed on the data using the Statgraphics Centurion XVI.II software package (StatPoint Technologies, Inc., Warrenton, Va., U.S.A.). Fisher's least significant difference (LSD) test was used to evaluate mean difference values ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Impact of extrusion temperature on flour properties

The moisture content of the extruded flours ranged from 10.25 – 12.67 g/100 g (Table 1), a typical moisture that ensures appropriate storage stability. The water binding capacity ranged from 1.02 – 10.07 g/g dry matter (DM), and was lowest for the commercial wheat flour (1.02 g/g DM). With increasing temperature during extrusion, WBC increased up to ~10 g/g DM for F110. Between 80 and 110 °C a fourfold increase in WBC was observed, which can be attributed to the gelatinisation of wheat starch that starts at approx. ~75 °C (Goesaert et al., 2005). The absence of significant differences between the F110 and F150 flours indicate that the starch was almost completely gelatinised.

Table 1. Physicochemical and pasting properties of control wheat flour (CF) or with 50% control flour and 50% flour extruded at different temperatures: 50°C (F50); 80°C (F80); 110°C(F110); 150°C (F150). Mmax is maximum torque in the pasting experiment, T(Mmax) is the corresponding temperature, and Mend is torque at the end of the experiment.

Sample	Moisture content [g/100 g]	Water binding capacity [g/g dry matter]	M_{max} [mN.m]	$T(M_{max})$ [ºC]	M_{end} [mN.m]
CF	$10.88^{bc} \pm 0.08$	$1.02^{c} \pm 0.06$	$1.61^{a} \pm 0.02$	$91.1^{a} \pm 0.01$	$1.90^{a} \pm 0.02$
F50	$12.67^{a} \pm 0.75$	$1.61^{c} \pm 0.15$	$1.03^{b} \pm 0.01$	$91.1^{a} \pm 0.02$	$1.44^{b} \pm 0.03$
F80	$11.24^{b} \pm 0.53$	$2.40^{b} \pm 0.33$	$0.59^{c} \pm 0.01$	$91.1^{a} \pm 0.12$	$0.83^{c} \pm 0.03$
F110	$10.01^{c} \pm 0.34$	$10.07^{a} \pm 0.33$	$0.50^{d} \pm 0.01$	$86.0^{b} \pm 1.22$	$0.52^{d} \pm 0.00$
F150	$10.25^{bc} \pm 0.69$	$9.86^{a} \pm 0.78$	$0.42^{e} \pm 0.00$	$81.7^{c} \pm 0.08$	$0.44^{e} \pm 0.00$

Mean values in a column with different superscripts differ significantly ($p < 0.05$) according to the LSD multiple range test. Values for moisture content and WBC are mean ± standard deviation of (n = 3) determinations. Values for pasting properties are mean ± half deviation range (n=2).

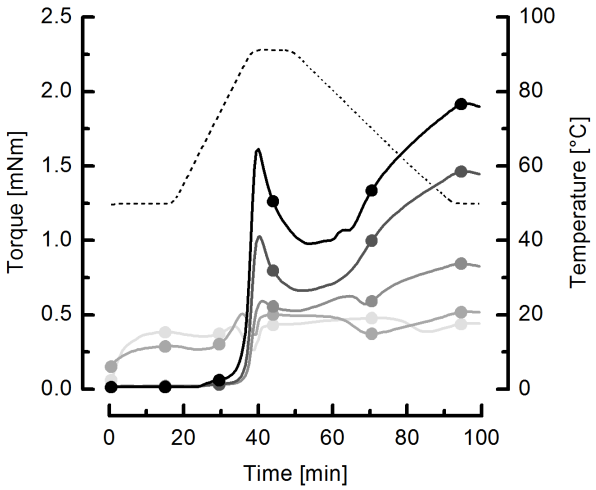

Figure 1. Pasting profiles of control wheat flour (● CF) and flour extruded at different temperatures: 50°C (● F50); 80°C (● F80); 110°C (● F110); 150°C (● F150).

Fig. 1 shows the pasting profiles of the flour suspensions. The control flour, F50 and F80 show a typical pasting behaviour with a torque that increases at a specific gelatinisation temperature, a viscosity breakdown and a setback during subsequent cooling (Table 1). It is also evident that the extrusion process clearly influences the pasting properties of the modified flour. For F110 and F150 the initial torque was significantly higher, indicating that the starch is at least partially gelatinised by the high temperature that was applied during extrusion (see also the effects on WBC). Hence, the solubilised amylose increases the viscosity of the suspension already at 50 °C.

The maximum torque M_{max} was highest for CF and decreased for flours that were produced with increasing extrusion temperature. Generally, the starch granules swell and their increasing volume causes a rise of the torque in the pasting experiments. High temperature during extrusion and the respective mechanical stresses already results in partial gelatinisation and fragmentation of starch granules (Wang et al., 1993). In addition, the temperature at M_{max} decreases from 91.1 °C for CF, F50 and F80 to 81.7 °C for F150 the loss of starch granule integrity presumably is causing the decrease in pasting temperature (Ye et al., 2018).

The increase of the measured torque during cooling (Fig. 1) is caused by the reorganisation and interaction of solubilised amylose molecules. Much lower final viscosities are reached for F110 and F150 with their highly pre-gelatinised starches, and additional effects may come from the reduced molecular masses of amylose and amylopectin through fragmentation that also impact the gelation behaviour of starch suspensions (Politz et al., 1994).

Fig. 2 shows the microstructure of the control flour and the flours extruded at different temperatures. Maltese crosses can be observed in the polarized light images because of the semicristalline structure of the starch granules. CF showed the greatest number of these crosses, followed by F50, F80 and F150. The effect of birefringence and hence the Maltese cross patterns decreased with the severity of the extrusion

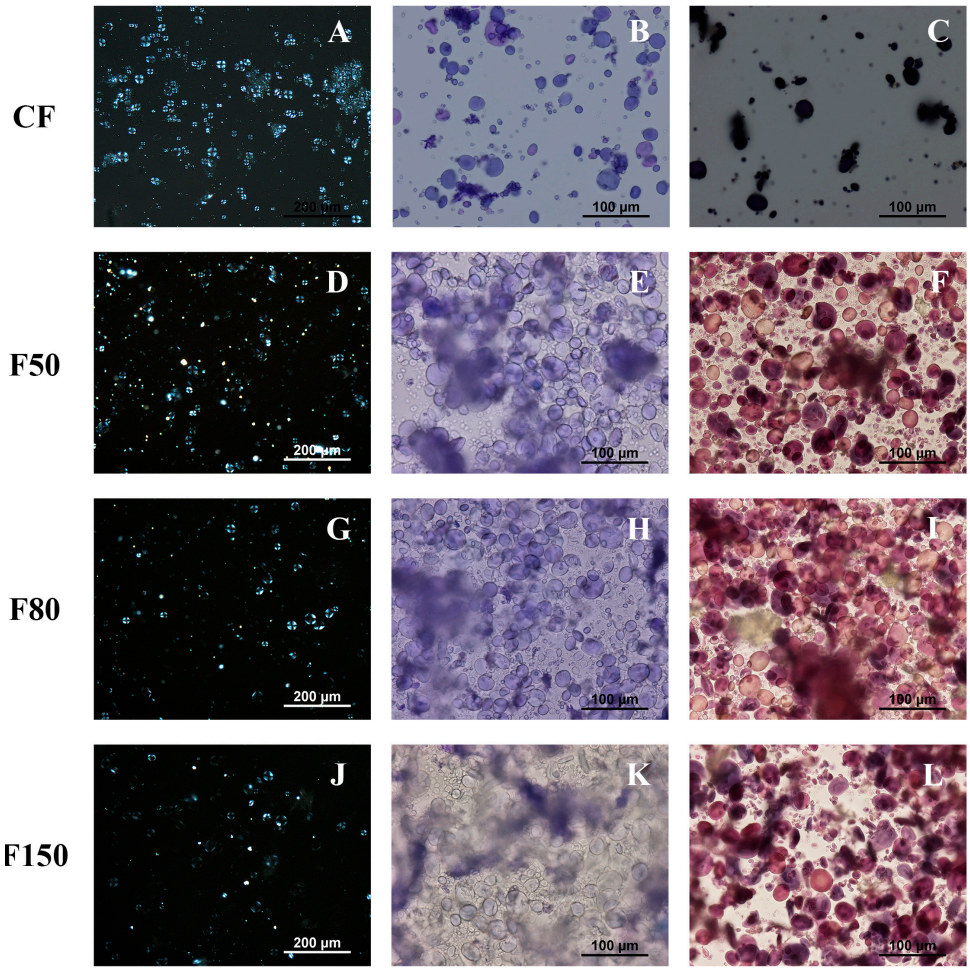

Figure 2. Light microscopy images of control wheat flour (CF) and flour extruded at different temperatures: 50°C (F50); 80°C (F80); 150°C (F150). Polarized light (A, D, G and J), toluidine blue staining (B, E, H and K) and iodine staining (C, F, I and L).

treatment, because of the starch granule degradation that occurs during the process of pre-gelatinisation induced by extrusion (Altan et al., 2009; Chevallier et al., 2000).

The dying with toluidine blue and iodine allowed the observation of two different populations of starch granules in CF, namely larger and smaller ones. The extruded flours contain larger disaggregated granules than the control flour because of the loss of granular integrity

derived from the applied temperatures and the mechanical shearing during extrusion. With higher treatment temperature, the proportion of deformed starch granules increased due to the progressive gelatinisation and swelling of the starch granules, and more starch compounds were released to the matrix. It is important to highlight that, for the treatment at 150 °C, a lot of granules are disintegrated; in fact, empty granules can be seen in the image K, meanwhile in the treatment at 80 °C (image H, Fig. 2) the granules are only partially disintegrated, and part of the amylose and amylopectin still remains inside the granule. The leaching of starch components and the swelled structure of F150, corresponding to a highly pre-gelatinised starch, are presumably related to the higher initial torque in pasting experiments (Fig. 1).

3.2. Muffin batter properties

The viscosity curves of muffin batters are shown in Fig. 3. Apparent viscosity at a shear rate of 10/s ranged between 4.7 and 16.5 Pa·s, with the control batter exhibiting the lowest viscosity over the investigated shear rate range. Viscosity of muffin batters increased when regular

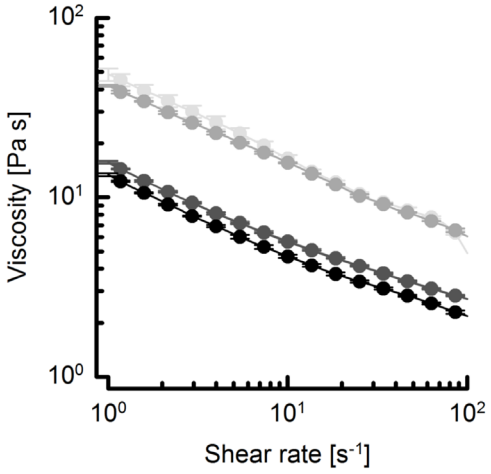

Figure 3. Flow curves at 25°C of muffin batters made with control flour (● CB) or with 50% control flour and 50% flour extruded at different temperatures: 50°C (● B50); 80°C (● B80); 110°C (● B110); 150°C (● B150).

wheat flour was partly replaced by extruded flour, and this increase was higher at higher extrusion temperature and thus a higher degree of starch pre-gelatinisation. As also observed for WBC and pasting behaviour, the changes in solubility and gelation properties of pre-gelatinised starch are visible in the pronounced increase of batter viscosity between B80 and B110.

Fig. 4 depicts the results of the simulated baking experiments. At the beginning of the experiments, the complex modulus G* as a measure of batter stiffness decreased with increasing temperature until G* reached a minimum between 61 and 73 °C. Subsequently, batter stiffness increased up to 100 °C, primarily caused by starch gelatinisation and protein denaturation. This increase is most pronounced for the control batter, having the highest content of native wheat flour. The replacement of wheat flour with extruded flour increases the content of pre-gelatinised starch. B110 and B150 show a higher initial G*, which is in accordance with the results of the pasting experiments. Such a higher batter stiffness may inhibit bubble growth, resulting from the gas release of the leavening agent, before structure setting at higher temperature (Struck, Gundel, et al., 2016; Struck, Plaza, et al., 2016).

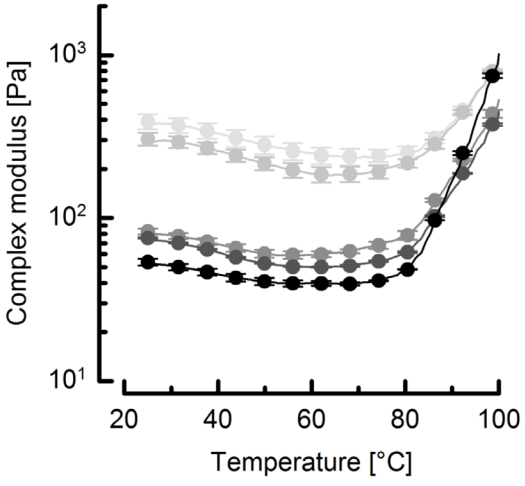

Figure 4. Temperature sweeps in oscillatory measurement (1 Hz, strain 0.05 %) of muffin batters made with control flour (● CB) or with 50% control flour and 50% flour extruded at different temperatures: 50°C (● B50); 80°C(● B80); 110°C (● B110); 150°C (● B150).

The microstructure of the muffin batters stained with Nile Red and Rhodamine B is shown in Fig. 5. Starch granules of different sizes appear in black, fat in green, and protein and dispersed carbohydrate appear in red-orange. Beside this, fibre particles can be observed in intense orange.

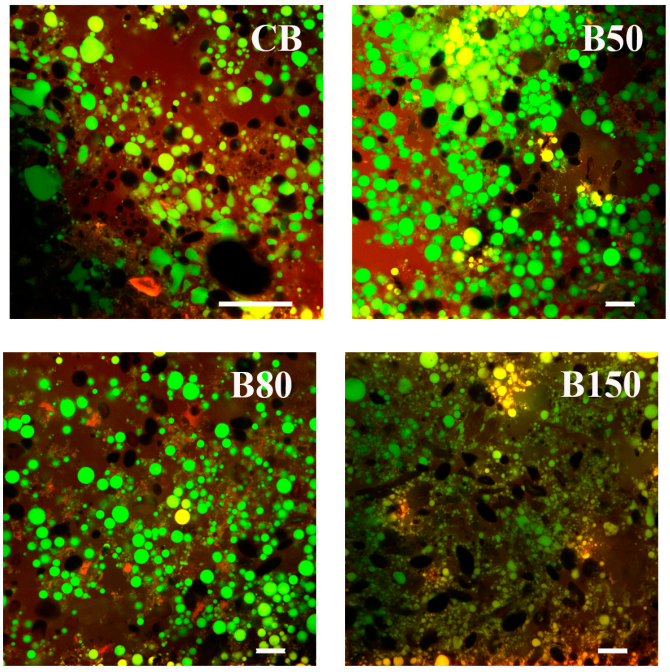

Figure 5. Confocal Laser Scanning Microscopy images (40X) of muffin batters made with control flour (CB) or with 50% control flour and 50% flour extruded at different temperatures: 50°C (B50); 80°C (B80); 150°C (B150).

Micrographs of the batters show a continuous matrix composed mainly of egg, milk and flour proteins. Starch granules are distributed in the matrix, and the fat exhibits a globular structure. Rodríguez-García et al. (2012) previously described a similar structure for cake batters. The description is also in accordance with Chevallier et al. (2000), who presented dough structure as a suspension of proteins, starch–protein associations, and isolated starch granules in a liquid continuous phase

based on an emulsion of lipids in a sugar solution. The fat is more homogenously distributed in batters with extruded flours (F50, F80 and F150) than in batters with CF. The globule size decreased with increased flour extrusion temperature. This could be due to the dilution of proteins during extrusion treatment, therefore acting as emulsifiers by forming a film around oil droplets and preventing structural changes such as coalescence or creaming, as observed by Sharma et al. (2016) when formulating muffins with extruded flour. Moreover, the extrusion treatment induced starch granule breakdown and the subsequent lixiviation of the components from the granule, favouring the formation of a more consistent and compact network that allows a more homogeneous distribution of the fat fraction. The lixiviation of starch granule components to the matrix in the flours extruded at 150 °C is connected to the high viscosity of muffin batter elaborated with this flour (Fig. 3).

3.3. Characterisation of baked products

Four formulations of muffins were prepared: control muffin (CM) and the muffins where 50% of the wheat flour was replaced by wheat flour extruded at 50, 80 or 150°C (M50, M80 and M150). Flour extruded at 110 ºC was not used for muffin elaboration as it exhibited pasting and rheological properties similar to flour extruded at 150 °C.

The textural parameters for the muffins are presented in Table 2. As regards hardness, there were no significant differences ($p > 0.05$) between the CM and the samples M80 and M150; however, the sample M50 was significantly harder ($p < 0.05$) than the others, except M150. Thus, replacement with flour extruded at 50 °C resulted in harder muffins but the use of higher extrusion temperatures did not cause differences to the control muffin. This behaviour may be caused by the higher content of pre-gelatinised starch in muffins made with extruded flour. The swelling of starch granules occurs at lower temperature and the thermosetting stage, in which the batters set when heated, arises

Table 2. Texture properties of the crumb of muffins made with control flour (CM) or with 50% control flour and 50% flour extruded at different temperatures: 50°C (M50); 80°C (M80); 150°C (M150).

Sample	Hardness [N]	Cohesiveness	Springiness
CM	$1.15^{a} \pm 0.23$	$0.69^{a} \pm 0.03$	$0.90^{a} \pm 0.02$
M50	$1.32^{b} \pm 0.32$	$0.69^{a} \pm 0.03$	$0.89^{a} \pm 0.03$
M80	$1.16^{a} \pm 0.19$	$0.69^{a} \pm 0.03$	$0.88^{a} \pm 0.02$
M150	$1.21^{ab} \pm 0.28$	$0.69^{a} \pm 0.03$	$0.88^{a} \pm 0.02$

Mean values in a column with different superscripts differ significantly ($p < 0.05$) according to the LSD multiple range test. Values for texture parameters are mean ± standard deviation of (n = 9) determinations.

before the complete gas is released by the action of the leavening agents. This is in accordance with the simulated baking experiments, and was also observed by Hesso et al. (2014) for cakes. Additionally, Sharma et al. (2016) proposed that the increase in hardness caused by extruded flour might be attributed to its low gas retention ability. However, Martinez et al. (2013) observed no changes for bread hardness with increasing extrusion temperature of the flour for a substitution when only 5% of the flour was replaced by extruded flour, probably due to the low percentage of substitution.

Regarding cohesiveness and springiness, muffins with and without flour replacement were not significantly different ($p > 0.05$) between them. Thus, the extrusion treatment did not affect these two parameters.

Consumer acceptability of muffins with blackcurrant and the different extruded and non-extruded flours is shown in Table 3. There were no significant differences ($p > 0.05$) among all the formulations for none of the attributes; the consumers did not discriminate between reference muffins and those with substitution by extruded wheat flour.

Sharma et al. (2016) also studied the substitution of wheat flours for extruded starch in muffins. The results showed that muffins with extruded flour had lower sensory scores than the reference. Román et

Table 3. Mean consumer acceptability of muffins made with control flour (CM) or with 50% control flour and 50% flour extruded at different temperatures: 50ºC (M50); 80°C (M80); 150°C (M150).

Sample	Appearance	Texture	Taste	Overall acceptability
CM	6.5 [a] ± 1.7	6.2 [a] ± 1.8	6.4 [a] ± 1.6	6.3 [a] ± 1.7
M50	6.7 [a] ± 1.4	5.9 [a] ± 1.8	6.4 [a] ± 1.8	6.3 [a] ± 1.7
M80	6.7 [a] ± 1.3	5.9 [a] ± 1.8	5.9 [a] ± 2.0	6.0 [a] ± 1.8
M150	6.6 [a] ± 1.4	6.2 [a] ± 1.6	6.1 [a] ± 1.8	6.2 [a] ± 1.6

Means in the same attribute without a common letter are significantly different (P<0.05) according to the LSD multiple range test.

al. (2015) substituted fat in a cake formulation with extruded wheat flour, and observed a decrease in acceptability. However, our results demonstrate that muffins formulated with extruded flours (M50, M80 and M150) present good acceptability results (over 5.9 points in the different attributes), this could be because all the muffins were prepared with blackcurrant pomace and this ingredient could be masking the presence of the extruded flour in the formulation.

3.4. Glucose release during *in vitro* digestion

In vitro tests were performed to study starch digestibility and glycaemic response for flours CF, F50 and F150 and for the muffins CM, M50 and M150. Fig. 6A and 6B show the glucose release due to the enzymatic hydrolysis of the starch granules in flours and baked products, respectively. The results show that starch hydrolysis in the flours increases with increasing extrusion temperature but, in the muffins, differences between the formulations are negligible.

The parameters obtained after fitting the starch hydrolysis curves using the first-order model described by Goñi et al. (1997) and Butterworth et al. (2012) are shown in Table 4. Regarding the flour, the kinetic constant (k), which refers to the rate of hydrolysis in the early stage, was higher for F50 ($p < 0.05$), followed by F150 and CF. Nevertheless, the concentration reached at the equilibrium (C_∞) significantly increased ($p < 0.05$) with extrusion temperature. AUC, HI

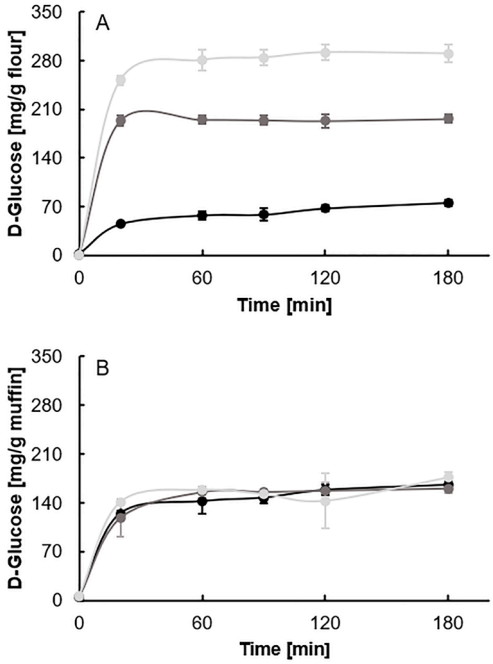

Figure 6. In vitro digestibility of starch in different flours (A): CF (●): control wheat flour; F50 (●): flour extruded at 50 ºC; F150 (●): flour extruded at 150 ºC and in different muffins (B): CM (●): muffin with control flour; M50 (●): muffin with 50% control flour and 50% flour extruded at 50 ºC; M150 (●): muffin with 50% control flour and 50% flour extruded at 150 ºC

Table 4. Kinetics of the in vitro starch digestibility. CF: control wheat flour; F50: flour extruded at 50 ºC; F150: flour extruded at 150 ºC, CM: muffin prepared with control flour; M50: muffin with 50% control flour and 50% flour extruded at 50 ºC; M150: muffin with 50% control flour and 50% flour extruded at 150 ºC.

Product	Sample	k (1/min)	C_∞ (g/100 g)	AUC 180	HI	pGI
Flour	CF	$0.02^{a} \pm 0.01$	$80.55^{a} \pm 2.80$	$10718.13^{a} \pm 1147.19$	$23.34^{a} \pm 2.50$	$52.52^{a} \pm 1.37$
	F50	$0.21^{c} \pm 0.04$	$192.50^{b} \pm 0.29$	$33708.85^{b} \pm 129.65$	$73.40^{b} \pm 0.28$	$80.01^{b} \pm 0.15$
	F150	$0.11^{b} \pm 0.03$	$288.30^{c} \pm 15.15$	$49076.00^{c} \pm 1796.07$	$106.87^{c} \pm 3.91$	$98.38^{c} \pm 2.15$
Muffin	CM	$0.06^{a} \pm 0.01$	$168.8^{a} \pm 1.65$	$27537.58^{ab} \pm 285.28$	$59.96^{ab} \pm 0.62$	$72.63^{ab} \pm 0.34$
	M50	$0.08^{a} \pm 0.04$	$161.28^{a} \pm 6.16$	$26450.30^{a} \pm 477.87$	$57.6^{a} \pm 1.04$	$71.33^{a} \pm 0.57$
	M150	$0.08^{a} \pm 0.01$	$175.80^{a} \pm 7.21$	$29338.43^{b} \pm 907.27$	$63.87^{b} \pm 1.95$	$74.77^{b} \pm 1.07$

Means in the same attribute without a common letter are significantly different ($p < 0.05$) according to the LSD multiple range test. Values for the kinetics of the in vitro digestion are mean ± standard deviation of (n = 3) determinations.

and pGI followed the same trend. This means that glucose release from the granule is faster when the degree of starch pre-gelatinisation is more pronounced which, in turn, is affected by extrusion temperature. These results are in agreement to the results obtained by Martinez et al. (2014) in which the susceptibility to enzymatic hydrolysis increased with the severity of the treatment.

However, for the baked products, there were no significant differences ($p > 0.05$) for k and C_∞ between the different formulations. Most of the glucose released in muffins was produced during the first 20 min of the digestion process. However, AUC and thus HI and pGI showed significant differences ($p < 0.05$) between the muffins elaborated with extruded flours (M50 and M150) but there were not significant differences ($p > 0.05$) for these parameters if compared to the control muffin.

Therefore, even though the *in vitro* digestion of the flours presented more glucose release with higher extrusion temperatures; in the *in vitro* digestion of muffins all the formulations presented similar glucose release in comparison with the control. This has been reported previously by Mofasser Hossain et al. (2017), which observed that increasing the percentage of blackcurrant in cookies resulted in a significant ($p < 0.05$) decrease of glucose release during *in vitro* digestion compared to the control. It has been widely proven that polyphenols may have the possibility to modify the bioavailability of nutrients through the inhibition of digestive enzymes. In particular, berry extracts are rich in polyphenols that may inhibit the action of α-amylase and α-glucosidase (Bordenave et al., 2014; Grussu et al., 2011; Hogan et al., 2010; McDougall et al., 2005; Zhu, 2015). In our work, the total phenolic content for blackcurrant pomace is 63.55 mg GAE/g DM, that compared with the content in the green apple (Álvarez et al., 2016), which is one of the most consumed fruit in Spain (Saura-Calixto & Goñi, 2006) could be considered as a great source of polyphenols. Thus, the addition of blackcurrant pomace may be the reason that, unlike flour, at high extrusion temperatures, the glucose release in the muffins and therefore the HI and pGI in the

in vitro digestion were very similar compared to the control due to the inhibition of digestive enzymes by the blackcurrant polyphenol composition.

Grussu et al. (2011) and Lo Piparo et al. (2008) described the inhibition mechanism as a complex formation between polyphenols and α-amylase; it was hypothesized that polyphenols bind to the active site of the enzyme, preventing its interaction with the starch substrate and thus reducing the glycemic response. Therefore, the addition of blackcurrant has a hypoglycaemic effect in muffins counteracting the higher glucose release caused by the disrupted structure of starch granule in the extruded flours.

4. CONCLUSIONS

The results showed that the more severe the extrusion treatment, the higher is the disruption of starch granules and thus starch pre-gelatinisation. A 50% substitution of regular wheat flour by pre-gelatinised flours slightly affected textural properties of muffins but, from a sensory point of view, consumers did not discriminate among the different muffins. The *in vitro* digestion results demonstrate that the addition of blackcurrant has an inhibitory effect on starch digestive enzymes, thus reducing glucose release from pre-gelatinised starch. Therefore, the use of extruded flour did not interfere on textural properties of the muffins and the addition of blackcurrant pomace can be used to counteract the hyperglycaemic effect caused by the flour extrusion on the *in vitro* digestion.

5. CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

6. ACKNOWLEDGEMENTS

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Capítulo 3

Uso de bagazo de frutos rojos en batidos.

Sinergia entre tratamiento no térmico y polifenoles del bagazo.

Optimizing high pressure processing parameters to produce milkshakes using chokeberry pomace

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A B S T R A C T

High hydrostatic pressure is a non-thermal treatment of great interest because of its importance for producing food with additional or enhanced benefits above their nutritional value. In the present study, the effect of high hydrostatic pressure processing parameters (200–500 MPa; 1–10 min) is investigated through response surface methodology (RSM) to optimize the treatment conditions, maximizing the phenol content and antioxidant capacity while minimizing microbiological survival, in milkshakes prepared with chokeberry pomace (2.5%–10%). The measurement of fluorescence intensity of the samples was used as an indicator of total phenolic content and antioxidant capacity. The results showed that the intensity of the treatments had different effects on the milkshakes. The RSM described that the greatest retention of phenolic compounds and antioxidant capacity with minimum microbiological survival were found at 500 MPa for 10 min and 10% (*w*/*v*) chokeberry pomace. Therefore, this study offers the opportunity to develop microbiologically safe novel dairy products of high nutritional quality.

Keywords: antioxidant capacity; microbial inactivation; image analysis; high pressure processing; total phenolic content

1. INTRODUCTION

Nowadays, consumers show increasing preference for foods with additional or enhanced benefits beyond their basic nutritional value. These benefits come from the composition, e.g., bioactive compounds, which may have long-term health effects. There is convincing evidence of the cardioprotective effects for frequent consumption of fruits and vegetables high in fiber, micronutrients, and several phytochemicals. Specifically, the association between flavonoids and an increased cardiovascular health has been proven in berries (Basu et al., 2010). Producing berry-based juice generates by-products, comprising peel and

seeds, having a high nutritional value because of their polyphenol and fiber content. Berry by-products can be a value-added food ingredient (de Souza et al., 2019; Rodríguez-Werner et al., 2019; Tańska et al., 2016) and recent studies show that the enrichment of food products with these by-products is feasible (Diez-Sánchez et al., 2019; Ospina et al., 2019; Quiles et al., 2018). In this context, chokeberry (*Aronia melanocarpa*) pomace can be used, as chokeberry is one of the richest plant sources of phenolic phytochemicals, including procyanidins and anthocyanins (Kähkönen et al., 2001; Kulling & Rawel, 2008) which are related to effectiveness in several pathological conditions where damage is caused by uncontrolled oxidative processes (Chrubasik et al., 2010).

Previous studies have used dairy products and pomace for the production of yogurts with apple pomace (Issar et al., 2017; Wang et al., 2019, 2020) or fermented milk with grape pomace (de Souza de Azevedo et al., 2018). However, in the work of Issar et al. (2017) and de Souza et al. (2018), polyphenols and fiber were extracted, respectively, and added to milk for product preparation. Wang et al. (2019, 2020) incorporated the apple pomace directly into the dairy matrix. Regarding berry pomace, Ni et al. (2018) formulated yogurts with aqueous berry extracts from salal berry and blackcurrant pomace. In this study, we propose the incorporation of the pomace directly into the milk using high hydrostatic pressure (HPP) to help polyphenols being extracted into the matrix.

HPP is a method to preserve food and has the potential to retain or improve the bioaccessibility and bioavailability of nutritional and antioxidant compounds because of microstructural modifications (Vázquez-Gutiérrez et al., 2013). HPP retains the nutritional and sensory quality of food products, but there is a concern related to food safety (San Martín et al., 2002). In this context, high pressures have been effective at inactivating vegetative cells when sufficient intense pressure is applied (Welti-Chanes et al., 2005).

Thus, the present study aimed to prepare milkshakes enriched with polyphenols by adding chokeberry pomace to the milk and using HPP to improve polyphenols extraction from the pomace. The effect of HPP parameters such as time and pressure on total phenolic content (TPC), antioxidant capacity (AC), and the microbiological inactivation in milkshakes with different concentrations of chokeberry pomace will be studied. To define the best processing conditions, response surface methodology (RSM) was used to maximize the TPC and the AC results while minimizing the microbiological survival.

2. MATERIALS AND METHODS

2.1. Sample preparation and HPP treatments

Döhler GmbH (Darmstadt, Germany) provided fresh chokeberry pomace. The pomace was dried at 70 °C for 3 h and milled in a ZM 100 ultracentrifuge mill (Retsch GmbH, Haan, Germany) at 14,000 r.p.m. using a 0.5 mm sieve (Reißner et al., 2019). Reconstituted skimmed milk powder (Corporación Alimentaria Peñasanta S.A., Siero, Spain) was selected for chokeberry pomace inclusion.

Different concentrations of chokeberry powder were added to skimmed milk samples to give final chokeberry pomace concentrations of 2.5%, 6.25%, and 10% (*w*/*v*). The samples were poured into 50 mL polypropylene tubes that were introduced into polyethylene bags and heat-sealed (MULTIVAC Thermosealer, Switzerland) before undergoing HPP treatment. HPP treatments were performed in a unit with a 2.35 L vessel volume and maximum operating pressure of 600 MPa (High Pressure Food Processor, EPSI NV, Belgium). The samples were pressurized at 200, 350, and 500 MPa, at 18–22 °C, for 1, 5.5, and 10 min, using a compression rate of 300 MPa/min and a decompression time < 1 min (Pina Pérez et al., 2007; Saucedo-Reyes et al., 2009). Other parameters, pressure intensity, pressurization time, and temperature

were automatically controlled. Once the treatment was completed, the samples were taken from the vessel, immersed in an ice-water bath, and refrigerated (3 ± 1 °C) until use. Before each analysis, both microbiological and chemical, the samples were filtered with paper filter previously sterilized using an autoclave. The microbiological cultures and microscopic observations were immediately conducted after the filtration, while the samples for the TPC and AC determination were stored by deep-freezing at −80 °C until use.

2.2. Total phenolic content

The total phenolic content (TPC) was determined according to the method described by Singleton et al. (1999), with some modifications. The treated chokeberry pomace milk (5 mL) was homogenized in an Ultraturrax with 25 mL of 96% ethanol. The homogenate was centrifuged (4122 x *g*, 30 min, 4 °C) and filtered, and the supernatant was stored. This treatment was repeated on the leftover pellet using 25 mL of 96% ethanol to extract more supernatant, then added to the first supernatant; the total supernatant was brought up to 100 mL. After, 6 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent (1:1 (*v*/*v*)) were added to an aliquot of 1 mL of the ethanolic extract. After three minutes, 1 mL of sodium carbonate solution (20% (*w*/*v*)) (Panreac Química SLU, Castellar del Vallès, Barcelona, Spain) and 1.5 mL of distilled water were added. The mixture was vortexed and kept at room temperature (~25 °C) in a dark room for 90 min. Absorbance was measured at 765 nm using a spectrophotometer (series 1000, model CE 1021; CECIL Instruments Ltd., Cambridge, UK) with results expressed as mg of Gallic Acid Equivalents (GAE)/100 mL. Total phenolic extractions were made in triplicate.

2.3. Antioxidant capacity

The antioxidant capacity (AC) was measured by a ferric reducing antioxidant power assay (FRAP) (Benzie & Strain, 1996; Pulido et al.,

2000). Extracts were obtained in the same way as for TPC determination. Distilled water (30 µL), the sample (30 µL), and FRAP reagent (900 µL) were placed in the cuvette. The cuvettes were incubated for 30 min in a water bath at 37 °C and the absorbance was measured at 595 nm. A calibration curve was obtained using different concentrations of Trolox in 96% ethanol. The results were expressed as µmol Trolox/mL of sample. Three separate extractions were made for each treatment and the measurements were performed in triplicate.

2.4. Chokeberry microstructure

The microstructure analysis was carried out following Hernández-Carrión et al. (2015) with some modifications. For the study of the chokeberry microstructure, a Nikon Eclipse E80i microscope (Nikon, Tokyo, Japan) was used. The autofluorescence of the phenolic compounds in the samples was observed using a mercury arc lamp with a tetramethyl rhodamine filter (λ_{ex} = 543/22 nm, λ_{em} = 593/40 nm) as the excitation source. Samples were visualized using 10× and 20× objective lenses. The micrographs were stored at a 1280 × 1024-pixel resolution using the microscope software (NIS-Elements F, Version 4.0, Nikon, Tokyo, Japan). Analysis of the fluorescence intensity was conducted with the ImageJ software.

2.5. Microbiological analysis

The effect of HPP treatment was evaluated on natural contaminating flora (aerobic mesophilic microorganisms, molds, and yeasts) and on *Listeria monocytogenes* serovar 4b (Murray et al., 1926) (CECT 4032) as pathogen microorganism (Farber & Peterkin, 1991). The growth media used for the spreading of samples was plate count agar (Scharlau Chemie S. A., Spain) for mesophilic aerobic; potato dextrose agar (Scharlau Chemie S. A., Spain) for molds and yeasts; and tryptic soy agar (Scharlau Chemie S. A., Spain) for *L. monocytogenes*. The incubation conditions were 48 h at 30 °C, 120 h (5 days) at 24 °C, and 48 h at 37 °C, respectively.

L. monocytogenes was artificially inoculated in the sample. The stock vials containing *L. monocytogenes* at a concentration ca. 10^9 cfu/mL were generated following the methods described by Saucedo-Reyes et al. (2009) and Pina-Pérez et al. (2009). Before HPP treatment, vials were inoculated in the chokeberry-skimmed milk samples at a final concentration of 10^8 cfu/mL. The counts for evaluating microorganism inactivation were performed before and after each HPP treatment. Two aliquots (0.1 mL) were taken from each sample, diluted with buffered peptone water (Scharlau Chemie S. A, Spain), and plated in the respective agar. Two replicas of each treatment were obtained, and three repetitions of each treatment condition was performed. The survival fraction $S = N/N_0$ and the level of inactivation Log_{10} (N/N_0) were evaluated for each repetition.

2.6. Experimental design and statistical analysis

RSM was used to optimize the preservation process and to investigate the simultaneous effects of pressure, time, and chokeberry powder concentration on TPC, AC, and microbiological inactivation of the prepared samples. For the chokeberry-milk matrix, a face-centered central composite design was used with three levels (maximum, minimum, and central) and three independent factors, namely pressure (200 to 500 MPa), time (1 to 10 min), and chokeberry pomace concentration (2.5 to 10% (*w*/*v*)), resulting in 16 combinations (Table 1). The central point of the three variables was replicated twice to assure the reproducibility and stability of the results. All the experiments were randomized. A quadratic model was obtained with regression coefficients associated with the linear, quadratic, and interaction effects. A *t*-test determined their significance through the *p*-value generated.

The non-significant terms ($p > 0.05$) were deleted from the second-order polynomial model after an ANOVA test, and a new ANOVA was performed to find the coefficients of the final equation for better accuracy (Barba et al., 2014). The experimental design and the data

analysis were performed using the Statgraphics® Centurion XVII software (Statpoint Technologies, Inc., Virginia, USA).

Table 1. Experimental design matrix for studies conducted.

Run	Pressure (MPa) (X_1)	Time (min) (X_2)	Chokeberry Pomace (% (w/v))
1	500	1	2.5
2	350	10	6.25
3	500	5.5	6.25
4 [a]	350	5.5	6.25
5	500	10	10
6	350	5.5	2.5
7	350	5.5	10
8	350	1	6.25
9	200	10	10
10	500	10	2.5
11	200	5.5	6.25
12	200	10	2.5
13	350	5.5	6.25
14	200	1	10
15	200	1	2.5
16	500	1	10

[a] Central point.

3. RESULTS AND DISCUSSION

3.1. Effect of HPP on TPC, AC, and microbial counts of chokeberry milkshakes

Effects of HPP treatments on the TPC, AC, and microbiological survival fraction are shown in Table 2. The analyses were conducted on untreated and treated samples to observe differences with HPP treatment. Results for molds and yeasts are not shown because there were no changes in any treatments.

Table 2. Effect of HPP and chokeberry pomace on TPC, AC, and the microbial survival fraction.

Pressure (MPa)	Time (min)	Chokeberry Pomace % (*w*/*v*)	TPC (mg GAE/100 mL)	AC (µmol Trolox/mL)	Inactivation Log_{10} (N/N_0)
0	0	2.5	53.02 ± 0.14	6.06 ± 0.14	-
		6.25	73.92 ± 3.17	9.27 ± 0.20	-
		10	121.16 ± 0.31	14.89 ± 0.30	-
200	1	2.5	49.39 ± 2.24	5.02 ± 0.34	0.01 ± 0.08
		10	130.20 ± 3.46	17.3 ± 1.08	−0.18 ± 0.04
	5.5	6.25	136.79 ± 8.06	11.79 ± 0.99	−0.20 ± 0.08
	10	2.5	50.54 ± 4.64	4.80 ± 0.16	0.06 ± 0.12
		10	132.85 ± 2.31	13.98 ± 0.49	−0.11 ± 0.06
350	1	6.25	84.32 ± 2.59	10.13 ± 0.79	−0.20 ± 0.06
	5.5	2.5	42.45 ± 2.89	4.77 ± 0.39	−0.25 ± 0.14
		6.25 [a]	78.54 ± 3.39 [a]	7.58 ± 0.41 [a]	−0.21 ± 0.19 [a]
		6.25	106.14 ± 4.28	8.54 ± 0.03	−0.33 ± 0.09
		10	124.96 ± 3.78	16.45 ± 0.10	−0.55 ± 0.05
	10	6.25	101.92 ± 5.70	16.53 ± 0.78	−0.33 ± 0.32
500	1	2.5	54.14 ± 0.61	5.7 ± 0.45	−0.44 ± 0.05
		10	155.28 ± 2.07	16.36 ± 0.35	−0.67 ± 0.08
	5.5	6.25	97.17 ± 7.09	11.33 ± 2.03	−0.87 ± 0.06
	10	2.5	58.36 ± 2.97	6.69 ± 0.19	−3.63 ± 0.08
		10	134.17 ± 1.57	14.79 ± 0.76	−4.02 ± 0.15

[a] Central point; HPP: High Pressure Processing; TPC: Total Phenolic Content; AC: Antioxidant Capacity; N: final cell concentration; N_0: initial cell concentration.

TPC concentration in untreated samples with 2.5%, 6.25%, and 10% (*w*/*v*) of chokeberry pomace is 53.02 ± 0.14, 73.92 ± 3.17, and 121.16 ± 0.31 mg GAE/100 mL, respectively. Furthermore, the AC results for samples 2.5, 6.25, and 10% (*w*/*v*) of chokeberry pomace, are 6.06 ± 0.14, 9.27 ± 0.20, and 14.89 ± 0.30 µmol Trolox/mL, respectively. As expected, the TPC and AC results are higher with higher pomace concentrations.

In treated samples, the highest result for TPC is 155.28 ± 2.07 mg GAE/100 mL with 500 MPa during 1 min and 10% (*w*/*v*) pomace addition; the lowest is 42.45 ± 2.89 mg GAE/100 mL with 350 MPa during 5.5 min and 2.5% (*w*/*v*) pomace addition. Yet, the highest AC is 17.3 ± 1.08 µmol Trolox/mL for the treatment of 200 MPa during

1 min with 10% (*w*/*v*) pomace addition. The treatment that obtained the lower AC matches the one that obtained the lower TPC (350 MPa during 5.5 min with 2.5% (*w*/*v*) pomace addition).

The lowest results for TPC and AC are obtained for the intermediate pressure and not for the lowest as expected, yet these low results are similar to other treatments at different processing conditions but with the same pomace concentration (2.5%). In contrast, the higher value results for TPC and AC are obtained for milkshakes with 10% of chokeberry. When comparing the results of the treatments at each concentration, values were higher as the pomace concentration increased. In addition, comparing the results of treatments at each concentration with its untreated counterpart, samples 6.25 and 10% show an increase in TPC and AC, influenced by the pressures and times studied. However, for samples at 2.5%, this effect is less acute, affected only by high pressures (500 MPa).

Thus, the results are affected by all the factors in this study (pressure, time, and concentration), but mainly the pomace concentration. The HPP treated foods are either unaffected or have increased TPC and/or extractability following treatments with high pressures (Tokuşoğlu, 2016). Andrés et al. (2016) found increases of 6.6% and 4.2% in TPC values for fruit smoothies treated at 450 and 600 MPa, respectively. Corrales et al. (2008) showed that treating at 600 MPa enhanced the anthocyanin extraction and its AC in grape by-products than with conventional extraction methods. Liu et al. (2016) found treatments at 200 MPa, for 5 and 10 min, led to an increased TPC of 14.24% and 14.35% in wild *Lonicera caerulea* berry, respectively, however, for treatments at 500 and 600 MPa there was a significant decrease of TPC. In contrast, other authors found HPP had little effect on phenolic content. Barba et al. (2010) observed TPC to be relatively resistant to the effect of processing in tomato purées. Hurtado et al. (2017) did not observe differences in AC values between untreated and treated red fruit-based smoothies for treatments at 350 MPa, 10 °C, and 5 min. Patras et al. (2009) found that phenol content in HPP treated

strawberry purées was relatively resistant to the effect of processing at 400 and 500 MPa, only showing an increase in treatments at 600 MPa for TPC and AC. Therefore, the results obtained with HPP depend of several conditions, such as the matrix in which they are applied, and the severity of the treatment and it is necessary to study the behavior of different samples with these treatments.

In fluorescence microscopy, the intensity value of a pixel is related to the number of fluorophores present at the corresponding area in the particle. Thus, the digital images can be used to extract the intensity values to determine the local concentration of fluorophores in a specimen (Waters, 2009). In our case of study, the images in Figure 1 show the pomace particles dispersed into the milk matrix.

To analyze the fluorescence intensity, the images corresponding to the lower (200 MPa, 1 min, 2.5%), central (350 MPa, 5.5 min, 6.25%), and higher (500 MPa, 10 min, 10%) treatments were selected. The particle with greater intensity was selected to generate intensity profiles (Figure 1A-C). A line (shown in yellow) was drawn across the particle, and a plot (graph) was generated to show the intensity values of the pixels along the line (Figure 1D–I). In addition, Figure 1J-L shows the relation between the percentages of particles at each fluorescence intensity interval.

The fluorescence intensity for the isolated particles is higher in the medium (Figure 1K) and high treatments (Figure 1L) than in the low treatment (Figure 1J). Comparing the background intensity, corresponding to the liquid phase of the sample, fluorescence increases as the severity of the treatment increases. Several authors (Gao et al., 2016; Hernández-Carrión et al., 2014; Vázquez-Gutiérrez et al., 2013) have described the cell wall degradation and breakage in plant tissue after HPP, leading to a leaching of contents from the pomace cells (included polyphenols) to the milk acting as a liquid medium (Corrales et al., 2008; Gao et al., 2016). In addition, Gonzalez & Barrett (2010) described that treatments at pressures above 220 MPa were

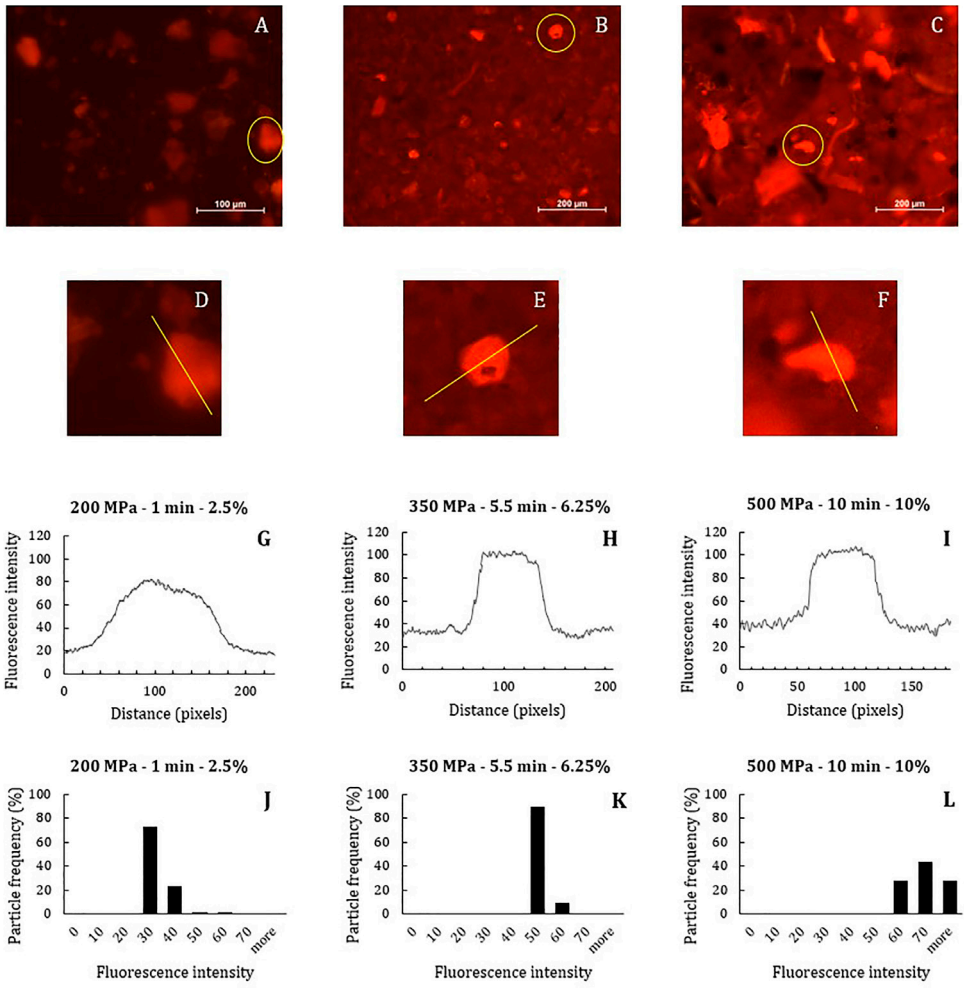

Figure 1. Fluorescence intensity of: 200 MPa, 1 min, 2.5% (**A**,**D**,**G**,**J**); 350 MPa, 5.5 min, 6.25% (**B**,**E**,**H**,**K**); and 500 MPa, 10 min, 10% (**C**,**F**,**I**,**L**).

responsible for the breakage of the membrane structure because of protein unfolding and interface separation. Therefore, as higher pressures are applied, the phenolic contents are being released to the medium due to the membrane breakage, giving as a result higher values of fluorescence intensity. The particle frequency plots show that the frequency of particles at high intensities rises with the severity of the treatment. These results agree with the results of TPC and AC,

i.e., higher fluorescence intensities correspond to the treatments that obtained the higher TPC and AC results. Therefore, measurement of fluorescence intensity can be an indicator for TPC and AC in this type of sample. Further research is needed to prove if the analysis is usable in other matrices.

Besides the effect of HPP on the polyphenols, there could be a microbial inactivation because of changes induced in the microbial cells. These changes include alterations in the cell membrane, effects on proteins, and effects on the genetic mechanism of microorganisms (Patterson, 2005; Ritz et al., 2002; Welti-Chanes et al., 2005). Seen in microbiological inactivation results in Table 2, treatments at 200 MPa during 1 min with 2.5% (*w*/*v*) of pomace and at 200 MPa during 10 min with 2.5% of pomace do not show microbial inactivation. At 2.5% pomace concentration and low pressure (200 MPa), longer treatment time is not enough for microbial inactivation. Muñoz-Cuevas et al. (2013) also observed this behavior. Still, it is necessary to reach a minimum treatment intensity (500 MPa, 10 min) to obtain significant *L. monocytogenes* inactivation. At this condition, an increase in chokeberry pomace concentration from 2.5% to 10% (*w*/*v*) increases microbial inactivation from 3.63 to 4.02 Log reductions.

Thus, increasing the pressure and treatment time results in an increase in the lethal effect of HPP treatment. This effect relates to food composition, technological parameters, and the factors acting in synergy (Ferreira et al., 2016; Possas et al., 2017).

Besides the effect of treatment conditions, several authors have described the high antimicrobial capacity of berry fruits and their by-products (Bartkiene et al., 2019; Cisowska et al., 2011; Nohynek et al., 2006) and the synergistic effect between natural substances and high pressure treatments (Evrendilek & Balasubramaniam, 2011; Pina-Pérez et al., 2009; Vurma et al., 2006). Despite the evidence found in the literature, except the treatments of 500 MPa, 10 min with 2.5% and 10% (*w*/*v*) pomace, the inactivation values are lower than similar treatments

with other products and microorganisms. For example, Evrendilek & Balasubramaniam (2011) concluded that samples of ayran (yogurt drink) treated at 600 MPa during 5 min had reduced in the levels of *L. monocytogenes* and *L. innocua* by more than five log units ($p < 0.05$) at ambient temperature. Nevertheless, Gervilla et al. (1997) and Black et al. (2007) have described a baroprotective effect that milk has on the cells. Thus, this effect could counteract the antimicrobial effect of chokeberry pomace, explaining the low inactivation levels found for *L. monocytogenes* in this study. To prove this effect, an experiment was conducted where the central point of the design (350 MPa, 5.5 min in milk with 6.25% (*w*/*v*) of chokeberry pomace) was used as a treatment to compare the inactivation reached in four different matrices: (i) peptone water with *L. monocytogenes*, (ii) milk with *L. monocytogenes*, (iii) peptone water with *L. monocytogenes* and chokeberry pomace, and (iv) milk with *L. monocytogenes* and chokeberry pomace. Results tested the baroprotective effect of milk and are shown in Figure 2.

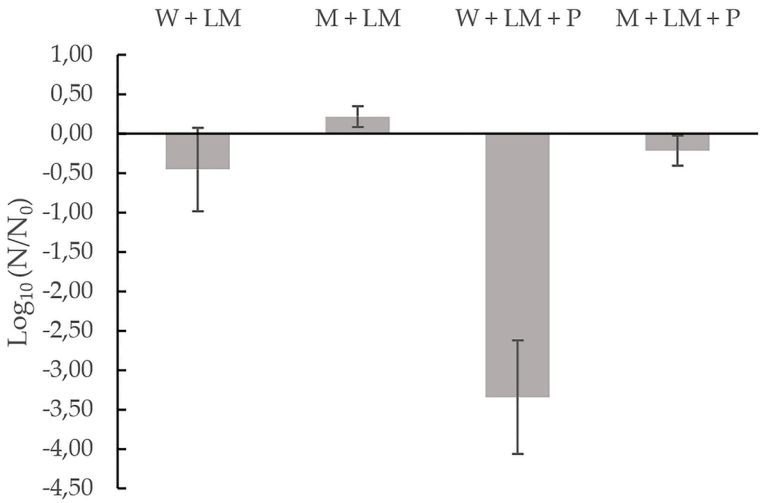

Figure 2. Inactivation level of ingredients' different combinations: peptone water (W), milk (M), inoculated Listeria monocytogenes (LM), and pomace (P).

In samples without milk (W + LM and W + LM + P), the number of surviving cells is lower than with milk samples (M + LM and M + LM + P), and more prominent when pomace is added. Apart from the protective effect of milk, an increase is seen in the efficacy of HPP against *L. monocytogenes* when pomace is present in the sample. Thus, these results can describe the synergistic effect of pomace, HPP, and the protective effect of milk on *L. monocytogenes*. Still, there is microbial inactivation with some treatments, even with the protective effect of milk on the microbiological cells.

3.2. Processing parameter optimization and their effect on the safety and quality of the formulated matrix

The best processing conditions for treating chokeberry milkshakes when HPP is combined with added by-products with antimicrobial and antioxidant properties (chokeberry pomace) were studied by RSM. This methodology uses a sequence of designed experiments to obtain an optimal response.

First, the estimated effects of each factor (pressure, time, and concentration) and their interactions were analyzed (Figure 3). The response function for the factors and the adjusted regression coefficient (R^2 adjusted), showing the percentage of variation in the response explained by the fitted model, is shown in Equations (1)–(3) (pressure (P), time (t), chokeberry pomace concentration (C)). The value of the adjusted R^2 close to one indicates a high correlation between the experimental and fitted values.

$$TPC = 25.6475 + 11.2687 \times C \qquad R^2adj = 0.85 \quad (1)$$

$$AC = 2.08477 + 1.38395 \times C \qquad R^2adj = 0.80 \quad (2)$$

$$\mathrm{Log}_{10}\left(\frac{N}{N_0}\right) = -0.160277 + 0.000618685 \times P + 0.285165 \times t - 0.00123019 \times P \times t \qquad R^2adj = 0.76 \quad (3)$$

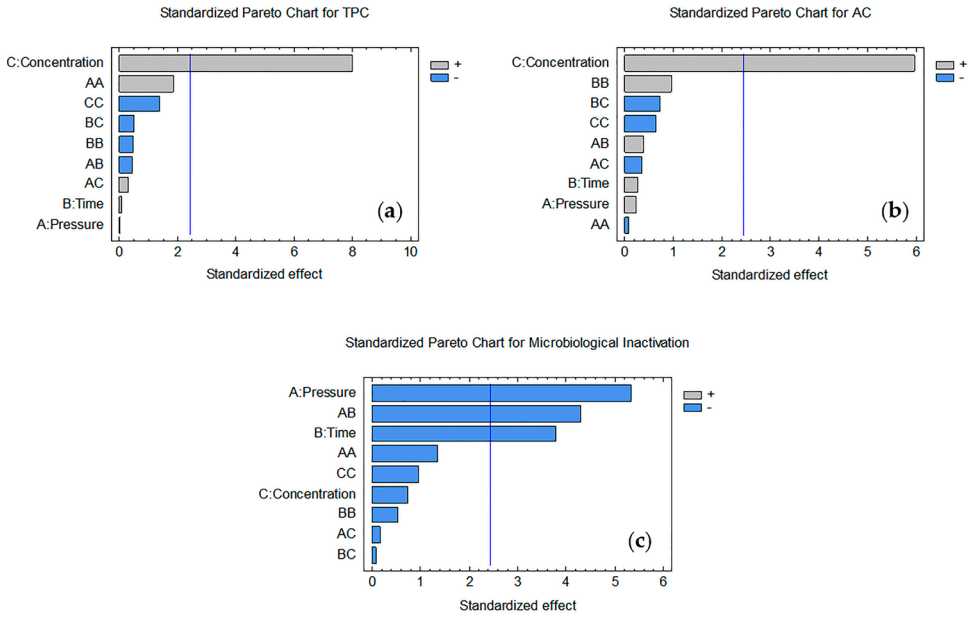

Figure 3. Estimated effects of each factor (pressure, time, and concentration) and their interactions with TPC (**a**), AC (**b**), and *L. monocytogenes* inactivation (**c**) where + or - means a positive or negative relation between factor (pressure, time or concentration) and response (TPC, AC or *L. monocytogenes* survival fraction), respectively. A: pressure, B: time and C: concentration. The combination of letters (AA, BB, AB …) refer to the interactions carried out in the analysis.

Figure 3 shows the pareto chart for TPC, AC, and microbial inactivation. This chart determines the magnitude and the importance of the effects. The bars that extend beyond the line correspond to effects that are statistically significant with a 95.0% confidence level. The factor "pomace concentration in milkshakes" is the only factor that significantly affects TPC and AC concentration. However, the results in Table 2 show TPC and AC are influenced by all the factors, including time and pressure. Chokeberry pomace has been reported as a berry fruit with high phenolic content (Kulling & Rawel, 2008). Thus, though it could exist with the effect of pressure and time, the results could be masked by the natural high phenolic content.

The low effect of treatment conditions on TPC and AC could be explained by using milk as a liquid medium. High pressure processing

induces physicochemical and technological changes in milk properties. When HPP is applied to milk, the casein micelles are disintegrated into casein particles of smaller size, which is accompanied by an increase in casein and calcium phosphate levels in the serum phase of milk and by a decrease in both non-casein nitrogen and serum nitrogen fractions (Chawla et al., 2011; Welti-Chanes et al., 2005). In addition, interactions between polyphenols and milk proteins have been previously described by other researchers (Bordenave et al., 2014; Jakobek, 2015; Zhang et al., 2014). In our work, these interactions could be favored by the changes in the casein structure due to HPP treatment, which would lead to the formation of complexes that restrict the accessibility of analysis, leading to lower AC and TPC and a non-significant effect of treatment conditions (pressures and time). Tadapaneni et al. (2012) also observed this effect in strawberry-based beverages treated with HPP at pressures ranging from 200 to 600 MPa. They saw, when formulated with milk instead of water, the beverage presented reduced levels of AC and anthocyanins because of complexes forming between polyphenols and milk proteins. Therefore, as the effect of concentration is so pronounced in RSM and polyphenol–milk protein interactions may exist, decreasing the AC and TPC, the effect of the other parameters is much lower, leading to a non-significant effect of time and pressure.

In contrast, pressure and time are the parameters with a significant effect on the microbiological inactivation. Thus, the chokeberry pomace concentration added to the milkshake, does not have a significant effect on the inactivation results. These results confirm the hypothesis explained above (Figure 2); there is an antimicrobial effect of berry pomace. However, it is masked with the protective effect of milk on *L. monocytogenes* cells, giving a lower inactivation result than with similar treatment conditions in products with a natural antimicrobial agents, yet without milk (Evrendilek & Balasubramaniam, 2011). Despite the protective effect of milk on microorganisms, adding chokeberry pomace could help achieve higher inactivation levels than HPP without the pomace.

Once the estimated effect and its interaction were analyzed, the response optimization was carried out. The results show that the optimized factors are 500 MPa for 10 min in milk with 10% (*w*/*v*) of chokeberry pomace (Table 3). This treatment condition ensures the maximum TPC and AC with the minimum microbiological survival.

The optimum treatment conditions are the same as those of the experimental design. When comparing the results in Table 2 with the predicted values in Table 3, we see that experimental results are like the predicted values through optimization. Therefore, the RSM is proven to be a reliable tool to predict the behavior of the sample studied in terms of AC, TPC, and microbial inactivation.

Table 3. Predicted and limit response values for optimum treatment conditions.

Response	Predicted	Lower 95.0% Limit	Upper 95.0% Limit
TPC (mg GAE/100 mL)	138.33	125.68	150.99
AC (μmol Trolox/mL)	15.92	14.13	17.72
Log_{10} (N/N_0)	−3.15	−3.96	−2.34

4. CONCLUSIONS

The fluorescence intensity measurement of microstructure images can be an indicator of the TPC and AC of the samples. Microstructure images showed that, with intense treatment, there is leaching of the polyphenolic substances into the milk because of cell structure breakage. For the microbiological inactivation, the results showed that the pomace had antimicrobial properties, but they were partially masked by the interactions between milk proteins and the polyphenols available, and that higher levels of inactivation were achieved at high pressures and long treatment times. The RSM results showed that TPC and AC were only affected by the pomace concentration added to the milkshake, because the high pomace concentration and the polyphenol–milk protein interactions could mask the effect of pressure and time.

Although the efficiency of HPP inactivation on *L. monocytogenes* has been proven, further research is needed for products without milk to study the effect of chokeberry pomace treated with HPP on the TPC, AC, and microbial inactivation.

5. AUTHOR CONTRIBUTIONS:

Formal analysis, D.R.; Investigation, E.D.-S; Methodology, A.M.; Supervision, A.Q.; Writing – review & editing, I.H. All authors have read and agreed to the published version of the manuscript.

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8. CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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Capítulo 4

Digestión *in vitro*.

Interacciones entre polifenoles del bagazo de frutos rojos y principales macronutrientes de los alimentos.

Interactions between blackcurrant polyphenols and food macronutrients in model systems: *in vitro* digestion studies

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A B S T R A C T

Blackcurrant pomace, rich in fiber and polyphenols, can be used as added-value ingredient for food formulation. However, the bounding of polyphenols to pomace and the interactions that take place with food nutrients modify polyphenol bioaccessibility. This work studied the interactions between polyphenols and the main macronutrients in foods, and the changes that occurred during *in vitro* digestion, using model systems. Model systems were formulated with (i) water, (ii) wheat starch, (iii) olive oil, (iv) whey protein, and (v) a model combining all the ingredients. Polyphenols were added from two sources: as pomace and as a polyphenolic pomace extract. Interactions between polyphenols and macronutrients were studied using light microscopy; total phenolic content (TPC) and antioxidant capacity (AC) were determined before and after the *in vitro* digestion process. Lastly, the bioaccessibility of the samples was calculated. Polyphenols incorporated into the model systems as pomace, increased their bioaccessibility if compared to polyphenols added as extract. For single-nutrient model systems formulated with pomace, the bioaccessibility was higher than when the system contained all the nutrients. Of all the components studied, the greatest effect on bioaccessibility was observed for proteins.

Keywords: bioaccessibility; total phenolic content; microstructure; dietary fiber; antioxidant capacity

1. INTRODUCTION

By-products of the industries of fruit juice processing, also known as pomaces, comprise the remains of skins and seeds and are rich in bioactive compounds. These bioactive compounds include dietary fiber, polyphenols, carotenoids, and other phytochemicals (Coman et al., 2020).

Polyphenolic compounds are characterized by the presence of one or more hydroxyl groups linked to aromatic rings and can vary in their chemical structure and properties, ranging from simple molecules, such as phenolic acids, to highly polymerized molecules, such as proanthocyanidins; this structure gives them their antioxidant capacity (AC). Several studies relate the AC of polyphenols with prevention of certain types of cancer (Gu et al., 2020; Sharma et al., 2017) and other diseases, such as type 2 diabetes (Gowd et al., 2018). This protective activity has led to the study of the benefits of its consumption for health (Durazzo et al., 2019; Fraga et al., 2019; Joseph et al., 2016; Wootton-Beard & Ryan, 2011).

Berry pomace is a valuable source of dietary fiber with associated phenolic compounds, which could be a good alternative for its use as a value-added ingredient in functional foods. The main anthocyanins detected in pomaces from blackcurrant are cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, and cyanidin 3-xyloside (Reißner et al., 2019). The further use of pomace in food formulations contributes to improve the sustainability of the agri-food processing chain. Moreover, their great variety, effect on health, and relative ease to obtain make berry pomace polyphenols the ideal bioactive compounds to produce functional foods. However, polyphenols in pomaces are linked to the fiber matrix. This association can be conducted by hydrogen bonds formed between polyphenol hydroxyl groups and the fiber hydroxyl groups, or between polyphenol hydroxyl groups and the fiber oxygen atom of the glycosidic linkages of polysaccharides. Van der Waals forces are also possible because both polyphenols and dietary fibers possess polarizable molecules. Furthermore, hydrophobic interactions can take place between the hydrophobic sites of polysaccharides and the aromatic rings of polyphenols. Finally, they can be linked by covalent bonds, such as ester bonds, between the phenolic acids and polysaccharides (X. Liu et al., 2020; Quirós-Sauceda et al., 2014).

When these value-added ingredients are incorporated into food matrices, it is necessary to consider that the hydrophobic aromatic ring

and the hydrophilic hydroxyl groups of the polyphenols interact with different macromolecules from food ingredients. These interactions cause reversible interactions through non-covalent forces, such as Van der Waal forces, hydrogen bonding, and hydrophobic interactions, and irreversible interactions through covalent bonding (Bordenave et al., 2014; González-Aguilar et al., 2017; Jakobek, 2015; Le Bourvellec & Renard, 2012; Renard et al., 2001). The polyphenol reactivity toward these forces will depend on structural factors, including the molecular weight, conformational mobility, flexibility, projection of the hydroxyl groups, the presence of galloyl groups, or the polyphenol's affinity for water (Le Bourvellec & Renard, 2012).

To optimize the food formulation, it is important to study the digestibility of its components. The efficacy in the absorption through the gastrointestinal tract of the polyphenols is affected by several factors, such as the food matrix, solubility, digestibility, bioaccessibility, molecular structures, or metabolizing enzymes (Bermúdez-Soto et al., 2007; Rodríguez-Roque et al., 2015; Wojtunik-Kulesza et al., 2020). Moreover, adding the polyphenols as a pomace or extract also influences their bioaccessibility. Although *in vivo* digestions best represent the digestion of food in real conditions, *in vitro* digestions are a cheaper, reproducible, and faster alternative for obtaining first hypotheses regarding the digestion of a certain food or nutrient (Bohn et al., 2018; Carbonell-Capella et al., 2014). Some *in vitro* studies describe interactions between polyphenols and the different macronutrients from foods (Bordenave et al., 2014; Jakobek, 2015), such as carbohydrates (Amoako & Awika, 2016; Zhu, 2015), lipids (Ortega et al., 2009), and proteins (Arts et al., 2002; Gallo et al., 2013; Ozdal et al., 2013).

In the simulated *in vitro* digestion, the process is divided into three key steps, which are the oral phase (mouth), gastric phase (stomach), and small intestine (jejunum). In the oral phase, the macrostructure breakdown and bolus formation take place due to the mechanical action and the enzyme activity within the food matrix. The latter is limited to

food rich in carbohydrates, in which starch is minimally hydrolyzed by the α-amylase enzyme (Martinez-Gonzalez et al., 2017; Palafox-Carlos et al., 2011; Pinto et al., 2017). This phase is where the bioconversion begins (Braga et al., 2018)—the glycosidase starts to hydrolysate the glycosidic flavonoids—but its effectiveness depends on the sugars present in the molecule; e.g., glucose conjugates are rapidly hydrolyzed in contrast to rhamnose conjugates (Wojtunik-Kulesza et al., 2020). The gastric phase occurs in an acidic environment with the presence of the pepsin enzyme. Due to low pH conditions, the anthocyanins transform to a stable species, which is the optimum for preserving the anthocyanins' natural structure, as flavium cations, avoiding its degradation (Bermúdez-Soto et al., 2007; Braga et al., 2018). In these conditions, the polymerized polyphenols with a high molecular weight (insoluble) are hydrolyzed to monomers or aglycones (soluble) due to pH changes (Kamiloglu & Capanoglu, 2014). In the small intestine, the polyphenols are sensitive to the mild alkaline conditions; thus, their stability decreases, being transformed into different structural forms with different chemical properties. Therefore, after the intestinal digestion, new anthocyanin degradation products (such as phenolic acids) and other forms of anthocyanins (such as chalcones) are generated (Y. Liu et al., 2013). Finally, the insoluble fraction would reach the colon where they are absorbed unaltered (through the epithelium) or metabolized by colonic microbiota (Correa-Betanzo et al., 2014).

When functional foods are prepared using value-added ingredients, such as polyphenols, their interaction with the different food macronutrients must be considered. Moreover, these interactions can be modified during the digestion process by the conditions and reactions that occur throughout it. Thus, the objective of this work was to study the interactions between polyphenols and macronutrients in different model systems and the implications of these interactions in the bioaccessibility of the polyphenols. For this purpose, two sources of polyphenols were used: dried berry pomace, where the polyphenols are bounded to fiber, and a polyphenolic extract obtained from blackcurrant pomace.

2. MATERIALS AND METHODS

2.1. Materials

The ingredients used to prepare the model systems were dried blackcurrant (*Ribes nigrum*) pomace (BCP), supplied by the Institute of Natural Materials Technology (Technische Universität Dresden, Germany) and prepared by drying the fresh pomace at 70 °C for 2 h and milling it in a ZM 100 ultracentrifuge mill (Retsch GmbH, Haan, Germany) at 14,000 rpm using a 1 mm sieve (Reißner et al., 2019). The blackcurrant extract (BCE) was prepared from BCP, olive oil (Consum, Valencia, Spain), native wheat starch (C*Gel, Cargill BV, Amsterdam, Netherlands), and whey protein isolate from milk (Harrison Sport Nutrition S.L, Granada, Spain). All the enzymes (α-amylase from porcine pancreas, pepsin from porcine gastric mucosa, porcine bile extract, lipase from porcine pancreas, and pancreatin from porcine pancreas) used in the *in vitro* digestion analysis were supplied by Sigma-Aldrich (Spain).

2.2. Polyphenol extraction from blackcurrant pomace

To obtain the polyphenolic extract from dried BCP, first an extraction was conducted for 120 min at 60 °C in 60% ethanol, in agitation and darkness with a 1:8 sample-to-solvent ratio (MohdMaidin et al., 2019). After the extraction, vacuum filtering was conducted, and the ethanol was removed with rotary evaporation. The resulting aqueous extract was freeze-dried (Telstar, Azbil group, Terrassa, Spain) to obtain a polyphenol-rich extract powder (BCE).

2.3. Model systems preparation

The consumption data in Spain of the different macronutrient are 5.5 g of carbohydrates, 4 g of fat, and 4 g of protein per 100 g of consumption

(Ruiz et al., 2016). According to this information, five types of model system were prepared with (i) water, (ii) wheat starch as a standard for carbohydrates, (iii) olive oil as a standard for fats, (iv) whey protein as a standard for proteins, and (v) a model combining all the ingredients described (water, wheat starch, olive oil, and whey protein). Five grams of dried pomace (BCP) or 0.275 g of polyphenol-rich extract (BCE) (corresponding to the equivalent amount of polyphenols in 5 g of pomace) were added to the different model systems; ten model systems were studied.

The models were prepared as follows. The water model system was prepared by dissolving 5 g of BCP or 0.275 g of BCE in 100 mL of distilled water for 30 min and the obtained systems were called BCP-Wa and BCE-Wa, respectively. For the carbohydrate model, 5.5 g of starch were gelatinized in distilled water (up to 100 mL) for 40 min at 65 °C, with constant agitation, and after cooling the sample, BCP or BCE was added, obtaining BCP-S and BCE-S respectively. For the fat model, 4 g of olive oil were mixed with water (up to 100 mL) during 1 min using a homogenizer (T18 digital ULTRA-TURRAX, IKA, Staufen, Germany) at 13,000 rpm, and BCP or BCE was added, obtaining BCP-O and BCE-O, respectively. For the protein model, 4 g of whey protein were dissolved in distilled water (up to 100 mL) and BCP or BCE was added, obtaining BCP-WP and BCE-WP, respectively. Finally, for the fifth model system, 5.5 g of starch were gelatinized in 70 mL of distilled water, as described before. When it was cooled, 4 g of oil and 4 g of whey protein were added in distilled water up to 100 mL. Lastly, the solution was mixed for 1 min in the homogenizer at 13,000 rpm and BCP or BCE was added, obtaining BCP-All and BCE-All, respectively.

2.4. Microstructure

The microstructure analysis was conducted following Hernández-Carrión et al. (2015), with some modifications. A Nikon ECLIPSE 80i light microscope (Nikon Co., Ltd., Tokyo, Japan) was used working in

the bright field and fluorescence modes. The autofluorescence of the phenolic compounds in the samples was observed using a mercury arc lamp with a tetramethyl rhodamine filter (λ_{ex} = 543/22 nm, λ_{em} = 593/40 nm) as the excitation source. Samples were visualized using 10× and 20× objective lenses. The images were captured and stored at 1280 × 1024 pixels using the microscope software (NIS-Elements F, Version 4.0, Nikon, Tokyo, Japan).

2.5. Analytical determinations

To determine the content of phenolic compounds and its AC, the model systems were centrifuged for 20 min at 5000× *g* and 4 °C and then filtered (Whatman® Grade 4, Fisher Scientific S.L., Madrid, Spain). The supernatant was used for the analysis.

2.5.1. Total Phenolic Content (TPC)

TPC was determined by the Folin–Ciocalteu (F-C) assay according to the procedure described by Singleton et al. (1999), with some modifications. In test tubes, 6 mL of double-distilled water and 1 mL of the supernatant were added (1 mL of double-distilled water for the blank). Then, 0.5 mL of Folin–Ciocalteu reagent (1:1 (v/v)) was added. After 3 min, 1 mL of sodium carbonate solution (20% (w/v)) and 1.5 mL of distilled water were added and vortexed. The mixture was kept at room temperature (≈25 °C) in a dark room for 90 min. Absorbance was measured at 765 nm. The absorbances obtained were related to a calibration curve, which was prepared using different concentrations of gallic acid. The results were expressed as mg of Gallic Acid Equivalents (GAE) per 100 g of sample. The analysis was made in triplicate.

2.5.2. Antioxidant Capacity (AC)

The AC was measured by the ferric reducing antioxidant power assay (FRAP) described by Benzie and Stain (1996) and Pulido et al. (2000) with slight modifications. Following the order described below, 30 µL distilled water, 30 µL extract, and 900 µL FRAP reagent were added in 1.5 mL cuvettes. Distilled water was used as blank. The cuvettes were incubated in a bath at 37 °C for 30 min and the absorbance was measured at 595 nm. The absorbances obtained were related to a calibration curve, which was prepared using different concentrations of Trolox. The results were expressed as µmol Trolox per g of sample. The analysis was made in triplicate.

2.6. Simulated *in vitro* digestion process

An *in vitro* gastrointestinal tract model was used to simulate the biological fate of the ingested samples following the methodology described by Minekus et al. (2014), with modifications (Eriksen et al., 2017; Gómez-Mascaraque et al., 2017). Three phases were simulated: oral, gastric, and intestinal.

The digestion process was conducted in a "Carousel 6 Plus" reaction station (Radleys, United Kingdom). To mimic human physiological conditions, the analysis was conducted with a controlled temperature (37 °C) and agitation (150 rpm), and without light. Both the gastric and intestinal step were performed in a N_2 atmosphere to mimic human physiological reduction of oxygen levels during digestion (Eriksen et al., 2017).

Solutions of simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were prepared according to the compositions described by Minekus et al. (2014). First, for the oral stage, 5 mL of the sample were added in the digestion flask. Then, 4 mL of SSF + α-amylase, 19 µL of $CaCl_2$, and 0.981 mL of distilled water were added. The pH of the saliva was adjusted to 7. The oral digesta was

under agitation for 2 min at 37 °C. Second, for the gastric phase, 8 mL of SGF + pepsin and 4 µL of $CaCl_2$ were added. The pH was adjusted to 3 using 1M HCl, and the volume of distilled water necessary for a total volume of 10 mL was added. The mixture was incubated at 37 °C for 1 h under agitation without oxygen. Third, for the intestinal stage, 5.3 mL of SIF + pancreatin, 40 µL of $CaCl_2$, 5.3 mL of SIF + bile salts (Gómez-Mascaraque et al., 2017), and 5.3 mL of SIF + lipase were added. The pH was adjusted to 7 using 1M HCl or 1M NaOH. Once the pH was adjusted, the volume of distilled water necessary for a total volume of 30 mL was added; the pH was readjusted to 7. Finally, for the filtration process, the final digestion mixture was centrifuged (27,641× *g*, 20 min, 4 °C) and filtered (Whatman® Grade 4). The residue was the non-digested fraction, and the filtered solution was the soluble fraction available for absorption. The samples were stored at −80 °C until further analysis. The digestions were conducted twice.

2.7. Bioaccessibility

The bioaccessibility (BA) is defined as the amount of an ingested nutrient available for absorption in the gut after digestion and is calculated using Equation (1) (Ortega et al., 2011; Quatrin et al., 2020; Rodríguez-Roque et al., 2015).

Bioaccessibility (%) = (TPC of the soluble fraction / TPC of fresh samples) × 100

2.8. Statistical analysis

A categorical multifactorial experimental design with two factors, namely, the source of polyphenol (pomace, BCP or extract, BCE) and model system, was performed on the values of TPC and AC. Analysis of variance (ANOVA) was performed on the data. Least significant difference (LSD) Fisher's tests were used to evaluate the mean value differences ($p < 0.05$) using XLSTAT 2014 statistical software (Microsoft, Mountain View, CA, USA).

3. RESULTS AND DISCUSSION

3.1. Microstructure of the model systems

Figure 1 shows the light microscopy (LM) and fluorescence (FL) images of the fresh (non-digested) systems.

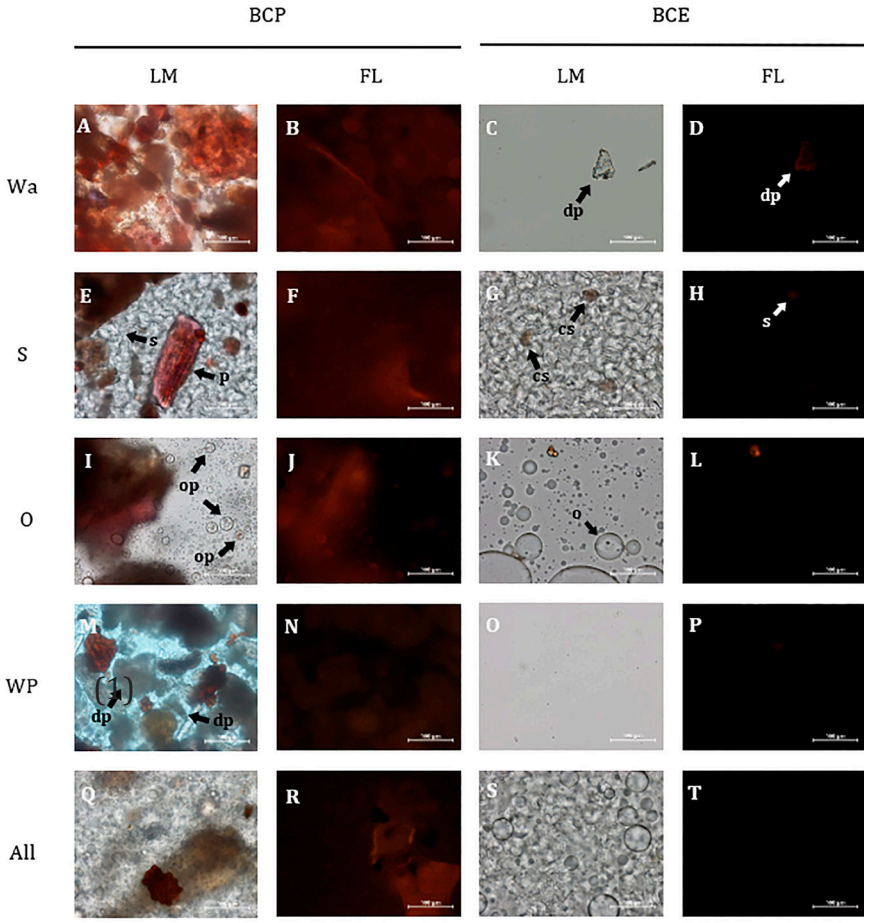

Figure 1. Light microscopy (LM) and fluorescence (FL) pictures of the model systems at 100 μm. BCP: blackcurrant pomace; BCE: blackcurrant extract; Wa: water; S: starch; O: oil; WP: whey protein; and All: water, starch, oil, and whey protein. BCP-Wa (A, B), BCE-Wa (C, D), BCP-S (E, F), BCE-S (G, H), BCP-O (I, J), BCE-O (K, L), BCP-WP (M, N), BCE-WP (O, P), BCP-All (Q, R) and BCE-All (S, T), where first letter between parenthesis correspond to LM images and the second to FL images. Arrows: p (pomace), s (starch granules), cs (colored starch granules), op (oil globules with pomace), o (oil droplets), and dp (discolored pomace).

The BCP-Wa model system (Figure 1A) comprises pomace particles of different sizes and shapes distributed in a continuous phase of water. These particles are mostly reddish and brownish. Blackcurrant pomace is rich in polyphenols, mainly anthocyanins (Reißner et al., 2019), and the aglycone form of anthocyanins are auto fluorescent (Drabent et al., 1999; Rakić et al., 2015). The intensity of the intrinsic fluorescence of the pomace is observed in Figure 1B. In the BCE-Wa system (Figure 1C), the extract was dissolved in the continuous phase and coloration cannot be observed, nor was any important autofluorescence observed (Figure 1D).

In the BCP-S systems (Figure 1E), the pomace particles are distributed in the continuous phase formed by partially gelatinized starch granules. In these systems, pomace particles maintain their characteristic reddish coloration (Figure 1E) and autofluorescence (Figure 1F). In BCE-S, where the extract was added to the starch system, some colored gelatinized granules can be observed (Figure 1G), and a slight fluorescence (Figure 1H) is also detected. This could be indicating interactions between the starch polymers and polyphenols.

In BCP-O systems (Figure 1I), the pomace particles maintain their reddish coloration and some oil globules can be observed, containing small pomace particles inside. Moreover, in these systems, both particles and globules show autofluorescence (Figure 1J). However, this cannot be observed in BCE-O systems (Figure 1K and L). Thus, while pomace is interacting with the oil, the extract is diluted into the liquid media, and interactions with oil are not produced.

When the pomace is incorporated into a system with protein (BCP-WP and BCP-All, Figure 1M and Q, respectively), discolored pomace particles distributed in the continuous phase can be observed. This can be due to the lixiviation of phenolic compounds from the pomace to the continuous phase. In these systems, pomace particles present low levels of autofluorescence (Figure 1N and R) if compared with systems prepared with starch and oil. However, neither color nor fluorescence

are observed in the BCE model systems (Figure 1O, P, S and T). Strong protein–polyphenol interactions could be favoring the extraction of the polyphenols from the pomace toward the medium, becoming part of the continuous phase. However, the polyphenols in the extract could be dissolved into the aqueous medium, preventing color or autofluorescence from being observed.

3.2. Total Phenolic Content (TPC) and Antioxidant Capacity (AC) of the model systems: analysis of macronutrient–polyphenol interactions

A two-way ANOVA was performed for the TPC and AC results. In both analyses, the results show significant interactions ($p < 0.05$) between the two factors: source of polyphenol (BCP or BCE) and model system (Wa, S, O, WP, and All). In addition, each factor presented a significant effect. The results can be seen in Figure 2.

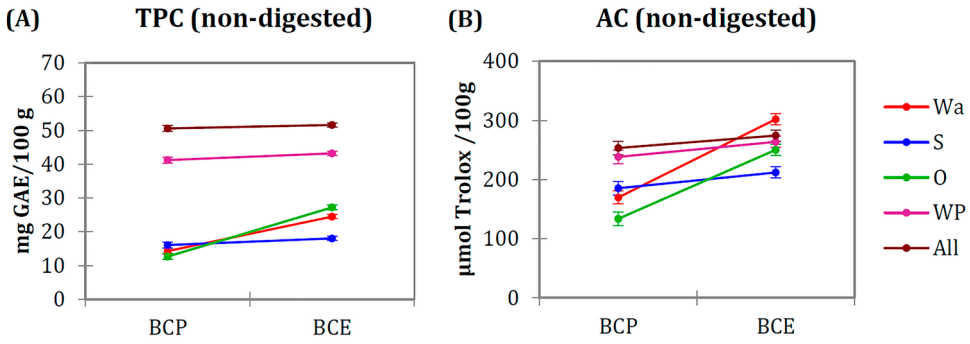

Figure 2. Interaction plot between the source of polyphenol and model system for (**A**) total phenolic content (TPC) and (**B**) antioxidant capacity (AC). BCP: blackcurrant pomace; BCE: blackcurrant extract; Wa: water; S: starch; O: oil; WP: whey protein; and All: water, starch, oil, and whey protein.

When comparing the use of the pomace (BCP) or the extract (BCE) for TPC results (Figure 2A), samples prepared with BCP gave significantly ($p < 0.05$) lower values than those prepared with BCE for the model systems

prepared with water (BCP-Wa and BCE-Wa) or oil (BCP-O and BCE-O). Regarding the different model systems, the formulations with the highest values for both BCP and BCE were those prepared with protein (BCP-WP and BCE-WP, and BCP-All and BCE-All). For the starch models, BCP-S did not present significant differences ($p > 0.05$) with the water model (BCP-Wa) and the oil model (BCP-O), but BCE-S did ($p < 0.05$).

As it can be observed, for the AC results (Figure 2B), that formulations with O (BCP-O and BCE-O) and formulations with Wa (BCP-Wa and BCE-Wa) presented the same trend as the TPC results. In addition, formulations with WP in their composition (BCP-WP and BCE-WP, and BCP-All and BCE-All) also presented the same trend but the difference between the results for these formulations and the remaining model systems decreased when compared with TPC. The lowest results for AC corresponded to BCP-O and the highest to BCE-Wa.

The different TPC and AC results for BCP-Wa and BCE-Wa (Figure 2) can be explained because, in the BCP, the polyphenols are associated with the pomace fiber compounds through covalent interactions or weak non-covalent interactions (hydrogen bonds, Van der Waal forces, and hydrophobic interactions), avoiding its extraction for its determination (Quirós-Sauceda et al., 2014; Saura-Calixto, 2011). In contrast, for BCE, its behavior is different. Phenolic compounds from BCE are dissolved into the water because besides the distinctive aromatic rings, these compounds have a substantial number of hydroxyl groups that gives them a highly polar structure, allowing its solubilization in water. Thus, for BCP, the phenolic compounds are bound to the pomace whereas the phenolic compounds in BCE are free to be dissolved, as shown in the schematic representation of Figure 3. In Figure 1A, the red-colored pomace particles can be observed, whereas in Figure 1C there are discolored particles. It confirms that polyphenols from pomace are not readily available for interaction and that polyphenols in the extract are soluble into the media and free to interact with other compounds or macromolecules.

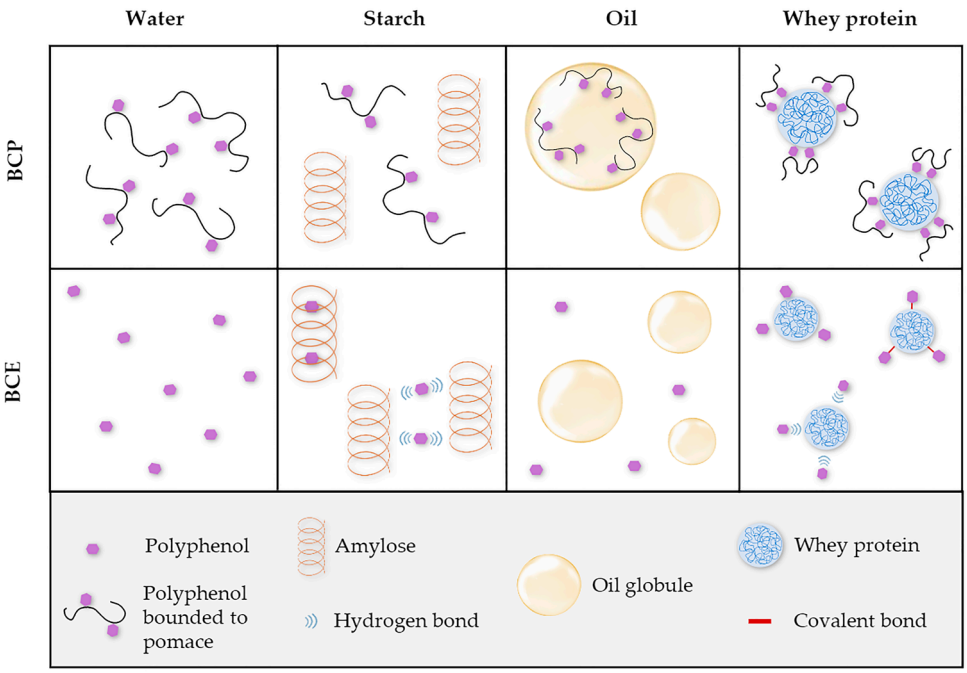

Figure 3. Schematic representation of the polyphenol–macronutrient interactions. BCP: blackcurrant pomace; BCE: blackcurrant extract.

Regarding the TPC and AC results for starch formulations, BCP-S and BCP-Wa models show no differences between them. Thus, incorporating starch in BCP systems did not cause critical changes. However, BCE-S presented lower results ($p < 0.05$) than the BCE-Wa. It could mean there are interactions between the starch and the free polyphenols from BCE because of their high availability to interact. Colored starch granules can be observed in Figure 1G, which could confirm the existence of these interactions. Some authors, such as Amoako and Akiwa (2016) and Zhu et al. (2015), have described the interactions between starch and polyphenols. Two types of interactions between amylose and polyphenols are described: a V-type amylose inclusion complex, which is driven by hydrophobic interactions, and a non-inclusion complex, driven by the hydrogen bounds between the polyphenol hydroxyl groups and the hydrophilic part of the amylose chains (Camelo-Méndez et al., 2016; Chai et al., 2013; Kan et al., 2020;

Zhu, 2015). A schematic representation of the amylose–polyphenol interactions is shown in Figure 3. The inclusion complex may not be possible with the bulky-sized polyphenols because of the limitation of the size of the cavity in the amylose helix, but possible with the low-molecular-weight polyphenols (Chai et al., 2013; Zhu, 2015). Thus, as the extract comprises polyphenols of different chemical structure and size, both types of interactions may occur simultaneously: inclusion-type interactions for small-sized polyphenols and the non-inclusion type for bigger polyphenol molecules.

When comparing the BCP and BCE values of the oil systems (BCP-O and BCE-O), the results are higher when oil is formulated with BCE (Figure 2), probably because of the hydrophilic nature of the free polyphenols that causes their dissolution in the aqueous medium, avoiding its interaction with oil. In addition, an effect of micellarization of phenolic compounds with oil can be produced in the BCP-O systems, *i.e*., phenolic compounds bounded to pomace fiber are within a micelle covered by oil, as described by Ortega et al. (2009). Some components of pomace, as seeds and peels, have a hydrophobic nature that allows the micellarization. This effect is showed in the images of Figure 1E, in which encapsulated pomace fractions into the oil globules can be observed (schematically represented in Figure 3). Therefore, if polyphenols are participating in the micelles, the phenolic content and the antioxidant capacity are reduced.

The formulations with the highest TPC and AC values for both BCP and BCE were those prepared with protein (BCP-WP and BCE-WP, and BCP-All and BCE-All) (Figure 2). The interaction between polyphenols and milk proteins can be caused by both non-covalent and covalent forces (Gallo et al., 2013; Jakobek, 2015; Le Bourvellec & Renard, 2012; Ozdal et al., 2013; Yildirim-Elikoglu & Erdem, 2018). Non-covalent interactions involve weak associations, specifically a combination of hydrogen bonds and hydrophobic interactions. The latter are reversible interactions that would involve aromatics rings of polyphenols and hydrophobic sites of proteins, whereas hydrogen bonding occurs

between hydrogen-acceptor sites of the proteins and the hydroxyl groups of the polyphenols. These interactions will be determined by the molecular weight, conformational mobility, and flexibility, as well as by the relation between the donor/acceptor hydrogen bonds in the proteins and polyphenols (Jakobek, 2015; Le Bourvellec & Renard, 2012). In addition, protein and polyphenols can bind through covalent bonds. These bonds are formed between O-quinones, coming from the oxidation of BCP or BCE polyphenols and nucleophilic groups of proteins, such as -NH_2 and -SH; these interactions are irreversible. Most common interactions are the non-covalent types, such as hydrogen bonding and hydrophobic forces, which can lead to a complex formation between WP and polyphenols (Jiang et al., 2018; Kardum & Glibetic, 2018; Meng et al., 2021). In addition, the great molecular weight and high polarity of most polyphenols from BCP and BCE favors the interactions because of the high number of binding sites (Gallo et al., 2013; Yildirim-Elikoglu & Erdem, 2018). Both types of interactions—non-covalent and covalent—are represented in Figure 3.

In the BCP-WP and BCP-All model systems (Figure 1M and Q), the particles from the BCP are discolored, probably because of the release of the polyphenols from the pomace matrix to the medium. The non-covalent hydrophobic interactions between the aromatic ring of polyphenols and the hydrophobic sites of the proteins, which leads to the complexation with whey proteins, could force the release of polyphenols from the pomace. This effect is also observed in Figure 1N and R because there is a low fluorescence intensity. This is because the fluorescence phenomenon is given by the aromatic rings, and if there is a complex formation where the aromatic ring is involved, the fluorescence intensity is lower. However, despite this complex formation, the TPC values obtained for all the systems prepared with protein are high (Figure 2A) because the hydroxyl groups still possess their reducing capacity. In addition, it is also necessary to consider that the high TPC results could be related to the detection of the aromatic amino acids with reducing capacity present in the whey protein, detected by the

Folin–Ciocalteu reagent (Corrochano et al., 2018; Everette et al., 2010; Mann et al., 2015).

Regarding formulations with protein and BCE (BCE-WP and BCE-All), a complex formation by non-covalent interactions through hydrogen bonding is also likely. These interactions are because of the higher availability of the free phenolic extract compounds to interact, which facilitates the interaction with the globular WP.

3.3. Total phenolic content, antioxidant capacity, and bioaccessibility of the digested model systems.

Figure 4 shows there are significant interactions ($p < 0.05$) between the different model systems and using pomace or extract for both the TPC and AC analysis. In addition, each factor has a significant effect.

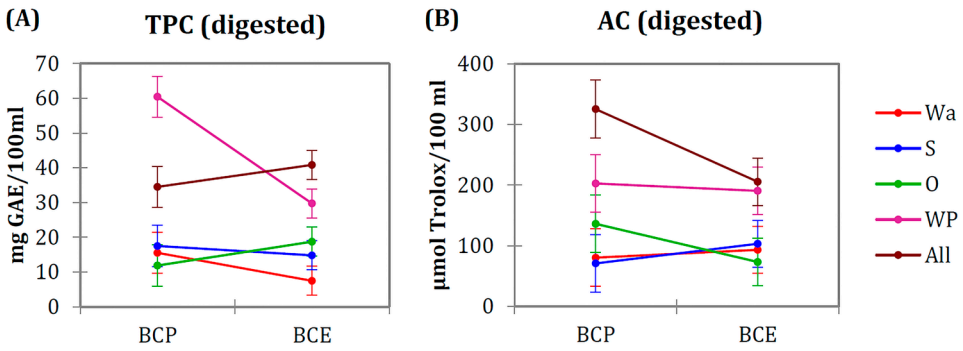

Figure 4. Interaction plot between the source of polyphenol and model system for (**A**) total phenolic content (TPC) and (**B**) antioxidant capacity (AC). BCP: blackcurrant pomace; BCE: blackcurrant extract; Wa: water; S: starch; O: oil; WP: whey protein; and All: water, starch, oil, and whey protein.

In terms of the differences between using BCP or BCE as a polyphenol source, for TPC (Figure 3A), no significant differences ($p > 0.05$) were observed between the water models (BCP-Wa or BCE-Wa) and the models formulated with starch or oil (BCP-S, BCE-S, BCP-O, and BCP-S). However, when WP was used as an ingredient, the TPC was

lower ($p < 0.05$) when the system was formulated with BCE, whereas when all the ingredients were added to the model, the TPC was higher ($p < 0.05$). Finally, results for models with protein in their formulation (BCP-WP, BCE-WP, BCP-All, and BCE-All) were the highest.

For AC values (Figure 4B), and regarding the differences between using BCP or BCE, the formulations with all the ingredients (BCP-All and BCE-All) presented significant ($p < 0.05$) differences, with values for BCP-All higher than from BCE-All. The rest of the formulations did not show significant differences ($p > 0.05$) whether BCP or BCE were used. In addition, formulations with BCP did not show differences ($p > 0.05$) between the BCP-Wa model and models BCP-S and BCP-O. Furthermore, a similar trend than for TPC results can be observed, with the models formulated with protein being the ones with the highest results. However, for the BCE results in systems elaborated with protein (BCE-WP and BCE-All), the results are the highest ($p < 0.05$).

The bioaccessibility results (Figure 5) allow to understand the changes that occurred in the interactions between the macronutrients and polyphenols induced by the conditions throughout the *in vitro* digestion process. The release, stability, and solubility of the polyphenols is related to the food matrix composition and the polyphenols' chemical structure (Pineda-Vadillo et al., 2016). The bioaccessibility of each class of polyphenol is affected by the molecular weight, glycosylation (aglycones are more hydrophilic and thus more easily absorbed), the interactions between the polyphenols and food components, and also by its different pH transformations (Karaś et al., 2017; Wojtunik-Kulesza et al., 2020).

Figure 5 shows that the samples formulated with BCP had the highest bioaccessibility results, which exceeded the 100% level for the BCP-Wa, BCP-S, and BCP-WP samples, or were near to 100% for BCP-O.

Thus, bioaccessibility results above 100% could mean there is a release of polyphenols from the pomace during the *in vitro* digestion process, allowing them to be available for further absorption into

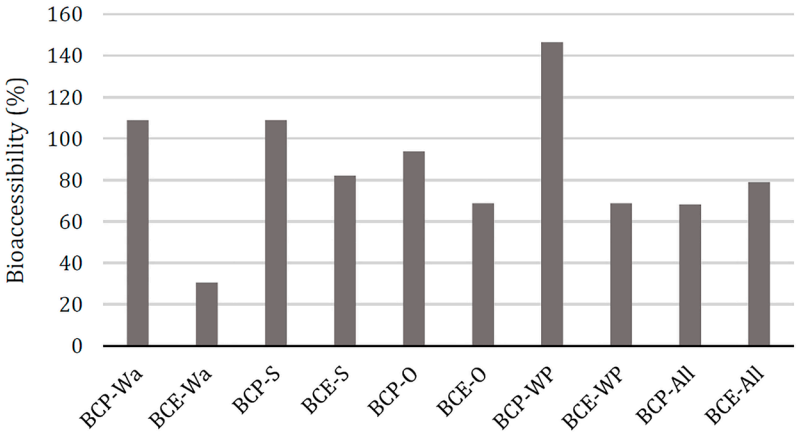

Figure 5. Bioaccessibility results from all the model systems. BCP: blackcurrant pomace; BCE: blackcurrant extract; Wa: water; S: starch; O: oil; WP: whey protein; and All: water, starch, oil, and whey protein.

the systemic circulation. Moreover, during the digestion process, the phenolic compounds are released from the food matrix in the upper part of the gastrointestinal tract because of the polyphenol solubilization into the intestinal fluids at physiological conditions, where they become available for intestinal absorption (Quirós-Sauceda et al., 2014). At the end of the gastrointestinal tract, there would be two fractions of phenolic compounds: the accessible phenolic compounds with a low molecular weight, which may be partially absorbed through the small intestine mucosa, and the non-accessible phenolic compounds, which comprises high-molecular-weight polyphenols or low-molecular-weight-polyphenols bonded to dietary fiber and/or trapped into the fiber matrix core (Palafox-Carlos et al., 2011; Quirós-Sauceda et al., 2014; Saura-Calixto, 2011).

However, the models with BCE, especially the one formulated only with water (BCE-Wa), had the lowest results compared with the rest of models. Several authors (Bermúdez-Soto et al., 2007; Correa-Betanzo et al., 2014; Y. Liu et al., 2013) have described a decrease in bioaccessibility

of polyphenols, particularly in anthocyanins, after the digestion process. This may be due because the polyphenols in the extract are free and dissolved into the medium once the simulated digestion starts, being more susceptible to changes and therefore to their degradation. Specifically, under the conditions during the simulated digestion (mainly changes in pH and enzyme action), the red flavylium cation is transformed into less stable forms, such as the pseudobase, quinoidal base, and chalcone (Bermúdez-Soto et al., 2007).

Despite BCP-O not having a BA over 100%, its result is 94%; so, although there is not a significant release of polyphenols during *in vitro* digestion, important polyphenol degradation is not occurring either. Gu et al. (2020) proposed a mechanism of anthocyanin protection, which is one of the main phenolic compounds of blackcurrant pomace, based on the anthocyanin incorporation into the lipid phase of the micelles (bile salts from digestion emulsify lipids and break into micelles under the actions of lipase before absorption). Thus, the oil may exert a protective effect on polyphenols.

The highest BA results were for BCP-WP. As described in Section 3.2, a protein–polyphenol complex is formed through hydrophobic interactions. This complexation could have a protective role over the polyphenols, avoiding their transformation by alkaline pH or their oxidation into O-quinones by reactive oxygen species (Kardum & Glibetic, 2018; Tagliazucchi et al., 2016). In addition, during the digestion process, a release of polyphenols from the pomace because of gastrointestinal conditions is produced. Therefore, new complexes may form, and WP would exert a protective effect on the polyphenols from blackcurrants during *in vitro* digestion. As described by Meng et al. (2021) and Tagliazucchi et al. (2016), milk proteins could avoid autoxidative reactions through the gastrointestinal tract, acting as a carrier for the delivery of antioxidant compounds.

Finally, BCP-All presented the lowest values compared with other models with BCP. Here, all the ingredients are used in the formulation,

giving the possibility of multiple interactions between the ingredients and the phenolic compounds. This effect could form different complexes or aggregates of high molecular weight, which would avoid their absorption through the epithelium (Correa-Betanzo et al., 2014). Thus, polyphenols embedded into the complex could reach the colon unaltered, where the phenolic compound may be absorbed intact or metabolized by colonic microbiota. Furthermore, polyphenols can stimulate beneficial bacteria and inhibit pathogenic bacteria, thus resulting in great importance for colon health (Jakobek & Matić, 2019).

4. CONCLUSIONS

When polyphenols are incorporated into the model systems as blackcurrant pomace, their bioaccessibility is increased if compared to the polyphenols added from the extract source. The bounding of polyphenols to pomace and the interactions that take place with the nutrients of the model systems influence the proportion of polyphenols that can be available for absorption in the initial stages of intestinal digestion. However, the polyphenols in the extract are dissolved into the aqueous medium, which makes them more susceptible to changes, decreasing their bioaccessibility. When the model systems are formulated with pomace and only one nutrient, the bioaccessibility is higher than when the system contains all the nutrients. Of all the components studied, the greatest effect on bioaccessibility is observed for proteins due to the protein–polyphenol complex formation. Although the data obtained with these model systems cannot be directly extrapolated to human *in vivo* conditions, they could be a helpful approach for determining the effects of the food matrix on polyphenol bioaccessibility. Further *in vivo* investigations would be helpful to better comprehend the fate of polyphenols in the last steps of the digestion process.

5. AUTHOR CONTRIBUTIONS

Conceptualization, I.H.; methodology, E.D.-S. and A.Q.; investigation, E.D.-S. and A.Q.; writing—original draft preparation, E.D.-S.; writing—review and editing, I.H. and A.Q.; supervision, I.H. and A.Q; funding acquisition, I.H. All authors have read and agreed to the published version of the manuscript.

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8. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Discusión general de los resultados

La tesis abarca distintas estrategias para el diseño de alimentos a partir del aprovechamiento del bagazo generado como subproducto de la industria de elaboración de zumos de frutos rojos.

La incorporación de fibra en productos horneados complejos con elevado número de componentes, como el bizcocho, puede dar lugar a cambios en la estructura y distribución de las burbujas de aire y por lo tanto en la textura. Por ello, y debido a que el bagazo tiene alto contenido en fibra, se hizo un trabajo previo en el que se formularon bizcochos reemplazando parte del aceite por fibra insoluble, fibra soluble o una mezcla de ambas.

La formulación de los bizcochos con diferentes tipos de fibras dio lugar a una reducción de la viscosidad aparente, la cual provocó que una mayor cantidad de aire fuera incorporado durante la etapa de mezclado en forma de burbujas. Este efecto se pudo observar en los resultados del micro-horneado, ya que, así como en el control estas burbujas fueron creciendo de manera controlada para dar lugar a una estructura uniforme, en los bizcochos formulados con fibra soluble y con la mezcla de ambas, las burbujas aumentaron en número y crecieron adoptando una estructura irregular. En cuanto a los bizcochos con fibra insoluble, estos presentaron un mayor porcentaje de burbujas grandes en las temperaturas más altas del micro-horneado. Estas burbujas se pudieron observar en el análisis de la macroestructura de la miga. Por lo tanto, la reducción en la viscosidad aparente también dio lugar a una menor estabilidad de las burbujas ya que aumentó la movilidad de las mismas, favoreciendo su coalescencia y por lo tanto la formación de alveolos más grandes en el bizcocho.

La adición de fibra modificó las características texturales en comparación con el control, principalmente, en el caso del bizcocho formulado con fibra insoluble, el cual presentó los valores más altos en dureza, masticabilidad y esponjosidad. Pese a que los bizcochos formulados con fibra insoluble tuvieron mayor cantidad de aire y burbujas de mayor tamaño, lo que dio lugar a valores altos de

esponjosidad, la dureza fue mayor y la matriz mucho más compacta, tal y como se observó en las imágenes de FESEM. La mayor compactación de la matriz pudo ser debida a la sustitución de parte del aceite por la fibra insoluble.

Durante la digestión *in vitro,* solo los bizcochos formulados con fibra soluble presentaron una menor liberación de glucosa a los 180 minutos. Al tener la fibra soluble capacidad de absorción de agua, compite con el almidón por el agua disponible. Esto dio lugar a bizcochos con una menor gelatinización del almidón, lo que disminuyó la liberación de glucosa durante la digestión *in vitro.*

Por último, en cuanto a la aceptabilidad, fue el bizcocho formulado con fibra insoluble el que obtuvo una menor calificación, mientras que, los consumidores no encontraron diferencias entre el resto de los bizcochos.

Por lo tanto, la incorporación de fibra insoluble en bizcochos dio lugar a cambios estructurales que fueron percibidos por el consumidor de forma negativa. Debido a que el bagazo de frutos rojos posee una gran cantidad de fibra, principalmente fibra insoluble, sería conveniente establecer estrategias de mejora para contrarrestar los efectos negativos sobre la estructura y textura en productos horneados como bizcochos y magdalenas.

Una de las estrategias de mejora planteadas fue estudiar, de manera instrumental y sensorial, la influencia de la incorporación de diferentes impulsores químicos con diferentes ratios de liberación de CO_2 (ácido cítrico, pirofosfato ácido de sodio 10 y glucono-δ-lactona) en combinación con bicarbonato encapsulado y no encapsulado en bizcochos formulados con bagazo de frutos rojos.

El pirofosfato ácido de sodio es el impulsor que tarda más tiempo en comenzar a liberar gas, por ello cuando la viscosidad de las masas aumentó (temperaturas cercanas a la solidificación de la masa), estas fueron capaces de retener una gran cantidad de CO_2 formando burbujas grandes. La incorporación de pirofosfato dio lugar a estructuras más

aireadas y con una menor dureza. En cambio, el ácido cítrico comienza rápidamente a liberar CO_2 una vez está en contacto con el bicarbonato, lo cual dio lugar a la formación de burbujas más pequeñas y por consiguiente a la menor incorporación de aire en la masa. Por lo tanto, se obtuvieron bizcochos con valores más altos de dureza. Por último, las formulaciones con glucono-δ-lactona, al ser este un impulsor que necesita ser hidrolizado para reaccionar con el bicarbonato (antes y después de la hidrólisis hay liberación de CO_2), presentaron una estructura con burbujas de diferente tamaño, y dieron lugar a bizcochos de dureza intermedia.

Además de esto, la incorporación de bicarbonato encapsulado provocó una ralentización de la reacción de liberación de gas, ya que a los 70 °C es cuando el recubrimiento del bicarbonato empieza a fundirse. Asimismo, la estructura de la miga fue más compacta y la cantidad de aire en la masa fue menos, por lo que la esponjosidad del bizcocho se vio afectada.

En cuanto a los resultados sensoriales, el método de *Flash Profile,* mostró que las formulaciones elaboradas con glucono-δ-lactona y bicarbonato encapsulado fueron caracterizadas por los panelistas con atributos negativos como duro, aceitoso y rancio, siendo además los que peor calificación hedónica obtuvieron. Sin embargo, el uso de pirofosfato, independiente del tipo de bicarbonato usado, dio lugar a unos bizcochos caracterizados positivamente con atributos como esponjoso y dulce. Se observó por lo tanto que el uso de diferentes impulsores puede contrarrestar los efectos negativos de la fibra sobre las características de estructura y textura. En este caso fue el pirofosfato el impulsor que dio unas características deseables y aceptadas por el consumidor.

Como una segunda estrategia de mejora se propuso el uso de harinas pregelatinizadas mediante extrusión con el objetivo de mejorar la calidad estructural y sensorial del producto.

Se formularon magdalenas con una sustitución del 50% de harina de trigo por harina de trigo pregelatinizada por extrusión a diferentes temperaturas (50, 80 y 150 °C). La incorporación de las harinas pregelatinizadas provocó un aumento en la viscosidad de las masas; este aumento fue mayor al aumentar la temperatura de extrusión. La harina sometida a una mayor temperatura de extrusión presentó gránulos de almidón con mayor grado de gelatinización, pérdida de integridad y mayor capacidad de retención de agua.

Esta pérdida de la integridad del gránulo provocada por la temperatura de extrusión favoreció la accesibilidad de las enzimas degradativas del almidón a los gránulos durante el proceso de la digestión *in vitro* de las harinas, produciendo un aumento de la liberación de glucosa. Sin embargo, este efecto se vio contrarrestado debido a que los polifenoles del bagazo presente en las magdalenas tuvieron un efecto inhibitorio sobre las enzimas digestivas.

Por lo tanto, la adición de bagazo en magdalenas elaboradas con harina pregelatinizada dio buenos resultados como estrategia de mejora, ya que, por una parte, permitió mantener las propiedades texturales apreciadas por el consumidor en este tipo de productos y, por otra parte, los polifenoles presentes en el bagazo contrarrestaron el aumento de la liberación de glucosa que produce la harina pregelatinizada durante la digestión *in vitro*.

Una vez analizado el efecto de la incorporación de bagazo de frutos rojos en productos horneados, se planteó analizar otras características del bagazo. En concreto, se estudió si los polifenoles presentes en el bagazo presentaban actividad antimicrobiana. Estos polifenoles se encuentran adheridos a la fibra del bagazo, por ello, es conveniente someter el bagazo a algún tratamiento que facilite su extracción sin que sean degradados, para que puedan llevar a cabo esta acción antimicrobiana. Como tratamiento de extracción de polifenoles se seleccionó las altas presiones hidrostáticas. Las altas presiones es una tecnología emergente, no térmica, que está siendo muy empleada

en el ámbito de la alimentación. Esta tecnología permite asegurar la inactivación microbiológica a la vez que mantener la calidad nutricional y sensorial de los alimentos.

Para comprobar esta hipótesis, se reformularon batidos lácteos con diferentes proporciones de bagazo de frutos rojos y se optimizaron los parámetros del tratamiento por altas presiones hidrostáticas (tiempo, presión) con el fin de conseguir un producto rico en polifenoles, capaz de inhibir el crecimiento de microorganismos sin sacrificar el contenido fenólico y su capacidad antioxidante. El bagazo fue inoculado con *Listeria monocytogenes* para evaluar el efecto antimicrobiano.

Los resultados mostraron que el contenido fenólico y la capacidad antioxidante se ven afectados por la presión y el tiempo de tratamiento, pero especialmente, por la concentración de bagazo. En cambio, la presión y el tiempo son los parámetros con efecto significativo sobre la inactivación de *L. monocytogenes*. Esta diferencia se atribuyó a que la leche tuvo un efecto protector sobre *L. monocytogenes* al existir interacciones entre las proteínas de la leche y los polifenoles del bagazo, por lo que, pese a la existencia de efecto antimicrobiano del bagazo, este se vio ligeramente enmascarado. Las condiciones de tratamiento que consiguieron la máxima efectividad antimicrobiana asegurando el máximo contenido fenólico y capacidad antioxidante fueron 500 MPa durante 10 min en batidos con una concentración de bagazo de 10%.

Por lo tanto, se pudo observar como el tratamiento de altas presiones en batidos con bagazo de frutos rojos tiene un claro efecto antimicrobiano y reduce la supervivencia de posibles microorganismos patógenos como *L. monocytogenes*. Adicionalmente, dicho tratamiento aumenta en general la capacidad antioxidante y el contenido en compuestos fenólicos totales.

Una vez establecidas las estrategias de mejora de textura en bizcochos y magdales, y estudiado el efecto sinérgico entre el bagazo y las altas presiones en batidos, se quisieron conocer las posibles interacciones entre los polifenoles del bagazo y los diferentes macronutrientes de los

alimentos, y como estas afectan a la bioaccesibilidad de los polifenoles. Para ello se utilizaron sistemas modelo basados en los principales macronutrientes: (i) sistema modelo con almidón, (ii) sistema modelo con aceite, (iii) sistema modelo con proteína y (iv) sistema modelo con almidón, aceite y proteína. Además, para comprender las interacciones de los polifenoles en función de si están unidos o no a la fibra de bagazo, se adicionó a los sistemas modelo el bagazo en polvo o un extracto de los compuestos fenólicos del bagazo.

Se estudió el contenido fenólico y la capacidad antioxidante en los sistemas modelo formulados con bagazo y con extracto. Las principales diferencias encontradas entre el uso de uno u otro fueron debidas a que en el bagazo los polifenoles se encuentran adheridos a la fibra mientras que los polifenoles del extracto se encuentran disueltos en el medio. En cuanto a las interacciones entre los polifenoles (bagazo o extracto) con los sistemas modelo, en los sistemas modelo elaborados con almidón se pudieron observar interacciones de los gránulos de almidón con el extracto, que pudieron ser debidas a dos tipos de interacciones: formación de un complejo de inclusión de los polifenoles con la amilosa del almidón o asociación de la amilosa con los fenoles mediante puentes de hidrógeno. En los sistemas modelo formulados con aceite, se observó que el extracto, al encontrarse solubilizado en el medio acuoso, no interaccionó con el aceite. En cambio, sí que hubo interacciones entre el aceite y el bagazo al producirse un efecto de micelarización. En cuanto al uso de la proteína en los sistemas modelo, tanto en los elaborados con bagazo como con extracto, se observaron interacciones que podrían ser debidas fundamentalmente a la formación de complejos con las proteínas mediante interacciones hidrofóbicas no covalentes.

Finalmente, se observó que la bioaccesibilidad de los polifenoles aumentó cuando se incorporaron en forma de bagazo en vez de en forma de extracto. Esto fue debido a la unión de los polifenoles al bagazo y a las interacciones entre el bagazo y los nutrientes de los sistemas modelo. Estas interacciones influyen en la cantidad de polifenoles que pueden estar disponibles para su absorción en las etapas iniciales

de la digestión intestinal. Sin embargo, cuando los polifenoles se incorporaron como extracto, estos se disolvieron en el medio acuoso, lo que los hizo más susceptibles a los cambios que se producen durante la digestión, disminuyendo su bioaccesibilidad. Además, en este estudio se observó que cuando los sistemas modelo fueron formulados con el bagazo y un solo nutriente, la bioaccesibilidad de los compuestos fenólicos fue mayor que cuando se encontraron todos los nutrientes en el sistema modelo.

Conclusiones

Las principales conclusiones que se extraen de la presente tesis son:

- La incorporación de fibra soluble o una mezcla de soluble e insoluble en la formulación de bizcochos, como reemplazo de grasa, permite mantener la estructura, textura y aceptación de los bizcochos en mayor medida que la fibra insoluble.

- El uso de impulsores químicos con diferentes ratios de liberación de CO_2 provoca cambios en la distribución del tamaño de las burbujas en la masa, dando lugar a diferentes estructuras de la miga que se correlacionan con los resultados de textura. Concretamente, el pirofosfato ácido de sodio permite mejorar las características texturales de bizcochos elaborados con bagazo de frutos rojos, dando lugar a bizcochos más esponjosos y con una buena aceptabilidad.

- El uso de harinas pregelatinizadas como sustitución de harina en magdalenas formuladas con bagazo de frutos rojos no produce cambios importantes en los parámetros de textura ni en la aceptabilidad por parte de los consumidores en comparación con una formulación estándar. Por tanto, el uso de estas harinas es una buena estrategia de mejora de características texturales en productos horneados ya que los efectos negativos de la fibra del bagazo sobre la textura se ven enmascarados. La digestión *in vitro* de harina extrusionada produce un aumento de la liberación de glucosa en comparación con la harina sin tratar, debido al estado de pregelatinización del almidón. Sin embargo, en las magdalenas, la acción de inhibición enzimática de los polifenoles del bagazo permite contrarrestar este efecto.

- El tratamiento de batidos formulados con bagazo de frutos rojos favorece la liberación de compuestos fenólicos al medio. De este modo aumenta el contenido fenólico y la capacidad antioxidante de los batidos. Además, el tratamiento potencia la actividad antimicrobiana de los polifenoles. Las condiciones de tratamiento que consiguieron la máxima efectividad antimicrobiana asegurando el máximo contenido fenólico y capacidad antioxidante fueron 500 MPa durante 10 min en batidos con una concentración de bagazo de 10%.
- El estado en el que se encuentren los polifenoles -unidos a la fibra del bagazo o disueltos en el medio- tiene un papel muy importante en cuanto a las interacciones producidas entre los polifenoles y los macronutrientes presentes en los sistemas modelo y la bioaccesibilidad tras la digestión *in vitro*. Los sistemas modelo formulados con bagazo y un solo nutriente, presentan una mayor bioaccesibilidad de los compuestos fenólicos que cuando se encuentran todos los nutrientes en el sistema modelo.