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Mejoras tecnológicas para el incremento de la funcionalidad de antioxidantes y probióticos Contribución a la sostenibilidad de la industria agroalimentaria.

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Valencia, julio de 2018

Fdo. Noelia Betoret Valls	Fdo. Cristina Barrera Puigdollers	Fdo. Lucía Seguí Gil	Fdo. Maria Ester Betoret Valls

A mis padres, quienes me inculcaron
el valor del esfuerzo y el sacrificio.

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Resumen

En el sistema actual de producción intensiva de alimentos surge la necesidad de considerar la sostenibilidad de las industrias agroalimentarias. El uso correcto de las tecnologías, una mejor gestión de los subproductos y el empleo de materias primas alternativas a las convencionales pueden contribuir con este cometido. En esta tesis se estudia la forma en que algunas tecnologías contribuyen a la mejora de la funcionalidad de alimentos con alto contenido en antioxidantes y probióticos. El tema se aborda desde dos enfoques: por un lado, la aplicación de las tecnologías existentes para la mejora de la funcionalidad de los alimentos, ya sea mediante la incorporación de ingredientes o bien a partir de la formación de estructuras; y, por otro lado, el aprovechamiento de fuentes alternativas de ingredientes alimentarios con elevado contenido en compuestos bioactivos. Las tecnologías estudiadas en esta tesis han sido el tratamiento con altas presiones de homogeneización (HPH), la encapsulación mediante HPH, la impregnación a vacío (IV) y el secado por aire caliente.

Se estudió el efecto combinado de la aplicación de HPH (20, 50, 100 y 150 MPa) y la adición de trehalosa (10, 20 y 30 g/ 100 g) en zumo de mandarina con y sin *Lactobacillus salivarius* spp. *salivarius* sobre las principales propiedades fisicoquímicas y funcionales del zumo. Concretamente, se evaluó la distribución del tamaño de partícula, la pulpa suspendida, la turbidez, los parámetros de impregnación del zumo en manzana, la capacidad antirradical y el contenido en fenoles y flavonoides del zumo de mandarina. Por otra parte, en el zumo inoculado con *Lactobacillus salivarius* spp. *salivarius* se evaluó la hidrofobicidad del microorganismo, y su viabilidad durante 10 días de almacenamiento a 5 ºC. Los resultados obtenidos mostraron que el tratamiento por HPH redujo el tamaño medio de la distribución de las partículas suspendidas en el zumo, mejoró la estabilidad de la nube y aumentó el contenido en fenoles totales y flavonoides. La

incorporación de trehalosa en una cantidad de 20 g/ 100 g mejoró la actividad antioxidante determinada por el método ABTS. Los resultados en el zumo de mandarina con *L. salivarius* constataron que, tanto la aplicación de presiones de homogeneización como la adición de trehalosa mejoraron la hidrofobicidad del probiótico y su supervivencia tras el almacenamiento.

De forma adicional, la encapsulación de las células microbianas mediante presiones de homogenización permite mejorar la viabilidad de *Lactobacillus salivarius* spp. *salivarius* en zumo de mandarina. En los discos de manzana impregnados con el zumo de mandarina incluyendo las células microbianas encapsuladas no se observó una mejora en la supervivencia del probiótico durante las operaciones de IV y de secado con aire a 40 ºC, aunque la encapsulación si que mejoró su resistencia al proceso de digestión *in vitro*. Se estudió el almacenamiento a 5 ºC durante 10 días del zumo y durante 30 días de los discos de manzana a temperatura ambiente, determinándose en ambos casos el efecto de la encapsulación sobre la supervivencia de *L. salivarius* y sobre su resistencia al proceso de digestión *in vitro*. Los resultados obtenidos tanto en el zumo de mandarina como en los discos de manzana impregnados, muestran que el probiótico encapsulado presentó mayor supervivencia durante el almacenamiento y el proceso de digestión *in vitro* que el probiótico sin encapsular.

En relación al aprovechamiento de otras fuentes para la obtención de nuevos ingredientes alimentarios con elevado contenido en compuestos bioactivos, se seleccionaron 11 azúcares de caña no refinados como alternativa al azúcar blanco y se analizaron sus propiedades fisicoquímicas y antioxidantes. Se confirmó que los azúcares de caña no refinados presentaron compuestos con actividad antioxidante y que su contenido guardaba relación con el grado de refinado del producto. Los azúcares no refinados constituyen una fuente natural de antioxidantes y la sustitución del azúcar blanco por los azúcares no refinados supondría un gran aporte de compuestos antioxidantes a la dieta. Por último se

aborda el aprovechamiento de subproductos para la obtención de polvos a partir de bagazo de arándano mediante secado por aire caliente y posterior triturado. Se ha analizado el efecto de la temperatura de secado (60 y 70 ºC) y de la intensidad del triturado sobre las propiedades fisicoquímicas y funcionales de los mismos. El secado disminuyó la capacidad antioxidante del bagazo de arándano pero no hubo un efecto significativo de la temperatura. La acción mecánica del triturado, redujo de manera significativa el contenido en fibra del polvo. Además, se demostró que el tamaño de partícula y el contenido en fibra de los polvos influyeron de forma decisiva en las propiedades de interacción con el agua y con el aceite, lo que condiciona en gran medida su estabilidad y su aplicación como ingrediente en la formulación de alimentos.

Abstract

In the current system of intensive food production, there is the need to consider sustainability in agri-food industries. The correct use of technologies, a better management of by-products and the use of alternative raw materials can contribute to this objective. In this thesis, the way in which some technologies can improve the functionality of food with a high content of antioxidants and probiotics are studied. This is addressed from two approaches: on the one hand, the application of existing technologies for the improvement of food functionality, either through the incorporation of ingredients or from the formation of structures; and, on the other hand, the use of alternative food ingredients sources with high content in bioactive compounds. The technologies studied in this thesis have been the treatment with high homogenization pressures (HPH), encapsulation by HPH, vacuum impregnation (VI) and hot air drying.

Combined effect of HPH treatment (20, 50, 100 and 150 MPa) and the addition of trehalose (10, 20 and 30 g / 100 g) in mandarin juice with and without *Lactobacillus salivarius spp. salivarius* on the main physicochemical and functional properties were studied. In particular, particle size distribution, suspended pulp, cloudiness and juice impregnation parameters in the apple were evaluated, as well as the antiradical capacity, total phenols content and flavonoids of mandarin juice. Furthermore, hydrophobicity of *Lactobacillus salivarius* spp. *salivarius* inoculated in the juice and its survival during 10 days of storage at 5ºC were evaluated. It was obtained that HPH treatment reduced the particle size distribution of suspended particles in juice, improved cloud stability and increased total phenols content and flavonoids. Trehalose addition in the amount of 20 g / 100 g increased the antioxidant activity determined by the ABTS method. Results of the mandarin juice with *L. salivarius* evidenced that both the application of homogenization pressures

and the addition of trehalose improved the hydrophobicity of the probiotic and its survival after storage.

Additionally, microbial cells encapsulation by means of homogenization pressures improved the *Lactobacillus salivarius* spp. *salivarius* survival in mandarin juice. In apple discs impregnated with mandarin juice including microbiane cells encapsulated, it was not observed an improvement in probiotic survival after vacuum impregnation and air drying at 40 ºC, although encapsulation does improve the resistance to *in vitro* digestión. Storage for 10 days in mandarin juice and for 30 apple discs at 5 ºC was studied, determining in both the effect of the encapsulation on *L. salivarius* survival and on its resistance to *in vitro* digestion. Results obtained in the mandarin juice show that the encapsulated probiotic had a greater survival during storage and during the *in vitro* digestion than the non- encapsulated one. In apple discs, the encapsulation had no effect on probiotic survival after IV and air drying at 40 °C, although it improved its resistance to the *in vitro* digestion process.

In relation to the use of other sources to obtain new food ingredients with high content of bioactive compounds, 12 non refined sugar cane were selected as alternatives to white sugar and their physicochemical and antioxidant properties were analyzed. It was confirmed that the no refined sugar cane presented compounds with antioxidant activity and that their content was related to the degree of refining of the product. The no refined sugars are a natural source of antioxidant compounds and the substitution of white sugar by no refined sugars would have a great contribution to the diet of antioxidant compounds. Finally, the use of by-products to obtain powders from blueberry pomace by using hot air drying and subsequent grinding is considered. The effect of the drying temperature (60 and 70 °C) and grinding intensity on the physicochemical and functional properties was analyzed. Drying decreased the antioxidant capacity of the blueberry pomace regardless the temperatura. The

mechanical action of the grinding significantly reduced the fibre content of the powder. In addition, it was demonstrated that particle size and fibre content of powders had a decisive influence on interaction with water and oil properties, which largely conditioned its stability and its application as an ingredient for food formulations.

Resum

En el sistema actual de producció intensiva d'aliments sorgeix la necessitat de considerar la sostenibilitat en les indústries agroalimentàries. L'ús correcte de les tecnologies, una millor gestió dels subproductes i l'ús de matèries primeres alternatives a les convencionals poden contribuir amb esta comesa. En aquesta tesi s'estudia la forma en què algunes tecnologies contribueixen a la millora de la funcionalitat d'aliments amb alt contingut en antioxidants i probiótics. El tema s'aborda des de dos enfocaments: d'una banda, l'aplicació de les tecnologies existents per a la millora de la funcionalitat dels aliments, ja siga per mitjà de l'incorporació d'ingredients o bé a partir de la formació d'estructures; i d'altra banda, l'aprofitament de fonts alternatives d'ingredients alimentaris amb elevat contingut en compostos bioactius. Les tecnologies estudiades en aquesta tesi han sigut el tractament amb altes pressions d'homogeneïtzació (HPH), l'encapsulació per mitjà de HPH, la impregnació a buit (IV) i l'assecat per aire calent.

Es va estudiar l'efecte combinat de l'aplicació de HPH (20, 50, 100 i 150 MPa) i l'addició de trehalosa (10, 20 i 30 g/ 100 g) en suc de mandarina amb i sense *Lactobacillus salivarius* spp. *salivarius* sobre les principals propietats fisicoquímiques i funcionals del mateix. Concretament, es va avaluar la distribució de la grandària de partícula, la polpa suspesa, la turbidesa i els paràmetres d'impregnació del suc en poma, així com la capacitat antirradical, el contingut en fenols i flavonoids del suc de mandarina sense el microorganisme. A més, en el suc inoculat amb *Lactobacillus salivarius* spp. *salivarius* es va avaluar l'hidrofobicitat del microorganisme, així com la seua viabilitat durant 10 dies d'emmagatzemament a 5 ºC en el suc. Es va obtindre que el tractament per HPH va reduir la distribució de la grandària de partícula de la polpa, va millorar l'estabilitat del núvol i va augmentar el contingut en fenols totals i flavonoids i a més, que la trehalosa va millorar l'activitat antioxidant determinada pel

mètode ABTS quan es va afegir 20 g/ 100 g. Els resultats en el suc de mandarina amb *L. salivarius* van constatar que, tant l'aplicació de pressions d'homogeneïtzació com l'addició de trehalosa van millorar l'hidrofobicitat del probiòtic i la seua supervivència després de l'emmagatzenament.

De forma addicional, l'encapsulació de les cèl·lules microbianes mitjançant pressions d'homogenización permet millorar la viabilitat de Lactobacillus salivarius spp. salivarius en suc de mandarina. En els discos de poma impregnats amb el suc de mandarina incloent les cèl·lules microbianes encapsulades no es va observar una millora en la supervivència del probiótic durant les operacions d'IV i d'assecat amb aire a 40 °C, encara que l'encapsulació si que va millorar la seua resistència al procés de digestió *in vitro*. Es va estudiar l'emmagatzematge a 5 °C durant 10 dies del suc i durant 30 dies dels discs de poma, determinant-se en tots dos casos l'efecte de l'encapsulació sobre la supervivència de *L. salivarius* i sobre la seua resistència al procés digestiu *in vitro*. Els resultats obtinguts tant en el suc de mandarina com en els discs de poma impregnats, mostren que el probiótic encapsulat va presentar major supervivència durant l'emmagatzematge i el procés de digestió *in vitro* que el probiótic sense encapsular.

En relació a l'aprofitament d'altres fonts per a l'obtenció de nous ingredients alimentaris amb elevat contingut en compostos bioactius, es van seleccionar 12 sucres de canya no refinats com a alternativa al sucre blanc i es van analitzar les seues propietats fisicoquímiques i antioxidants. Es va confirmar que els sucres de canya no refinats presentaren compostos amb activitat antioxidant i que el seu contingut guardava relació amb el grau de refinat del producte. Els sucres no refinats constituïxen una font natural d'antioxidants i la substitució del sucre blanc pels sucres no refinats presentaria una gran aportació de compostos antioxidants a la dieta. Finalment s'aborda l'aprofitament de subproductes per a l'obtenció de pols a partir de bagàs de nabiu per mitjà d'assecat per aire calent i posterior triturat. S'ha

analitzat l'efecte de la temperatura d'assecat (60 i 70 ºC) i de la intensitat del triturat sobre les propietats fisicoquímiques i funcionals dels mateixos. L'assecat va disminuir la capacitat antioxidant del bagàs de nabiu però no va haver-hi un efecte significatiu de la temperatura. L'acció mecànica del triturat, va reduir de manera significativa el contingut en fibra de la pols. A més, es va demostrar que la grandària de partícula i el contingut en fibra de les pols van influir de forma decisiva en les propietats d'interacció amb l'aigua i amb l'oli, que condicionen en gran manera la seua estabilitat i la seua aplicació com a ingredient en la formulació d'aliments.

ESTRUCTURA DEL DOCUMENTO DE TESIS DOCTORAL

La siguiente tesis doctoral se presenta en compendio de artículos. Se plantea una introducción general, los objetivos de la tesis y el plan de trabajo. A continuación la sección de resultados se divide en tres capítulos. En cada capítulo se incluye una pequeña introducción y las conclusiones generales derivadas del mismo.

El **primer capítulo** titulado "**Sostenibilidad y tecnologías en la industria agroalimentaria**" contiene dos capítulos de libro publicados en editorial internacional, que presentan una revisión bibliográfica acerca de la sostenibilidad en relación con las tecnologías empleadas en la industria agroalimentaria. El capítulo afronta la forma en que estas tecnologías aportan una mejora en la funcionalidad de los componentes bioactivos y cómo esto contribuye a la sostenibilidad de los procesos agroalimentarios.

El **segundo capítulo,** titulado "**Tecnologías para la mejora de la funcionalidad de antioxidantes y probióticos",** incluye cuatro artículos científicos publicados o en proceso de publicación en revistas incluidas en el "Science Citation Index". En este apartado se estudian la aplicación de presiones de homogeneización, la encapsulación, la impregnación a vacío, el secado por aire caliente y la adición de trehalosa, como estrategias para mejorar la funcionalidad de compuestos con actividad antioxidante y/o microorganismos con efecto probiótico. El efecto de estas tecnologías se ha evaluado en un alimento líquido como el zumo de mandarina y/o en otro sólido obtenido a partir de manzana y zumo de mandarina.

El **tercer capítulo** se titula "**Fuentes alternativas de ingredientes alimentarios de alto valor funcional**" e incluye dos artículos, uno publicado y el otro en proceso de publicación. En este apartado se evalúa la funcionalidad de dos ingredientes naturales: el azúcar no refinado de caña y un polvo obtenido a partir de bagazo de arándano. Ambos se consideran como ejemplo de ingredientes cuyo uso extendido en alimentos puede contribuir a una mejora de la sostenibilidad de los procesos agroalimentarios.

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1. INTRODUCCIÓN

En los últimos años, los desequilibrios ambientales originados por la producción intensiva de alimentos y el uso masivo de recursos han provocado un aumento del interés hacia la valorización de subproductos y la promoción de procesos y materias primas más sostenibles. Al mismo tiempo y desde hace más de una década, ha quedado establecida la relación entre la alimentación y la salud. Los alimentos funcionales son una realidad y con ellos se ha impuesto la necesidad de asegurar, aumentar y demostrar su efecto preventivo de enfermedades crónicas y/o desórdenes fisiológicos. En esta tesis doctoral se estudian diferentes estrategias tecnológicas para mejorar las propiedades funcionales de los alimentos preservando la sostenibilidad de los procesos.

El desarrollo sotenible es considerado como aquel que asegura las necesidades del presente sin comprometer las del futuro. Las siguientes aproximaciones al concepto de sostenibilidad son las que se han abordado en la tesis doctoral.

1. Sostenibilidad derivada de la reducción de residuos y aprovechamiento de subproductos agroalimentarios que contienen compuestos de interés funcional.

La obtención de ingredientes de alto valor añadido a partir de estos subproductos permitiría reducir los residuos generados en la industria agroalimentaria, minimizar el daño ambiental de los procesos y reducir el coste de la gestión, mejorando, con todo ello, la sostenibilidad del sistema alimentario. La industria agroalimentaria genera gran cantidad de residuos que por su naturaleza suponen una fuente rica en compuestos bioactivos,

tales como vitaminas, fibra y minerales. La obtención de polvos a partir de los subproductos es una opción cada vez más interesante por la versatilidad de sus aplicaciones y la facilidad de almacenamiento y distribución.

2. Sostenibilidad derivada de la aplicación de tecnologías existentes para la mejora de la funcionalidad de los alimentos.

Los conceptos de *biodisponibilidad* y *bioaccesibilidad* han adquirido especial relevancia en la producción y el procesado de los alimentos, así como para determinar los efectos nutricionales y sobre la salud de los compuestos bioactivos (Manach et al., 2005). La Food and Drug Administration (FDA) define la biodisponibilidad como la tasa en la que un nutriente o medicamento es absorbido por el organismo y está disponible en el punto de acción (Shi y Le Maguer, 2000). Este concepto está asociado con la eficiencia de absorción y la utilización metabólica del nutriente ingerido (Gregory et al., 2005). A su vez, la biodisponibilidad depende de la bioaccesibilidad entendida como la cantidad de un nutriente ingerido que está disponible para la absorción en el intestino después de su digestión (Hedrén et al., 2002). En todos los casos, la bioaccesibilidad y la biodisponibilidad están condicionadas por las características estructurales de la matriz alimentaria lo que determina en gran medida la eficiencia de los procesos de digestión física, enzimática y química (Boyer y Liu, 2004).

Diferentes estudios demuestran que para aumentar la funcionalidad de los alimentos pueden utilizarse las siguientes

estrategias: (a) mediante la incorporación de ingredientes que ejercen un efecto protector sobre la estructura del alimento y reducen el impacto causado por determinadas condiciones de proceso; (b) mediante la creación de nuevas estructuras protectoras o utilizando la propia estructura de los alimentos como medio protector de los componentes bioactivos.

A continuación se explican las tecnologías empleadas en esta tesis con el fin de mejorar la funcionalidad de determinados compuestos con capacidad antioxidante y/o microorganismos con efecto probiótico.

Por una parte, la aplicación de altas presiones de homogeneización (HPH) es una tecnología que inicialmente se planteó como alternativa a los tratamientos térmicos para la destrucción de microorganismos y la inactivación de enzimas (Daoudi, 2004; Caminiti et al., 2011). El equipo consta de una bomba de desplazamiento positivo y una válvula de homogeneización. El líquido se lleva a alta presión en una unidad llamada intensificador de presión y luego se fuerza a través del espacio de la válvula de unos pocos micrómetros de ancho. Como consecuencia, el fluido alcanza una alta presión y velocidad y se produce un incremento de su temperatura de entre 14 y 16 ºC por cada 100 MPa según Thiebaud et al., (2003). El efecto del tratamiento HPH depende de la presión aplicada, de ahí sus diferentes aplicaciones. Tiene especial interés la aplicación de HPH en zumos, cuya calidad y aceptación esta condicionada por la estabilidad de la nube. La aplicación de HPH ejerce un impacto sobre la microestructura del zumo que produce una mejora en la

estabilidad de la nube. Además, se ha comprobado que esto implica un aumento de la biodisponibilidad y la bioaccesibilidad de algunos compuestos con actividad antioxidante. Asimismo, la liberación de sustancias bioactivas también puede promover su reacción con otros componentes, dando como resultado nuevos compuestos con diferente actividad (Velazquez-Estrada et al., 2013). Los efectos descritos la convierten, entre otras aplicaciones, en una tecnología idónea para ser utilizada como pretratamineto del líquido de impregnación en el proceso de impregnación a vacío cuando éste se utiliza para la obtención de alimentos funcionales (Betoret et al., 2012).

La hidrofobicidad del los microorganismos probióticos es una propiedad de los mismos relacionada con la capacidad del microorganimo de adherirse e interactuar con la pared intestinal. Es por tanto una propiedad beneficiosa que permite a los microorganimos colonizar con mayor facilidad el tracto intestinal. Al inducir cambios en la membrana plasmática de los microorganismos probióticos, mediante el tratamiento de HPH con presiones moderadas, sobre alimentos fluidos que contengan probióticos, se puede mejorar dicha propiedad y por tanto su efecto probiótico (Burns et al., 2008).

Adicionalmente, las presiones de homogeneización (aplicadas entre 60 y 100 MPa) se pueden emplear con la finalidad de encapsular microorganismos con efecto probiótico y mejorar su viabilidad (Burns et al., 2008). La encapsulación es una estrategia que permite la protección de una amplia gama de compuestos de interés biológico, desde pequeñas moléculas y proteínas (enzimas,

hormonas, vitaminas, etc) hasta células bacterianas, levaduras o de origen animal (Thies, 2005). La principal ventaja de la encapsulación por HPH es que permite realizar la operación a nivel industrial en un proceso en continuo Además es una tecnología mucho más económica que otras empleadas de forma más extendida para la encapsulación, como el spray drying.

Por otra parte, la impregnación a vacío (IV) es una operación de transferencia de materia entre un sólido poroso y un medio líquido en el que este se encuentra sumergido. La aplicación de un gradiente de presiones al sistema permite el intercambio del gas ocluido en la estructura porosa por líquido externo. Mediante esta tecnología se pueden incorporar en tejidos vegetales crioprotectores, estabilizantes, enzimas, inhibidores del pardeamiento enzimático y compuestos bioactivos (minerales, vitaminas, probióticos…) a tejidos vegetales. La propia estructura del alimento puede ejercer un efecto protector sobre los compuestos incorporados y originar interacciones sinérgicas entre los compuestos incorporados y el alimento sólido que aumenten la funcionalidad del mismo. Los alimentos resultantes de la impregnación a vacío poseen una actividad del agua elevada, por lo que deben estabilizarse para aumentar su vida útil. En este sentido, la operación de deshidratación resulta adecuada. Sin embargo, las condiciones de proceso, en la mayoría de los casos, pueden originar daños irreversibles que afecten a la estructura celular, cambios en las estructuras químicas de compuestos de interés nutricional y reacciones que disminuyan el valor nutricional de compuestos de interés funcional, tales como

antioxidantes, vitaminas, etc. (Betoret et al., 2015). Para mitigar estos efectos se pueden añadir ingredientes que prevengan la degradación de los compuestos bioactivos como la trehalosa o bien crear estructuras que mantengan la funcionalidad de los mismos.

Con todo lo dicho, la reducción de residuos y el aprovechamiento de subproductos que contienen compuestos de interés funcional así como la aplicación adecuada de la tecnología, contribuyen a la sostenibilidad desde sus tres dimensiones: ambiental, social y económica. Ambiental porque los procesos y las materias primas utilizadas en el desarrollo de alimentos tienden a ser cada vez más respetuosos con el medio ambiente, con el uso eficiente de recursos como el agua y la energía, con menos producción de residuos, mejorando su reutilización y el uso de materiales biodegradables. Social, derivada del uso de los recursos locales y el fomento de la actividad y creación de empleo a nivel rural y local. Además los alimentos funcionales mejoran la biodisponibilidad y bioaccesibilidad de los nutrientes y claramente contribuyen a mejorar la salud pública. La mejora de la salud pública implica una reducción de los costes en el sistema de atención sanitaria, y la eficiencia del uso de recursos repercute en la reducción de costes de producción, lo que contribuye con la dimensión económica del término. En la figura 1 se presenta de forma esquemática la relación entre la tecnología y las tres dimensiones de la sostenibilidad descritas en este apartado.

Figura 1. Impacto de las tecnologías empleadas en esta tesis sobre la sostenibilidad global de la industria agroalimentaria.

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2. OBJETIVOS Y PLAN DE TRABAJO

2.1. Objetivos

Los objetivos generales plateados en esta tesis son:

1. Evaluar el efecto que tecnologías de procesado como la HPH, la encapsulación, la IV y el secado, y la adición de ingredientes como la trehalosa, ejercen sobre compuestos bioactivos como antioxidantes y microorganismos con efecto probiótico. Estas tecnologías se aplicarán a diferentes matrices alimentarias: un líquido con partículas suspendidas como el zumo de mandarina y un sólido con estructura celular como la manzana.

2. Evaluar la funcionalidad y el efecto de las condiciones del proceso de obtención de dos ingredientes con poder antioxidante: el azúcar no refinado de caña y un polvo obtenido a partir de bagazo de arándano. Ambos se consideran como ejemplo de ingredientes cuyo uso extendido en alimentos puede contribuir a una mejora de la sostenibilidad de los procesos agroalimentarios

Para la consecución del primer objetivo general se han planteado los siguientes objetivos específicos:

1.1 Realizar una revisión de cómo algunas tecnologías utilizadas en la industria agroalimentaria pueden mejorar la funcionalidad de los alimentos contribuyendo a la sostenibilidad en la industria agroalimentaria.

1.2 Estudio del efecto de las presiones de homogeneización y la adición de trehalosa sobre las propiedades funcionales y antioxidantes de zumo de mandarina.

1.3 Estudio del efecto de las presiones de homogeneización y la adición de trehalosa sobre las propiedades funcionales de zumo de mandarina con probióticos.

1.4 Evaluación del efecto de la encapsulación de *Lactobacillus salivarius* spp. *salivarius* en zumo de mandarina mediante presiones de homogeneización sobre la resistencia gastrointestinal y viabilidad microbiana durante 10 días de almacenamiento.

1.5 Evaluación del efecto de la encapsulación de *L. salivarius* spp. *salivarius* incorporado en manzana mediante IV y estabilizada mediante secado a 40 ºC sobre la resistencia gastrointestinal y viabilidad microbiana durante 30 días de almacenamiento.

Para la consecución del segundo objetivo general se han planteado los siguientes objetivos específicos:

2.1. Determinar las propiedades fisicoquímicas y funcionales de diferentes azúcares de caña no refinados encontrados en el mercado.

2.2. Estudiar el efecto de las condiciones de secado y triturado sobre las propiedades fisicoquímicas y funcionales de un polvo obtenido a partir de bagazo de arándano.

2.2. Plan de trabajo

A continuación se detalla el plan de trabajo para cada uno de los objetivos planteados.

Para la consecución del objetivo 1:

1. Revisión bibliográfica.
2. Estudio del efecto de las presiones de homogeneización y la adición de trehalosa sobre las propiedades funcionales y antioxidantes de zumo de mandarina, (figura 2).
    a. Obtención de zumo a partir de mandarinas frescas (*var.* ortanique). Congelación del zumo hasta su uso.
    b. Adición de trehalosa (0, 10 ó 20 g/100 g zumo) y homogeneización de los zumos a 0, 20, 50, 100 y 150 MPa.
    c. Caracterización físico-química de los zumos obtenidos: pH, Brix, a_w, tamaño de partícula, parámetros de impregnación, pulpa suspendida y turbidez.
    d. Caracterización funcional de los zumos: contenido en fenoles totales, flavonoides y capacidad antioxidante.
3. Estudio del efecto de las presiones de homogeneización y la adición de trehalosa sobre las propiedades funcionales de zumo de mandarina con probiótico, (figura 2).
    a. Estudio de la actividad inhibitoria de *Lactobacillus. salivarius* spp. *salivarius* contra *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Escherichia coli* y *Salmonella enteriditis*.

b. Adición de trehalosa (0, 10 ó 30 g/100 g zumo), inoculación del zumo de mandarina con *Lactobacillus salivarius* spp. *salivarius* e incubación (37 ºC/ 24 h).
c. Homogeneización a 0, 20 y 100 MPa.
d. Determinación de pH, Brix, acidez, viscosidad, pulpa suspendida y turbidez de los zumos obtenidos.
e. Recuento de *Lactobacillus salivarius* spp. *salivarius* (Log UFC/mL) en los zumos de mandarina obtenidos tras 1, 2, 3, 7 y 10 días de almacenamiento a 5 ºC.
f. Determinación de la hidrofobicidad del probiótico incubado en diferentes medios:
    i. Caldo MRS y zumo de mandarina con 0, 10 y 30% de trehalosa y homogenaizado a 0, 20 y 100 MPa.
    ii. Caldo MRS con 0, 10 y 30% de trehalosa y homogenaizado a 0, 20 y 100 MPa y añadido a zumo de mandarina.

4. Evaluación del efecto de la encapsulación de *Lactobacillus salivarius* spp. *salivarius* mediante presiones de homogeneización en la supervivencia en zumo de mandarina durante 10 días de almacenamiento y resistencia gastrointestinal, (figura 3).
    a. Determinación del nivel de presión más favorable para la encapsulación de *L. salivarius* spp. *salivarius.*
    b. Encapsulación de *L. salivarius* spp. *salivarius* mediante HPH a 70 MPa.
    c. Adición de las capsulas al zumo de mandarina. Zumo con *L. salivarius* encapsulado.

d. Inoculación de zumo de mandarina con *L. salivarius* spp. *salivarius.* Zumo con *L. salivarius* no encapsulado.

e. Determinación de Brix, pH, a_w, distribución del tamaño de partícula y las propiedades reológicas del zumo de mandarina con *L. salivarius* encapsulado y sin encapsular.

f. Evaluación del efecto de la encapsulación sobre la supervivencia de *L. salivarius* spp. *salivarius* en los zumos durante 0, 1, 3, 7 y 10 días de almacenamiento a 5 ºC.

g. Evaluación del efecto de la encapsulación sobre la supervivencia de *L. salivarius* spp. *salivarius* a la simulación gastrointestinal *in vitro* a los 0, 1, 3, 7 y 10 días de almacenamiento a 5 ºC.

5. Efecto de la encapsulación de *L. salivarius* spp. *salivarius* introducido mediante presión de vacío en manzana y estabilizada mediante secado por aire caliente a 40 ºC. Viabilidad durante 30 días de almacenamiento y resistencia a condiciones gastrointestinales simuladas, (figura 3).

   a. Encapsulación de *L. salivarius* spp. *salivarius* mediante HPH a 70 MPa.

   b. Adición de las capsulas al zumo de mandarina. Zumo con *L. salivarius* spp. *salivarius* encapsulado

c. Inoculación de zumo de mandarina con *L. salivarius* spp. *salivarius.* Zumo con *L. salivarius* no encapsulado.
d. Impregnación a vacío de rodajas de manzana con los zumos obtenidos.
e. Deshidratación de las rodajas de manzana mediante secado por aire caliente a 40ºC durante 24 h.
f. Determinación de humedad, pH y a_w a los 1, 7, 14, 21 y 30 días de almacenamiento.
g. Determinación del contenido de *L. salivarius* spp. *salivarius* en los discos de manzana deshidratados con el probiótico encapsulado y sin encapsular a los 1, 7, 14, 21 y 30 días de almacenamiento.
h. Evaluación del efecto de la simulación gastrointestinal sobre la supervivencia de *L. salivarius* spp. *salivarius* en los discos de manzana deshidratados con el probiótico encapsulado y sin encapsular a los 1, 7, 14, 21 y 30 días de almacenamiento.

Para la consecución del objetivo 2:

6. Evaluación de las propiedades fisicoquímicas y antioxidantes de azúcares de caña no refinados como alternativa al azúcar blanco, (figura 4).
   a. Prospección del mercado: visita a diferentes puntos de venta (comercios especializados y supermercados)

para el acopio de endulzantes no refinados procedentes de la caña de azúcar.

b. Caracterización fisicoquímica de los azúcares comerciales: humedad, a_w, pH, perfil de azúcares (sacarosa, glucosa y fructosa) y color.

c. Caracterización de las propiedades antioxidantes de los azúcares comerciales. Determinación de fenoles totales, flavonoides y capacidad antioxidante.

d. Selección de los productos que presentaron mejores propiedades.

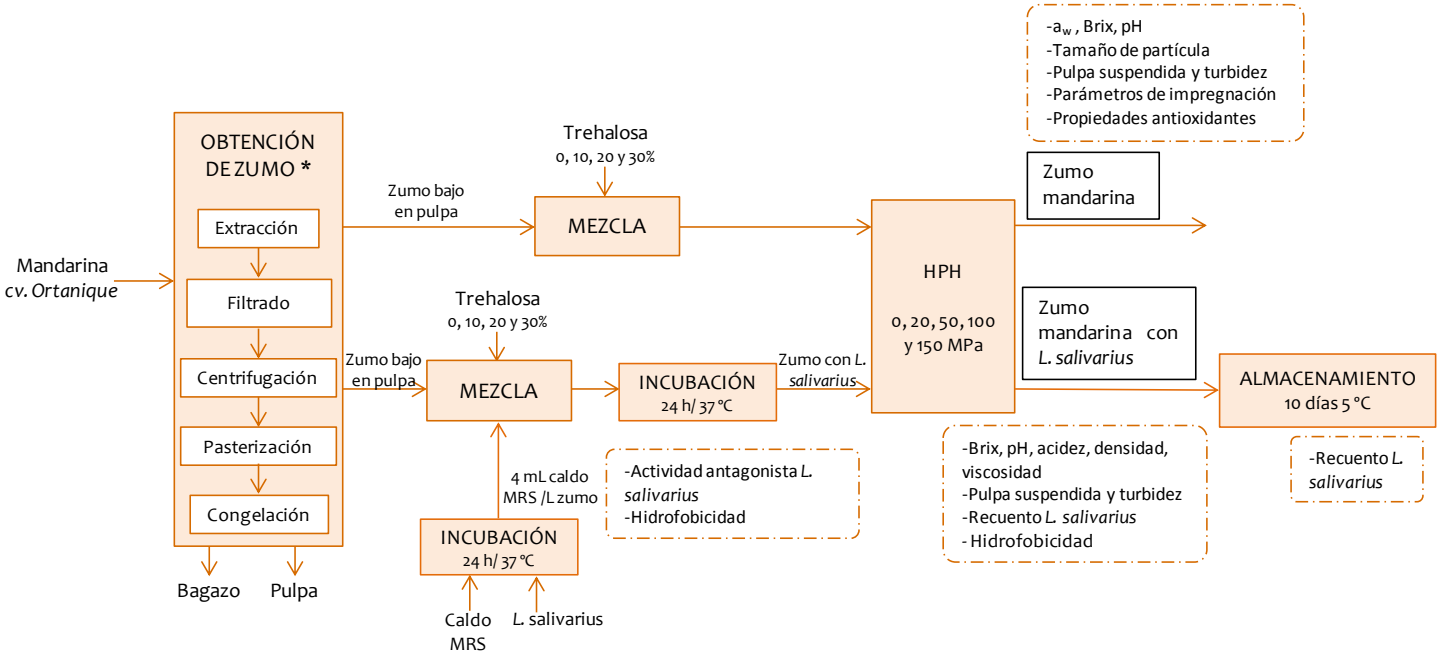
e. Estudio del efecto de diferentes tratamientos térmicos (las combinaciones resultantes de tiempo - temperatura 10, 30 y 60 min- 60, 80 y 100 ºC) sobre la capacidad antioxidante de los productos seleccionados.

7. Evaluación de la temperatura de secado más adecuada para la obtención de un polvo de arándano estable y con un elevado contenido en compuestos con actividad antioxidante procedente de bagazo arándano. Determinación de la estabilidad durante el almacenamiento de 20 semanas, (figura 5).

   a. Obtención del bagazo a partir de zumo de arándano congelado de calidad industrial.

   b. Caracterización fisicoquímica y funcional del bagazo de arándano fresco: humedad, a_w, sólidos solubles, capacidad antioxidante y color.

c. Secado del bagazo con aire caliente a 60 y 70 °C hasta conseguir una actividad de agua inferior a 0,3.
d. Caracterización fisicoquímica y funcional de los bagazos secos a las dos temperaturas.
e. Selección de la temperatura de secado más adecuada según su efecto sobre las propiedades antioxidantes y el color.
f. Triturado del bagazo secado a 70 ºC a dos velocidades distintas con el fin de obtener polvo de arándano a dos granulometrías diferentes.
g. Caracterización de los polvos de arándano en términos de humedad, a_w, sólidos solubles, capacidad antioxidante, color y fibra.
h. Estudio de la estabilidad a lo largo del almacenamiento durante 20 semanas en una cámara con humedad relativa controlada.



(*) Obtención de zumo de mandarina bajo en pulpa de acuerdo con la patente WO/2007/042593 titulada "Method of obtaining refrigerated pasteurized citrus juices" (Izquierdo et al., 2007).

Figura 2. Diagrama de flujo seguido en la evaluación del efecto del tratamiento con HPH (altas presiones de homogeneización) y la adición de trehalosa sobre las propiedades tecnológicas y funcionales del zumo de mandarina con y sin *L. salivarius* spp. *salivarius*.

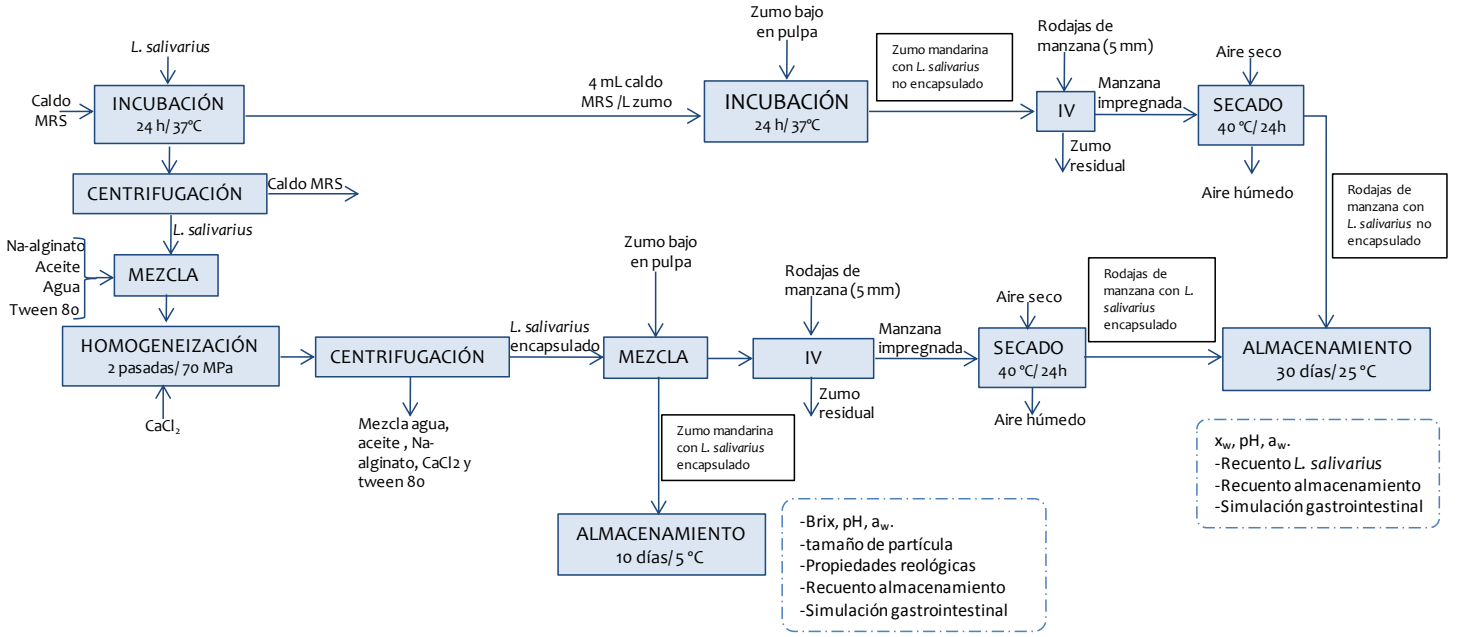


Figura 3. Diagrama de flujo seguido en el estudio de efecto de tratamiento con HPH y la encapsulación sobre la viabilidad y resistencia gastrointestinal *in vitro* del mismo en zumo de mandarina y en manzana impregnada con este. IV: Impregnación a vacío.

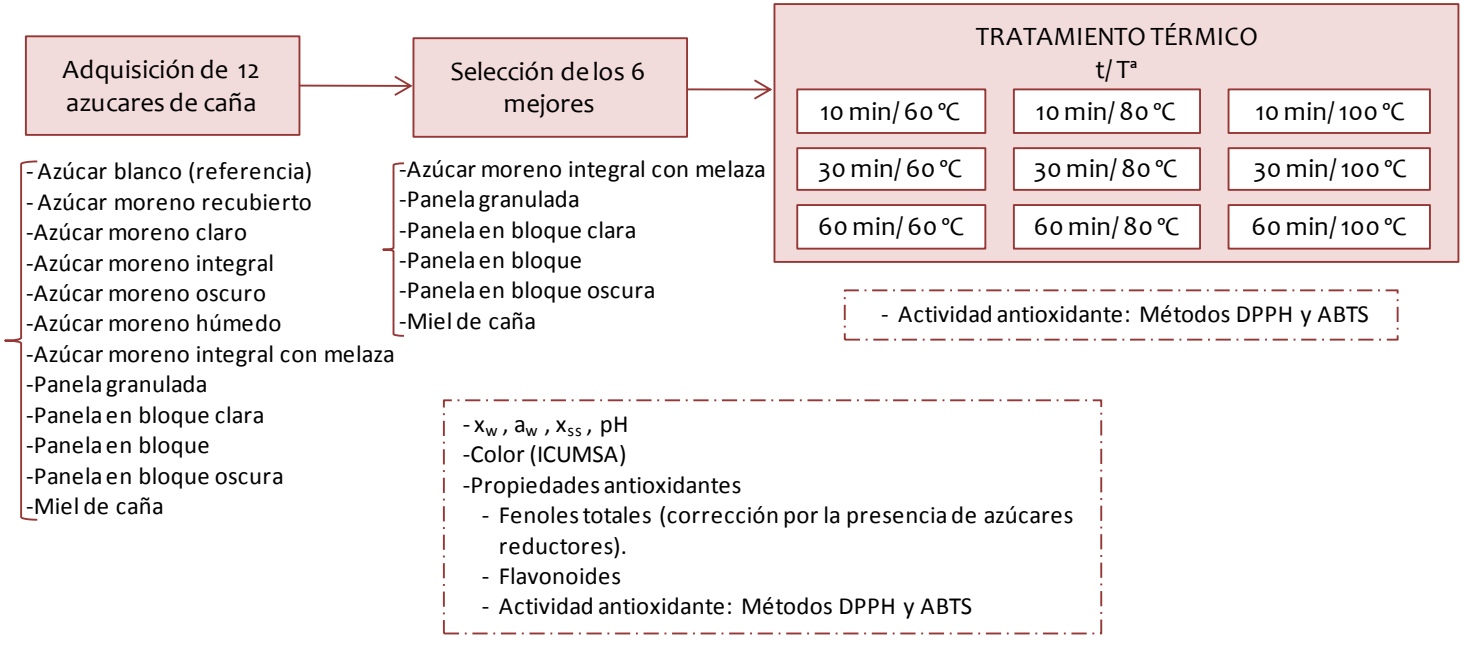


Figura 3. Estudio de las propiedades fisicoquímicas y antioxidantes de azúcares no refinados derivados de la caña de azúcar.

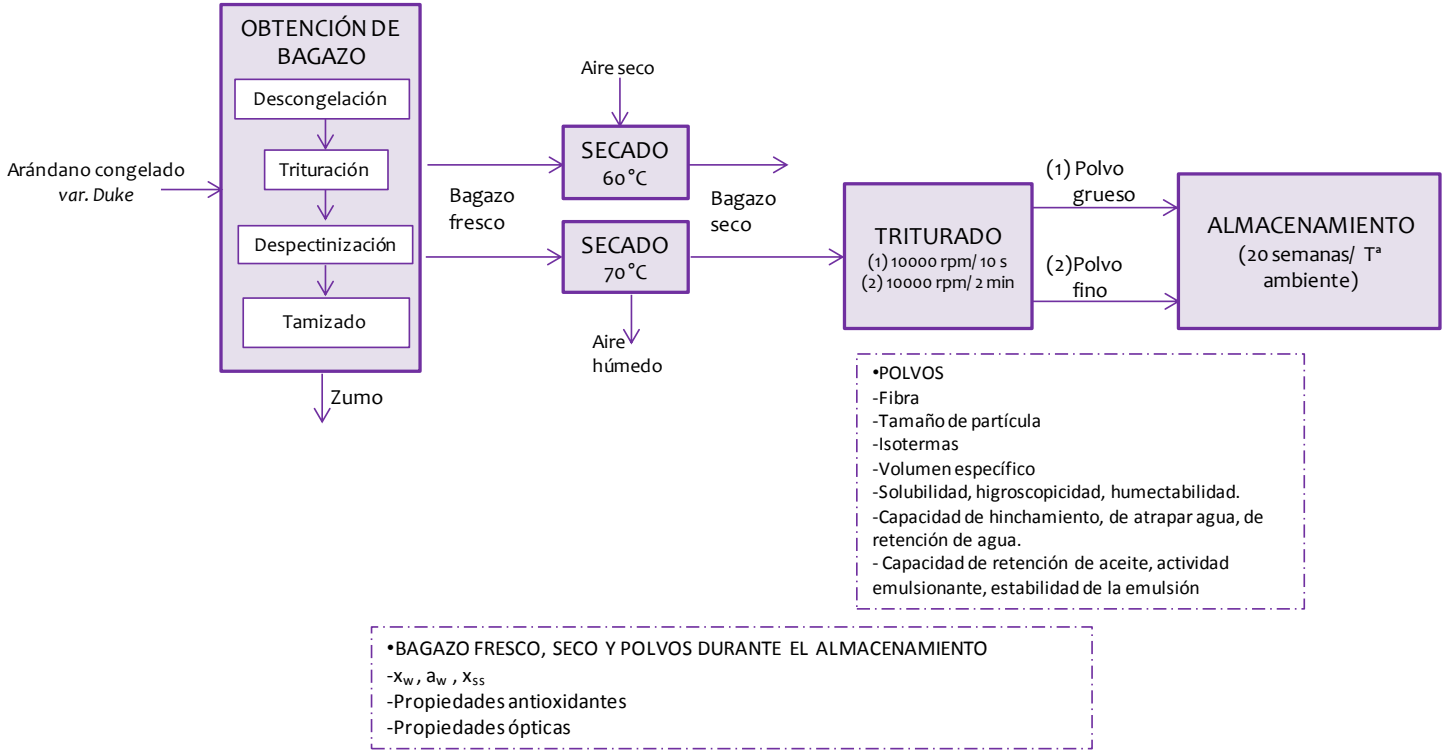


Figura 5. Diagrama de flujo para la evaluación del efecto de la temperatura de secado, del triturado y del almacenamiento sobre las propiedades fisicoquímicas y funcionales del polvo de bagazo de arándano.

3. RESULTADOS

3.1. Sostenibilidad y tecnologías en la industria agroalimentaria

La gestión adecuada de los procesos alimentarios puede contribuir a lograr procesos más sostenibles desde las tres dimensiones implícitas en el concepto: ambiental, social y económica. La investigación en alimentos va dirigida al desarrollo de productos alimenticios saludables, de calidad y seguros, adaptados a las necesidades del consumidor y, cada vez más, elaborados mediante procesos que garanticen la funcionalidad de los compuestos bioactivos.

La sostenibilidad en el sistema de producción de alimentos puede mejorarse con el uso corecto de las tecnologías en los procesos alimentarios. En este capítulo se presenta una revisión sobre las tecnologías empleadas en la industria agroalimentaria en relación a la sostenibilidad de los procesos, haciendo hincapié e la forma en que pueden contribuir a una mejora en la funcionalidad de los compuestos bioactivos. Una parte del capítulo se centra en las operaciones de deshidratación, explicando cómo la implementación de diferentes estrategias en los procesos de secado puede disminuir el efecto negativo sobre los componentes bioactivos, aumentando la calidad de los alimentos deshidratados.

El resultado de la revisión ha sido publicado en dos capítulos de libro de editorial internacional.

- Betoret, E., Calabuig-Jiménez, L, Betoret, N., Barrera, C., Seguí, L., Fito, P. (2016). Sustainable Innovation in Food Science and Engineering. In *Innovation Strategies in the Food Industry*. Elsevier. http://dx.doi.org/10.5772/61766

- Betoret, E., Calabuig-Jiménez, L, Barrera, C., Dalla Rosa, M. (2016). Sustainable drying technologies for the development of functional foods and preservation of bioactive compounds. In *Sustainable Drying Technologies*. InTech. ISBN 978-953-51-4786-2.

En el primer capítulo de libro titulado "Sustainable innovation in food science and engineering", las tecnologías empleadas para el desarrollo alimentos funcionales se clasifican en tres grupos. El primer grupo está formado por las tecnologías tradicionalmente utilizadas en el procesado de alimentos, tales como la formulación y mezcla, y el cultivo y cría. El segundo grupo está constituido por las tecnologías que aprovechan la estructura natural de los alimentos o ayudan a formar otras nuevas para evitar el deterioro de los compuestos fisiológicamente activos, como la microencapsulación, la formación de películas y recubrimientos comestibles y la impregnación a vacío. Finalmente, se consideran en su conjunto las tendencias más recientes, destinadas a diseñar alimentos funcionales personalizados como la nutrigenómica.

Una industria agroalimentaria sostenible previene, minimiza y valoriza los residuos para mejorar su gestión. La formulación y la mezcla permiten enriquecer los alimentos con ingredientes que se pueden obtener a partir de subproductos, con lo que se reduce el impacto ambiental debido a los desechos generados por la industria agroalimentaria y a su vez se reducen los costes derivados de la gestión de los mismos. Con respecto al

cultivo y cria, la biotecnología permite obtener cultivos más capaces de satisfacer la creciente demanda de alimentos de una forma más sostenible, al mejorar el uso de recursos no renovables como el suelo y el agua. En cuanto a las tecnologías empleadas para formar una estructura que proteja los componentes bioactivos, su contribución a la sostenibilidad se deriva de la protección que estas estructuras ofrecen frente al deterioro de los compuestos bioactivos y su consecuente aumento de la funcionalidad en los productos finales.

En el segundo capítulo de libro, que tiene por título "Sustainable drying technologies for the development of functional foods and preservation of bioactive compounds", se realiza una revisión sobre el uso de las tecnologías de secado y las condiciones que preservan en mayor medida las propiedades funcionales de los alimentos, asegurando la sostenibilidad del proceso global. El principal desafío de la operación de secado es eliminar el agua del alimento de la manera más eficiente, sin comprometer la calidad del producto, con un impacto mínimo en el medioambiente y con el menor coste posible. El diseño de la tecnología y sus condiciones de proceso para su mayor eficiencia es el enfoque fundamental para un desarrollo más sostenible.

La operación de deshidratación de alimentos debe de realizarse para producir un producto de buena calidad con el más alto nivel de retención de nutrientes. Las estrategias discutidas para preservar o incluso aumentar la funcionalidad de los productos alimenticios durante el secado se han clasificado en tres

grupos: (1) adición de ingredientes que reducen la degradación de compuestos bioactivos, (2) creación de elementos estructurales que protegen/ mantienen la funcionalidad de los compuestos bioactivos y (3) la prevención de reacciones que causan degradación de compuestos bioactivos y promueven la formación de otros con efecto funcional.

Sustainable Innovation in Food Science and Engineering

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Abstract

Nowadays, the sustainability of a product, a process or a system is assessed according to three dimensions: environmental, social and economic. Sustainability challenges occur at all stages in the food system from production through processing, distribution and retailing to consumption and waste disposal. Consequently the promotion of organic and local food is not the only way there is other possibility that implies to continue the production hegemony, emphasizing biotechnology and technological panaceas.

Increasing research is being focused on the development of healthy, quality and safety food products adapted to consumer's needs and more environmentally friendly processes, that is, processes consuming energy more efficiently, generating less waste and emitting less greenhouse effect gases, among other features.

This chapter contains detailed information about some measurements taken by the food industry to ensure the supply of essential nutrients to as many individuals as possible assuring the global sustainability. More specifically, the contribution of some techniques employed in the development of functional foods, such as formulation and blending, cultivation and breeding, microencapsulation, edible films and coatings application, vacuum impregnation and nutrigenomics, to increase the sustainability of the feeding process, is discussed.

Keywords

Sustainability, food production, functional food, biotechnology, environmental.

1. Introduction

Sustainability means meeting the needs and aspirations of the present without compromising the ability of future generations to meet theirs. To achieve food and agricultural, traditionally, the system has been directed towards promotion of organic and local food. As explained by Spiertz (2010), there is other possibility that implies to continue the production hegemony, emphasizing biotechnology and technological panaceas.

Global food production allows to obtain the most safety and with higher quality food than ever before but at a great cost. Current food supply systems not only deliver insufficient food but are economically and environmentally unsustainable, lacking in

resilience, inequitable and risk a human health disaster. A food system in which nearly one billion people are under-nourished and 1.5 billion are over-weighted is at the very least testament to a massive system failure. The need for urgent action is widely recognised (Godfray *et al.*, 2010; Royal Society, 2009; MacMillan and Benton, 2014). A host of environmental problems such as greenhouse gas (GHG) emissions, deforestation, desertification, eutrophication and biodiversity loss are exacerbated through current food system activities (Garnett, 2011).

Nowadays, the sustainability of a product, a process or a system is assessed according to three dimensions: environmental, social and economic. Sustainability challenges occur at all stages in the food system from production through processing, distribution and retailing to consumption and waste disposal. There are increasing demands from policy makers, stakeholders and public interest groups for research to adopt more integrated perspectives in pursuit of more holistic solutions (Defra, 2003; Kates *et al.*, 2001). Integrated perspectives are particularly called for to improve understanding of the mutual interaction between technological change and the economic, social and environmental contexts in which it occurs. The promise is held out for holistic solutions combining adaptations in socio-technical systems, rather than single-minded technological responses. As explained by OECD (2004) the development of a sustainable agri-food system places responsibilities on both the natural and the social sciences. While advances in basic and strategic biological research have greatly expanded the potential to produce nutritious food in an

efficient and environmentally sustainable manner, social and economic factors will determine the uptake and value of this research, as well as its future direction (Lowe *et al.*, 2008).

Sustainable food production stands at the intersection of several growing needs. Primarily the needs of consumers for improved food security and safety, as well as more sophisticated needs. Secondly the quest for economic sustainability of food production, based on cost reduction and increased product differentiation. Third, the growing concern for reversing the over exploitation of natural resources, waste generation, and the contribution to climate change (Fava *et al.*, 2013).

Functional foods are foods that beneficially affect one or more target function in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and wellbeing and/or reduction of health risk, and it is consumed as a part of a normal food pattern (not a pill, a capsule or any form of dietary supplement) (European Commission, 2010). Many diseases are strictly related with diet as well as lifestyle, and concern to the society because of their prevalence. Functional foods can help to prevent or improve those diseases thus contributing directly to public health. In Betoret *et al.*, (2011) the technologies used to develop functional foods are classified in three groups. The first group is formed by the technologies traditionally used in food processing, formulation and blending and cultivation and breeding. The second group is constituted by the technologies forming a structure to prevent the deterioration

of physiologically active compounds, i.e. microencapsulation, edible films and coatings and vacuum impregnation are part of this group. Finally, the third group is formed by those technologies, recent ones, aimed to design personalized functional foods. In this book chapter the technologies described in Betoret *et al.*, (2011) have been overviewed emphasizing the sustainability innovations achieved in each case as well as its industrial application.

2. Formulation and blending

Formulation and blending constitutes the most common, traditionally used, simply and cheap methodology to develop functional foods and has been widely used in food processing. Its use in functional food development has a long history for the successful control of deficiencies (Burgi *et al.*, 1990; FAO & WHO, 2006; Betoret *et al.*, 2011). In more recent years, the emergence of dietary compounds with health benefits offered an excellent opportunity to improve public health and thus, this category of compounds received much attention from the scientific community, consumers and food manufacturers. The list of dietary active compounds (vitamins, probiotics, bioactive peptides, antioxidants…) is endless and the type of final products obtained is growing steadily (Wildman, 2006).

All these natural compounds used in the formulation of new food or functional products can be obtained from food producing industries by-products (which differ from waste in that they have not been widely exposed to environmental contamination) (Fava *et*

al., 2013). Vegetable, cereal and fruit processing by-products and waste are typically rich in proteins, sugars and lipids and contain particular aromatic and aliphatic complex compounds. Thus they are cheap abundant sources of value-added bio-based chemicals and materials. Indeed, after specific pretreatments with physical and biological agents followed by tailored recovery procedures, they might provide specific natural antioxidants, antimicrobial agents, vitamins, among others, along with macromolecules (e.g. soluble fibres), bioactive oligosaccharides, oligopeptides, and pigments.

A sustainable Agro-Food industry recognizes that waste prevention, minimization and valorization, rather than 'end of pipe', are the required solutions for waste management. The legislation at the European level is promoting the use of these solutions.

3. Cultivation and breeding

Plant and animal breeding techniques have been used since the beginning of human history. The evaluation and selection of different breeds started with the domestication of animal and plant species around 12.000 years ago, which was led by the wish to obtain desired traits, dictated by social, nutritional and environmental needs with no understanding of the molecular processes involved (National Research Council, 1989). In cases where agronomic and breeding approaches cannot achieve significant improvement of food products, biotechnology offers a useful alternative (Zhao and Shewry, 2011). In the last decades,

with the use of molecular biology tools and the development of genetically modified plants, the biotechnology turns into a useful technology which offers an additional way to improve the traits of foods (GMO). A GMO is 'an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination' (EU Directive 2001/18). In the plant sciences, attention was directed at increasing yields by enhancing soil fertility, reducing pests and developing new genetic varieties. By contrast, in animal science is mainly focused on health, nutrition and breeding (Lyson, 2002).

In plant biotechnology, the focus has been mostly on producing genetically modified crops that are resistant to insects, viral pathogens, and herbicides (Barling *et al.*, 1999; Hails, 2000; Deisingh and Badrie, 2005). In food manufacture, biotechnology has been mostly applied to enzyme production by microorganisms (Barling *et al.*, 1999). Even so, there are experiments to produce crops with enhanced nutritional and health benefits to obtain enriched crop products as 'functional foods' and 'nutraceuticals' (Zhao and Shewry, 2011; Betoret *el at.*, 2011), and with the capacity to produce pharmaceuticals ("pharming"). The metaphor of 'crops becoming factories, producing vaccines, plastics, industrial starches, and feed supplements and enzymes' captures the trajectory of this type of research (Vergrart and Brown, 2008).

In the last decades population has grown considerably in the world. Projections for human population growth suggest that by 2050, more than 9 billion people will inhabit the earth (United

Nations, 2015), hence is needed to produce more food to satisfy food security to world population. As Ban Ki-moon´s remarks at the Food and Agriculture Organization of the United Nations High-Level Conference on World Food Security "Food production needs to rise by 50 percent by the year 2030 to meet the rising demand" (Ki-moon, 2008). This context shows a big challenge to the food production systems and to agriculture, which can be faced using plant and animal breeding techniques as biotechnology. The challenge of a global food security in a sustainable way requires the intensification of knowledge-intensive approaches and the use of modern agrotechnologies and biotechnologies (Spiertzt *et al.*, 2010). Experts assert that biotechnology innovations will triple crop yields without requiring any additional farmland, saving valuable rain forests and animal habitats; can reduce or eliminate reliance on pesticides and herbicides that might contribute to environmental degradation and can preserve precious ground soils and water resources (Lyson, 2002).

The integration of biotechnology in the sustainable framework has been shown by Ervin *et al.*, (2010), where is suggested that production and environmental benefits are the two legs of the sustainability stool. Genetically modified crops generally showed progress in reducing agriculture's environmental footprint and improving farmers profits (herbicide-resistant and insect-resistant crop varieties). But this option of biotechnology has paid insufficient attention to the integrated and systemic requirements of sustainable agriculture. In particular, the consideration of socio-economic distributive or equity effects

into any assessment of sustainability (Ervin *et al.*, 2010). Fundamental changes are required in the way public and private research, and technology development and commercialization should be structured (Kvakkestad, 2009). Strong efforts and coordination between stakeholders are needed and combined with science to obtain good results in the wide term of sustainability (Ervin *et al.*, 2010; Spierzt *et al.*, 2010).

The plea is to maximize the potential of the plant genome sciences in contributing significantly to human health, energy security and environmental stewardship. Strikingly, food production is not listed as one of the major challenges; however, climate change and the world food crisis bring a 'sense of urgency' in the debate on meeting the demands of a growing global and wealthier population.

Biotechnology and plant breeding, agriculture crop management and government policies contribute to counteract the growth demand (Tester *et al.*, 2010). To satisfy the growing demand it will be necessary to make use of the best science and technology to raise crop productivity (Pardue, 2010). So far, a combination of advanced plant breeding, systems innovations, development of best practices and legislation turned out to be effective in developing more environment- friendly agricultural systems that are profitable, ecologically safe and socially acceptable (Spierzt *et al.*, 2010).

The issue of genetically modified crops (GM crops) has been highly controversial since the introduction of the recombinant

DNA technology in the 1970's (Singer and Soll, 1973; Berg *et al.*, 1974; Devos *et al.*, 2007). It is more intense in European Union, basing on their precautionary principle (Vergragt and Brown, 2008; Ervin *et al.*, 2010; Spiertz, *et al.*, 2010). The public objections had numerous causes, including the concerns about the risk assessment, the ethics and equity issues, power relations and the mistrust of technocrats and public authorities (Vergragt and Brown, 2008).

Biotechnologies have the potential to revolutionize virtually all aspects of society, from how, where, when and by whom food is produced, processed and consumed to how dietary changes might be used to treat illness and disease. In the field of food science and agriculture, biotechnologies have the potential to satisfy the growing demand of food over the world with sustainable agriculture. Biotechnologies will participate in farm and agriculture changes improving the sector in a sustainable manner (social, economic and environmental approach), to guarantee food security in the world.

4. Microencapsulation

Microencapsulation is the envelopment of small solid particles, liquid droplets or gases in a coating (Thies, 1987). Microencapsulation is based on the embedding effect of a polymeric matrix, which creates a microenvironment in the capsule able to control the interactions between the internal part and the external one (Borgogna *et al.*, 2010). This technology allows the protection of a wide range of materials of biological

interest, from small molecules and protein (enzymes, hormones,…) to cells of bacterial, yeast and animal origin (Thies, 2005). Thanks to microencapsulated ingredients, many products that were considered technically unfeasible are now possible (Gharsallaoui *et al.*, 2007).

Such versatile technology is widely studied and exploited in high technological fields for application ranging from cell therapy to drug delivery (Smidsrød and Skjak-Braek, 1990). There are numerous industrial applications of microencapsulation. Some examples are carbonless paper, "scratch and sniff" fragrance samples, "intelligent" textiles, controlled release of drugs, pesticides, cosmetic active agents and functional foods. Martins *et al.*, (2014) illustrates the distribution, in percentage, of microencapsulation over different fields of applications showing that the sector with highest level is the drug sector (68%), followed by the food (13%) and cosmetic ones (8%).

In the food industry, Shahidi and Han (1993) proposed six reasons for applying microencapsulation:

i. to reduce the core reactivity with environmental factors; to decrease the transfer rate of the core material to the outside environment;
ii. to promote easier handling; to control the release of the core material;
iii. to mask the core taste; and finally to dilute the core material when it should be used in only very small amounts.

The application for the productions of high value foods and nutraceuticals results very interesting and is being developed fast. Some examples are described in Betoret *et al.*, (2011). In this regard the main challenge of technology is to maintain the active form in the food during preparation, processing, until the time of consumption and deliver it in the appropriate specific site of the organism.

It is absolutely necessary that the technologies used for functional food development are sustainable. The research studies related to microencapsulation and sustainability are growing significantly although are still scarce. In this regard, the published studies are focused mainly in four main research directions:

1. Wall (matrix) materials for microencapsulation.
2. Microencapsulating materials.
3. Processes for microencapsulation.
4. Innovative applications.

The selection of wall material for microencapsulation is an important part of the process. Each material possesses unique emulsifying and film forming properties and from its macro and microstructural properties will depend the capacity to form small-sized, physical stable microcapsules. There is a need for selection, development and characterization of biodegradable, biocompatible, safe and environmental friendly materials suitable for utilization as encapsulating agents.

In recent years, researchers have focused on the utilization of secondary plant materials in coatings. Plant materials are mainly made up of three types of biopolymers: cellulose, lignin and hemicellulose. Various agricultural residues such as corn fiber, corn peel and sugar beet, contain about 20-40 % hemicellulose, making it the second most abundant polysaccharide in nature (Tatar *et al.*, 2014). It is now agreed that hemicellulose is valuable due to its adhesive, thickening, stabilizing and emulsifying (Yadav *et al.*, 2009), and film forming properties (Hansen and Plackett, 2008). Ebringerovà (2006) demonstrated that hemicellulose produces stable foams and oil/water type emulsions due to presence of small amount of lignin and proteins, acting as the hydrophobic centers, and due to film forming effects. Yadav *et al.* (2009) deduced that the corn fiber gum is a better emulsifier that gum arabic in an oil-in-water emulsion system. On the other hand, McPherson *et al.*, (2006) evaluated the application of hemicellulose hydrolysate from corn hull as coating. They found that the hemicellulose was more efficient than the gum arabic. Tatar *et al.*, (2014) tested the effectiveness of hemicellulose-based coating isolated from corn wastes and showed that hemicellulose can be used in combination with gum arabic in coatings to be microencapsulated by spray drying method. Although the chemical and physical properties of hemicellulose-based or derived products were studied (Ebringerovà, 2006; Hansen and Plackett, 2008), there is still need to investigate the potential of hemicelluloses in encapsulation or coating applications (Celebioglu *et al.*, 2012). The

use of plant proteins as encapsulating materials represents another trend for renewability and sustainability in microencapsulation technology. In this regard, vegetable proteins used as a wall material in microencapsulation include soy protein isolate, pea protein isolate and cereal proteins (Gharsallaoui *et al.*, 2011; Nesterenko *et al.*, 2012; Tang and Li, 2013a, 2013b), among which soy protein isolate was most frequently applied, possibly due to its widely commercial availability. Many previous works have indicated that relative to sodium caseinate or whey proteins, the microencapsulated products with vegetable proteins as the encapsulating materials exhibit comparable or even better encapsulation efficiency, and higher stability against oxidation (Charve and Reineccius, 2009; Kim and Morr, 1996; Rascón *et al.*, 2011). Gharsallaoui *et al.*, (2011) reported a novel system using pea protein isolate-stabilized emulsions, to encapsulate lipophilic ingredients by spray drying. Liu *et al.*, 2014 compared the microencapsulating potential of phaseolus legumes, including red bean, kidney bean and mung bean with soy protein isolate and showed that all the tested proteins exhibited similar emulsifying properties but the interfacial properties noting that more studies should be conducted on these proteins to warrant their application as wall materials in spray-drying microencapsulation.

In the case of microencapsulated materials, the sustainability of the technology is based on the use of bioactive compounds extracted from food processing by products. To date certain classes of phenolics such as procyanidins of grape seeds (Zhang *et al.*, 2007), polyunsaturated fatty acids ω-3 from marine

by-products (Ferraro *et al.*, 2010), phenolics from pomegranate peel (Çam *et al.*, 2014), polyphenols from star fruit (*Averrhoa carambola*) pomace (Saikia *et al.*, 2015) and polyphenols from *Vitis vinifera* grape wastes (Aizpurua-Olaizoa *et al.*, 2016) are some examples of bioactive compounds extracted from food by products that have been microencapsulated. Polyphenols have a poor long-term stability, as they are affected by pH variation, presence of metal ions, light, temperature, oxygen, and enzymatic activities (Bakowska *et al.*, 2003). Moreover, due to low water solubility, they often present a poor bioavailability (Munin and Edwards-Lévy, 2011) and they are unstable in alkaline conditions encountered in biological fluids (Dube *et al.*, 2010).

Microencapsulation can be achieved by a wide range of methods or techniques, providing isolation, entrapment, protection or controlled release of sensitive or reactive materials from/across the surrounding matter (Martins *et al.*, 2014). The adequate microencapsulation method will depends on the specific molecular structure of the compound to be encapsulated and on the specific characteristics of the wall material as well as on the desired functionality of the obtained ensemble in each case. The different types of microcapsules and microspheres are produced from a wide range of wall materials (monomers and/or polymers) and by a large number of different microencapsulation processes such as: spray-drying, spray-cooling, spray-chilling, air suspension coating, extrusion, centrifugal extrusion, freeze-drying, coacervation, rotational suspension separation, co-crystallization, liposome entrapment, interfacial polymerization, molecular

inclusion, etc. (Desai and Park, 2005; Gibbs *et al.*, 1999; Gouin, 2004; King, 1995; Shahidi and Han, 1993). The current industrial scenery is founded on compromises based on the needs of the industrial processes developed to satisfy both the increasing market requirements and the mandatory rules in sustainable productions such as raw material/energy savings, respect of environmental constraints of industrial-scale processes (Charpentier, 2007). New microencapsulation technologies are relentlessly devised and invented by academics and industrial researchers: in 2002 over 1000 patents were filed concerning various microencapsulation processes. Some of these new processes have very little industrial relevance because of the extremely high cost-in-use, difficult scale-up, and/or narrow applicability range. However, some of these processes stand out as being promising, sensible and likely to be scaled up in the near future for the encapsulation of active ingredients (Tran *et al.*, 2011; Gouin,2004). Dalmoro *et al.*, (2012) reviewed the new approaches to the microencapsulation processes focusing on the emerging ultrasonic atomization technique and presented fundamentals and novel aspects of the technology emphasizing the advantages in terms of intensification and low energy request. Amongst the various techniques developed to encapsulate food ingredients spray drying is the most common technology due to its low cost and availability of equipment (Gharsallaoui *et al.*, 2007). Aghbashlo *et al.*, (2012) applied the exergy analysis using second law of thermodynamics to obtain a quantitative measure regarding the efficiency, losses and performance for

microencapsulation of fish oil using different wall materials and drying air temperatures. Authors concluded that the use of exergy analyses it is a good way to manage and improve the sustainability of the process.

The microcapsules produced can be used in the development of functional foods but there are emerging other interesting applications regarding the sustainability of the field. For example, Lamppa *et al.*, (2014) reviewed the nutritional delivery systems emerging from the drug delivery field aimed at reducing waste in food and beverage and eliminating waste in food and beverage packaging. Authors presented the microencapsulation as one technology able to functionalize nutrients powders by decreasing the dissolution times and improving the consumer experience by modulating particle size, porosity, and hydrophilicity and by preventing degradation of the bioactive compound before it reaches the desired functional location. Takei *et al.*, (2008) used the microencapsulated *Lactobacillus delbrueckii* spp. *bulgaricus* NBRC 13953 as soil bioamendments and evaluated the effect of preparation parameters in the emulsion system on the survival activity of the encapsulated bacteria. Furthermore, the soil application demonstrated that the microcapsules are effective in the removal of root-knot nematode.

5. Edible films and coatings

As pointed out by Attila E. Pavlath at the 24th National Meeting & Exposition of the American Chemical Society (ACS, 2013), the use of edible films and coatings has grown in the last 30

years from 10 to more than 1,000 companies in the business, with annual sales exceeding $100 million. The great success of these products is due to the fact that they can be applied in almost any sector of the food industry to meet requirements related to the marketing of safe foods, with high quality and enhanced nutritional value, as well as being cost-effectively and environmentally friendly (Gennadios *et al.*, 1997). Because coatings are applied and formed directly on the food product, whereas films are applied after being formed separately, their composition, manufacture, properties and uses are not exactly the same (Bourtoom, 2008). Regarding edible films and coatings contribution to sustainable economic and social progress, it takes place trough different ways.

First edible coatings date from the 12th century and consisted of layers of molten wax applied on the surface of citrus fruits with the aim of preserving them for later consumption (Pavlath and Orts, 2009). Extending shelf-life is still nowadays one of the main objectives of scientific research and industrial application of edible films and coatings on the surface of several foods. As stated in a recent study (FAO, 2011), about one third of the edible parts of food produced for human consumption gets lost or wasted, basically for not meeting food safety and/or quality standards. Food losses involve a waste of land, water, energy and several inputs used in production, so any technique reducing effectively these losses will also contribute to the more efficient use of natural resources.

In the particular case of fruits and vegetables, losses in post-harvest are estimated to range between 5 and 25% in developed countries, and between 20 and 50% in developing countries, depending on the type of product (Pérez-Gago *et al.*, 2008). Cold and storage in controlled and/or modified atmosphere are usually employed in slowing down the senescence process. However, the result of applying these techniques is not always homogeneous which, in addition to its relatively high cost, makes it necessary the search for new storage alternatives. In general terms, it is more usual for this kind of products applying an edible coating than a film and formulations combining hydrocolloids (polysaccharides or proteins) and lipid compounds are reported to be the most appropriate (Olivas *et al.*, 2008). On one hand, hydrocolloids provide selective permeability to CO_2 and O_2 while lipids confer resistance to water vapor migration. Although emulsions based on natural waxes are the most commercially applied products due to their effectiveness in citrus and apples, it is increasing the use of cellulose ethers (such as carboxymethylcellulose, hydroxypropylcellulose, and methylcellulose) as ingredients in coatings for fruits and vegetables (Han, 2014; Pérez-Gago and Rhim, 2014). In recent years, research is focused on the development of edible coatings with antimicrobial activity for the control of microorganisms that cause post-harvest diseases. Apart from the use of hydrocolloids with certain antimicrobial properties, such as chitosan or aloe vera, incorporating ingredients with antifungal properties, such as certain food grade additives, natural extracts and antagonistic

to fungal pathogen, has been reported to be a good alternative to the use of synthetic chemical fungicides in whole fruits (Pérez-Gago, 2015). In addition, packaged or coated products are more protected losses of volatile compounds and nutrients, as well as from physical damage caused by physical impact, pressure, vibrations, and other mechanical factors, which are especially prevalent when handling grapes, cherries, stone fruits, berries and fresh cut products. In the particular case of the latter ones, food grade additives are usually included to the film or coating formulation in order to prevent browning or tissue softening.

Although losses and waste of meats, poultry and sea foods account for half of the ones for fruits and vegetables (FAO, 2011), using edible coatings on such highly perishable products has intensified recently. Indeed, weight losses taking place during storage under refrigeration, freezing or vacuum conditions have been successfully reduced by applying edible coatings with good moisture barrier properties as is the case of lipid-based coatings (Gennadios *et al.*, 1997). In the same way, rancidity and brown coloration, respectively, caused by lipid and myoglobin oxidation are reduced by using edible coatings of low oxygen permeability.

According to their ability to extend the shelf-life of several foods, edible films and coatings contribute in the food globalization process that involves a larger supply of products to vary and improve consumer's diets. Beyond the conservation of each food nutritional properties, new edible coatings arise with the aim of improving them. On one hand, coatings are applied on

the surface of several foods to reduce oil uptake during frying (García *et al.*, 2002; Suárez *et al.*, 2008). Moreover, edible films and coatings are used as physiological active ingredients carriers (Salgado *et al.*, 2015). In fact, a wide range of natural occurring antioxidants such as essential oils and plant extracts (basil, thyme, cinnamon leaf and tea tree essential oil among others), as well as pure compounds (like ascorbic acid and □-tocopherol) have been incorporated into edible films and coatings to improve their bioactive properties (Bonilla *et al.*, 2012; Bonilla *et al.*, 2013; Perdones *et al.*, 2014; Sánchez-González *et al.*, 2009). In a lesser extent, the incorporation of probiotics into functional edible films and coatings has been studied for the development of probiotic breads (Soukoulis *et al.*, 2014) and probiotic fresh-cut fruits (Tapia *et al.*, 2007).

Finally, edible films and coatings play an important role in reducing the environmental impact of the feeding process. On one hand, they contribute to the revalorization of those industrial by-products that meet the necessary conditions to be included in their formulation. This is the case of starch and cellulose obtained from different vegetables, chitosan from crustacean, carrageenan and protein extracted from seaweed, whey protein from the dairy industry, gelatin from slaughterhouses and tanneries, soybean and sunflower proteins from oilcakes and keratin from feathers (Salgado *et al.*, 2015). On the other hand, the use of edible films and coatings as primary packaging may simplify the total packaging structure and therefore, the overall utilization of synthetic materials (Han, 2009). Apart from food applications, the

use of edible films for agricultural purposes, as grocery bags or in place of cushioning foams could also reduce the accumulation of non-renewable and non-biodegradable materials derived from petroleum.

6. Vacuum impregnation

Vacuum impregnation is a mass transfer operation between a porous solid matrix immersed in a liquid media. The creation of a pressure gradient between the porous and the liquid media origins a degasification of the porous structure in the first step of the process, and secondly an important input of liquid from the external media to the solid matrix (Fito et al., 1996). A great amount of research work have been reported in the last fifteen years, and some of them transferred to the food processing industry (Zhao and Xie, 2004; Radziejewska-Kubzdela et al., 2014). Because of the perishability and the short shelf life of vegetal tissues that compromise the sustainability of natural resources, vacuum impregnation has a relevant interest on them.

By means of vacuum impregnation, it has been possible to include inside a food porous structure, mainly a vegetal tissue, cryoprotectans, stabilizers, enzymes, inhibitors of enzymatic browning and physiologically active compounds (e.g. minerals, vitamins, probiotics or antioxidants among others). This way, it has been possible to enhance freezing tolerance, to increase stability and shelf life of minimally processed fruits or vegetables, to improve kinetics and efficiency of processes in which a solute, like sugar or salt, has to be homogenously distributed inside a

food, and to develop nutritionally fortified products. In any case, vacuum impregnation makes possible food quality and safety without high incomes, contributing to the sustainability of food processing system. Additionally, some results have proved the possibility to effectively reduce the incidence of chronic disease including vacuum impregnated foods specifically formulated on diet, with the derivative reduction of costs on medical public systems.

Xie and Zhao (2004) used a high-fructose corn syrup (50%) or high-methylated pectin (3%) to impregnate strawberries prior to the freezing process. They found that vacuum impregnation resulted in a strengthened structure and reduced water drip in thawed strawberries. High-fructose corn syrup decreased the amount of frozen water in tissue, while high-methylated pectin penetrated into intracellular spaces protecting fruit against freezing damage. An example for the application of vacuum impregnation to inhibit enzymatic browning may be provided by a study by Perez-Cabrera et al., (2011). During the impregnation of pears the authors used an isotonic solution containing enzymatic browning inhibitors (ascorbate; 4-hexylresorcinol; EDTA; citrate) with or without an addition of calcium lactate. In the study reported by Derossi et al., (2010), it was observed that the reduction of pH values was greater during vacuum impregnation of pepper slices than in the case of blanching at atmospheric pressure. Related with the improvement in the distribution of a solute within a food, Tamer et al., (2013) reported the duration decrease of debittering process in olive fruits impregnated with

NaOH (1.5%) and NaCl (3%). In the case of drying, vacuum osmotic dehydration in a hypertonic solution may result in a reduction of raw material moisture content and a shortening drying time, thus contributing to an improvement of dried material quality. Pallas et al., (2013) reported a significant reduction of drying time as a result of vacuum osmotic dehydration of rabbiteye blueberries in a sucrose solution (60 Brix).The use of vacuum impregnation allows the introduction of other structure-forming compounds, e.g., polyamines, into the plant tissue. Polyamines exhibit properties similar to those of calcium ions. They may bind with cell walls and pectins found in the middle lamella (Kramer et al., 1991). On the other hand, they may inhibit the synthesis of ethylene in damaged tissue and reduce the activity of enzymes responsible for its softening (Kramer et al., 1989). The use of vacuum impregnation technique to introduce these compounds to the tissue of strawberries was tested by Ponappa et al., (1993).

Regarding the impregnation with physiologically active compounds such as probiotics or antioxidants, Betoret et al., (2003) applied vacuum impregnation of apples with apple juice supplemented with *Saccharomyces cerevisiae* or milk inoculated with *Saccharomyces cerevisiae* and *Lactobacillus casei*. Impregnation facilitated the effective introduction of probiotics to apple tissue, providing the content of microorganisms in the product after convection drying (air drying) at 10^6–10^7 CFU/g. This is equivalent to the level of bacteria in dairy products. Similarly, Krasaekoopt and Suthanwong (2008) obtained the level

of microorganisms in fruit after air drying at 10^7 CFU/g during the vacuum impregnation of guava and papaya fruits using *L. casei*, which makes this product probiotic food.

The current state of knowledge on the biological activity of flavonoid compounds clearly indicates that their positive effect on the human organism results mainly from antioxidant properties. The capacity of flavonoids to scavenge reactive oxygen species (ROS) and chelate transition metals may have a significant role in pathological conditions (e.g., inflammations, atherosclerosis, diabetes, neurodegenerative diseases or cancer), which are accompanied by oxidative stress (Hanasaki et al., 1994; Yao et al., 2004). Betoret et al., (2012) applied vacuum impregnation to introduce homogenized mandarin juice with low pulp content to apple snacks. The authors obtained the content of hesperidin in 40 g of the enriched product equivalent to that in 250 mL of mandarin juice. In a continuation of studies by Betoret et al., (2012) apple snacks vacuum impregnated with mandarin juice were administered to obese children in order to alleviate inflammatory conditions and improve the antioxidant capacity of the organism (Betoret et al., 2012; Codoñer-Franch et al., 2013). A considerable improvement was observed in systolic blood pressure and the lipid profile following the treatment period. The authors concluded that the addition of the product to diet contributed to an alleviation of oxidative stress and the inflammatory condition, as well as several other risk factors connected with atherosclerosis in the examined obese children.

The most controversial aspect of the application of vacuum impregnation in the food industry is the great amount of residual liquid generated as a consequence of the process. Different solutions can be applied in order to assure the environmental and economic sustainability. Some of them are:

1. To use as an impregnation liquid by-products generated in other food processes. Vegetable, cereal and fruit processing by-products are typically rich in proteins, sugars and lipids and contain particular aromatic and aliphatic complex compounds. When it has a suitable composition for vacuum impregnation purpose, could be used directly. Otherwise, components of interest could be extracted and incorporated to the impregnation liquid.
2. To reuse the impregnation liquid. Some studies show that it is possible to reuse the same liquid medium in different cycles of impregnation. The impregnation liquid composition is hardly affected by the output of native liquid during the first stage of the operation (Castagnini *et al.*, 2015). Regarding the microbiological quality of impregnation liquid along reuse, it can be controlled by a cold treatment like a high pressure homogenization.
3. To use the impregnation liquid waste to formulate new food products. Ingredients obtained from fruit and cereals by-products might have great potential and market opportunities in the modern society where the consumption of "ready to eat" products with health promoting properties is increasing. The possibility of using the liquid waste to

produce new food products, including fruit juice beverages and snacks, self-stable fillers for bakery products, fibre-enriched bakery products is being investigated (Fava *et al.*, 2013).

Additionally, after specific pre-treatments with physical and biological agents followed by tailored recovery procedures, they might provide specific natural antioxidants, anti-microbial agents, vitamins, among others, along with macromolecules (e.g. soluble fibres), bioactive oligosaccharides, oligopeptides, and pigments. Further, some of the compounds occurring in the hydrolysates resulting from the by-products pretreatment can be transformed into more sophisticated molecules like flavours and fermentation products, throughout tailored biotechnological processes (Wynan, 2003; Laufenberg et al., 2003). All these natural compounds can be combined in the formulation of new food products with the attempt to close the circle within large fruit and cereal food industries and/or to create new synergies between fruit/cereal processing industries and food producing industries.

7. Nutrigenomics

Nutrigenomics, also called nutritional genomics, considers the interactions between foods or dietary supplements, proteome (the sum total of all proteins), and a metabolome (the sum of all metabolites) on an individual's genome, and the consequent downstream effects on their phenotype (Debusk *et al.*, 2005, Ferguson, *et al.*, 2010). Consequently, nnutritional genomics has the potential to provide tailored nutrition advice to populations or

to individuals. It recognizes that what is appropriate dietary advice for one individual may be inappropriate, or actually harmful, to another.

In nutrition terms, few will question the repeatedly documented link between diet, an environmental factor, and the risk and incidence of a number of the chronic diseases of today. The acceptance of such associations has contributed significantly to the formulation of current public health nutrition policies, which, although based on credible evidence, have not been shown to benefit equally those who follow them.

Functional foods may be a mechanism through which optimal dietary advice can be tailored to a population's needs. But to do this link in a sustainable way, it is essential that individuals from all the relevant professions have active input integrating the new knowledge into appropriate training programs. Input through the food industry into new functional foods development will also be necessary (Ferguson, 2009). Food and nutrition professionals have the responsibility to ensure that all groups of society are given appropriate nutrition advice and the resources are used in an optimal way.

Experts and other stakeholders predict that nutrigenomics will deliver improvements in public health by identifying genetically determined differences in how diet impacts on chronic disease, both in terms of food and food components as a cause of disease, and as a preventative or curative agent, although there is uncertainty regarding the concrete form such developments will

take (Komduur *et al.*, 2007). There is little evidence that societal benefits and sustainability (e.g. reduced health service costs) increase acceptance of nutrigenomics, though some stakeholders have assumed this (Frewer *et al.*, 2011).

8. Conclusions

Current food supply systems and practices are economically and environmentally unsustainable and the need for urgent action is widely recognized. Traditionally, regarding to sustainability, the agri-food system has been directed towards promotion of organic and local food, but there is other possibility that implies emphasizing biotechnology and technological panaceas. Management of food processes in an adequate way can contribute to achieve a full sustainability concept with three dimensions implied: environmental, social and economical. Environmental because the processes and the raw materials used in the development of functional foods are more and more environmentally friendly with less waste production, enhancing reuse and biodegradable products. Social because functional foods contribute clearly to improve public health and economical because the improvement of public health implies reducing the rising costs of the health care system.

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Sustainable drying technologies for the development of functional foods and preservation of bioactive compounds

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Abstract

Nowadays, the sustainability of a product, a process or a system is assessed according to three dimensions: environmental, social and economic. Sustainability challenges occur at all stages in the food system from production through processing, distribution and retailing to consumption and waste disposal. The promotion of organic and local food is not the only way to reach the sustainability. There is other possibility that implies to continue the production hegemony.

Increasing research is being focused on the development of healthy, quality and safety food products adapted to consumer's needs and more environmentally friendly processes, that is, processes consuming energy more efficiently, generating less waste and emitting less greenhouse effect gases.

Drying technology is applied in the food industry not only for preservation but also to manufacture foods with certain characteristics. Drying technology operations need to be precisely

controlled and optimized in order to produce a good quality product with the highest level of nutrient retention and flavor together with microbial safety.

This chapter contains detailed information about some measurements taken by the food industry to ensure the supply of bioactive nutrients to as many individuals as possible assuring the global sustainability. More specifically, the contribution of some drying techniques employed in the development of functional foods to increase the sustainability of the feeding process, is discussed.

Keywords: *sustainability, functional foods, drying, bioactive compounds, structure,*

1. Introduction

Sustainability means meeting the needs and aspirations of the present without compromising the ability of future generations to meet theirs. As a result of environmental imbalances caused by intensive production and massive use of resources, to achieve food and agricultural sustainability, traditionally, the system has been directed towards promotion of organic and local food, but this is not the only way, as explained in (Spiertz et al., 2010) there is other possibility that implies to continue the production hegemony, emphasizing biotechnology and technological panaceas.

Nowadays, the sustainability of a product, a process or a system is assessed according to three dimensions: environmental,

social and economic. Sustainability challenges occur at all stages in the food system from production through processing, distribution and retailing to consumption and waste disposal. The development of a sustainable agri-food system places responsibilities on both the natural and the social sciences (OECD, 2004). While advances in basic and strategic biological research have greatly expanded, the potential to produce nutritious food in an efficient and environmentally sustainable manner, social and economic factors will determine the uptake and value of this research as well as its future direction (Lowe et al., 2008).

Food processing can be defined as the set of operations which allow manufacturing, preservation and distribution of food products from suitable raw materials. The improvement of the food products is now directed towards ensuring nutritional and specific functional benefits. Regarding the process improvement it is directed to ensure the quality and safety of environmentally friendly food products, prepared optimizing the resources used, minimally affecting or even enhancing their nutritional and beneficial characteristics (Betoret et al., 2015).

Sustainable food production stands at the intersection of several growing needs. Primarily, the needs of consumers for improved food security and safety as well as more sophisticated needs. Secondly, the quest for economic sustainability of food production based on cost reduction and increased product differentiation. Third, the growing concern for reversing the over-

exploitation of natural resources, waste generation, and the contribution to climate change (Fava et al., 2013).

Functional foods are foods that beneficially affect one or more target function in the body, beyond an adequate nutritional effects, in a way that is relevant to either an improved state of health and wellbeing and/or reduction of risk of disease, and it is consumed as a part of a normal food pattern (not a pill, a capsule or any form of dietary supplement) (European Comission, 2010). Many diseases strictly related with diet and lifestyle concern to the society because of its prevalence. Functional foods can help to prevent or improve those diseases thus contributing directly to public health. But the functional effect of a food or food component depends on the active component gaining access to the functional target site. Foods are mostly complex mixtures of macro and micro components organized in a structure that can trap active compounds, modulating their release or inhibiting their activity. The selection and development of both appropriate food matrix and technological process, able to maintain the active molecular form until the time of consumption is the key step for the success of a specific functional food (Betoret et al., 2015).

This chapter contains detailed information about some measurements taken by the food industry to ensure the supply of essential nutrients and bioactive compounds to as many individuals as possible assuring the global sustainability. More specifically, the contribution of some drying techniques employed in the development of functional foods to increase the sustainability of the feeding process, is discussed.

2. Drying operation

Drying is an energy intensive well-studied unit operation in process engineering to reduce moisture content in the food matrix to a level that is safe for storage and transportation, to avoid microbial multiplication, slow down/inactivate microbial activity and the associated product quality deterioration. It involves the removal of water from a wet feedstock by inducing phase changes of water from solid or liquid into a vapor phase via the application of heat (except in the case of osmotic dehydration during which the water is removed without a change in phase by the diffusion of liquid water from solid foods to an osmotic solution through an osmotic pressure difference). The process of drying food materials is extremely complex, involving coupled transient mechanisms of heat, mass, and momentum transfer processes accompanied by physical, chemical, structural and phase change transformations (Sabarez, 2012; Sabarez, 2014).

Drying is applied in the food industry not only for preservation but also to manufacture foods with certain characteristics. The nature of the process along with the food structural characteristics results in a very marked effect on the quality characteristics of the final product. There are many different methods of drying food materials, each with their own advantages and disadvantages for particular applications. A vast number of dryer designs reported in the literature are due to the differences in the physical attributes of the product, modes of heat input, operating temperatures and pressures, quality specifications on the dried product and so on (Sabarez, 2016). The

methods most commonly employed for biotechnological and food products include freeze drying, spray drying, convective drying, vacuum drying, microwave drying, osmotic drying and combinations thereof (reviewed in Sabarez, 2016; Walters et al., 2014). Overall, the quality characteristics of the final product are significantly affected by the process conditions and the way it is conducted. Thus, drying operations need to be precisely controlled and optimized in order to produce a good quality product with the highest level of nutrient retention. The changes caused to the food properties include discoloring, aroma loss, textural changes, nutritive value, and changes in physical appearance and shape (Quirijns, 2006). Conditions of drying have a great effect on quality attributes of dried product. For example, higher drying temperature reduces the drying time but may result in poor product quality, heat damage to the surface and higher energy consumption (Ho et al., 2002). On the other hand, mild drying conditions with lower temperature may improve the product quality but decrease the drying rate thus drying period is lengthened.

The problems of drying are diverse as various food materials with very diverse physical/chemical properties need to be dried at different scales of production and with very different product quality specifications (Mujumdar & Wu, 2010). The materials preserved by dehydration vary a lot, since fruits and vegetables to probiotic microorganisms and animal products in the food area, but also other biological materials with important physiological activities, such as human blood cells and insulin.

As described in (Betoret et al., 2015), in most cases drying involves the application of different temperature conditions (e.g. in the case of freeze-drying the temperature applied can be -30 or -80 ºC, and in the case of other methods such as air drying or spray drying the temperatures can be 45-80 ºC or 125-140 ºC respectively) that cause irreversible damage due primarily to:

- Changes in cellular structures (e.g. cell wall, cell membrane) constituting biological tissues and the induction of changes in key properties responsible for product functionality (e.g. cell membrane permeability, mechanical strength of the wall membrane assembly, etc.).
- Changes in the chemical structures responsible for the biological value of nutritious components (e.g. protein, fat). The structural changes also cause changes in the technological functionality that these compounds give to the food to which they belong.
- Reactions, mainly oxidation, than decrease the functional value of nutritive compounds (e.g. vitamin, antioxidant).

The major challenge is to remove water from the material in a most efficient way with better product quality, minimal impact on the environment and at the lowest capital and operating costs of the process. Today's increased competition due to globalization, together with the growing consumer demand for better quality products, coupled with the need for eco-friendly and sustainable processes to maintain competitiveness with minimal impact on the environment, will continue to seek innovations in the drying process (Sabarez, 2016).

3. Strategies to increase the functionality of food products in drying processes

One important part of the sustainability to point out is the minimization of residues on the bioactive compounds recovery from the food waste. During bioactive compounds recovery from food waste is common to carry out a drying operation in order to concentrate these ones and use the minimum quantity of solvent. A lot of papers have been written studying the optimal exploitation and revalorization of food waste extracting the maximum quantity of bioactive compounds and minimizing the environmental impact. Some examples of articles/reviews published are those from Galanakis (2012) and San Martin et al. (2016). However, in this book chapter we focus on the contribution of functional foods to global sustainability concept. In this way, the principal strategies to increase the functionality of food products during drying as indicated in Sabarez (2016) can be divided into three groups. These strategies can be applied regardless of bioactive compound source either being naturally present in the food matrix or derived from food waste recovery:

1. Addition of ingredients that protect the degradation of bioactive compounds.
2. Creation of structural elements that protect/maintain the functionality of bioactive compounds.
3. Prevention of reactions causing a degradation bioactive compounds and promotion of those that result in a functional effect.

3.1 Addition of ingredients that protect the degradation of bioactive compounds

As mentioned earlier, drying operation involves removing large amounts of intracellular and extracellular water from food matrixes that results in structural and biochemical changes that at the end can affect the functionality of bioactive compounds. The bioactive compounds to protect vary a lot, since probiotic microorganisms to other important biological compounds such as red blood cells and insulin. As a result, a variety of protectants have been added to the drying media in order to protect the viability of those bioactive compounds. Following this strategy the researchers not only aim reducing the degradation of bioactive compounds during drying but also even increasing their functionality.

Regarding to probiotic microorganisms protection during drying, a lot of literature can be found. The probiotic microorganisms are dried in order to extend their viability in dried form or during their incorporation into functional foods. Several works show that properly dried microorganisms remain viable during long-term storage at room temperature (Zayed & Roos, 2004). However, the stresses suffered during processing may lead to significant losses in viability and functionality. As explained by Iaconellia et al. (2015) the stresses applied on microorganisms by drying processes can be divided into two main categories: the mechanical stresses, mainly localized to the cell membrane, and the intracellular accumulation of reactive oxygen species that causes damage to cell proteins, lipids and nucleic

acids. Structural changes can lead to membrane deformation that with fast dehydration-rehydration processes results in membrane permeabilization leading to cell death (Schwab et al., 2007; Dupont et al., 2010; Lemetais et al., 2012). Moreover, reduced water activity induced phase transitions from crystalline to a gel in cell membrane (Milhaud, 2004) which may lead to leakage and cell death (Potts, 2001).

A variety of protectants have been added to the drying media before freeze-drying or spray-drying to protect the viability of probiotics during dehydration, including skimed milk powder, whey protein, trehalose, glycerol, betaine, adonitol, sucrose, glucose, lactose and polymers, such as dextran and polyethylene glycol (Morgan et al., 2006). The beneficial effects of the protectants, seems to be related to their protective effect on proteins and cell membranes (Leslie et al., 1995).

As reviewed by (Meng et al., 2008) drying injuries to the cell depends on probiotic strain, drying method and conditions of processing.

Some examples of new protectants and applications in the area of functional foods developments are described below. In most of studies, not only the survivability of the probiotic cells is considered but also their functionality measured in terms of enzyme activity, acid tolerance and hydrophobicity.

The benefit of disaccharide protectants such as cellobiose, lactose and sucrose, for maintaining viability and b-glucosidase activity of *Bifidobacterium infantis* UV16PR during freeze-drying and storage in different food matrices was evaluated (Basholli-

Salihua et al., 2014), concluding that at 10% concentration both trehalose and cellobiose significantly enhanced enzyme activity, viability and acid tolerance.

Resistant starch was found to protect *Lactobacillus plantarum* CIF17AN2 during drying process and could potentially protect it from gastric acid and bile exposures (Hongpattarakere & Uraipan, 2015). In the same way, whey protein isolate (WPI) was able to protect *Lactobacillus plantarum* A17 in the encapsulation process. A unique layer-by-layer electrostatic mechanism involved in encapsulation of A17 at pH 7 was found responsible for higher survival of cells (Khem et al., 2016).

The capability of different fibre preparations to protect the viability and stability of *Lacobacillus rhamnosus* during freeze-drying, storage in freeze-dried form and after formulation into apple juice and chocolated-coated breakfast cereals was studied (Saarela et al., 2006). The stability of freeze-dried *L. rhamnosus* cells at 20 °C was higher in chocolate-coated breakfast cereals compared to low pH apple juice. As in freeze-drying stability, wheat dextrin and polydextrose proved to be better carriers than oat flour in chocolate-coated breakfast cereals. In the development of probiotic chocolate, as reviewed by (Konaret al., 2016), the lipid fraction of cocoa butter was shown to be protective for bifidobacteria.

Regarding other bioactive compounds, trehalose seems to be the most studied protectant. For example, trehalose has shown to have a protective effect on insulin structure, probably via substitution of hydrogen bonds, while the mild surfactant, sodium

deoxycholate, was more protective on the native structure of insulin and, therefore, results in high bioactivity mainly due to resistance to the frozen concentration and interface denaturation in a concentration-dependent manner (Yong, et al., 2009). Intracellular trehalose has been shown to be necessary for successful stabilization of the membrane during freeze drying of liposomes and cells (Crowe et al., 1985). In the same way, trehalose loaded red blood cells lyophilized in the presence of liposomes demonstrated high survival and low levels of methemoglobin during 10 weeks storage at 4 ºC in the dry state. A detailed investigation on the liposome size revealed that extruded egg yolk phosphatidylcholine vesicles with an average diameter of 270 nm are the most effective in inhibiting hemoglobin release. Smaller vesicles could access membrane disruptions and be responsible for membrane repair, which was reflected in reduced hemoglobin leakage (Kheirolomoom et al., 2005).

Sometimes, the addition of key ingredients can not only help to reduce the degradation of bioactive compounds but increase their functionality. It was demonstrated that the addition of a cationic amphiphilically modified dextran could act as excipient in drug delivery nanocarriers of dry power inhalation and significantly increase the drug functionality and its effect (Varghese et al., 2015). In the same way, a dry powder phage K preparation for oral delivery to control *Staphylococcus aureus* using alginate-whey protein microspheres was developed (Tang et al., 2015). The results showed that maltose provided the best protection to encapsulated phage K during drying. Both the microsphere size

and polymer concentrations in the encapsulation matrix were important factors determining the degree of protection against stomach acids.

3.2 Creation of structural elements with protective effect

Creation of structural elements with protective effect like encapsulation by using spray drying to create a protective structure, the application of drying operation to form edible films and coatings and the use of vacuum impregnation and its subsequent drying are strategies which can reduce the negative effect of dehydration on biomolecules, protect and even improve the functional value of the food.

3.2.1 Encapsulation

Encapsulation is defined as a technology of packaging solids, liquids, or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Vilstrup, 2004; Desai & Jin Park, 2005). The main objective of encapsulation is to protect the core material from adverse environmental conditions such as moisture, heat, oxygen or other extreme conditions. Thereby encapsulation can contribute to: increase the shelf life of the product; increase functionality, promoting the controlled liberation of the encapsulated bioactive compound in the target site (Shahidi & Han, 1993) and keep its properties protecting its bioactive compounds. Extend its shelf life and hence reduce food losses is related with a waste of land, water, energy and several inputs used in production, so any technique reducing effectively these losses will also contribute to

the more efficient use of natural resources and therefore sustainability.

Regarding encapsulation technologies, spray drying is an economical, flexible, continuous operation, which produces particles of good quality. For this reason it is the most widely used as microencapsulation technique in the food industry. Encapsulation with spray drying is typically used for the preparation of dry, stable food additives and flavors, and to protect functional ingredients such as polyphenols and probiotics (Betoret at el., 2015).

In most of cases the capsule is mainly made of polysaccharides, proteins and its combinations for the microencapsulation of antioxidant components and probiotics. Some polysaccharides such as inulin and polydextrose may act as prebiotic, as are not hydrolyzed by human digestive enzymes, and have been used to protect probiotic bacteria during spray drying and storage (Avila-Reyes et al., 2014).

Recently food industry byproducts have raised considerable interest for their use as encapsulant because of being a sustainable source of material. In Chiou & Langrish (2007) demonstrated in its studies that milled citrus fiber can be used as a replacement for maltodextrin type carriers to encapsulate hibiscus extract. Also, whey protein is an excellent encapsulating material due to their emulsification, gelation and film forming properties. Denaturing the whey protein ensures higher tensile property and lower oxygen permeability which protects the probiotic cell from adverse gastrointestinal conditions (Perez-Gago

et al., 2001; Rajam et al., 2012). Concretely, microencapsulation of *Lactobacillus plantarum* with fructooligosaccharide and denatured whey protein as wall material was found to be most effective in maintaining the viability of bacteria after drying, during storage and in simulated gastric and intestinal conditions (Rajam & Anandharamakrishnan, 2015). Reconstituted skim milk has demonstrated to behave as a protective carrier for improving the survival ratio of lactic acid bacteria (LAB) after spray drying. Such protective effects has been attributed to calcium, which might enhance the heat resistance of LAB cells, and proteins, which lead to a mild temperature variation rate that is beneficial to cell survival (Zheng et al., 2015). Other example of food derived protein able to protect probiotics from hot temperatures is derived from flaxseed (*Linum usitatissimum L.*) and its mucilages, in reference (Bustamante et al., 2015) have demonstrated its efficiency as wall materials for microencapsulation by spray drying of *Lactobacillus acidophilus* La-05.

3.2.2 Edible films and coatings

Recently, the interest in high-quality food products, increase shelf life and reduce environmental impact has promoted the development of edible and biodegradable polymer films and coatings. Extending shelf life is still nowadays one of the main objectives of scientific research and industrial application of edible films and coatings on the surface of several foods.

Its use in multiple food-packaging applications has emerged as an environmental friendly technology with regard to their film-forming properties. Is considered an edible film or coating any

material used for enrobing (i.e., coating or wrapping) various food to extend shelf life of the product that may be eaten together with food or without further removal (Pavlath & Orts, 1999). Edible film or coating can control moisture transfer, gases exchange, lipid migration and/or oxidation processes. An edible coating is a thin layer of edible material formed as a coating on a food product, while an edible film is a preformed, thin layer, made of edible material, which once formed can be placed on or between food components (McHugh, 2000).

Edible films are obtained from food-grade suspensions and are usually molded as solid sheets onto inert surfaces. They are dried and put into contact with food as wrappings, pouches, capsules, bags, or casings through further processing (Falaguera et al., 2011). Biopolymer edible films can be formed via two basic technologies: dry and wet processes. In a dry process, the biopolymer relies on the thermoplastic behavior exhibited by some proteins and polysaccharides at low moisture levels in thermo-compression molding and extrusion. And in wet process biopolymers are dispersed or solubilized in a film-forming solution (solution casting), and drying steps to make the film matrix (Rhim & Ng, 2007), solvent removal is required to achieve solid film formation and control its properties (Hernandez-Izquierdo & Krochta, 2008). In this case, most of the times drying operation is applied to form the structure not to obtain dried foods as in aforementioned cases. Those drying operations are generally with air flow at moderate temperatures ranging from 30ºC to 60ºC, depending on the characteristics of the product. When the edible

film is applied in a dehydrate product drying temperature can be higher; in reference Tavera-Quiroz et al., (2015) applied an edible film in an apple snack enriched with FOS and *Lactobacillus plantarum* with methylcellulose, acid citric and sorbitol at different temperatures ranging from 50 ºC to 140 ºC during a range of 3 to 90 minutes.

Edible films and coatings contribute to the revalorization of some industrial by-products which are included in their formulation. This is the case of starch, cellulose and hemicellulose from plant origin, chitosan from crustacean, gums, carrageenan and protein extracted from seaweed, whey protein from the dairy industry, gelatin from slaughterhouses and tanneries, plant-based proteins as soybean and sunflower proteins from oilcakes and keratin from feathers (Hansen & Plackett, 2008; Jiménez et al., 2012; Elsabee & Abdou, 2013; Salgado et al., 2013). The use of by-products contribute to reduce the waste and hence to increase the sustainability of the process.

In addition, edible films and coatings can act as carriers of functional bioactive compounds as antioxidant or/and with antimicrobial properties, bacteria with probiotics effect or antimicrobial and other components which rise in value the product increasing food's shelf life and protect their physicochemical properties while maintaining their mechanical integrity and handling characteristics (Salgado et al., 2013; Rhim & Ng, 2007; Silva-Weiss et al., 2013).

3.2.3 Vacuum impregnation

Vacuum impregnation is a mass transfer operation where a liquid medium is introduced into a solid porous food structure due to pressure gradients created (Fito, 1994; Fito & Pastor, 1994). The liquid amount impregnated into the food matrix depends on the food structure (pore size, distribution, morphology and porosity) and on the vacuum force applied (time and intensity).

Vacuum impregnation can be considered as a useful technology to introduce solutes into the structural food matrix to modify their composition. Generally is applied to add bioactive compounds to achieve a technological and/or nutritional functionality (Betoret et al., 2015). In most cases this technological operation is used as a pre-treatment for other operations such as frying, drying and freezing due to its effectiveness in reduce enzymatic and browning reactions, without using antioxidants, as a result of removing oxygen from the food matrix (Alzamora et al., 2000). Vacuum impregnation with a subsequent drying operation is a good combination to obtain stable and enriched functional foods. This operation can be use also to mitigate drying effect introducing in the food matrix compounds with protector effect as sugars, sugar alcohols and non-reducing sugars. Functional compounds added into the food matrix are more protected from oxygen and other degradation factors than the free functional compound itself; hence functional properties and shelf life are improved, even synergies between some bioactive components can occur and enhance its functionality. Has been demonstrated that bioactive compounds provided by foods can have synergic effect, for example

hesperidin is more efficient in combination with ascorbic acid (Garg et al., 2001). In reference, Betoret et al., (2012) was developed a probiotic apple snack impregnated with mandarine juice and enriched with *Lactobacillus salivarius* spp. *salivarius.* The inclusion of the probiotic into a food matrix by vacuum impregnation demonstrated a protection against degradation reactions and at the same time the new structure could permit the liberation of the bioactive compound in the target site hence improving its functionality.

3.3 Prevention of reactions causing a degradation of bioactive compounds and promotion of those that result in a functional effect

Because of the decrease in the moisture content during drying, most of the nutrients present in the food undergo substantial concentration, thus increasing its nutritional value. However, other more sensitive nutrients are irreversibly transformed and/or destroyed during the dehydration step mainly due to the effect of light, oxygen, heat and the presence of sensitizers. The extent of such changes would depend not only on the processing conditions, but also on the sensitivity of each particular compound, their interaction with other food components and the protection conferred by structural matrices, such as cells or microcapsules. From deteriorative reactions occurring during drying of foodstuff, those having a chemical basis are basically oxidation and Maillard reactions. Lipids, vitamins, carotenoids and phenolic compounds

are particularly sensitive to oxidation which, in turn, can take place enzymatically or non-enzymatically.

Lipid oxidation leads to the development of the typical aroma of many meat products, but also to the formation of unpleasant odors and flavors. From a nutritional point of view, oxidation may affect the fatty acid composition and fat quality of meat and fish products. Significant decrease in long chain polyunsaturated fatty acids (LC-PUFA) was reported during dry-cured ham processing (Gilles, 2009). Also the exposure to light and oxygen during sun drying and controlled oven drying induced a noticeable reduction of the most important Ω-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in both lean and fat fishes (Telahigue et al., 2013). In addition, free radicals and peroxides originated during lipid oxidation are closely related to the pathology of some cancers, arteriosclerosis, arthritis, neurodegenerative diseases and the aging process (Packer & Ong, 1998). Moreover, oxidized lipids can react with proteins and other food components and reduce their nutritional quality and safety (Lupano, 2013). Regarding the application of a salting process, brine contact with fish has also been reported to enhance lipid oxidation of the highly unsaturated lipids, which is directly related to the production of off flavor, protein denaturation and texture changes (Aubourg & Ugliano, 2002). Specific techniques reported to prevent lipid oxidation in fish oil processing include microencapsulation and the application of natural food additives with antioxidant capacity like rosemary extracts, α-tocopherol or polyphenols from grape pomace (Baik et al., 2004). Among simpler

technical proposals for reducing lipid oxidation during fish and meat drying, those focused on reducing the exposure to oxygen in the drying chamber and, to a lesser extent, the drying temperature are of particularly interest. To this end, satisfactory results have been reported from vacuum drying and ultrasonic vacuum drying (Başlar et al., 2014), microwave drying (Darvishi, 2013), ultrasound assisted drying (Awad, 2012), freeze drying (Babić, 2009) and low-pressure superheated steam drying. Although there is little evidence about the impact of such techniques on the lipid profile of treated products, one intuits that those treatments resulting in more porous structure entail greater risk of damage by oxidation during further storage. Also in fruits and vegetables, such techniques have been applied with satisfactory results in reducing pigments, vitamin C, phenolic compounds and other minor ingredients losses due to oxidation.

Carotenoids are natural pigments synthesized by plants and microorganisms. Their importance in human nutrition and health is mainly due to their capability to inhibit oxidative reactions. This property is particularly high in the case of lycopene, closely followed by α-carotene and □-carotene and, to a lesser extent, zeaxanthin (Bohm et al., 2002). Carotenoids may be free in the lipid phase of the food, forming complexes with proteins, bound to carbohydrates or as fatty acid esters. Carotenoids oxidation can be indirectly catalysed by lipoxygenase, the enzyme responsible for the peroxides formation from lipid oxidation of unsaturated fatty acids, and results in important colour changes and losses in antioxidant activity. Isomerization is also involved in carotenoids

loss during food dehydration. Indeed, naturally occurring carotenoids are in all-trans form, which is the most stable to heat treatments chemical form. Thermal treatments applied during food processing promote isomerization of trans-carotenoids to their cis-form, mainly on the 9-cis and 13-cis types, which is not entirely clear to adversely affect their ability to scavenge free radicals (Bohm et al., 2002). As reported in Hiranvarachat et al. (2008), 13-cis-β-carotene occurs in carrots as the product reaches 60 °C, when submitted to hot air drying, or even lower temperature, when applying vacuum drying and low-pressure superheated steam drying. Although the antioxidant activity is unaffected in this case, the conversion of trans-□-carotene in any of its cis-isomers might imply a notably decrease in its activity as vitamin A precursor (Chen et al., 1995). Negative effects of isomerization are usually offset by an increase in bioavailability. All-trans forms naturally existing in foods are linear, long and rigid molecules, whereas their cis isomers are shorter molecules that can be more easily solubilised, absorbed and transported at a cellular level (Periago et al., 2001). Even the irreversible degradation of carotenes by oxidation could be compensated by this increase in bioavailability (Hedrén et al., 2002). For this purpose, losses during processing should be minimized by one of the alternative to conventional drying techniques mentioned above. Preventing the loss of cellular integrity also contributes to diminish the incidence of oxidation, as well as some pretreatments, such as blanching and osmotic dehydration. Blanching benefits are attributed to enzyme inactivation, while

osmotic treatments for a short period in the presence of sucrose at 30-40 °C have been reported to encourage these phytochemicals generation (Heredia et al., 2009).

Other food components having beneficial health effects due to their high antioxidant and antimicrobial activity, and therefore being susceptible to oxidation, include polyphenols and ascorbic acid. Phenolic compounds and vitamin C are known to prevent free radicals formation and to reduce molecular damage on DNA, lipids and proteins, which is directly related to a decrease in the incidence of cancer and coronary diseases. They also play a decisive role in the colour and flavour of certain fruits and vegetables. Most of the polyphenols are present in foods as esters, glycosides or polymers, that is, as forms that can not be absorbed (Quiñones et al., 2012). However, as previously mentioned for other functional compounds, structural and chemical changes taking place during fruits and vegetables drying can contribute in increasing their bioavailability during further consumption. In general, reducing the contact with oxygen in the drying chamber by reducing the drying time reduces losses in phenolic compounds, but due to its greater sensitivity to high temperatures reducing the vitamin C losses might imply a noticeable decrease in the drying temperature (Wojdyło et al., 2009). In spite of these considerations, certain fruits and vegetables show an increase in their ability to scavenge free radicals after drying in adverse conditions (López et al., 2010), which has been explained in terms of the generation of new compounds with higher antioxidant activity as the ones resulting from the Maillard reaction.

The Maillard reaction or non-enzymatic browning reaction is the chemical reaction that occurs between compounds with a primary amine function and compounds with carbonyl groups, which generate different flavours and brown colour (Tamanna & Mahmood, 2015). This reaction is accelerated under alkaline conditions, intermediate moisture content ($0.55 < aw < 0.75$) and high temperatures, but it is also observed under refrigeration (Lupano, 2013). The type of compounds involved also influences the reaction rate, as well as the presence of certain metals. Logically meat and fish products, with a particularly high protein content, are the most susceptible to experience such reaction. However, by-products of the Maillard reaction have been also found in low in lysine products, such as fruits and vegetables. Adverse effects associated with this reaction include alteration of the organoleptic properties and decrease of the nutritional value since essential amino acids, mainly lysine, and certain vitamins, such as K and C, are generally involved. In addition, some of the compounds formed in the Maillard reaction are toxic or mutagenic. This is the case of high carboxymethyl lysine that promotes diabetes and cardiovascular diseases, and some recognized as a probable human carcinogen compounds, such as acrylamide and hydroxymethylfurfural (Tamanna & Mahmood, 2015). On the contrary, melanoidins formed at the last stage of the Maillard reaction are non digestible compounds having antioxidant and antimicrobial activity against pathogenic microorganisms of the colon (Lupano, 2013). Non-enzymatic browning in foods also includes caramelization reaction, but it

involves only sugars or polyhydroxycarboxylic acids and usually requires more drastic conditions. Since the pyrolysis of sugars starts at above 110 °C, caramelization incidence in foodstuff drying is not as worrisome as compared to other chemical reactions.

4. Energetic considerations

Drying is probably the most energy intensive process of the major industrial process it consumes large amounts of energy and releases significant amount of carbon oxides to the environment (Mujumdar, 2006). In an energy intensive industry like heating or drying, improving energy efficiency by 1% could result as much as 10% increase in profit (Beedie, 1995). Any small improvement in energy efficiency in food drying process will lead to a sustainable development to global energy perspective.

Condition of drying air has a great effect on the quality attributes of dried product. Thus, one of the key issues of drying technology is to reduce the cost of energy sources to increase the efficiency of drying facilities for good quality of dried products. On the other hand, the design of an energy-intensive system for lower cost and higher efficiency is one of the essential approaches for sustainable development (Aghbashlo et al., 2013).

There are a lot of studies modelling drying operation. Most of times, the models are directed to analyze heat and mass transfer in order to improve the quality of final products obtained. With the aim to evaluate the drying operation, there are a lot of studies directed to analyze the energy used during process in order to

optimize the drying method and contribute to the sustainability of the process. It is necessary to combine all process variables (drying process, installation design, time, temperature and product characteristics…) to minimize energetic and product losses.

Usually, an energy analysis is carried out in most of studies. The energy analysis is a basic and traditional approach to estimate various energy conversion processes (Nazghelichi et al., 2011). The energy analysis is based on the first law of thermodynamics, which expressed the principle of the conservation of energy. According to (Singh, 1977), energy analysis is useful in quantitative evaluation of energy requirements of energy generating and delivery systems and in the detection of mode and evaluation of energy loss. However, it provides no information about the irreversibility aspects of thermodynamic processes. The energy analysis is unable to distinguish the different qualities of energy such as heat quality which is dependent on the heat source temperature.

The exergy based analysis and subsequent optimization of drying processes is having a growing interest among the researchers. Exergy is the maximum amount of work obtainable from a stream of matter, heat or work when some matter is brought to a state of thermodynamic equilibrium with the common components of natural surroundings by means of reversible processes, and is a measure of the potential of a stream to cause change, as a consequence of not being completely stable relative to the reference environment (Dincer, 2002; Pandey et al.,

2012). The exergetic performance assessments not only distinguish the magnitudes, location and causes of irreversibilities in the plants, but also enables (Kaushik et al., 2011; Siva Reddy et al., 2012). The main objective of exergy analysis of drying systems is to provide a clear picture of the process, to quantify the sources of inefficiency, to distinguish the quality of energy consumption, to select optimal drying conditions and to reduce the environmental impact of drying systems. The exergy analysis is being applied to more and more products. In recent years, some articles have been published combining both energy and exergy calculations in order to have a more completed analysis and sustainability evaluation of the process (Aviara et al., 2014).

5. Conclusions

The development of functional foods can clearly contribute to the global concept of sustainability. The negative effects related to the application of extreme temperatures in drying operations can be minimized by incorporating ingredients that protect structural elements, creating protective structures and avoiding degradation reactions. Management of drying processes in an adequate way can contribute to prevent bioactive compounds losses, maintain and even increasing the functionality of dried products.

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3.1.1. Conclusiones

Tradicionalmente se ha considerado que la agricultura ecológica y el fomento de la producción de alimentos a nivel local son las estrategias a seguir para asegurar las sostenibilidad de los productos alimentarios. Sin embargo, existen una gran variedad de estragegias a nivel tecnológico que pueden contribuir con éxito a la sostenibilidad en su sentido más amplio, dirigiéndose hacia procesos más sostenibles que a su vez mejoren la funcionalidad de los alimentos. A través de la revisión bibliográfica realizada se ha podido constatar cómo, cada vez más, se están estudiando tecnologías existentes con el objetivo de mitigar los efectos negativos que ocasiona el procesado tradicional. En particular, aparecen como destacadas estrategias tales como la adición de ingredientes que reducen la degradación de compuestos bioactivos, la creación de elementos estructurales que protegen la funcionalidad de los compuestos bioactivos y la prevención de reacciones que causan degradación de compuestos bioactivos.

3.2. Tecnologías para la mejora de la funcionalidad de antioxidantes y probióticos

El presente capítulo incluye varios trabajos en los que se evalúa el efecto de diferentes operaciones unitarias (aplicación de presiones de homogeneización, encapsulación, impregnación a vacío y secado por aire caliente), y de la adición de trehalosa como ingrediente protector, sobre las propiedades funcionales de diferentes matrices alimentarias (zumo de mandarina y discos de manzana), sin y con microorganismo probiótico encapsulado y sin encapsular.

La aplicación de las presiones de homogenización (HPH) en la industria alimentaria ha aumentado en los últimos años. Aunque generalmente se utiliza para mejorar la estabilidad de alimentos fluidos y como alternativa a los tratamientos térmicos, el tratamiento mediante HPH tiene un efecto importante sobre los compuestos bioactivos de los alimentos y su biodisponibilidad. Los efectos de este tratamiento dependen principalmente de la presión aplicada. A bajos niveles de presión (hasta 30 MPa) los compuestos bioactivos no se ven afectados, en cambio a presiones comprendidas entre 200 y 300 MPa pueden degradarse los compuestos con actividad antioxidante. Por el contrario, al aplicar presiones de homogenización intermedias (entre 30 y 150 MPa) se rompen estructuras y se liberan compuestos retenidos en la microestructura del alimento, pudiendo aumentar su biodisponibilidad (Velazquez-Estrada, et al., 2013, Betoret et al., 2012). Por otro lado, la trehalosa puede ejercer un efecto protector sobre las estructuras celulares, proteínas y lípidos frente a la degradación y/o desnaturalización causadas por la deshidratación,

los fenómenos osmóticos, la oxidación, el frío o el calor (Elbein et al., 2003, Higgashiyama, 2002).

En este contexto, se planteó evaluar el efecto de la combinación de la aplicación de presiones moderadas de homogeneización (20, 50, 100 y 150 MPa) y la adición de trehalosa (0, 10 y 20 g/100 g) como ingrediente protector de la estructura, sobre propiedades tecnológicas tales como la estabilidad de la nube y el tamaño de partícula, así como la capacidad antirradical, y el contenido en fenoles y flavonoides totales del zumo de mandarina. El tratamiento por HPH redujo el tamaño de la pulpa, mejoró la estabilidad de la nube y aumentó el contenido en fenoles totales y flavonoides. La trehalosa mejoró la actividad antioxidante determinada por el ABTS cuando se añadío en cantidades de 20 g/100g.

Con respecto a la aplicación de presiones de homogenización sobre microorganismos probióticos, la aplicación de presiones comprendidas entre 200 y 400 MPa produce un descenso notable en la carga microbiana, mientras que la aplicación de presiones de homogeneización moderadas, entre 30 y 150 MPa, inducen cambios en las membranas celulares que pueden mejorar la funcionalidad de probióticos y aumentar su hidrofobicidad (Capra et al., 2009). Adicionalmente, la incorporación de ciertos ingredientes como la trehalosa puede mejorar la resistencia de los microorganismos probióticos a condiciones de estrés y, en consecuencia, su supervivencia durante el procesado y posterior almacenamiento (Colaço y Roser, 1994). Es por ello que también

se planteó como objetivo evaluar el efecto de la presión de homogenización (0, 20 y 100 MPa) y la adición de trehalosa (0, 10 y 30 g/100 g) sobre las propiedades fisicoquímicas, hidrofobicidad de *Lactobacillus salivarius* spp. *salivarius* así como su viabilidad durante 10 dias de almacenamiento en zumo de mandarina. Este microorganismo se escogió por su probado efecto probiótico y también por su efecto frente a la infección ocasionada por *Helicobacter pylori,* y por la experiencia previa del grupo de investigación en el que se ha realizado esta tesis. A partir de este estudio se constató que tanto la aplicación de presiones de homogeneización como la adición de trehalosa mejoraron la hidrofobicidad del probiótico y su supervivencia tras 10 días de almacenamiento.

Los microorganismos probióticos favorecen el equilibrio de la flora intestinal ejerciendo un efecto beneficioso en la prevención de diferentes enfermedades y/o desórdenes fisiológicos, tales como enfermedades infecciosas, inflamatorias del tracto gastointestinal, algunos tipos de cáncer, o la obesidad (Rostami et al., 2018, Rouxinol-Dias, et al., 2016, Subramanyam, 2017). Las operaciones tecnológicas, la matriz alimentaria, las condiciones y el tiempo de almacenamiento, así como el paso por el tracto gastrointestinal, pueden mermar la viabilidad de los microorganismos con potencial efecto probiótico (Ranadheera, 2010 y Ying et al., 2010). Algunas técnicas, como la encapsulación, se plantean como una estrategia para mejorar la viabilidad de microorganismos. Entre los diferentes métodos de encapsulación, la aplicación de altas presiones de homogeneización es más económica y permite

trabajar a nivel industrial en un proceso en continuo. Es por esto que se planteó como objetivo evaluar la viabilidad de las presiones de homogeneización como tecnología para encapsular microorganismos probióticos y cuantificar su efecto sobre la supervivencia durante el almacenamiento y la resistencia gastrointestinal del microorganismo durante este tiempo. Los resultados obtenidos en zumo de mandarina muestran que el probiótico encapsulado presentó mayor supervivencia durante el almacenamiento y el proceso de digestión *in vitro* que en el caso del probiótico sin encapsular. En los discos de manzana, en los que el probiótico se habría incorporado mediante impregnación a vacío, la encapsulación apenas tuvo efecto sobre la supervivencia del probiótico tras el secado de las manzanas con aire a 40 ºC, aunque nuevamente aumentó su resistencia al proceso de digestión *in vitro*.

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Improvement of technological properties and antiradical capacity of mandarin juice by means of homogenization pressure and trehalose addition.

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Highlights

HPH reduces particle size and releases antioxidant components of mandarin juice.
HPH enhances cloud stability and phenols and flavonoids content.
Protective effect of trehalose results in an increase of the juice impregnated.
Trehalose acts as a secondary antioxidant protecting antioxidants of the juice.

Abstract

Increasing food products functionality ensuring nutritional and specific functional benefits is a challenge of today's food industry. Improving food functionality may be achieved not only with the use of specific ingredients but also with a proper management of food process technologies. The aim of this research was to quantify the effect of the combination of a non-thermal and emerging technology, High Pressure Homogenization (HPH) at 20, 50, 100 and 150 MPa, and a structural protective ingredient (trehalose at 10% and 20% w/w) on the technological properties and antiradical

capacity, phenols and flavonoids, of mandarin juice. The different juices obtained were characterized by measuring pH, a_w, total soluble solids, vacuum impregnation capacity, particle size, suspended pulp and cloudiness. As for their antioxidant properties, total phenolic content, flavonoids, and antioxidant capacity by the ABTS and DPPH methods were measured. The most relevant effects were a reduction of particle size, an increase in juice cloud stability and in phenols and flavonoids availability as a consequence of the physical changes induced by HPH into the suspended particles. Regarding the protective effect of trehalose, a modification of the impregnation properties of the apple tissue was observed.

Keywords:

Homogenization pressure, trehalose, antioxidant properties, particle size, cloudiness, mandarin juice.

1. Introduction

Sustainability of the agri-food industry is a priority challenge for the European Union. It is focused mainly in an improved management of food processes directed to exploitation and valorization of by-products as well as to increase the functionality of the food products ensuring nutritional and specific functional benefits. Both objectives require a proper management of the processing technologies emphasizing the use of emerging technologies with an improved effect on bioactive compounds

(Betoret et al., 2015). This means to preserve the active molecular form until the time of consumption or to induce molecular changes that increase its bioactivity, and deliver the active form to the physiological target within the organism. With regard to the use of different technologies to improve the functional value of foods, Wojodylo et al. (2014) showed that drying treatments may release bound phytochemicals from a cellular matrix to make them more bioaccesible and, additionally, vacuum-microwave drying has a positive influence on contents of the compounds from the quercetin in sour berries. García- Parra et al., (2018) increased the content of carotenoids in pumpkin by the application of moderate-intensity pulsed electric field. Moreover, the negative impact of some processing operations can be minimized by incorporating ingredients that protect structural elements or create protective structures. For example, a variety of protectants are usually added to the drying media before freeze-drying or spray-drying to prevent probiotics damage during dehydration, including skimmed milk powder, whey protein, trehalose, glycerol, betaine, adonitol, sucrose, glucose, lactose and polymers, such as dextran and polyethylene glycol (Morgan et al., 2006).

High pressure homogenization (HPH) is a non-thermal technology, which was initially used as an alternative to thermal processing. However, the impact of HPH treatment on the food product depends to a great extent on the pressure applied, for which different applications may be derived by modifying the working pressure. With regard to its effect on the bioactive compounds contained in fruit juices, HPH has an impact on the

microstructure of suspended particles, influencing the bioaccessibility of bioactive compounds retained in them, and therefore their bioavailability Velazquez-Estrada et al., (2013). Some changes impinged to the cell walls and membranes, such as breakdown or permeability increase, can favour the release of compounds making them more bioaccesible; in addition, this release of bioactive substances can also promote their reaction with others, resulting in novel bioactive compounds. In particular, Velazquez-Estrada et al., (2013) showed that high homogenization pressures (between 200 and 400 MPa) caused a degradation of components with antioxidant activity in orange juice and cranberry juice, although the treatment improved the content on bioactive compounds and the antioxidant properties of orange juice, as compared to thermal pasteurization. On a lower range of pressures, Betoret et al., (2012) demonstrated that the application of a HPH treatment up to 30 MPa (more accepted as standard homogenization pressure) in mandarin juice does not produce a significant improvement in the antioxidant properties of the juice but it does improve some physicochemical and technological properties such as yield, viscosity and colour. Furthermore, homogenization technology can be very useful as a pre-treatment prior other technological operations such as vacuum impregnation, where reduction of particle size enhances juice penetration in the food matrix (Betoret et al., 2009).

The addition of specific ingredients in order to preserve the food structure or its components can also be used to improve food functional properties. Accordingly, benefits and applications of

trehalose have been widely studied. Trehalose is a non-reducing sugar, stable under heat and acid environment, which increases many organisms tolerance to stress (Crowe et al., 1984; Li et al., 2010). It protects proteins, lipids and cellular membranes from inactivation, degradation and denaturalization caused by dehydration, osmotic phenomena, oxidation, cold and heat (Elbein et al., 2003; Higashiyama, 2002). In particular, Elbein et al. (2003) suggested that trehalose protects from oxygen radicals damage due to it action as a free radical scavenger. On the other hand, trehalose ability to stabilize structures has been attributed to its capability of binding the double bounds of the acyl chains (Oku et al., 2003). The acyl group is present in the main mandarin juice flavonoids, for which its antiradical capacity could be affected by the presence of trehalose. Moreover, it needs to be taken into account that the addition of trehalose as a protective ingredient to the mandarin juice juice could have effect on the response of the solid matrix when the juice is incorporated to it by vacuum impregnation.

Therefore, the aim of this research was to evaluate the effect that the combination of a non-thermal emerging technology (high pressure homogenization at 20, 50, 100 and 150 MPa) together with a structural protective ingredient (trehalose at 10% and 20% w/w) have on the technological properties and antiradical capacity, phenols and flavonoids content of a low pulp mandarin juice.

2. Materials and methods

2.1. Food materials and sample preparation

Mandarin juice was obtained from mandarin cv. *Ortanique* (hybrid *Citrus sinensis* x *Citrus reticolata*) from a cooperative located in Benaguacil (Valencia, Spain). To produce the juice, mandarins were washed with tap water, drained and squeezed with a citrus juicer (Citromatic Deluxe MPZ 22, Braun). Pulp was separated from the obtained juice by a mesh with a sieve opening of 0.7 mm. Juice with reduced pulp content was then centrifuged at 3650 x g for 5 minutes at 5 ºC (Medifriger BL, P-Selecta, Spain) and frozen at -18 ºC in sterilized glass jars until the different analyses.

When appropriate, 10% and 20% (w/w) food-grade trehalose (TREHA™, Cargill Ibérica, Barcelona, Spain) was added to the thawed juice. Juices with and without trehalose were subjected to homogenization at 20, 50, 100 or 150 MPa in a pilot-plant scale homogenizer (GEA Niro Soavi Panda PLUS, Parma, Italy) and refrigerated at 5ºC until the analysis.

Apples (cv. *Granny Smith*), used in vacuum impregnation experiments, were purchased in a local market and cut into 5 mm thick slices. Peel and seeds were removed with two cylindrical cutters, obtaining apple discs of 20 mm internal diameter and 65 mm external diameter.

2.2. Physicochemical characterization

Total soluble solids, water activity and pH

Total soluble solids (TSS) were determined by measuring the Brix degrees (º Brix) with a refractometer (ABBE ATAGO 3T, Japan) at 21 (± 1) ºC. Water activity was obtained with a dew point hygrometer (Aqualab 4TE; Decagon devices, Pullman, WA, USA, ± 0.003). pH was determined with a digital pH-meter (Mettler Toledo Gmbh., Schwerzenbach, Switzerland).

Particle size

A Mastersizer 2000 equipment (Malvern Instruments, Worcestershire, UK) was used to determine particle size. This equipment uses laser diffraction with a short wavelength blue light with backscatter detection to measure particle size in the range 0.02 to 2000 μm. The refraction indexes used for the juices were 1.73 and 1.33 for the cloud and the dispersed phase, respectively. The absorption index of cloud particles was 0.1 (Correding et al., 2001). Results of particle size distribution are based in volume measurements, so that the values given were obtained as the percentage of the volume of the particles with a specific diameter with respect to the total volume of all particles in the distribution. Particle size was then characterized by two different equivalent diameters: the volume-weighted mean diameter (D [4,3]; De Brouckere mean Diameter) and the surface area mean diameter (D [3,2]; Sauter mean diameter); as well as by

the percentiles of the distribution: d_{10}, d_{50} and d_{90} (Instruments, M., 2007).

Suspended pulp and cloudiness

Suspended pulp was measured by centrifugation of 10 mL of juice at 366 x g (for unstable pulp content) or 3000 x g (for more stable pulp content) for 10 min at 25 ºC (Medifriger BL, P-Selecta, Spain). Pulp was obtained by the difference between the initial volume and the volume of the supernatant at each centrifugal force (FMC Food Tech, 2005). Turbidity was obtained from the transmittance (T%) of the supernatant measured with an UV/Visible spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, England) at 650 nm.

2.3. Impregnation parameters

Impregnation capacity of the different juices was tested on a pilot-plant scale equipment specifically designed for this purpose (Fito et al., 1996). Apple discs were submerged in the impregnation liquid and a 50 mbar vacuum pressure was applied during 10 min. After that, atmospheric pressure was restored while sample remained immersed in the liquid for 10 min more. The evolution of sample weight was registered during the experiment according to a previously described procedure (Fito, 1994). Results obtained from this experiment allowed to calculate the volume fraction of the initial sample that is filled with the external liquid at the end of the vacuum step (X_1) or the atmospheric pressure step (X), as well as the volume fraction of the initial sample that is deformed

after both the vacuum (γ_1) or the atmospheric pressure step (γ), and the apple effective porosity ε_e.

2.4. Total phenols and flavonoids

Total phenols content of the juice samples was measured with a modified Folin- Ciocalteau method (Wolfe et al., 2003; Singleton et al., 1999). Measurements were performed on a 1:5 (v/v) dilution of the juice in distilled water. First, 0.125 mL of the diluted sample were added to 0.5 mL of distilled water in a spectrophotometry cuvette. Then, 0.125 mL of Folin-Ciocalteau reagent (Sigma-Aldrich) were added to the mixture and let it react for 6 min in dark conditions. Finally, 1.25 mL of a 7% (w/v) sodium carbonate solution and 1 mL of distilled water were added. After 90 min of reaction, absorbance was measured at 760 nm in a spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, England). Gallic acid (purity ≥ 98%, Sigma-Aldrich) was used as a standard. Results were expressed in mg of Gallic Acid Equivalents (GAE) per 100 mL of juice without trehalose.

Flavonoid content was determined by means of the aluminium chloride colorimetric method (Luximon- Ramma et al., 2005). In this case, 1.5 mL of a 1:5 (v/v) sample juice dilution was mixed with 1.5 mL of aluminium chloride solution (2% in methanol). The mixture was shaken and allowed to react for 10 min. Subsequently, the absorbance was read at 368 nm. Quercetin (purity ≥ 95%, Sigma-Aldrich) was used as a standard. Results were then given in mg of quercetin equivalents (QE) per 100 mL of juice without trehalose.

2.5. Antioxidant activity

Antioxidant activity of juices, with and without trehalose and subjected to different homogenization pressures, was measured evaluating their radical scavenging abilities by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) and 2,20-azobis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS) methods.

DPPH method (Brand-Williams et al., 1995) consisted of mixing 30 μL of a 1:20 (v/v) juice: water dilution with 970 μL of methanol and a 2 mL of a DPPH-methanol solution (0.1 mM). Absorbance was read at 517 nm after 60 min of reaction with a spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, England). Results were then expressed in mg of Equivalent Trolox (ET) per L of juice without trehalose.

The ABTS or TEAC method (Trolox Equivalent Antioxidant Capacity) was applied as explained by Re et al., (1999). This method assesses the capacity that the antioxidants present in the sample have to scavenge the radical cation ABTS+ compared to the antioxidant standard trolox. The radical ABTS+ was released by reacting 7 mM of ABTS with potassium persulfate (2.45 mM) during 16 hours at room temperature in dark conditions. A solution of ABTS+ in phosphate buffer (pH 7.4) was prepared to reach an absorbance of 0.700 ± 0.02, at 734 nm (Helios Zeta UV/Vis, Thermo scientific). Then 90 μL of the sample were added to 2910 μL of this solution and the absorbance of samples was read after 1, 3 and 6 min of reaction. Results were given in mg of Equivalent Trolox (ET) per L of juice without trehalose.

2.6. Statistical analysis

All determinations were done in triplicate. Analysis of variance test (ANOVA) was performed using Statgraphics centurion XVI software with a 95% confidence level (p-value ≤ 0.05).

3. Results and discussion

3.1. Effect of trehalose and high pressure homogenization on the physicochemical properties of mandarin juice

Water activity, moisture content and total soluble solids (TSS) of low pulp mandarin juice with and without trehalose and homogenized at different pressures are given in table 1. The statistical analysis of the experimental data concluded that homogenization pressure did not significantly affect any of these physicochemical parameters. On the contrary, and as it was expected, addition of trehalose to the mandarin juice produced a significant increase in the TSS and the corresponding decrease in the water activity values. pH was slightly increased as a result of the addition of trehalose.

Particle size distribution of juice samples are shown in figure 1. As it can be observed, juice homogenization caused a significant reduction of particle size in all cases, for any applied pressure. All the analyzed juices were characterized by a bimodal distribution of particle size. In the case of homogenized samples, these showed a bimodal distribution with one pick around 0.1-0.2 μm and another one below 100 μm (70-80 μm), the smaller size particles

being the most abundant ones. Smaller particles in the non-homogenized juice were in the range of bigger particles in homogenized juices (70-80 μm), the other pick being close to 1000 μm. Trehalose addition did also influenced particle size distribution, especially in non homogenized juices, which experimented a displacement of the distribution towards bigger sizes, as well as a significant increase in the ratio of bigger particles.

Table 1. Water activity, total soluble solids (ºBrix) and pH of mandarin juice subjected to HPH treatments and trehalose (Tre) addition. Mean ± standard deviation of three replicates.

Tre (%)	HPH (MPa)	Water activity (a_w)	TSS (º Brix)	pH
0	0	0.988±0.003^{e}	13.63±0.06^{a}	3.397±0.015^{c}
	20	0.986±0.003^{e}	14.47±0.06^{c}	3.40±0.02^{c,d}
	50	0.987±0.003^{e}	14.43±0.06^{c}	3.350±0.010^{b}
	100	0.986±0.003^{e}	15.03±0.15^{d}	3.283±0.015^{a}
	150	0.987±0.003^{e}	14.03±0.06^{b}	3.39±0.02^{c}
10	0	0.979±0.003^{c}	21.50±0.17^{e}	3.400±0.017^{c,d,e}
	20	0.980±0.003^{c,d}	21.42±0.03^{e}	3.433±0.006^{e,f}
	50	0.981±0.003^{c,d}	21.47±0.06^{e}	3.430±0.010^{e,f}
	100	0.982±0.003^{d}	21.43±0.06^{e}	3.39±0.03^{c,e,f}
	150	0.981±0.003^{c,d}	21.43±0.06^{e}	3.430±0.010^{d,e,f}
20	0	0.973±0.003^{a,b}	28.03±0.06^{f}	3.410±0.010^{e,f}
	20	0.972±0.003^{a}	28.50±0.02^{h}	3.443±0.006^{f}
	50	0.972±0.003^{a}	28.47±0.06^{h}	3.430±0.010^{e,f}
	100	0.974±0.003^{a,b}	28.50±0.02^{h}	3.430±0.010^{e,f}
	150	0.975±0.003^{b}	28.33±0.06^{g}	3.423±0.015^{e,f}

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

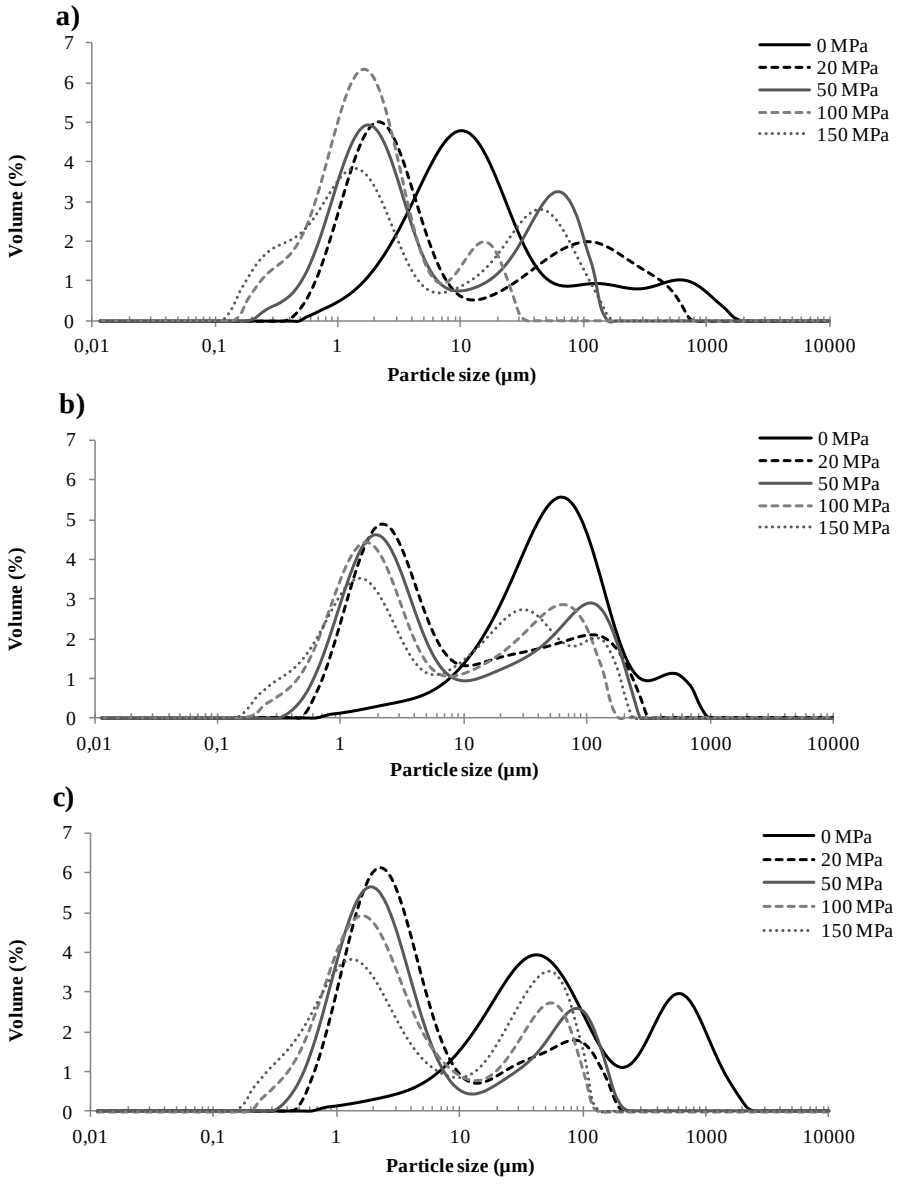

Figure 1. Distribution of particle size on the mandarin juices at different concentration of trehalose and processed at different homogenization pressures. a) juices with 0% of trehalose, b) juices with 10% of trehalose, c) juices with 20% of trehalose.

Table 2 shows some characteristic parameters that describe particle size distribution of the juices. The existing differences between D [4, 3] and D [3, 2] values evidence, in all cases, the presence of particles with different shape and size. Differences in the amount of suspended pulp of juice as a consequence of the application of various homogenization pressures, and the presence of non solubilised chemical components of different nature can explain this result. Coherently with the distributions shown in figure 1, it can be observed that D [4, 3] and D [3, 2] are higher in non homogenized juices than in the homogenized ones. The effect of trehalose over particle size distribution, when non pressure is applied, results in a significant effect in all the characteristic parameters of the distribution.

Table 2. Values of D [4,3]), (D [3,2]), d_{10}, d_{50} and d_{90} characteristic parameters that describe particle size distribution of the juices treated at different pressures and with different percentages of trehalose. Mean ± standard deviation of five replicates.

Trehalose (%)		0 MPa	20 MPa	50 MPa	100 MPa	150 MPa
D (4,3)	0	108±34^{d}	26±4^{a,b,c}	27±6^{a,b,c}	2.9±0.2^{a}	19±3^{a,b}
	10	57±28^{c}	38±7^{b,c}	29±3^{a,b,c}	36±25^{b,c}	24±3^{a,b}
	20	310±51^{e}	19±7^{a,b}	20±2^{a,b}	17±2^{a,b}	18±3^{a,b}
D (3,2)	0	6.3±0.4^{a}	2.27±0.08^{a}	1.9±0.3^{a}	0.89±0.12^{a}	1.04±0.05^{a}
	10	12±5^{a}	3.1±0.3^{c}	2.42±0.04^{a}	1.92±0.07^{a}	1.63±0.06^{a}
	20	24±4^{b}	2.48±0.19^{a}	2.08±0.08^{a}	1.66±0.05^{a}	1.42±0.03^{a}
d_{10}	0	2.62±0.13^{b}	0.990±0.017^{a}	0.79±0.07^{a}	0.35±0.08^{a}	0.353±0.015^{a}
	10	6±3^{c}	1.20±0.05^{a,b}	0.956±0.009^{a}	0.753±0.019^{a}	0.596±0.018^{a}
	20	11±2^{d}	1.08±0.03^{a,b}	0.883±0.015^{a}	0.68±0.010^{a}	0.523±0.006^{a}
d_{50}	0	11.1±1.2^{a}	2.84±0.13^{a}	3.1±0.8^{a}	1.45±0.08^{a}	2.2±0.2^{a}
	10	28±14^{b}	5±2^{a}	3.59±0.14^{a}	3.5±0.3^{a}	5.5±0.9^{a}
	20	74±18^{c}	3.1±0.3^{a}	2.69±0.17^{a}	2.51±0.14^{a}	2.97±0.19^{a}
d_{90}	0	417±148^{c}	93±12^{a,b}	79±9^{a,b}	8.8±1.0^{a}	60±6^{a,b}
	10	143±71^{b}	118±15^{b}	99±7^{a,b}	77.0±11.4^{a,b}	77±10^{a,b}
	20	959±133^{d}	68±22^{a,b}	75±7^{a,b}	59±7^{a,b}	59±10^{a,b}

D [4,3] (De Brouckere mean Diameter) the volume-weighted mean diameter; D [3,2]; (Sauter mean diameter) surface area mean diameter; and d_{10}, d_{50} and d_{90}, defined as the particle size which 10%, 50% and 90% of the distribution is below this size, respectively.

$^{a,b,c...}$Values with different superscript letters within the same parameter are significantly different ($p < 0.05$).

Results of **suspended pulp and cloudiness** of the juices are shown in table 3. Homogenization pressure had a significant impact on bigger or less stable pulp (precipitated at 366 x g), whereas it slightly affected more stable pulp (precipitated at 3000 x g). On the contrary, addition of trehalose did not have a clear effect on pulp stability, it suggesting that, even if trehalose modifies particle size it does not influence pulp/cloud stability.

Table 3. Obtained results of suspended pulp (%) and cloudiness (%) at two levels of centrifugation of the juices treated at different pressures and with different percentages of trehalose. Mean ± standard deviation of three replicates.

Trehalose (%)		0 MPa	20 MPa	50 MPa	100 MPa	150 MPa
Suspended pulp (%) at 366 x g	0	10.7±1.2^{d}	0.3± 0.6^{a,b}	0.00±0.00^{a}	0.3±0.6^{a,b}	0.00±0.00^{a}
	10	5±3^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.3±0.6^{a,b}	0.3±0.6^{a,b}
	20	11.3±1.2^{d}	0.7±1.2 ^{a,b}	1.3±1.2^{a,b}	0.00±0.00^{a}	2.00±0.00^{b}
Suspended pulp (%) at 3000 x g	0	2.7±1.2^{c,d,e}	0.7±0.6^{a,b}	1.00±1.00^{a,b}	2.00±0.00^{b,c,d}	0.00±0.00^{a}
	10	3.3±1.2^{d,e,f}	3.7±0.6^{e,f}	2.00±0.00^{b,c,d}	1.33±0.6^{a,b,c}	1.7±0.6^{b,c}
	20	4.7±1.2^{f}	3.3±1.2^{d,e,f}	1.3±1.2^{a,b,c}	1.3±1.2^{a,b,c}	0.00±0.00^{a}
Cloudiness (%) at 366 x g	0	21±2^{d}	8.85±0.15^{a}	13.0±0.2^{a,b}	8.6±0.7^{a}	17.84±0.17^{c}
	10	33±5^{e}	9.4 ±0.7^{a}	10.97±0.13^{a,b}	12.4±0.2^{a,b}	14.4±0.3^{b}
	20	24.0±0.8^{f}	15.63±0.04^{e}	17.8±0.5^{e}	21.4±0.2^{e}	24.0±0.2^{e}
Cloudiness (%) at 3000 x g	0	61.4±1.0^{h}	34.6±1.7^{b}	34.6±1.0^{b}	37±3^{c}	38.8±0.9^{d}
	10	72.0±0.2^{j}	47.6±0.5^{f}	53.5±0.3^{g}	43.4±0.6^{e}	38.17±1.10^{c,d}
	20	67.1±1.3^{i}	32.7±0.8^{a}	33.8±1.0^{a,b}	34.3±0.6^{a,b}	34.62±0.13^{b}

$^{a,b,c...}$Values with different superscript letters within the same parameter are significantly different ($p < 0.05$).

According to the transmittance values obtained as a measure of cloudiness, homogenization again has an impact in the results by reducing transmittance at both levels of centrifugation. This is

coherent with the reduction in particle size previously discussed and, consequently, an increase in cloud stability.

Changes in particle size distribution, suspended pulp and cloudiness produced as a consequence of trehalose addition or the application of homogenization pressures, could imply modifications in some technological applications of the juice. For example, Betoret et al. (2012) demonstrated the interest of using fruit juices to formulate natural functional foods by vacuum impregnation, showing the relevance of particle size and suspended pulp in the effectiveness of the operation. In the present work, vacuum impregnation parameters as a response of the applied variables were also studied (table 4).

As indicated, table 4 summarizes **vacuum impregnation properties** of the different juice samples. According to the model applied (Fito et al., 1994), the X value stands for the volume of liquid incorporated to the solid sample. As stated by Fito et al., (1996) this volumetric fraction is the result of all fluxes generated as a consequence of not only of pressure but also of water activity gradients between the impregnation liquid and the solid matrix. As it has been deduced previously (table 1), adding 10 or 20 g/100 g of trehalose to the juice formulation reduced its water activity below that of raw apple (0.984 ± 0.002 according to Martinez-Monzó et al., (1998). Therefore, overall mass changes undergone by the solid matrix during the impregnation procedure was not only due to the liquid inflow as a result of impregnation, but also to some water outflow promoted by water activity differences

between juices and raw apple. It was deduced then that X values reported in table 4 resulted lower to the real ones, for which they were corrected taking into account the amount of water lost (table 5). It can be observed that addition of trehalose significantly increased the amount of juice incorporated to the tissue, especially when non homogenization pressure was applied. Protective effect of trehalose over cellular structures (Miller et al., 1997) including membranes (Crowe and Crowe, 1982) might have modified the viscous-elastic properties of fruit tissue, thus affecting the deformation capacity and, as a result, the impregnation parameters (Fito et al., 1996; Salvatori et al., 1998).

Contrarily to what might be expected, the application of a homogenization pressure did not cause a significant increase in the amount of liquid incorporated. Apple porosity varies with cultivars and its porous form and size depends on the position relatively to the core (Mendoza et al., 2010). A mean pore size diameter of 12.07 ± 2.69 μm, a linear weighted average diameter of 109.0 ± 82.1 μm and a D [3,2] of 142.6 ± 95.6 μm, was reported for raw apple by Rahman et al., (2005). Taking into account the distribution of particle sizes in the homogenized and non homogenized juices, and a proper arrangement of the pores in the apple tissue, more than 50% of the particles will be able to go into

Table 4. Impregnation characteristic parameters of apple with juices with trehalose treated at different pressures as impregnation liquids. Mean ± standard deviation of three replicates.

	Trehalose	0 MPa	20 MPa	50 MPa	100 MPa	150 MPa
	0%	0.1055±0.0007^{d,e,f}	0.029±0.002^{c,d,e}	0.069±0.016^{c,d,e}	0.013±0,008^{b,c}	0.022±0.002^{c,d}
γ_1	10%	0.12±0.08^{e,f}	0.050±0.017^{c,d,e}	-0.079±0.006^{a,b}	-0.13±0.06^{a}	0.024±0.004^{c,d}
	20%	0.112±0.015^{d,e,f}	0.19±0.13^{f}	0.065±0.013^{c,d,e}	-0.017±0.005^{b,c}	0.026±0.017^{c,d}
	0%	-0.0060±0.0014^{f,g}	-0.042±0.004^{b,c,d,e,f}	-0.028±0.007^{d,e,f}	-0.050±0.007^{b,c,d,e,f}	-0.0130±0.0014^{e,f,g}
γ	10%	0.032±0.011^{g}	-0.036±0.006^{c,d,e,f}	-0.065±0.004^{b,c,d,e}	-0.0605±0.001^{b,c,d,e}	-0.077±0.003^{b,c,d}
	20%	-0.13±0.07^{a}	-0.09±0.04^{a,b,c}	-0.09±0.04^{a,b,c}	-0.08±0.03^{a,b,c,d}	-0.074±0.008^{b,c,d}
	0%	0.1360±0.0014^{e,f,g}	0.0780±0.0014^{a,b,c}	0.091±0.009^{a,b,c,d}	0.059±0.006^{a}	0.066±0.007^{a,b}
X_1	10%	0.17±0.03^{f}	0.114±0.005^{c,d,e}	0.0745±0.0007^{b,c}	0.093±0.004^{a,b,c,d}	0.122±0.004^{d,e,f}
	20%	0.088±0.011^{a,b,c,d}	0.16±0.06^{f,g}	0.078±0.005^{a,b,c}	0.096±0.006^{b,c,d}	0.099±0.004^{b,c,d}
	0%	0.217±0.016^{f,g}	0.205±0.006^{e,f}	0.2190±0.0014^{f,g}	0.232±0.004^{g}	0.236±0.012^{g}
X	10%	0.200±0.007^{d,e,f}	0.158±0.006^{b,c}	0.166±0.008^{c}	0.16±0.02^{b,c}	0.12±0.02^{a}
	20%	0.137±0.004^{a,b}	0.161±0.003^{b,c}	0.212±0.011^{f,g}	0.180±0.011^{c,d,e}	0.178±0.006^{c,d}
	0%	0.270±0.003^{c,d,e}	0.252±0.005^{b,c,d}	0.29±0.03^{d,e,f}	0.289±0.013^{d,e,f}	0.257±0.004^{b,c,d}
ε_e	10%	0.192±0.005^{a}	0.216±0.005^{a,b}	0.221±0.017^{a,b}	0.23±0.03^{a,b,c}	0.21±0.02^{a}
	20%	0.20±0.05^{a}	0.302±0.006^{e,f}	0.32±0.03^{f}	0.275±0.015^{d,e,f}	0.291±0.004^{d,e,f}

X (m^3 penetrated liquid/m^3 of sample), X_1 (m^3 penetrated liquid/m^3 of sample during impregnation stage), volumetric deformation as consequence of vacuum impregnation γ (m^3 variation of volume/m^3 of sample), γ_1 (m^3 variation of volume/m^3 of sample during impregnation stage) and effective porosity ε_e (m^3 of gas/m^3 of sample).

$^{a,b,c...}$Values with different superscript letters within the same parameter are significantly different ($p < 0.05$).

Table 5. Value of $X_{corrected}$ (m^3 penetrated liquid/m^3 of sample) correcting the osmotic effect of trehalose.

	Trehalose	0 MPa	20 MPa	50 MPa	100 MPa	150 MPa
$X_{corrected}$	0%	0.23 ± 0.02^{ab}	0.22 ± 0.03^{ab}	0.2192 ± 0.0013^{ab}	0.223 ± 0.015^{ab}	0.24 ± 0.12^{ab}
	10%	0.265 ± 0.019^{cd}	0.24 ± 0.03^{cd}	0.223 ± 0.013^{cd}	0.27 ± 0.08^{cd}	0.23 ± 0.03^{cd}
	20%	0.244 ± 0.018^{abcd}	0.29 ± 0.06^{abcd}	0.28 ± 0.04^{abcd}	0.25 ± 0.04^{abcd}	0.275 ± 0.008^{abcd}

$^{a,b,c...}$Values with different superscript letters are significantly different ($p < 0.05$).

the apple matrix by vacuum impregnation and application of homogenization pressures should increase this value to 90% of the particles of the juices. Since reported values do not allow deducing if the larger particles in the juices could have blocked the access to the pores of the apple matrix, it is not possible to clarify why the impregnation parameters shown in table 5 have been significantly affected by homogenization pressure.

3.2. Effect of trehalose addition and high homogenization pressure on the antioxidant properties of mandarin juice

Total phenol and flavonoid content of mandarin juices are shown in table 6. In juices without trehalose, it can be observed that homogenization at any pressure increases phenols but mainly flavonoids content. These results are similar to those obtained by Perez-Conesa et al., (2009) in tomato juice and by Velazquez-Estrada et al., (2013) for flavanone content in orange juice. It can be explained taking into account the homogenization effect on particle size of suspended particles and therefore in cloudiness of the juices. Considering the available knowledge on cloudiness and turbidity in fruit juices, it can be assumed that the turbid substances mainly consist of suspended proteinacious pectin particles (Grassing and Fauquemberge, 1996; Hilz et al., 2005). However, fractions of other type of cell wall material might also have contributed to part of the turbidity. The precise locations of flavonoids and phenolic compounds in mandarin fruit is in the

Table 6. Total phenol (mg GAE/ 100 mL juice without trehalose) and flavonoid (g QE/100 mL juice without trehalose) content of different juices. Mean ± standard deviation of three replicates.

	trehalose	0 MPa	20 MPa	50 MPa	100 MPa	150 MPa
Total phenol content (mg GAE /100 mL juice without trehalose)	0%	$62.7\pm0.9^{e,f}$	79.7 ± 0.8^{h}	71 ± 0.5^{g}	71.2 ± 1.9^{g}	71 ± 0.5^{g}
	10%	$55.0\pm1.4^{c,d,e}$	$56\pm4^{d,e}$	$59\pm3^{e,f}$	60 ± 3^{f}	61 ± 3^{f}
	20%	$48\pm2^{b,c,d}$	43 ± 2^{a}	$44.3\pm0.8^{a,b}$	$48.3\pm0.8^{c,d}$	$47\pm3^{b,c}$
Flavonoid content (mg QE /100 mL juice without trehalose)	0%	30.1 ± 0.2^{a}	58.0 ± 1.2^{a}	51.6 ± 1.4^{a}	49.8 ± 0.2^{a}	47.92 ± 1.19^{a}
	10%	32 ± 7^{e}	$31.2\pm1.6^{d,e}$	$29.3\pm0.7^{c,d,e}$	27.0 ± 1.3^{c}	$27.44\pm0.04^{c,d}$
	20%	11 ± 2^{b}	11 ± 3^{b}	12.5 ± 0.6^{b}	11.7 ± 0.5^{b}	13 ± 4^{b}

$^{a,b,c...}$Values with different superscript letters within the same parameter are significantly different ($p < 0.05$).

flavedo and albedo tissues, an important fraction of which is incorporated as suspended particles to the juice. As a consequence of cell wall disruption and particle size reduction when high homogenization pressures are applied, these compounds become more available (Arnao *et al.,* 2000).

Nevertheless, when trehalose is added, homogenization effect disappears, suggesting that trehalose establishes some type of interaction with flavonoid compounds. As described by Higashiyama, (2002), trehalose can act as antioxidant by protecting unsaturated fatty acids (UFA) by means of preventing: (1) the degradation of unsaturated linoleic and α-linolenic acids with aldehyde formation and, (2) the autooxidation of unsaturated acids with peroxide formation, as a result of a stoichiometric link of trehalose with the acyl chains of the UFA. In a different study, Oku et al., (2003 & 2005) confirmed this effect by analyzing with NMR spectra the interactions between trehalose and the fatty acids; in their work it is concluded that trehalose stabilizes the fatty acid structure by binding the double bounds of the acyl chains. In the present study, flavonoids, mainly hesperidin, narirutin and didymin, are the most abundant antioxidant compounds (Betoret et al., 2009). These flavonoids have an acyl group in their chemical structure, which can be a point trehalose linkage, as in the case of UFA. Thus, it is suggested that this reaction site would be occupied by trehalose, it not being available to react with the oxidant agent, trehalose acting as a secondary antioxidant.

The **capacity of antioxidant compounds** for scavenging free radicals was assessed by the reaction with stable reference radicals and expressed as mg Trolox Equivalents (TEAC) per 1L of a juice without trehalose (figure 2). Because of the synergistic effect that could exist between different antioxidants in the juice, the use of more than one assay was strongly recommended for the evaluation of the antioxidant activity of citrus juice by different assays (Plaza et al., 2011).

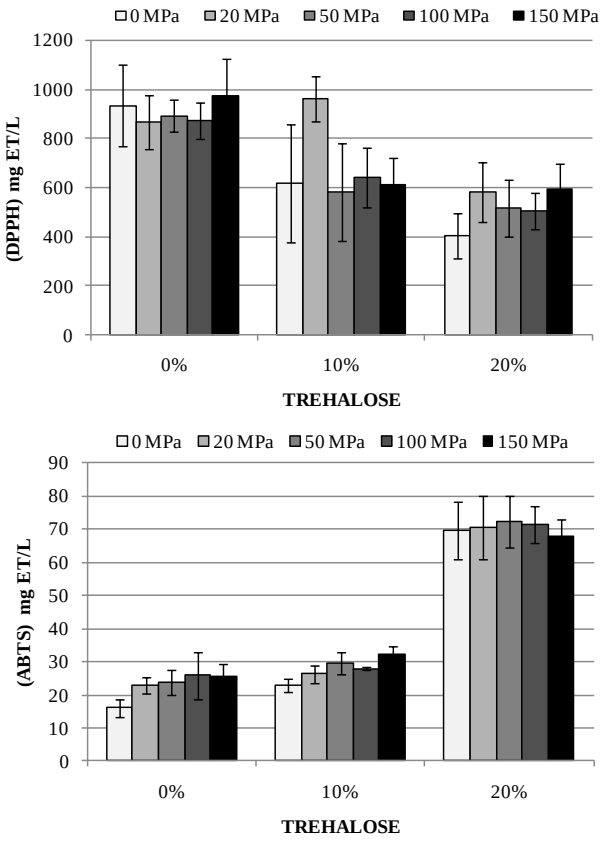

Figure 2. Antioxidant activity of samples with DPPH and ABTS method, expressed in mg of equivalent trolox per litre of mandarin juice without trehalose.

The ABTS-TEAC values were in the range of 16 and 72 mg/L while that obtained using DPPH method ranged from 405 to 960 mg/L. However, using the ABTS methodology, the differences between samples were more evident. In both cases, the homogenization treatment was not significant while the trehalose content was affecting the TEAC in a different way depending on the radical used. Trehalose addition decreased the antioxidant capacity determined by the DPPH method, regardless the amount added. In the case of the ABTS method, TEAC increased more than 150% when a 20% (w/w) of trehalose was added to the juice.

ABTS and DPPH methods are based on different mechanisms. Furthermore, the reactivity of the phenolic compounds is dependent on their chemical structures (Zhao et al., 2008). Therefore, spectra of antioxidants determined by DPPH and ABTS are partly different. It is known that DPPH radical reacts with polyphenols but not with phenolic acids, vitamin C or sugars (Kaneda et al., 1995). The nature of the most abundant antioxidant components in mandarin juice, would explain the differences between the two methods used. Antioxidants and antiradical activities of citrus fruit are mainly due to the hydrosoluble fraction containing polyphenols and vitamin C, as well as to the apolar fraction, including carotenoids (Byers & Perry, 1992; Gorinstein et al., 2001; Tripoli et al., 2007). Vitamin C is considered to be one of the most important nutrients found in citrus fruit, and it is an important water-soluble antioxidant that plays a crucial role in the suppression of superoxide radicals (Kaur & Kapoor, 2001), although it is easily degraded.

The more pronounced differences observed by the ABTS method when trehalose is added to the juice could be explained considering that trehalose would preferably link to the more hydrophobic components, such as flavonoids, and that trehalose itself can act as free radical scavenger (Elbein et al., 2003). Thus, when a 10% of trehalose is added all the sugar would be linked to the antioxidant molecules, whereas when increasing trehalose concentration (e.g. 20%), there would be an excess of trehalose that would be available to react with the $ABTS^{+}$ radical.

4. Conclusions

HPH has a significant impact on particle size, cloud stability and phenols and flavonoid content of the mandarin juice. In particular, particle size was reduced, cloud stability increased, and phenols and (mainly) flavonoids availability also increased. These effects were probably produced as a consequence of the physical changes introduced by HPH into the cell walls, such as breakdown or permeability increase. On the other hand, trehalose addition does not affect significantly the physicochemical properties and phenols and flavonoid content. However in the antioxidant capacity evaluation with ABTS method a significant increased was observed when 20% of trehalose is added. This concentration effect suggests a chemical interaction of trehalose with hydrophobic antiradical components, as previously suggested by other authors. A possible effect of saturation of acyl groups could explain this phenomenon.

Regarding the effect on the amount of juice incorporated into the apple by vacuum impregnation, the application of a homogenization pressure did not have a clear and significant effect. However, the protective effect that trehalose exerts on cellular structures would affect the viscous-elastic properties of fruit tissue, influencing the deformation capacity and modifying the impregnation properties of the apple tissue.

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Effect of high pressure processing and trehalose addition on functional properties of mandarin juice enriched with probiotic microorganisms.

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Key words: functional foods, probiotic, homogenization, cell hydrophobicity.

Highlights

L. salivarius has shown a positive effect against all food pathogens tested.

Homogenization at 20 MPa allows obtaining high quantities of microorganism.

Trehalose and homogenization influence stability of probiotic during storage.

In an optimum media homogenization pressures improves probiotic hydrophobicity.

In stress conditions trehalose interactions improve probiotic hydrophobicity.

Abstract

This work aimed to determine the effect of homogenization pressures and addition of trehalose on the functional properties of mandarin juice enriched with *Lactobacillus salivarius* spp. *salivarius*. Physicochemical and structural properties of mandarin juice were evaluated and related with quantity and stability of probiotic microorganism as well as with its hydrophobicity. Both food matrix and processing, affected functional properties of *L. salivarius* spp. *salivarius*. Homogenization pressures and trehalose addition affected quantity and stability of probiotic microorganisms during storage. 20 MPa and 20 MPa with 100 g/kg of trehalose allowed obtaining 10^6 colony forming units (CFU)/ml mandarin juice after ten storage days. In MRS growth, cell hydrophobicity increased with homogenization pressures, with values in range 67 – 98 %. Highest cell hydrophobicity was obtained in samples homogenized at 100 MPa. Under stress growth conditions, cell hydrophobicity values were in a range 30 – 84 %. In samples no homogenized, addition of trehalose resulted in an increased values of hydrophobicity, with highest levels in those samples with 100 g/kg of trehalose addition.

1. Introduction

Sustainable food production stands at the intersection of several growing needs. Firstly, the needs of consumers for improved food security and safety, as well as healthy needs. Secondly, the quest for economic sustainability of food production,

based on cost reduction and increased product differentiation. Third, the growing concern for reversing the over exploitation of natural resources, waste generation, and the contribution to climate change (Fava *et al.*, 2013).

Functional foods can help to prevent or improve some diseases thus contributing directly to the public health and global sustainability. Specifically, probiotic foods can help to prevent or improve the treatment of digestive system diseases and can suppose an alternative strategy to fight antibiotic excessive uses which produce antibiotic resistances and result in a high cost for the Health European System, waste generation and effluent contamination (Betoret *et al.*, 2016). In the development of a probiotic functional food, it is necessary to consider the effect of processing operations on the final product. Foods are mostly complex mixtures of macro and micro components organized in a structure that can trap active compounds, modulating their release or inhibiting their activity (Betoret, Betoret, Rocculi & Dalla Rosa, 2015). Food matrix, in its raw state or transformed during processing, can have a significant effect on the functionality of bioactive compounds. To choose an appropriate food matrix and technological process, as the key step for the success of a specific functional food, it is necessary to understand the establishment of some interactions between bioactive compounds, cellular structures and technological ingredients that contributing to a "barrier" formation can help to maintain the integrity of bioactive compounds preventing the action of some deterioration factors during processing or storage and ensuring

the active compound gaining access to the functional target site in the organism. Structure – property – process relationships approach can help developing probiotic functional foods allowing detecting strengths and weaknesses of the system in order to generate technologically feasible strategies that contribute to the success of a functional food (Betoret, Betoret, Rocculi & Dalla Rosa, 2015).

The objective of this research was to determine the effect of homogenization pressures and addition of trehalose on the functional properties of mandarin juice enriched with *Lactobacillus salivarius* spp. *salivarius,* a probiotic microorganism with potential effect against *Helicobacter pylori* infection.

2. Material and methods

2.1. Sample preparation

Ortanique fruit, a hybrid of tangerine and sweet orange (*Citrus sinensis* x *Citrus reticulata*) was provided by Rural S. Vicent Ferrer cooperative located in Benaguacil (Valencia), Spain. The preparation of the juices was carried out according to the patent WO/2007/042593 titled "Method of obtaining refrigerated pasteurized citrus juices" (Izquierdo, et al., 2007). The fruits were washed by immersing them in tap water, drained, and squeezed in an extractor ("GAM" MOD.SPA 1400 rpm, Cesena, Italy). Raw juice was centrifuged at 3645 x g during 5 min at 4 °C (Beckman Coulter AvantiTM J-25, California, United States), pasteurized at 63 ºC for 15 s (Roboqbo Qb8-3, Bologna, Italy), collected in sterile jars, and quickly frozen at -18 ºC until analyzed.

2.2. Mandarin juices with L. salivarius spp. salivarius

To obtain mandarin probiotic juices, 2 ml/l of de Man, Rogosa & Sharpe (MRS) Broth (VWR, Milan, Italy) with 9 log colony forming units (CFU)/ml *L. salivarius* spp. *salivarius* CECT 4063 (Spanish Type Culture Collection, Valencia, Spain) were transferred to mandarin juices following the procedure described by Betoret *et al.*, 2012. After incubation for 24 h at 37 ºC, the juices were homogenized with a Panda Plus pilot homogenizer (GEA Niro Soavi Panda PLUS, Parma, Italy) at 0, 20 and 100 MPa. In juice samples with trehalose (Cargill, Milan, Italy), an amount of 100 and 300 g/kg was added before homogenization and incubation steps.

2.3. Physicochemical characterization

Total soluble solids were measured as ºBrix with a digital refractometer (Pal-1; Atago Co., Ltd., Tokyo, Japan). Total titratable acidity was assessed by titration with 0.1 mol/l NaOH (Sigma Aldrich, Milan, Italy) and expressed as the percentage of citric acid. A potentiometer was used to measure pH (micropH Crison GLP21, Barcelona, Spain). The viscosity was determined by using a portable viscometer (Hydramotion Viscolite 700, York, UK). The values provided are the average of three replicates.

2.4. Suspended pulp and transmittance

Suspended pulp was evaluated by sample centrifugation at 365 x g during 10 minutes at 27 ºC (Amador, 2005). The supernatant was collected and evaluated its transmittance at 650 nm in spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). The values provided are the average of six replicates.

2.5. Characterization of L. salivarius spp. salivarius

Antagonist activity of *L. salivarius* spp. *salivarius* CECT 4063 was evaluated using the methodology described by Siroli *et al.*, 2015. Concretely, 0.5 ml of specific pathogen was inoculated in 10 ml of Brain Heart Infusion (BHI) soft agar (VWR, Milan, Italy) and transferred to the *L. salivarius* spp. *salivarius* petri dish. The antagonist activity was evaluated by the inhibition area created by the probiotic microorganism after incubation at 37 ºC for 24 h against pathogens associated with toxic infections or responsible of food degradation (Table 1). The target strains were chosen according to the literature Siroli *et al.*, 2015 and Pisano *et al.*, 2011. The values provided are the average of three replicates.

Bacteriocin production was evaluated by the inhibition area created by the supernatant after centrifugation at 13000 x g during 3 min at 4 ºC (Beckman Coulter AvantiTM J-25, California, United States) of *L. salivarius* spp. *salivarius* CECT 4063 boiled and neutralized, boiled but non-neutralized, filtered and non-neutralized against the food pathogens presented in Table 1. The values provided are the average of three replicates.

2.6. Microorganism counting

Juices homogenized at 0, 20, 100 MPa with 0, 100 and 300 g/kg of trehalose content, *L. salivarius* spp. *salivarius* CECT 4063 were stored during 0, 1, 2, 3, 7, 10 days at 4 ºC. Each day, a juice sample was taken and the number of probiotic microorganisms were counted on double layer MRS agar (VWR, Milan, Italy) following incubation for 24 h at 37 ºC. The values provided are the average of three replicates.

2.7. Hydrophobicity

L. salivarius spp*. salivarius* CECT 4063 hydrophobicity has been calculated following the methodology proposed by Vinderola & Reinheimer (2003) both in MRS Broth and in mandarin juices homogenized at 0, 20, 100 MPa with 0, 100 and 300 g/kg of trehalose content. Methodology was optimized to eliminate interferences in the measurement without affecting probiotic microorganism growth. The values provided are the average of six replicates.

2.8. Statistical analysis

A multi factorial ANOVA was carried out to determine the significant effect, with 95 % confidence level, of the process variables with the software STATISTICA 10.

3. Results and discussion

3.1. Characterization of L. salivarius spp. salivarius

The strain *L. salivarius* spp. *salivarius* CECT 4063 was chosen due to its demonstrated activity against *Helicobacter pylori* infection and because of the results obtained previously (Betoret *et al.*, 2012). To characterize the strain, both antagonist activity and bacteriocin production were evaluated.

Antagonist activity of *L. salivarius* spp. *salivarius* was evaluated against the most common pathogens responsible of food toxic infections or food degradation (table 1). *L. salivarius* spp. *salivarius* showed a positive effect against all food pathogens. Antagonist activity was always high with 6-10 mm or >10 mm inhibition halo for most of pathogens with low levels for *L.*

plantarum, *E. faecalis* and *S. enteritidis* in which the inhibition halo was 1-6 mm. In order to see if antagonist activity was a result of bacteriocin production, this one was evaluated. No inhibitory activity was detected in the cell supernatant boiled and neutralized, boiled and not neutralized, filtered and neutralized.

Table 1. Antagonist activity of L. salivarius spp. salivarius against most common food pathogenic and spoilage bacteria (Siroli et al., 2015; Pisano et al., 2011). The values provided are the average of three replicates.

		L. salivarius spp. *salivarius* CECT 4063 inhibition
L. monocytogenes	ATCC 13932	++++
L. monocytogenes	SCOTT A	++++
L. innocua	DSM 20649	++++
L. plantarum	V7B3	+
B. cereus	ATCC11966	+++
S. aureus	DSM 20231	+++
E. faecalis	ATCC29212	++
E. faecalis	EF37	+++
E. coli	DSM 18039	++++
E. coli	555	++++
S. enteritidis	E5	++

Legend: – (no inhibition); + (inhibition 1-3 mm); ++ (inhibition 3-6 mm); +++ (inhibition 6-10 mm); ++++ (inhibition > 10 mm).

3.2. Mandarin juice with L. salivarius spp. salivarius, physicochemical and structural characterization

Mandarin juices homogenized at 0, 20 and 100 MPa with 0, 100, 300 g/kg of trehalose were characterized by measuring brix, pH, and acidity (table 2). Trehalose addition had a significant effect ($p \leq 0.05$) on brix, pH and acidity values obtained. Density and viscosity in mandarin juice homogenized at 0, 20, 100 MPa and with trehalose addition in 0, 100, 300 g/kg were determined

(Table 2). Trehalose addition had a significant effect ($p \leq 0.05$) on both density and viscosity measurements. It was possible to observe an increase of both parameters with trehalose addition.

Fruit juices suspension contains cellular organelles and membranes, oil droplets, chromoplasts, fragments of cellular wall such as pectin, cellulose and hemicellulose, and functional compounds (Baker & Cameron, 1999). As observed in previous studies, homogenization operation associated to the juices production can have influence on the stability of suspended pulp and thus the functional compounds present in the cloud (Betoret, Betoret, Carbonell & Fito, 2009). Separated pulp by centrifugation at 365 x g and supernatant transmittance were measured in mandarin juice with probiotic microorganism homogenized at 0, 20, 100 MPa with 0, 100, 300 g/kg trehalose addition (table 2). As expected, and according to previous studies (Betoret, Betoret, Carbonell & Fito, 2009), separated pulp and transmittance levels decreased as homogenization pressures increased. This effect can be explained taking into account that homogenization pressures decrease particle size of pulp that tends to precipitate into more stable background pulp (Betoret, Betoret, Carbonell & Fito, 2009) and thus stabilizing cloud particles. In those juices with 100 g/kg trehalose addition it was possible to observe a decrease of separated pulp and transmittance values that it was even bigger in juices with 300 g/kg trehalose addition. This effect could be due to three main reasons: on one hand, samples with trehalose had less quantity of juice and on the other hand trehalose could

Table 2. Physicochemical characterization of mandarin juices with L. salivarius spp. salivarius. Values expressed as mean ± standard deviation. The values provided are the average of three replicates. In the case of separated pulp and transmittance the values provided are the average of six replicates.

Pressure MPa	Trehalose (g/kg)	°Brix ($g_{soluble\ solids}/g_{liquid\ phase}$)	pH	Acidity (g/l)	Density ($\cdot 10^3$) (g/l)	Viscosity ($\cdot 10^{-3}$) (Pa·s)
0	0	13.53 ± 0.06	3.87 ± 0.06	1.59 ± 0.05	1.06 ± 0.02^{c}	1.57 ± 0.06^{e}
0	100	21.40 ± 0.12	3.97 ± 0.06	1.37 ± 0.06	1.09 ± 0.03^{b}	2.23 ± 0.06^{d}
0	300	36.70 ± 0.12	4.30 ± 0.12	0.90 ± 0.08	1.17 ± 0.02^{a}	6.10 ± 0.12^{a}
20	0	13.03 ± 0.06	3.87 ± 0.06	1.53 ± 0.03	1.06 ± 0.02^{c}	1.7 ± 0.2^{e}
20	100	20.70 ± 0.12	3.93 ± 0.06	1.39 ± 0.05	1.09 ± 0.02^{b}	2.23 ± 0.06^{d}
20	300	35.8 ± 0.2	4.17 ± 0.06	0.9 ± 0.2	1.16 ± 0.04^{a}	4.7 ± 0.2^{c}
100	0	13.2 ± 0.2	3.8 ± 0.02	1.5 ± 0.2	1.05 ± 0.03^{c}	1.67 ± 0.06^{e}
100	100	20.90 ± 0.12	3.9 ± 0.02	1.4 ± 0.2	1.084 ± 0.013^{b}	2.2 ± 0.2^{d}
100	300	34.6 ± 0.4	4.2 ± 0.02	0.9 ± 0.2	1.16 ± 0.02^{a}	5.0 ± 0.2^{b}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Table 2 (Continuation). Physicochemical characterization of mandarin juices with L. salivarius spp. salivarius. Values expressed as mean ± standard deviation. The values provided are the average of three replicates. In the case of separated pulp and transmittance the values provided are the average of six replicates.

Pressure MPa	Trehalose (g/kg)	Separated pulp (ml/l)	Transmittance (%)
0	0	93.33 ± 0.06^{ab}	20.1 ± 1.2^{a}
0	100	83.33 ± 0.06^{c}	17.8 ± 2.2^{ab}
0	300	46.66 ± 0.06^{d}	7.5 ± 1.6^{c}
20	0	86.66 ± 0.06^{bc}	16.6 ± 1.3^{b}
20	100	83.33 ± 0.12^{c}	7.1 ± 0.8^{c}
20	300	2.00 ± 0.02^{e}	6.7 ± 0.3^{c}
100	0	10.00 ± 0.02^{a}	9.0 ± 2.7^{c}
100	100	8.00 ± 0.02^{c}	5.37 ± 0.08^{d}
100	300	2.00 ± 0.02^{e}	1.8 ± 0.2^{e}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

interact with cloud compounds stabilizing the suspension and maintaining juice cloudiness. Also trehalose addition leads to increased viscosity and therefore an increased resistance against sedimentation and resistance to movement.

3.3. Mandarin juice with L. salivarius spp. salivarius, quantity and stability of probiotic microorganisms

L. salivarius spp. *salivarius* growth was determined in juices homogenized at 0, 20, 100 MPa with 0, 100, 300 g/kg trehalose addition after 1, 2, 3, 7 and 10 storage days (Table 3).

The highest growth of probiotic microorganisms was obtained in juices homogenized at 20 MPa. Trehalose addition in 300 g/kg resulted in low levels of microorganism growth, probably due to the osmotic pressure created in the media. However, 100 g/kg of trehalose addition did not show significant differences when compared with juices without trehalose. When considering storage days, juices with 300 g/kg of trehalose addition presented lower microorganism content that remain stable during ten storage days between 5.5 – 3.5 log CFU/ml values. In samples with 100 g/kg of trehalose addition, levels of probiotic microorganism remained constant until third storage day, from which they start decreasing. In no homogenized samples, high levels of probiotic microorganisms quickly started decreasing with storage days. In samples homogenized at 20 and 100 MPa, there was an increase in probiotic microorganism at second storage day, probably due to the nutrients availability favored by the small sizes of cloud particles, decreasing from third storage day in samples homogenized at 100 MPa. Samples homogenized at 20 MPa had the highest *L. salivarius* spp. *salivarius* content, stable until seventh storage day.

Table 3. L. salivarius spp. salivarius content in mandarin juices during storage (log CFU/ml). Values expressed as mean ± standard deviation. The values provided are the average of three replicates.

Pressure	Trehalose	Storage days				
MPa	g/kg	1	2	3	7	10
0	0	8.10 ± 0.02^{abc}	7.8 ± 0.2^{b}	7.09 ± 0.06^{bcd}	5.84 ± 0.09^{d}	5.2 ± 0.2^{d}
0	100	7.7 ± 0.4^{cd}	8.54 ± 0.09^{a}	7.5 ± 0.09^{ac}	7.6 ± 0.02^{b}	6.29 ± 0.02^{b}
0	300	4.7 ± 0.2^{f}	4.0 ± 0.2^{c}	4.5 ± 0.02^{e}	3.5 ± 0.2^{f}	3.54 ± 0.09^{f}
20	0	8.39 ± 0.04^{ab}	8.58 ± 0.04^{a}	8.5 ± 0.9^{a}	8.45 ± 0.05^{a}	6.77 ± 0.12^{a}
20	100	8.19 ± 0.02^{b}	8.22 ± 0.12^{ab}	8.25 ± 0.12^{ad}	7.8 ± 0.3^{b}	6.8 ± 0.3^{a}
20	300	5.3 ± 0.2^{e}	4.3 ± 0.5^{c}	4.6 ± 0.8^{e}	4.8 ± 0.2^{e}	4.98 ± 0.03^{d}
100	0	7.46 ± 0.12^{d}	8.6 ± 0.2^{a}	8.24 ± 0.02^{a}	7.6 ± 0.2^{b}	5.80 ± 0.14^{c}
100	100	7.8 ± 0.2^{bd}	7.8 ± 0.02^{b}	7.8 ± 0.3^{ab}	6.81 ± 0.05^{c}	5.5 ± 0.3^{cd}
100	300	4.7 ± 0.2^{f}	4.5 ± 0.02^{c}	4.39 ± 0.12^{e}	4.7 ± 0.3^{e}	4.2 ± 0.2^{e}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

It seems that smaller size cloud particles together with sub-lethal homogenization pressures creates an optimal environment for *L. salivarius* spp. *salivarius* growth. In the same way, cloud stability created by compounds interaction made possible constant preservation of microorganism until seventh storage day. Addition of trehalose without creating an osmotic stress for the microorganism, reinforced juice cloud stability and maintained high microorganism levels until the tenth day of storage.

3.4. Functional properties of L. salivarius spp. salivarius. Determination of cell hydrophobicity and effect of processing technology

In probiotic microorganisms, cellular hydrophobicity has been related with the strain capacity to adhere and interact with intestine wall (Basson, Craig & Zhang, 2007; Burns et al., 2008). Hydrophobic strains have been described as more cellular and tissue invasive, being able to adhere to the intestine wall thus making possible a successive colonization. Surface hydrophobicity in *L. salivarius* spp. *salivarius* cells was calculated in MRS Broth medium homogenized at 0, 20, 100 MPa with 0, 100, 300 g/kg of trehalose addition (table 4).

Cell hydrophobicity of *L. salivarius* spp. *salivarius* in MRS Broth showed high results in all samples, with values in range 67 – 98 %. Cell hydrophobicity increased with homogenization pressures. Highest cell hydrophbicity was obtained in samples

Table 4. Cell hydrophobicity (%) of L. salivarius spp. salivarius incubated in MRS Broth. Values expressed as mean ± standard deviation. The values provided are the average of six replicates

Pressure (MPa)	Trehalose (g/kg)	Cell hydrophobicity (%)
0	0	66.9 ± 9.6^{gh}
0	100	90.4 ± 2.7^{ac}
0	300	80.0 ± 11.5^{de}
20	0	94.3 ± 0.8^{ab}
20	100	84.8 ± 6.9^{bcd}
20	300	73.0 ± 3.9^{efg}
100	0	98.2 ± 1.9^{efh}
100	100	71.9 ± 12.2^{df}
100	300	79.40 ± 1.12^{a}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

homogenized at 100 MPa. As observed in previous studies (Patrignani et al., 2009), sub-lethal homogenization pressures can change cellular structure of microorganism facilitating their adhesion to the digestive system. The effect of trehalose addition was different depending on levels of homogenization pressures applied. In samples no homogenized, the addition of trehalose increased cell hydrophobicity. However, in homogenized samples, trehalose addition decreased cell hydrophobicity. Specifically, in those samples homogenized at 20 MPa, trehalose addition of 100 and 300 g/kg resulted in lower hydrophobicity values, while in samples at 100 MPa there were not significant differences between hydrophobicity values calculated in 100 and 300 g/kg trehalose addition samples.

Table 5. Cell hydrophobicity (%) of L. salivarius spp. salivarius incubated in mandarin juices. Values expressed as mean ± standard deviation. The values provided are the average of six replicates

Pressure MPa	Trehalose g/kg	Cell hydrophobicity (%)
0	0	32.5 ± 7.9^{ef}
0	100	86.3 ± 4.6^{a}
0	300	55.2 ± 4.3^{d}
20	0	27.3 ± 1.5^{f}
20	100	27.3 ± 3.5^{f}
20	300	63.4 ± 5.8^{c}
100	0	37.3 ± 6.2^{e}
100	100	35.3 ± 6.2^{e}
100	300	73.6 ± 7.3^{b}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Cell hydrophobicity of *L. salivarius* spp. *salivarius* incubated in mandarin juices with 0, 100, 300 g/kg trehalose addition and then homogenized at 0, 20 and 100 MPa was calculated (table 5). Both homogenization pressures and trehalose addition had a significant effect ($p \leq 0.05$) on cell hydrophobicity. Analyzed samples showed values in a range 30 – 84 %. In samples no homogenized, addition of trehalose resulted in an increased values of hydrophobicity, with higher levels in those samples with 100 g/kg of trehalose addition. The tendency of the results was the same in samples homogenized at 20 and 100 MPa, with slightly higher values obtained for 100 MPa homogenization pressures. There were not significant differences in hydrophobicity values obtained in samples homogenized and 100 g/kg of trehalose

addition. However, 300 g/kg of trehalose addition resulted in higher levels of cell hydrophobicity.

As explained by Iaconelli et al., (2015) the measurement of bacterial hydrophobicity remains a good indicator to evaluate variation in bacterial surface properties, especially for an identical strain treated with different processes. The hydrophobicity of *L. salivarius* spp. *salivarius* incubated in mandarin juices was lower than that obtained in MRS Broth. The change in cell wall hydrophobicity could be a result of bacterial stress to certain culture conditions, such as low pH, high temperature and hyperosmotic stress (Lopez et al., 2000; Remeta et al., 2002). It seems that in those samples with 300 g/kg of trehalose addition, slightly higher pH and created interactions between trehalose, probiotic microorganisms and juices cloud, protected *L. salivarius* spp. *salivarius* and decreased its stress suffered during growing. Decreasing cloud particles size by homogenization, although could contribute to suspension stability, seemed not improve their hydrophobicity when they were growth in mandarin juices. High values obtained in samples no homogenized with 100 g/kg trehalose addition could be only explained taking into account the variability in cloud juice particles. Bigger particle sizes and less reactive points, could change the interactions created in the media, protecting better *L. salivarius* spp. *salivarius* from stress conditions. Trehalose is a disaccharide able to interact with various compounds, forming a glassy amorphous matrix around the tertiary structure of the proteins and phospholipids exerting a protective effect on various technological processes (Colaço &

Roser, 1994). There are a lot of studies demonstrating the ability of trehalose to interact with probiotic cell surface and showing its protecting effect during drying processes (Crowe, Carpenter, Crowe, & Anchordoguy, 1990). However, there is lack of studies in literature that evaluate the effect of trehalose and juices cloud interactions on hydrophobicity changes in probiotic microorganisms. This effect should be further investigated.

In order to avoid the stress suffered by probiotic microorganisms during incubation in mandarin juices that resulted in microorganism cellular surface changes, cell hydrophobicity of *L. salivarius* spp*. salivarius* incubated in MRS Broth with 0, 100, 300 g/kg of trehalose addition, homogenized at 0, 20 and 100 MPa and then transferred to mandarin juices, was calculated (table 6). All obtained values were in range 40 – 80 %, higher than those obtained with *L. salivarius* spp. *salivarius* incubated in mandarin juices and lower than those obtained in MRS Broth. It was possible to eliminate partially the microorganism growth stress created by low pH in juices that resulted in microorganism cellular surface hydrophobicity changes, and improve the values obtained. In this case, as in MRS Broth results, homogenization pressures and trehalose addition improved cell hydrophobicity.

Table 6. Cell hydrophobicity (%) of L. salivarius spp. salivarius incubated in MRS Broth and transferred to mandarin juices. Values expressed as mean ± standard deviation. The values provided are the average of six replicates

Pressure MPa	Trehalose g/kg	Cell hydrophobicity (%)
0	0	40.1 ± 6.9^{c}
0	100	72.1 ± 9.2^{a}
0	300	55.3 ± 3.4^{b}
20	0	42.9 ± 3.9^{c}
20	100	72.1 ± 0.3^{a}
20	300	77.0 ± 9.2^{a}
100	0	57.2 ± 11.8^{ab}
100	100	52.9 ± 7.6^{b}
100	300	55.9 ± 8.2^{b}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

4. Conclusions

Both food matrix and processing affected functional properties of *L. salivarius* spp. *salivarius*. In an optimal growth media, both homogenization pressures and trehalose addition improved cell hydrophobicity. Under stress growth conditions, trehalose addition improved cell hydrophobicity and influenced the effects promoted by homogenization pressures. It is necessary to further study trehalose interactions with juices cloud compounds together with microorganism cell surface to understand the mechanisms of action.

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High pressures homogenization to microencapsulate *L. salivarius* spp. *salivarius* in mandarin juice. Probiotic survival and in vitro digestion.

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Highlights

HPH at 70 MPa is a suitable technology to microencapsulate *L. salivarius* spp. s*alivarius.*

Capsules of *L. salivarius* spp. s*alivarius* interact with suspended pulp when added to mandarin juice.

Encapsulation effect on probiotic survival during in vitro digestion depends on the storage time.

After 10 days, the encapsulated probiotic content was higher than that for non-encapsulated one.

The encapsulation method efficiency and the stability of the coating could explain the results.

Abstract

High Pressure Homogenization (HPH) technology is considered to improve efficiency and up-scaling of probiotic microencapsulation. Microencapsulation method should increases the resistance of

microbial cells to processing conditions and gastrointestinal digestion. The aim of this work was to evaluate the effect of microencapsulation of *L. salivarius* spp. *salivarius* by HPH in mandarin juice on the physicochemical properties, particle size and rheology and the probiotic survival under simulated gastrointestinal conditions after 1, 3, 7 and 10 days of storage. Cells were microencapsulated forming an oil emulsion with sodium alginate by homogenization at 70 MPa for few seconds at room temperature. Juice was enriched with encapsulated and non-encapsulated *L. salivarius*. Particle size of juice with the encapsulated probiotic is similar to the capsule particle size distribution, thus revealing a possible aggregation of the microorganism with the juice particles. The digestion process results in 75% degradation of non-encapsulated against 50% in the case of the encapsulated probiotic.

Keywords:

High pressure homogenization, microencapsulation, probiotic, mandarin juice, digestion, storage.

1. Introduction

The importance of the microbiome in the incidence of a large number of diseases becomes evident; from infectious diseases to degenerative diseases, including cancer, obesity and even psychological diseases (Avershina et al., 2017; Auderson et al., 2017; Subramanyan et al, 2017; Rouxinol – Dias, 2016). Together with this, it has been demonstrated that food can influence

growth, viability and survival of microorganisms in gastrointestinal tract thus conditioning the human organism microbiota and therefore recommending probiotic food consumption (Kashtanova et al., 2016).

Dairy products are more suited to probiotic food development. However, due to the high prevalence of lactose intolerance, different non-dairy probiotic products such as fruit juices, cereal based breakfast products and baby foods have been developed in recent years (Anekella & Orsat, 2013; Chen & Mustapha, 2012; Rivera-Espinoza & Gallardo-Navarro, 2010). In any case, there is a need for designing new products which can deliver between 10^7-10^9 viable cells into the intestine by consuming approximately 100 g/day of the product (Rad et al., 2013).

Mandarin juice is quite appreciated by its functional properties due to the presence of antioxidants and phenolic compounds such as hesperidin, carotenoids and vitamin C (Putnik et al., 2017). Those bioactive compounds of mandarin juice have been related with a health promoting effect against cancer, hypertension, cardiovascular disorders, stroke and diabetes (Milella et al., 2011; Jedrychowski et al., 2010). Beside this, fermented citrus juices can have antibacterial activities (Hashemi et al., 2017). Concretely, *Lactobacillus salivarius* spp. *salivarius* has a demonstrated probiotic effect (Aiba et al., 1998) with antagonist properties against *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella enteriditis* (Betoret et al., 2017).

It has been demonstrated in numerous research works that not only food matrix, processing conditions and storage time, but also digestion process clearly influence the total amount of probiotic microorganisms able to reach the targeted tissue (Sagdic et al., 2012). Therefore, product formulation and process conditions should be directed to increase probiotic resistance to stress conditions and to improve viability, acid and bile tolerance, adhesion to intestinal epithelium, antimicrobial properties, antibiotic resistance and other functionality of probiotics that determine their efficacy in the gastrointestinal tract.

Microencapsulation is one of the most efficient strategies that has been considered in recent years to protect probiotic cells from degradation by adverse conditions, and to control their release under particular conditions (Martín et al., 2015). In fact, during the past few years, a number of food products containing encapsulated probiotics cells have been introduced into the market (Burgain et al., 2011). Although the most used microencapsulation techniques are extrusion, freeze and spray drying there is a need to develop more competitive technologies with industrial applications (Vinceković, 2017 et al., 2017; Mota et al., 2018). Burns et al. (2008) used high pressure homogenization (HPH) ranging between 60 and 100 MPa to increase *Lb. paracasei* A13 and *Lb. acidophilus* 08 viability in probiotic fermented milks and cheeses. Tabanelli et al. (2013) and Betoret et al. (2017) demonstrated that sub-lethal HPH treatment (performed at 50 MPa) improved functional properties of probiotic bacteria (such as hydrophobicity, auto-aggregation and resistance to biological

stresses) in different food matrixes and preserved their viability during refrigerated storage. Patrigniani et al. (2017) used HPH at 50 MPa to microencapsulate *L. paracasei* A13 and *L. salivarius* spp. *salivarius* CECT 4063 to produce functional fermented milks. This technology already implemented at industrial level to improve quality attributes of fruit juices could be used to microencapsulate probiotics and increase viability in citrus juices.

The aim of this research was to determine the effect of *Lactobacillus salivarius* spp. *salivarius* microencapsulation, by using high pressure homogenization, on the probiotic survival under simulated gastrointestinal conditions when incorporated into mandarin juice and stored. Physicochemical and technological properties of mandarin juice were also evaluated

2. Material and methods

2.1. Strain and food materials

Lactobacillus salivarius spp. s*alivarius* CECT 4063 was obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain).

Mandarin fruit cv. Ortanique (*Citrus sinensis* x *Citrus reticulata*) was provided by Rural S. Vicent Ferrer cooperative located in Benaguacil, Valencia, Spain. Juice preparation was done following the procedure described in WO/2007/042593. Fruits were washed, drained, squeezed ("GAM" MOD.SPA 1400 rpm, power 350W – monophase 220V, Cesena, Italy) filtered with 0.7 mm sieve, centrifuged at 3645 x g during 5 minutes at 5ºC (Beckman Coulter AvantiTM J-25, California, United States) and

pasteurized at 63ºC for 15 s (Roboqbo Qb8-3, Bologna, Italy) (Izquierdo *et al.,* 2007).

2.2. *Microencapsulation procedure*

To microencapsulate *L. salivarius* spp. *salivarius* the method described by (Ding & Shah, 2009) was followed with some modifications. A volume of 2 L of Man, Rogosa & Sharpe (MRS) Broth (Scharlab, Barcelona, Spain) containing 10^9 CFU/mL of *Lactobacillus salivarius* spp. *salivarius* was centrifuged at 7700 x g for 15 mins at 10ºC (Beckman Coulter AvantiTM J-25, California, United States) and suspended in 100 mL sterile water. A mixture of 25 mL of microorganism solution, 100 mL of sodium alginate (3%) (Sigma-aldrich, Steinheim, Germany), 1 mL of tween 80 (Sharlau, Sentmenat, Spain) and 200 mL of commercial sunflower oil was homogenized in two passes through the valve at 70 MPa and at room temperature with a homogenizer (Panda Plus Niro Soavi, Parma, Italy). The emulsion was broken with calcium chloride 0.1 M (Sigma-aldrich, Steinheim, Germany) and kept overnight at 4ºC to separate the phases. Microcapsules were isolated by centrifugation at 8000 rpm (7700 x g) for 15 minutes at 10ºC (Beckman Coulter AvantiTM J-25, California, United States).

2.3. Mandarin juice with probiotic microorganisms.

Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* was prepared following the methodology described in Betoret et al. (2012) by inoculation with 4 mL/L of MRS broth (Scharlab, Barcelona, Spain) containing 10^9 CFU/mL and maintained at 37ºC for 24 h. Prior to this step, the juice pH was

modified by adding 9.8 g/L of sodium bicarbonate (Hacendado, Novelda, Spain).

Mandarin juice with microencapsulated *L. salivarius* spp. *salivarius* was prepared by adding microcapsules prepared as described above into the juice at a ratio of 1.45 juice/microcapsules (w/w). The mixture was maintained in agitation at room temperature for 1 h.

2.4. Physicochemical characterization

Total soluble solid (ºBrix) was measured with a digital refractometer (DR 201-95 A.KRUSS OPTRONIC, Hamburg, Germany) at 20 ºC, and pH with a pH meter (Crison GLP21, Barcelona, Spain). A liquid pycnometer was used to determine the density. Water activity was measured using a dew point hygrometer (DECAGÓN Aqualab CX-2, Washington, United States). The values provided are the average of three replicates.

2.5. Particle size

Particle size was determined with a Mastersizer 2000 equipment (Malvern Instruments, Worcestershire, UK) following the methodology described by Betoret et al., (2009) with some modifications. The refractive indexes used were 1.73, 1.33 and 1.46 (Ciron et al., 2010), the absortion index of cloud particles were 0.1 (Correding et al., 2001) and 0.01 (Ciron et al., 2010) for non-encapsulated and encapsulated *L. salivarius spp. salivarius* mandarin juices respectively. Results were expressed as the volume-weighted mean diameter (D [4,3]), the surface area mean diameter (D [3,2]) and d_{10}, d_{50} and d_{90}, defined as the particle size which 10%, 50% and 90% of the distribution is below this size

respectively (Instruments, M., 2007). The values provided are the average of five replicates.

2.6. Rheological properties

Rheological properties were studied with a rheometer (Haake RheoStress 1, Thermo Electron Corporation, Kalsruhe, Germany) using a concentric cylinder (Z34 DIN Ti, Thermo Electron Corporation, Kalsruhe, Germany). Controlled shear rate experiments were done for 300 s with an increasing rate from 0 to 250s^{-1} at 20ºC. Parameters K (consistency index, Pa·s) and n (flow behaviour index, dimensionless) were obtained by regression adjusted to Ostwald-de-Waele model linearized as equation 1, where σ (Pa) is the shear stress, K is the consistency index, $\dot{\gamma}$ (s^{-1}) is the shear rate and n is the flow behavior index. HAAKE RheoWin Data Manager v.3.61.0004 software was used to process data. The values provided are the average of three replicates.

$$\sigma = K\dot{\gamma}^{n} \quad (1)$$

2.7. Microbial content

Mandarin juices with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius* were stored at 4ºC and microbial survival was evaluated at 0, 1, 3, 7 and 10 days. Microbial content was determined following the dilution method and growth in MRS agar (Scharlab, Barcelona, Spain) on double layer incubated during 24 h at 37 ºC. In juice with encapsulated *L. salivarius* spp. *salivarius* the first dilution was done in phosphate buffer solution (pH 7.4) maintained in agitation during 30 minutes. Values provided are the average of four replicates.

2.8. Gastrointestinal digestion.

In order to determine the effect of gastrointestinal digestion on the microorganism survival two variables were considered: t_i referred to a moment during the gastrointestinal digestion; T_i referred to the *L. salivarius* spp. *salivarius* content at different stages during the gastrointestinal digestion. A dilution 1:1 of the mandarin juice with 0.6% (w/v) pepsine (Sigma-aldrich, Steinheim, Germany) was adjusted with HCl 4M to pH 3 (t_1 - T_1). Sample was kept in an agitated bath at 37ºC for 90 minutes (t_2 - T_2). Phosphate buffer solution at pH 8 with 10% of bile (Sigma-aldrich, Steinheim, Germany) were added and mixed (t_3 - T_3). Finally, phosphate buffer solution at pH 8 with 0.3% of bile 0.1% pancreatine (Sigma-aldrich, Steinheim, Germany) was added and sample was incubated at 37ºC for 90 minutes (t_4 - T_4). Microorganism content was measured by plate count after each of the four stages considered for gastrointestinal digestion process described before. The results provided are the average of four replicates.

2.9. Statistical analysis

A multi factorial ANOVA was carried out to determine the significant effect of the process variables, at 95% confidence level using Statgraphics centurion XVI software (StatPoint Technologies, Virginia, US).

3. Results and discussion

3.1. Physicochemical characterization, particle size and rheological properties.

Minor proportion of mandarin juice together with the microcapsules incorporated were responsible for the minor total soluble solids content obtained in mandarin juice with encapsulated *L. salivarius* spp. *salivarius* (table 1).

Table 1. Physicochemical properties of the mandarin juice with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius*. Values expressed as mean ± standard deviation.

	Non encapsulated	Encapsulated
TSS (ºBrix)	13.63 ± 0.06[a]	9.8 ± 0.2[b]
pH	3.7 ± 0.01[a]	3.4 ± 0.01[b]
a_w	0.989 ± 0.003 [a]	0.994 ± 0.003 [a]
Density (g/mL)	1.060 ± 0.001[a]	1.033 ± 0.008[b]

* Values with different superscript letters in a row are significantly different ($p \leq 0.05$)

Particle size distribution of all samples ranged between 0.5 and 1500 μm (figure 1). The wideness of the distribution and the variability in the particle sizes obtained could be due to the presence of different cloud particles such as cellular organelles and membranes, oil droplets, chromoplasts, fragments of cellular wall such as pectin, cellulose and hemicellulose and functional compounds (Baker & Cameron, 1999). Fresh mandarin juice and mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* showed a bimodal distribution. Fresh mandarin juice showed a maximum peak at 7.6 μm and a minimum peak at 416.6 μm. Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* showed a maximum peak at 19.9 μm and a minimum peak at 724.4 μm. Despite of *L. salivarius* spp. *salivarius* microbial cells sizes varies between 1 and 8 μm (Kokkinosa et al.,

1998), their presence increased slightly the particle size distribution of mandarin juice. This result could evidence an interaction and aggregation of juice cloud particles promoted by the presence of microorganisms. Particle size distribution of the microcapsules was monomodal, with a maximum peak at 316.3 μm. The addition of the microcapsules to the mandarin juice changed the distribution from bimodal to monomodal with a maximum peak at 316.3 μm too. A possible aggregation of the microcapsules with the suspended particles of the mandarin juice could explain these results.

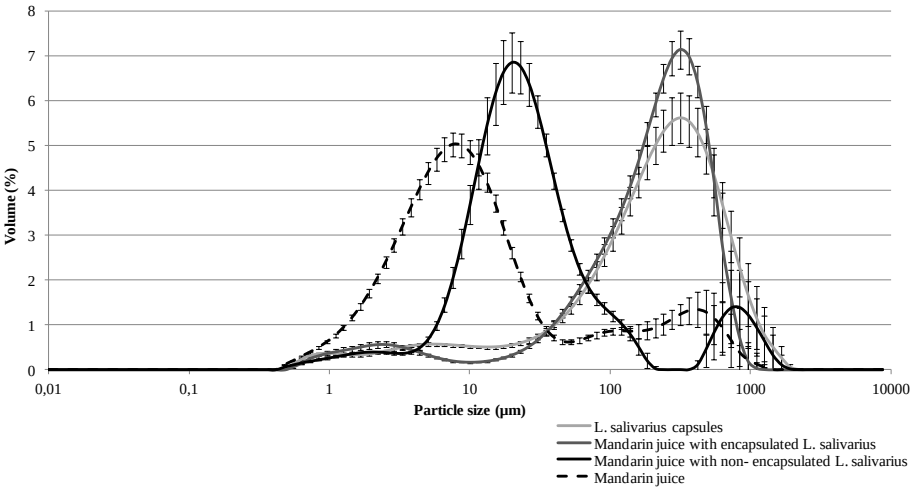

Figure 1. Particle size distribution for the capsules, the mandarin juice with encapsulated *L. salivarius* spp. *salivarius*, the mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* and the mandarin juice

Table 2 shows values of the main parameters that describe particle size distribution. Differences obtained between D(4,3) and D(3,2) values in both mandarin juices evidenced the existence of particles with high variability in shape and size. Particle size is an important parameter to be considered when mandarin juice

enriched with microcapsules is going to be consumed directly or when it is going to be used in other pretreatment operations such as vacuum impregnation. Microcapsules smaller than 100 μm are required in order to do not be perceived by the consumer (Hansen et al., 2002). In vacuum impregnation operation, a particle size smaller than the food matrix porous is required (Castagnini et al., 2015). Patrignani et al., (2017) showed that high pressure homogenization at 50 MPa allows obtaining microcapsules of *Lactobacillus* microorganisms such as *L. paracasei and L. salivarius* smaller than 100 μm. In our case, less than 50 % of the particles in the mandarin juice with encapsulated *L. salivarius* spp. *salivarius,* had a size smaller than 100 μm (figure 1). Nevertheless, results of d_{50} in mandarin juice with encapsulated *L. salivarius* spp*. salivarius* revealed that the microcapsules obtained by homogenization pressures were similar to those obtained by other traditional microencapsulation methods such as spray drying, spray cooling, spray chilling, extrusion, freeze-drying and coacervation (Desai & Park, 2005; Ding & Shah, 2009, Gibbs et al., 1999; Gouin, 2004; Shahidi & Han, 1993).

Table 2. Characteristic parameters that describe particle size distribution of mandarin juices and the capsules. Values expressed as mean±standard deviation.

	D [4,3]	D [3,2]	d_{10}(μm)	d_{50}(μm)	d_{90}(μm)
Mandarin juice	74± 30^{a}	5.9± 0.3^{a}	2.50±0.09^{a}	10± 0.8^{a}	208± 64^{a}
Mandarin juice with non-encapsulated *L. salivarius*	177± 83^{b}	13.9±1.3^{b}	8.4± 0.8^{b}	31± 17^{b}	577±295^{b}
Mandarin juice with encapsulated *L. salivarius*	265± 28^{c}	22± 3^{c}	29± 10^{c}	235± 20^{c}	543± 63^{b}
Capsules of *L. salivarius*	317± 46^{d}	20.9± 1.8^{c}	14.9± 3^{d}	240± 25^{c}	721±129^{c}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Microcapsules incorporation had an impact on mandarin juice rheological behavior. In fact, the rheological obtained curves showed that encapsulated *L. salivarius* spp. *salivarius* mandarin juice resulted in a more viscous fluid than non-encapsulated one (figure 2). Experimental data were fitted to the Ostwald-de-Waele model (table 3). A Newtonian behavior is generally observed for clarified and depectinated orange juices (Ibarz et al., 1994). In our case, both fluids resulted in a non-Newtonian pseudo plastic behavior (n<1) generally observed in complex fluids or polymer solutions in which viscosity decreases under shear strain. Rheological properties of the isolated microcapsules were not characteristic of a liquid because of the irregular aggregates formed.

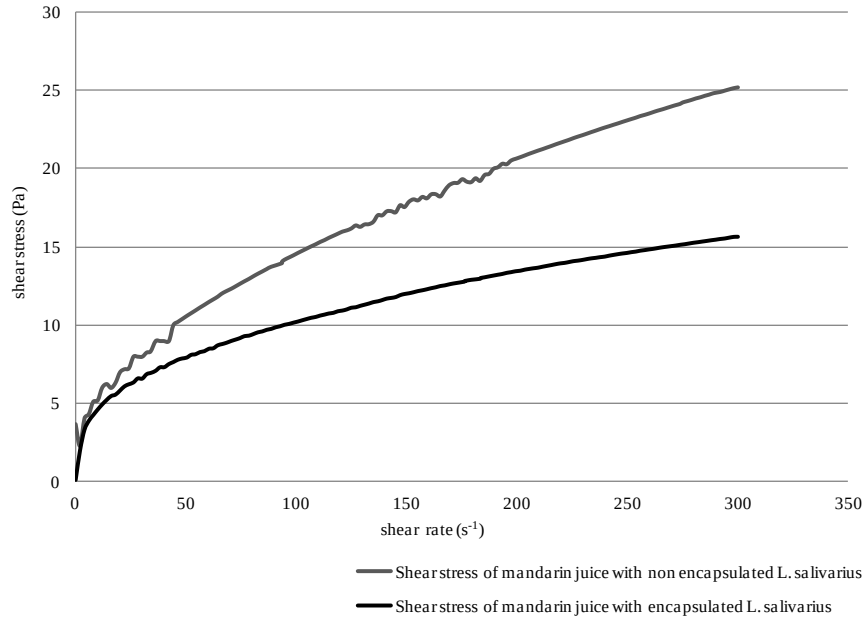

Figure 2. Rheogram of mandarin juice with encapsulated *L. salivarius* and mandarin juice with non-encapsulated *L. salivarius.*

Table 3. Rheological properties of mandarin juice with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius*. Values expressed as mean ± standard deviation

	Encapsulated	Non encapsulated
K (Pa·s)	1.96±0.07[a]	1.92±0.04[a]
n	0.376±0.007[a]	0.463±0.004[b]

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

3.2. Probiotic survival during storage and gastrointestinal digestion effect.

In order to have a probiotic effect or any other beneficial effect associated to the microorganism strain it is necessary, firstly, to maximize the microorganism content and its survival in the food matrix during all the processing and storage conditions; then, the microorganism needs to maintain its active form after the consumption and during all digestion steps until the targeted site in the organism where it will be able to interact, colonize and finally will exert its beneficial effect. As described in Betoret et al., (2016), Lactobacillus cells survival in mandarin juice is affected mainly by low pH, high temperature, hyperosmotic stress, nutrient bioavailability, cloud structure and stability.

Content of *L. salivarius* spp. *salivarius* encapsulated and non-encapsulated was determined in mandarin juice after 1, 3, 7 and 10 storage days. Results are shown in table 4 (T_0). Despite of differences obtained in both microorganism content at day 1, no significant differences were observed at 3 and 7 storage days. After 10 storage days, the content of encapsulated *L. salivarius* spp. *salivarius* was significantly higher than that obtained for

non-encapsulated one. It seems that entrapment of *L. salivarius* spp. *salivarius* by a microcapsule formed by homogenization pressures and with sodium alginate as a coating it is protective enough to increase significantly ($p \leq 0.05$) its survival in mandarin juice at 10 storage days.

L. salivarius spp. *salivarius* has been proved to have both, effect against *Helicobacter pylori* infection and probiotic (Messaoudi, et al., 2013, Zheng, et al., 2013). *L. salivarius* spp. *salivarius* probiotic effect could be improved when added to mandarin juice, because of a synergic effect between the flavanones of the juice and the probiotic bacteria (Pereira-Caro et al., 2015; Putignani & Dallapiccola, 2016). The precise mechanisms by which probiotic microorganisms have an effect against *Helicobacter pylori* infection are still unknown. A possible competition over the binding sites in the gastrointestinal tract between the probiotic and the bacteria and a posterior displacement by the probiotic is widely accepted. There are evidences that *L. salivarius* spp. *salivarius* colonizes the stomach and produce immunomodulatory factors which suppress inflammation caused by *H. Pylori* infection of the gastric epithelial cells (Aiba et al., 1998, Servin 2004, Panpetch et al., 2016). In this case, it will be necessary that *L. salivarius* spp. *salivarius* maintain its active form until the stomach where it will be able to compete with *Helicobacter pylori* bacteria and interact with gastric epithelial tissue in order to exert a positive effect against infection. Nevertheless, in order to have a probiotic effect will be necessary that *L. salivarius* spp. *salivarius* maintains its

active form until reaching the intestine where must be able to interact with intestine wall to carry out a subsequent colonization. In both cases, microcapsule function is twofold, on the one hand protecting *L. salivarius* spp. *salivarius* enough to resist unfavorable conditions during digestion process but on the other hand allowing the release at the appropriate time and point in the organism so that it can interact with the target tissue. Simulated gastrointestinal digestion was carried out in order to know the survival of *L. salivarius* spp. *salivarius* encapsulated and non-encapsulated in mandarin juice.

Table 4. *L. salivarius* spp. *salivarius* content (log CFU/l) of mandarin juice with and without the encapsulated microorganisms during *in vitro* digestion over ten days. Values expressed as mean ± standard deviation.

		Day 1	Day 3	Day 7	Day 10
Encapsulated	T_0	9.09 ± 0.03^{j}	7.93 ± 0.05^{h}	6.87 ± 0.05^{h}	6.64 ± 0.06^{g}
	T_1	8.419 ± 0.016^{i}	7.18 ± 0.05^{f}	6.92 ± 0.08^{h}	6.47 ± 0.04^{g}
	T_2	6.89 ± 0.02^{f}	6.34 ± 0.08^{d}	5.91 ± 0.04^{f}	$5.43\pm0.02^{e,f}$
	T_3	6.66 ± 0.04^{e}	6.22 ± 0.04^{d}	5.61 ± 0.05^{e}	4.59 ± 0.06^{d}
	T_4	3.93 ± 0.04^{b}	3.96 ± 0.07^{b}	3.96 ± 0.02^{c}	3.31 ± 0.07^{c}
Non-encapsulated	T_0	8.08 ± 0.05^{h}	7.53 ± 0.07^{g}	6.14 ± 0.04^{g}	5.65 ± 0.05^{f}
	T_1	7.32 ± 0.03^{g}	6.637 ± 0.014^{e}	5.66 ± 0.05^{e}	5.12 ± 0.04^{e}
	T_2	6.48 ± 0.09^{d}	5.892 ± 0.017^{c}	4.74 ± 0.07^{d}	4.52 ± 0.02^{d}
	T_3	4.56 ± 0.05^{c}	3.81 ± 0.04^{b}	3.56 ± 0.04^{b}	1.60 ± 0.12^{b}
	T_4	2.1 ± 0.2^{a}	1.9 ± 0.3^{a}	1.60 ± 0.12^{a}	0.9 ± 1.0^{a}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

The microbial content during gastrointestinal simulation is shown in table 4. T_0 means the initial content of *L. salivarius* spp.

salivarius in mandarin juice. T_1 and T_2 refer the microorganism quantity by simulated stomach conditions after pH change by HCl addition and peristaltic movements respectively. T_3 and T_4 are the counting of microorganism after the duodenal shock and intestinal juice mixing respectively. Statistical analysis revealed that all variables studied; the encapsulation, the specific moment in the simulated gastrointestinal digestion and the storage time had a significant effect ($p \leq 0.05$) on *L. salivarius* spp. *salivarius* content. Figure 3 shows the evolution of the microbial concentration (T_i/T_0) throughout the gastrointestinal digestion process (t_i) in the stored juices. Thus, probiotic resistance to the digestion process was influenced by juice storage time. During three storage days, the encapsulation of the probiotic increased its resistance from t_2. However, when the juice was stored for 7 and 10 days, the positive effect of the capsule on the microorganism survival was evident from t_1. In order to quantify the effect of the different factors, the percentage of accumulated degradation was calculated (table 5). Microorganism encapsulation caused a decrease in the degradation percentage from 8-9 % to 0-2 % when the juice was stored for 7 to 10 days. After mixing simulating peristaltic stomach movements, the accumulated degradation was independent of microencapsulation and storage time. The biggest differences were observed in the passage from the stomach to the intestine. Thus, the duodenal shock resulted in degradation percentages between 18 and 30 % in the mandarin juice with encapsulated *L. salivarius* spp. *salivarius.* Degradation percentages increased to 42 and 72 % in mandarin juice with non-

encapsulated microorganisms. Simulated gastrointestinal digestion resulted in losses around 50 % in encapsulated *L. salivarius* spp. *salivarius* increasing to levels of 75 - 85 % in non-encapsulated microorganisms. Similar results were obtained by Abbaszadeh et al., (2013). However, Gandoni et al. (2016) obtained lower rate survival in apple juices enriched with *L. rhamnosus* encapsulated and non.

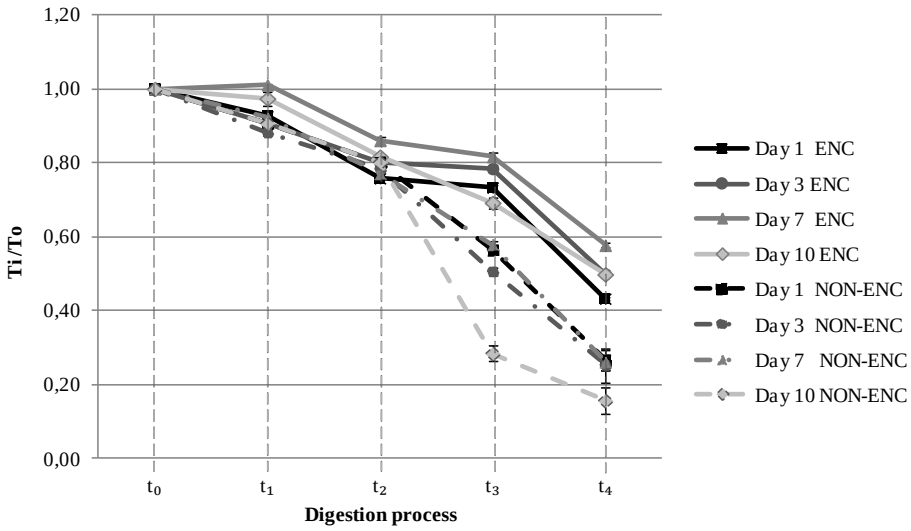

Figure 3. Evolution of encapsulated and non-encapsulated *L. salivarius* spp. *salivarius* in mandarin juice during the digestion process at 1, 3, 7 and 10 days.

The efficiency of the encapsulation method and the stability of the protective material could explain the obtained results. Ding and Shah (2009), observed a microencapsulating efficiency of 77 % when capsules were generated by a microfluidizer at 68 MPa. A similar efficiency in our method could explain the 20% of degradation, affecting non-encapsulated *L. salivarius spp. salivarius*, produced in the acid stages of the simulated

gastrointestinal digestion process. Beside this, the solubility of alginate salts at pH above 3.5 could leave encapsulated microorganims unprotected in the last stage of the gastrointestinal digestion. The values observed in t_2 and t_3 (figure 3) could be explained considering that non-encapsulated microorganisms have been degraded by the acidic conditions but microcapsules has not had enough time to be solubilized.

Table 5. Percentage degradation ($\Delta T_i = (T_i-T_0)/T_0$) of *L. salivarius* spp. s*alivarius* during *in vitro* digestion process over ten days. Values expressed as mean ± standard deviation.

		Day 1	Day 3	Day 7	Day 10
Encapsulated	ΔT_1	7.39±0.17^{a}	9.5±0.9^{a}	-0.8±0.4^{a}	2.6±1.9^{a}
	ΔT_2	24.2±0.03^{d}	20.0±0.7^{b}	14.0±1.0^{c}	18.3±0.5^{b,c}
	ΔT_3	26.7±0.6^{e}	21.6±0.9^{b}	18.4±1.0^{d}	30.8±1.5^{d}
	ΔT_4	53.4±0.5^{g}	44.9±1.0^{c}	42.8±0.9^{f}	48.9±1.6^{e}
Non encapsulated	ΔT_1	9.4±0.5^{b}	11.8±0.9^{a}	7.8±1.3^{b}	9.4±1.5^{a,b}
	ΔT_2	19.8±1.2^{c}	21.7±0.5^{b}	22.9±0.9^{e}	19.9±1.0^{c}
	ΔT_3	43.6±0.5^{f}	49.4±0.9^{d}	42.1±1.1^{f}	71.7±2^{f}
	ΔT_4	73.5±3^{h}	74.8±5^{e}	74.0±2^{g}	84.4±3^{g}

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

4. Conclusion

Microencapsulation by homogenization at pressures of 70 MPa with alginate as a coating seems to be a promising strategy to protect *L. salivarius* spp. *salivarius* during gastrointestinal digestion process and storage. The efficiency of the encapsulation method together with the stability of the protective material could explain the obtained results in the simulated gastrointestinal

digestion. The incorporation of encapsulated *L. salivarius* spp. *salivarius* into mandarin juice modified its physicochemical and technological properties creating a complex food matrix with new aggregates and interactions that will need to be analyzed in further studies.

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Effect of hot air drying on probiotic survival and in vitro digestion of L. *salivarius* spp. *salivarius* encapsulated with high pressures homogenization and incorporated into a food matrix.

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Abstract

High pressure homogenization allows encapsulating microorganism in continuous conditions with the advantage of its industrial up-scaling. Microencapsulation of probiotic microorganism may enhance their viability during food processing, storage and gastrointestinal passage. The aim of this work was to evaluate the effect of hot air drying on the probiotic survival and in vitro digestion of non-encapsulated and encapsulated *Lactobacillus salivarius* spp. *salivarius* by homogenization pressures included into an apple matrix and stored during 30 days. *Lactobacillus salivarius* spp. *salivarius* was encapsulated with Na- alginate as a coating by homogenization

Keywords:

Microencapsulation, hot air drying, high pressure homogenization, probiotic survival, gastrointestinal simulation.

1. Introduction

One of the challenges of the European Union is the growing concern about the sustainability of the agri-food system. An improved management of food processes and by-products

valorization with a consequent increase of food functionality contributes to the sustainability in the three dimensions of the term: environmental, social and economic. In this definition, also the contribution of functional foods and food processing to enhance some bioactive compounds is implicit. (Betoret et al., 2016, pp.149-165).

Most of the probiotic food is dairy products with the related issue of lactose intolerance and fat content. In recent years researchers have develop healthy probiotic food based on vegetables and fruits. For the development of a probiotic food is needed that formulation of the product and used technologies protect bacteria and favor the physiological effect on the target site (Chen et al., 2006). The viability of probiotic microorganism should be kept during manufacture, storage and the passage through the gastrointestinal tract in order to have a healthy effect (Champagne et al., 2005 and Ying et al., 2010). Along all those steps until the target site, bacteria are exposed to various stress factors such as food processing, presence of bile salts, osmotic and oxidative stress; their capacity to survive under these conditions are fundamental for their efficacy (Rossi et al., 2016).

The establishment of some interactions between bioactive compounds, cellular structures and technological ingredients that contributing to a "barrier" formation can help to maintain the integrity of bioactive compounds, such as probiotics (Betoret et al., 2015). Microencapsulation of probiotic bacteria can be a very useful strategy to maintain survival rates and viability higher

over the shelf life compared to non encapsulated cells (Burgain et al., 2011; Capela et al., 2006). Homogenization pressures can be used as a technology to microcapsulate probiotic bacteria (Ding & Shah, 2009). In addition, application of sub-lethal homogenization pressures to *Lactobacillus salivarius* spp*. salivarius* improves probiotic properties such as hidrofobicity, resistance to biological stresses and microorganism viability (Burns et al., 2015; Patrignani et al., 2017; Betoret et al., 2017). Besides many advantageous effects have been reported for improving the availability of antioxidant compounds and quality parameters (Velazquez- Estrada et al., 2013; Betoret et al., 2012)

Foods are mostly complex mixtures of macro- and micro-components, organized in a structure that can trap active compounds, modulating their release or inhibiting their activity (Chen, et al., 2006). Food matrix and its interactions with encapsulated and not probiotic microorganisms together with enzymatic and chemical reactions that occur during processing and storage are important factors to consider which exert an effect on the surveillance of microorganisms (Ranadheera, 2010; Kailasaphathy et al., 2008).

The aim of this work is to determine the effect of hot air drying on the probiotic survival and in vitro digestion of *Lactobacillus salivarius* spp. *salivarius* encapsulated by using homogenization pressures, included into an apple food matrix and stored during 30 days.

2. Material and methods

2.1. Strain and food materials

Lactobacillus salivarius spp. *salivarius* CECT 4063 was obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain).

Mandarin fruit cv. Ortanique (*Citrus sinensis* x *Citrus reticulata*) was provided by Rural S. Vicent Ferrer cooperative located in Benaguacil, Valencia, Spain. Juice preparation was done following the procedure described in WO/2007/042593 (Izquierdo et al., 2007). Fruits were washed, drained, squeezed ("GAM" MOD.SPA 1400 rpm, power 350W – monophase 220V, Cesena, Italy) filtered with 0.7 mm sieve, centrifuged at 3645 x g during 5 minutes at 5 ºC (Beckman Coulter AvantiTM J-25, California, United States) and pasteurized at 63 ºC during 15 seconds (Roboqbo Qb8-3, Bologna, Italy).

Apples (cv. Granny Smith) were obtained from a local market and were cut into slices of annular shape, 5 mm thick with 20 mm internal diameter and 65 mm external diameter from its vertical axis.

2.2. Microencapsulation

To microencapsulate *L. salivarius* spp. *salivarius* the method described by (Ding & Shah, 2009) was followed with some modifications. A volume of 2 L of Man, Rogosa & Sharpe (MRS) Broth (Scharlab, Barcelona, Spain) containing 9 Log CFU/mL of *L. salivarius* spp. *salivarius* was centrifuged at 7700 x g during 15 minutes at 10 ºC (Beckman Coulter AvantiTM J-25, California,

United States) and suspended with 100 mL of sterile water. A mixture of 25 mL of microorganism solution, 100 mL of sodium alginate (3%) (Sigma-aldrich, Steinheim, Germany), 1 mL of tween 80 (Sharlau, Sentmenat, Spain) and 200 mL of commercial sunflower oil was homogenized in two cycles at 70 MPa with a homogenizer (Panda Plus Niro Soavi, Parma, Italy). The emulsion was broken with calcium chlorine 0.1 M (Sigma-aldrich, Steinheim, Germany) and kept overnight at 4 ºC to separate the phases. Microcapsules were isolated by centrifugation at 7700 x g during 15 minutes at 10 ºC (Beckman Coulter AvantiTM J-25, California, United States).

2.3. Impregnation liquids

Mandarin juice with *L. salivarius* spp. *salivarius* encapsulated and not was used as impregnation liquid.

Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* was prepared following the methodology described in Betoret et al., 2012 by inoculation with 4 mL/L of MRS Broth (Scharlab, Barcelona, Spain) containing 9 Log CFU/mL and maintained at 37 ºC during 24 h. Previously, juice pH was modified by adding 9.8 g/L of sodium bicarbonate (Hacendado, Novelda, Spain).

Mandarin juice with microencapsulated *L. salivarius* spp. *salivarius* was prepared by adding microcapsules prepared as described above into the juice in a ratio of 1.45 juice/microcapsules

(w/w). The mixture was maintained in agitation at room temperature during 1 h.

2.4. Process to produce L. salivarius spp. salivarius enriched dried apple

To produce the dried apple with *L. salivarius* spp. *salivarius* microencapsulated and not, the methodology described at Betoret et al., (2012) was followed. Briefly, fresh apple rings were immersed into impregnation liquids and impregnation was carried out with a vacuum pressure of 50 mbar during 10 minutes. Then, atmospheric pressure was restored and samples were left submerged further 10 min. Impregnated samples were dried using an air drier (POL-EKO model CLW400 TOP, Controltecnica Instrumentación Científica, S.L., Madrid, Spain) at 40 ºC during 24 hours. The values provided are the average of three replicates.

2.5. Physicochemical characterization

Impregnated and dried apple discs were characterized in terms of pH, water activity and moisture content. To determine pH, a pHmeter (Crison GLP21, Barcelona, Spain) was used. Water activity was measured using a dew point hygrometer (DECAGÓN Aqualab CX-2, Washington, United States). Water content was quantified by vacuum drying at 60 ºC until a constant weight (method 20.013, AOAC, 1980). The values provided are the average of three replicates.

2.6. Microbial content

Microbial content was determined following the dilution method and growth in MRS agar (Scharlab, Barcelona, Spain) on double layer incubated 24 hours at 37 ºC. In samples with encapsulated *L. salivarius* spp. *salivarius* the first dilution was done in phosphate buffer solution (pH 7.4) maintained in agitation during 30 minutes. Values provided are the average of four replicates.

2.7. Gastrointestinal simulation

In order to determine the effect of gastrointestinal digestion on the microorganism survival two variables were considered: t_i referred to a moment during the gastrointestinal digestion; T_i referred to the *L. salivarius* spp. *salivarius* content at different stages during the gastrointestinal digestion. A dilution 1:1 of the mandarin juice with 0.6% (w/v) pepsine (Sigma-aldrich, Steinheim, Germany) was adjusted with HCl 4M to pH 3 (t_1 - T_1). Sample was kept in an agitated bath at 37ºC for 90 minutes (t_2 - T_2). Phosphate buffer solution at pH 8 with 10% of bile (Sigma-aldrich, Steinheim, Germany) were added and mixed (t_3 - T_3). Finally, phosphate buffer solution at pH 8 with 0.3% of bile 0.1% pancreatine (Sigma-aldrich, Steinheim, Germany) was added and sample was incubated at 37ºC for 90 minutes (t_4 - T_4). Microorganism content was measured by plate count after each of the four stages considered for gastrointestinal digestion process described before. The results provided are the average of four replicates.

2.8. Storage

Dried samples were stored in closed opaque plastic bags at room temperature and analyses were performed weekly during 30 days.

2.9. Statistical analysis

An ANOVA analysis was carried out to determine the significant effect of the process variables, with 95% of confidence level, using Statgraphics centurion XVI software (StatPoint Technologies, Virginia, US).

3. Results and discussion

3.1. Physicochemical characterization

Physicochemical characteristics of the impregnated and dried apple discs with *L. salivarius* spp. *salivarius* were evaluated during 30 days of storage (table 1). Generally, the physicochemical properties of dried apple with *L. salivarius* spp. *salivarius* encapsulated and not, were maintained similar during all the storage time. pH values of dried apple with encapsulated *L. salivarius* spp. *salivarius* were higher, showing less variability than that obtained in samples with non-encapsulated microorganisms. Samples with encapsulated *L. salivarius* spp. *salivarius* had less amount of mandarin juice impregnated than those samples with non-encapsulated microorganisms. Additionally, the encapsulation process could decrease the activity of *L. salivarius* spp. *salivarius* resulting in a lower fermentation

activity of the microencapsulated cells which would produce less acidic compounds (Bilenler et al., 2017; Ribeiro et al., 2014). At the end of the storage there were not differences between both samples.

The rate of food reactions and metabolomic activities of spoilage organisms are reduced with lower moisture content, being retarded and even inhibited with a water activity as or below 0.3 (Smith, 2008). In our case, despite obtained water activity is higher than 0.3, any moulds or harmful bacteria were developed during the storage. Our results were similar to that obtained previously by Betoret et al., (2012). Water activity values ranged between 0.48 and 0.54 in both cases, with more variability observed in samples with encapsulated *L. salivarius* spp. *salivarius* and a tendency to increase with storage time. In samples with non-encapsulated *L. salivarius* spp. *salivarius,* the values of water activity were maintained practically constant during 21 days from which had a tendency to increase. The same behaviour was observed for moisture content values. Air drying is a mass transfer operation that takes place in a complex and porous structure. The presence of oil coming from the emulsion to encapsulate *L. salivarius* spp. *salivarius* in the apple slices could difficult the water flux during drying, resulting in a less homogeneous product. An unequal distribution of water content during drying could cause further water migrations during storage, explaining then the differences observed between both samples.

Table 1. Physicochemical properties of the dried apple with encapsulated and non- encapsulated *Lactobacillus salivarius* spp. *salivarius* during the storage time. Mean ± standard deviation of three replicates.

	pH		a_w		Moisture(kg_{water}/kg_{dried})	
Day	Encapsulated	Non-encapsulated	Encapsulated	Non-encapsulated	Encapsulated	Non-encapsulated
1	3.44 ± 0.05^{ab}	3.21 ± 0.05^{a}	0.516 ± 0.002^{c}	0.516 ± 0.002^{c}	0.107 ± 0.002^{a}	0.128 ± 0.006^{ab}
7	3.48 ± 0.03^{abc}	3.16 ± 0.08^{a}	0.487 ± 0.006^{a}	0.516 ± 0.002^{c}	0.128 ± 0.012^{b}	0.124 ± 0.006^{ab}
14	3.39 ± 0.09^{a}	3.36 ± 0.08^{b}	0.534 ± 0.002^{d}	0.5003 ± 0.002^{a}	0.125 ± 0.003^{b}	0.117 ± 0.003^{a}
21	3.55 ± 0.12^{bc}	3.43 ± 0.04^{b}	0.544 ± 0.002^{e}	0.5116 ± 0.002^{b}	0.129 ± 0.006^{b}	0.12 ± 0.06^{a}
30	3.6 ± 0.02^{c}	3.6 ± 0.02^{c}	0.505 ± 0.002^{b}	0.5325 ± 0.003^{d}	0.132 ± 0.006^{c}	0.136 ± 0.003^{b}

Values with different superscript letters within the same column are significantly different ($p \leq 0.05$).

3.2. Effect of technological operations on probiotic survival

Microbial content of the encapsulated and non-encapsulated *L. salivarius* spp. *salivarius* at each intermediate product of the process are presented in figure 1. The content of encapsulated *L. salivarius* spp. *salivarius* in mandarin juice was managed to be the same as that obtained in samples with non-encapsulated microorganisms in order to compare its degradation during the processing. The obtained results in mandarin juice with *L. salivarius* spp. *salivarius* encapsulated and not, are similar to that obtained in previous studies (Calabuig- Jiménez et al., 2019). In the same way, the obtained results at different process stages in samples with non-encapsulated *L. salivarius* spp. *salivarius* are similar to that obtained in previous studies (Betoret et al., 2016; Betoret et al., 2012).

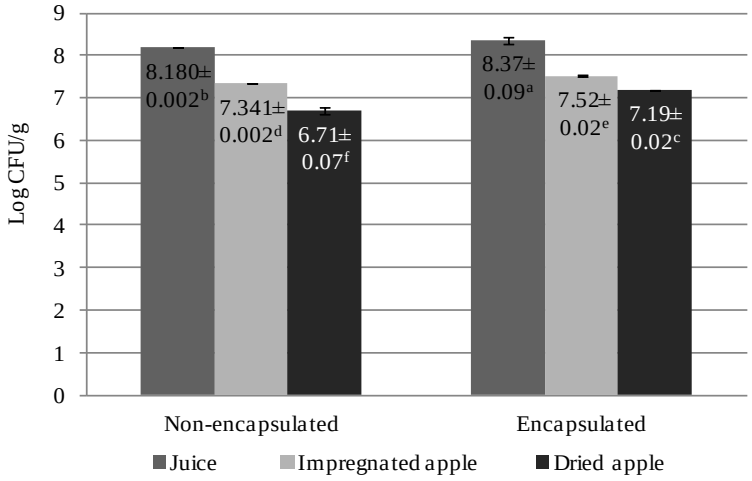

Figure 1. Microorganism content expressed in Log CFU/g of juice, impregnated apple and dried apple with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius*. Plotted results are the average ± standard deviation of four replicates.

Microorganisms' losses during vacuum impregnation operation were higher in samples with encapsulated *L. salivarius* spp. *salivarius*. In order to determine whether the vacuum impregnation was homogeneous and the main mechanism of action was the hydrodynamic, a theoretical estimation of the microorganism content was calculated using the equation 1.

$$x_{fIV} = \frac{x_{LIV} \cdot X \frac{\rho_{LIV}}{\rho_{fa}}}{1 + X \frac{\rho_{LIV}}{\rho_{fa}}} \quad (1)$$

Where,

m: mass (g); x: microorganism content; X: volumetric impregnation; ρ: density (g/cm^3); fIV: subscript refered to impregnated apple; fa: subscript refered to fresh apple; LIV: subscript refered to impregnation liquid.

Calculated and determined values were 8.7 ± 0.02 Log CFU/g$_{IV}$ - 7.52 ± 0.02 Log CFU/g$_{IV}$ and 7.62 ± 0.04 Log CFU/g$_{IV}$ - 7.341 ± 0.002 Log CFU/g$_{IV}$ in samples with encapsulated *L. salivarius* spp. *salivarius* and not, respectively. Similar obtained values, as in samples with non-encapsulated *L. salivarius* spp. *salivarius,* indicated that the vacuum impregnation was homogeneous and the main mechanism of action implicated in the mass transfer between the impregnation liquid and the apple slices was the hydrodynamic. In this case, the pressure gradients created in the system are the responsible of the impregnation liquid flux into the intracellular spaces of apple. Pressure levels applied during the vacuum impregnation operation in this study do not affect significantly microorganisms' survival. Thus, the differences observed between calculated and determined values in samples

with encapsulated *L. salivarius* spp. *salivarius* could be due to the vacuum impregnation operation was not homogeneous.

The content of *L. salivarius* spp. *salivarius* encapsulated and not, in dried apple samples was significantly different and enough high to have a potential probiotic effect (De Camps et al., 2013). In order to evaluate the real losses of *L. salivarius* spp. *salivarius* during drying, the microorganisms' content of dried apple (Log CFU/g_{dried}) (figure 1) was expressed in the same basis that the microorganisms content of impregnated apple (Log CFU/g_{IV}). Thus, the microorganisms' losses during drying in samples with *L. salivarius* spp. *salivarius* encapsulated and not were 6.473 ± 0.017 and 6.29 ± 0.011 Log CFU/g_{IV} respectively.

During air drying operation the most important change is the removal of water from sample. Heat damage, water losses linked to structural changes and oxidation reactions due to the air exposure affect both cellular plant tissues and microbial cells. Excessive heat unfolds the higher order structure of macromolecules such as protein and nucleic acid, breaks the linkage between monomeric units and eventually causes the destruction of the monomeric units (Corcoran et al., 2008; Santivarangkna et al., 2008). Water losses linked to structural modifications and oxidation reactions mainly affects the cytoplasmic membrane of microbial cells by changing its fluidity or the physical state as well as causing lipid peroxidation (Crowe et al., 1992 …). The resultant membrane leakage followed by the loss of life-essential cellular substances is fatal. Cells entrapped within the droplets formed by alginate would obtain additional

protection by the capsule. However, as according to Fu and Chen, 2011, the protection of cell viability during drying given by this type of microencapsulation is quite limited. The optimization of encapsulated cell survival during a drying process relies more on the material of carrier and the drying conditions. In this study a mild drying was employed, with an air temperature of 40 ºC (the limit for the survival of *L. salivarius* spp. *salivarius*) in order to limit drying stress in bacterial cells but more oxidation reactions could be promoted due to the long air exposure time.

3.3. Probiotic content during storage time

The content of *L. salivarius* spp. *salivarius* encapsulated and not, stored during 30 days at room temperature and maintained in closed opaque plastic bags, was determined (table 2). During the first 14 days of storage a decrease in 60 % of the microorganisms' content was observed. This results agree with Weinbreck et al., 2010 and Moumita et al., 2017 that observed a decrease of 3-5 log in the microorganism content encapsulated and not, after 14 days of storage. From this point, significant differences were observed between both samples, with an improvement in the microorganism survival in encapsulated samples of 39 versus 19 % of non- encapsulated at the end of storage.

During storage, cell survival is particularly affected when the food matrix has an elevated water activity (aw > 0.25) (Teixeira et al., 1995). Storage temperature and the presence of atmospheric oxygen might also contribute to reductions in viable cell amounts (Anal and Singh, 2007). Our results, showed up that capsules

were not able to protect significantly *L. salivarius* spp. *salivarius* from degradation reactions during the first 14 days of storage. Alginate is a porous material that is not able to isolate encapsulated microorganisms from water migrations. However, after 14 days of storage, capsules were able to protect *L. salivarius* spp. *salivarius* from degradation reactions.

Table 2. Microbial count (Log CFU/g_{dried}) of encapsulated and non-encapsulated dried apple during the storage time. Number in brackets indicates the survival in percentage respect the first day. Mean± standard deviation of four replicates.

	Encapsulated	Non- encapsulated
Day 1	7.19 ± 0.07^{a} (100)	6.71 ± 0.08^{b} (100)
Day 7	5.85 ± 0.12^{a} (81.3 ± 1.7)	5.26 ± 0.09^{b} (78.2 ± 1.4)
Day 14	3.03 ± 0.06^{a} (42.2 ± 0.9)	2.89 ± 0.09^{b} (43.1 ± 1.4)
Day 21	2.94 ± 0.03^{a} (40.9 ± 0.5)	2.37 ± 0.05^{b} (35.4 ± 0.7)
Day 30	2.78 ± 0.14^{a} (39 ± 2)	1.3 ± 0.2^{b} (19 ± 3)

Values with different superscript letters within the same column are significantly different ($p \leq 0.05$).

3.4. Gastrointestinal simulation

In order to exert a positive effect on the host, probiotic microorganisms should maintain their active form during digestion process, being able to survive the action of lytic enzyme and adverse pH until reaching the target point. Moreover, in the case of encapsulated microorganisms the capsule must be a protection from adverse conditions but should release them at the

appropriate time and place in the organism. The microbial content after each simulated gastrointestinal digestion is shown in table 3. T_0 refers to the initial content of *L. salivarius* spp. *salivarius* in mandarin juice. T_1 and T_2 refer the quantity of microorganisms after simulated stomach conditions, pH change by HCl addition and peristaltic movements respectively. T_3 and T_4 refer the counting of microorganism after the duodenal shock and intestinal juice mixing respectively. *L. salivarius* spp. *salivarius* has been demonstrated to have a potential effect against *Helicobacter pylori* infection. Thus, microorganism survival at gastroduodenal stage, in order to have a potential effect against *H. pylori,* and survival at intestinal step, in order to have a potential probiotic effect, are both key points to consider.

Taking into account number of total microorganisms, only impregnated and dried apple with encapsulated *L. salivarius* spp. *salivarius* at day 0 would reach enough quantity of microorganisms at both gastroduodenal and intestinal stages in order to have a potential beneficial effect. However, regardless of the absolute amount of microorganisms it is interesting to know the behavior of the *L. salivarius* spp. *salivarius* encapsulated and not, introduced into an apple matrix by impregnation and dried, at each stage of the gastrointestinal simulation process as well as the effect during the storage. The statistical analysis revealed that all variables studied; the encapsulation procedure, the single stage at the simulated gastrointestinal digestion and the storage time had a significant effect ($p \leq 0.05$) on *L. salivarius* spp. *salivarius* survival.

Table 3. Microbial content (Log CFU/g_{dried}) of encapsulated and non-encapsulated dried apple with *L. salivarius* at the beginning (To) and at each phase of the gastrointestinal simulation process (T_1 to T_4), over the storage time. Number in brackets indicates survival in percentage respect the initial content. Mean± standard deviation of four replicates.

		Day 0	Day 7	Day 14	Day 21	Day 30
Encapsulated	T_0	7.19 ± 0.07^{h_B} (100)	5.85 ± 0.12^{g_B} (100)	3.03 ± 0.06^{cd_B} (100)	2.94 ± 0.03^{f_B} (100)	2.83 ± 0.14^{f_B} (100)
	T_1	6.03 ± 0.09^{f_B} (83.7 ± 0.8)	5.58 ± 0.02^{f_B} (96 ± 2)	3.71 ± 0.07^{g_A} (122 ± 2)	2.67 ± 0.09^{e_B} (90.7 ± 3)	2.38 ± 0.09^{ef_B} (85.6 ± 1.3)
	T_2	5.81 ± 0.07^{e_B} (80.8 ± 0.4)	5.44 ± 0.06^{ef_B} (94 ± 3)	3.84 ± 0.04^{h_B} (127 ± 2)	2.32 ± 0.13^{c_A} (79 ± 4)	2.0 ± 0.2^{e_B} (70 ± 2)
	T_3	5.26 ± 0.02^{d_B} (73.2± 0.4)	3.99 ± 0.07^{c_B} (68 ± 2)	2.96 ± 0.06^{bc_A} (97.7 ± 0.9)	2.04 ± 0.12^{b_B} (69 ± 3)	0.8 ± 0.3^{ab_A} (29 ± 9)
	T_4	5.2 ± 0.2^{d_B} (72 ± 2)	4.20 ± 0.04^{c_B} (72 ± 2)	$3.09 \pm 0.12^{d,e_B}$ (102 ± 3)	1.41 ± 0.13^{a_A} (48 ± 4)	0.87 ± 0.19^{abc_B} (31 ± 6)
Non-encapsulated	T_0	6.71 ± 0.08^{g_A} (100)	5.26 ± 0.09^{e_A} (100)	2.89 ± 0.09^{b_A} (100)	2.37 ± 0.05^{cd_A} (100)	1.3 ± 0.2^{cd_A} (100)
	T_1	3.89 ± 0.08^{d_A} (58.1 ± 0.4)	4.5 ± 0.3^{d_A} (86 ± 6)	3.75 ± 0.06^{gh_B} (130 ± 4)	2.39 ± 0.13^{de_A} (105 ± 5)	1.0 ± 0.7^{bc_A} (77 ± 5)
	T_2	3.55 ± 0.06^{d_A} (52.9 ± 0.3)	4.5 ± 0.5^{d_A} (85 ± 9)	3.18 ± 0.03^{ef_A} (109 ± 3)	2.40 ± 0.06^{cd_B} (100 ± 0.8)	1.8 ± 0.3^{de_A} (138 ± 15)
	T_3	3.96 ± 0.04^{c_A} (59.1 ± 0.7)	2.75 ± 0.12^{a_A} (52 ± 2)	3.25 ± 0.05^{f_B} (112 ± 2)	2.0 ± 0.2^{b_A} (86 ± 7)	0.7 ± 0.8^{abc_B} (53 ± 62)
	T_4	1.9 ± 0.06^{a_A} (28 ± 0.6)	3.48 ± 0.05^{b_A} (66.2 ± 0.3)	2.67 ± 0.02^{a_A} (92 ± 2)	1.46 ± 0.06^{a_B} (61 ± 2)	0.3 ± 0.3^{a_A} (22± 25)

$^{abc...}$Values with different superscript letters within the same column are significantly different ($p \leq 0.05$).

$_{ABC...}$Values with different subscript letters within the same column shows significance of encapsulation factor ($p \leq 0.05$).

Generally, encapsulated *L. salivarius* spp. *salivarius* demonstrated higher resistance to gastrointestinal simulation as compared to their free form. Total microorganisms content and survival percentage of encapsulated *L. salivarius* spp. *salivarius* was higher than non-encapsulated one. Degradation tendency of the microorganisms encapsulated and not was different at each stage of the simulated gastrointestinal process as well as during the storage. Survival of encapsulated *L. salivarius* spp. *salivarius* was mainly affected by the acidic environment created at t_1 and the addition of bile at t_3. Moreover, survival of microorganisms decreased with storage time at gastrointestinal stages t_2, t_3 and t_4 but not at t_1 at which survival percentage remained practically constant. The results obtained in literature on the protective effect of alginate capsules against acidic environmental conditions are contradictory. While in some cases, the capsule created protects the microorganisms against acidic conditions in others capsule it does not provide any additional protection. Seems that the method used to make the capsule significantly influences the final result. In our case, the capsule conferred a limited protection. A porous capsule surface and its degradation during storage could explain the observed decrease in the *L. salivarius* spp. *salivarius* survival with storage time. Non- encapsulated *L. salivarius* spp. *salivarius* was affected by the acidic environment created at t_1 and the addition of lytic enzymes at t_4. In this case, survival of microorganisms decreased with storage time mainly at t_3.

It is remarkable the increase in microorganisms content observed at day 14 in encapsulated *L. salivarius* spp. *salivarius* and not, and at day 21 in non-encapsulated *L. salivarius* spp. *salivarius*. Upon sudden changes in temperature, osmotic pressure or pH, a microbial cell is able to adapt itself to the new environment by adjusting the metabolic flow and genetic expression (Santivarangkna et al., 2008). After the acidic stress conditions created around cells at pH 3.5 Jin et al., 2012 observed a significant increase in the acid tolerance response mechanism which would promote their growth when optimal conditions are restored.

4. Conclusion

Incorporation of encapsulated *L. salivarius* spp. *salivarius* using homogenization pressures into an apple structure by vacuum impregnation operation was successfully done. In spite of the microorganisms losses during hot air drying operation, the number of *L. salivarius* spp. *salivarius* in the impregnated and dried apple was enough high to have a potential beneficial effect.

Capsules were able to significantly protect *L. salivarius* spp. *salivarius* during the simulated gastrointestinal digestion. However, further fundamental studies on morphology and degradation of capsules during processing and storage would be necessary in order to enhance the microorganisms' protection and thus the industrial utility.

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3.2.1. Conclusiones

Como consecuencia del tratamiento por HPH y debido a la disminución del tamaño de particula, se mejoró la estabilidad de la nube del zumo. A su vez, este tratamiento mejoró la disponibilidad de los fenoles totales y de los flavonoides. Sin embargo, la adición de trehalosa al zumo provocó una disminución en el contenido en fenoles y flavonoides. Por lo contrario, la adición de un 20% de trehalosa a los zumos, aumentó la actividad antioxidante determinada por el método del ABTS, lo que sugiere una interacción entre la trehalosa y los compuestos antioxidantes como fenoles y flavonoides.

El zumo de mandarina con *Lactobacillus salivarius* spp. *salivarius* tratado con presiones moderadas (20 MPa) mejoró la viabilidad del probiótico durante el almacenamiento. La adición de trehalosa (10 %) mejoró, además, su hidrofobicidad

La encapsulación de *L. salivarius* spp. *salivarius* mediante la homogeneización a 70 MPa, mejoró su supervivencia durante el almacenamiento y su resistencia a la digestión *in vitro,* tanto en el zumo como en los discos de manzana impregnados con este. La encapsulación no tuvo efecto sobre la supervivencia del microorganismo durante la operación de IV y el secado por aire a 40ºC.

3.3. Fuentes alternativas de ingredientes alimentarios de alto valor funcional

La búsqueda de materias primas alternativas a las convencionales, obtenidas mediante procesos más sostenibles y que preserven los compuestos de mayor interés funcional, o bien, a partir del aprovechamiento de subproductos de la industria agroalimentaria, es otra de las formas de contribuir a la sostenibilidad de los procesos alimentarios.

En primer lugar, se aborda el estudio de las propiedades funcionales del azúcar de caña no refinado frente al azúcar blanco. Se trata de un producto sometido a un menor procesado y de consumo habitual en los países y comunidades en los que se cultiva, donde además, la agricultura y la producción de la caña consituyen el principal modo de vida para muchos de sus habitantes. El azúcar es una de las materias primas más utilizadas en el mundo, siendo el azúcar blanco o refinado el más comúnmente empleado en la industria alimentaria para la formulación de alimentos. A sus particulares características organolépticas hay que sumarle su acción conservante, sobre todo cuando se emplea en concentraciones elevadas, lo cual lo diferencia sustancialmente de otras alternativas endulzantes como los edulcorantes. Por otro lado, el azúcar blanco común tiene un coste significativamente menor al del azúcar de caña sin refinar, fundamentalmente por su producción a gran escala a partir tanto de la caña de azúcuar como de la remolacha azucarera. Sin embargo, el proceso necesario para obtener los cristales de sacarosa elimina la totalidad de los compuestos beneficiosos presentes en la caña de azúcar a través de un proceso que incluye etapas contaminantes, debido a la generación de

residuos de cal y ácido fosfórico. No obstante, su ingesta se ha relacionado con el desarrollo de desórdenes metabólicos y enfermedades asociadas tales como la obesidad y la diabetes tipo 2, por lo que se recomienda limitar la ingesta de este ingrediente, sobre todo en niños y en individuos con determinados riesgos. En este contexto, el azúcar de caña no refinado puede plantearse como una alternativa más saludable, dado que numerosos estudios avalan la presencia de compuestos antioxidantes en el jugo de caña del que proviene. En este capítulo, se presenta el estudio de las propiedades fisicoquímicas y antioxidantes de los productos derivados de la caña de azúcar. Se estudiaron 12 productos, entre ellos, azúcares morenos, panela granulada, panelas en bloque y miel de caña. Estos productos se evaluaron en términos de humedad, actividad del agua, sólidos solubles, pH, color, contenido en glucosa, fructosa y sacarosa, fenoles totales, flavonoides totales y actividad antioxidante por los métodos DPPH y ABTS. Se confirmó que los azúcares de caña no refinados presentan compuestos con actividad antioxidante y que su contenido está relacionado con el grado de refinado del producto. Los azúcares no refinados constituyen una fuente natural de antioxidantes. Además, dado el alto consumo de azúcar en la industria, la sustitución del azúcar refinado por los azúcares de caña no refinados presentaría un gran aporte de compuestos antioxidantes a la dieta.

Por otra parte, la industria agroalimentaria genera gran cantidad de residuos que implican un coste económico y medioambiental. En muchos casos, estos subproductos incluyen en

su composición una gran cantidad de compuestos de interés, como la fibra, los minerales, los compuestos antioxidantes y las vitaminas. Concretamente el arándano es muy rico en compuestos antioxidantes beneficiosos para la salud, destacando por su alto contenido en antocianinas. El bagazo de arándano, además es muy rico en fibra y contiene compuestos antioxidantes, principalmente antocianinas. Además de estos compuestos, la fibra le confiere propiedades de hidratación y emulsionantes que pueden resultar muy interesantes para la formulación de alimentos. Recientemente está cobrando gran importancia la obtención de polvos a partir de los subproductos de la industria agroalimentaria ya que presentan gran versatilidad en sus aplicaciones y gran facilidad en el almacenamiento y distribución. En este sentido, se ha obtenido polvo de bagazo de arándano. Para ello, se ha estudiado el efecto de la temperatura de secado del bagazo de arándano (60 y 70 ºC) y la intensidad de triturado (obteniendo un polvo de granulometría fina y otro de granulometría gruesa) sobre las propiedades funcionales de los polvos obtenidos. Los polvos se evaluaron en términos de humedad, actividad del agua, contenido en sólidos solubles, contenido en fibra, tamaño de partícula, color, isotermas, propiedades de interacción con el agua y de interacción con los lípidos (propiedades emulsionantes), capacidad antioxidante, contenido en fenoles totales y en antocianinas monoméricas así como el efecto de almacenamiento durante 20 semanas sobre las propiedades antioxidantes de los polvos. Los resultados obtenidos mostraron que el secado, independientemente de la temperatura,

disminuyó la capacidad antioxidante del bagazo de arándano, afectando sobre todo al contenido en fenoles totales y antocianinas monoméricas. Además, se demostró que el tamaño de partícula influye de forma decisiva en las propiedades del producto final. En particular, la acción mecánica del triturado, redujo de manera significativa el contenido en fibra del polvo, lo que influyó sobre las propiedades relacionadas con su interacción con el agua y las grasas.

Physicochemical and antioxidant properties of non-refined sugarcane alternatives to white sugar.

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Abstract

Antioxidant properties of commercial sugar cane derived products were analyzed to study their suitability for being used as functional ingredients. Different products, including cane honey, several jaggeries (from light to dark) and several brown sugars (with molasses, and light to dark) were selected from the market and analyzed in terms of physicochemical characteristics and antioxidant properties, and compared with white refined sugar (12 products in total). Moisture, water activity, total soluble solids, pH, color and sugar profile are reported. As for antioxidant properties, total phenols and flavonoid content, as well as antiradical ability of the products (DPPH• and the TEAC-ABTS methods) are given. All sugarcane products contained phenols and flavonoids and exhibited in vitro antioxidant activity, determined by degree of refining. Among the alternatives analyzed, jaggeries and cane honey showed the best antioxidant properties. Thermal treatment did not significantly affect the antioxidant capacity of sugarcane products, especially jaggeries. Since sugar and sugar-rich products are widely consumed worldwide, the use of non-refined sugarcane derivatives in reformulation of foods with increased nutritional properties is encouraged.

Keywords: sugars, sugarcane, jaggery, antioxidants, phenols, flavonoids.

1. Introduction

Table sugar, also known as white or refined sugar, is a white refined product extracted from sugar cane (70%) or sugar beet (30%), made of up to 99.9% of sucrose. Due to its high purity, its nutritional value is very poor and it is said to provide a high amount of "empty calories"; in addition, its consumption has also been related to a higher incidence of dental caries in occidental societies. Both aspects have contributed to the search of non-caloric and non-cariogenic alternatives such as sweeteners. Nevertheless, despite the increase in sweeteners consumption and their use in food formulation, sugar continues to be an essential part of our diet, not only appreciated for its flavor and its particular sweetening characteristics, but also for its contribution to food preservation (Harish Nayaka et al., 2005). Refined sugar is the sugar most widely consumed in Europe and North America. However, apart from refined sugar, non-refined sugarcane alternatives are nowadays available in the market. According to Galloway (2000), non-centrifugal sugar used to be the dominant form of sugarcane consumption before the large-scale production of refined sugar after 1700. At present, these sugars are still commonly consumed in South-America, Asia and Africa, and they have experienced a significant increase in the European market. In fact, non-refined sugars have increased in quantity, diversity and availability, this being a consequence of both the increasing interest for natural food and ingredients, as well as of globalization, multiculturalism and immigration mainly from Asian and South American countries.

In Europe and North America sugar is mostly appreciated for its sweetening properties, whereas sugarcane has been part of traditional medicine in the tropics and subtropics where it is produced. As an example, it is used in Ayurvedic medicine to treat different heath problems such as infections, bronchitis, cough, anemia, constipation, jaundice, general debility and heart or blood conditions (Kadam et al., 2008). During the last years, sugarcane has raised interest regarding its nutraceutic properties. Several studies have shown beneficial effects of sugarcane extracts on models *in vivo* in the stimulation of the immune response, protection against liver damage, intestinal function recovery, protection against some infections, anti-trombotic and anti-stress effects or growth stimulation (Koge et al. 2001; Noa et al., 2002; El-Abasy et al., 2003, 2004; Amer et al., 2004; Lo et al., 2005; Motobu et al., 2006; Yamauchi et al., 2006). On searching the origin of these beneficial effects, it has been found that sugarcane has a powerful antioxidant activity, and it is known that oxidative damage is involved in many human diseases such as cancer, cardiovascular diseases or other degenerative disorders. According to Kadam et al. (2008), the antioxidant properties of sugarcane juice could partially explain its therapeutic effects.

Antioxidant properties of sugarcane have been basically attributed to phenolic compounds, mainly flavonoids, phenolic acids and poliphenols. Food phenolic compounds, particularly flavonoids, are thought to play important roles in human health (Yao et al., 2004). However, these compounds are non-desired components in the sugar manufacturing process, and are

eliminated from the juice during refining. Other sugarcane processed products, such as molasses, may contain other antioxidants components as a result of Maillard reactions developing during processing. Some studies have proved the antioxidant properties of raw sugarcane, residual molasses or their extracts. Among other beneficial effects, antiradical capacity, inhibition of lipid peroxidation, protection against oxidative and radiation induced DNA damage, and *in vitro* antiproliferative activity against cancer cell lines have been reported (Duarte-Almeida et al., 2007; 2006; Guimaraes et al., 2007; Kadam et al., 2008). Nevertheless, most studies have focused on identifying antioxidant components in sugarcane extracts and their potential health benefits, whereas less attention has been paid to the characterization of non-refined commercially available sugars. Yet, it seems plausible that non-refined sugarcane products may preserve some of the raw material properties, and these are likely to depend on the refining degree of each product. In recent years, some authors have reported the antioxidant activity of cane brown sugars and molasses (Payet et al., 2005; Phillips et al., 2009); Harish Nayaka et al. (2009) also included a jaggery sugar in their study.

At present, many non-refined sugarcane products are available in markets and supermarkets. In particular, we identified different kinds of brown sugars (coated, boiled, light to dark), several jaggeries (light to dark, granulated or in block) and sugarcane honey; each of which has undergone different processing, and may have been refined to a different extent.

Despite available in many stores and supermarkets, consumption of these non-refined sugars is still marginal as compared with white sugar, let alone have they been proposed for food formulation. Although sugarcane extracts have been suggested as therapeutic agents, although sugarcane extracts have been pointed as therapeutic agents, the potential impact of non-refined cane sugars as a substitute for white sugar on formulated foods has not been evaluated yet.

In the present work, physicochemical and antioxidant properties of twelve commercial sugarcane derived products are analyzed to study their suitability for being used as functional ingredients. As a healthier sweetener, non-refined cane sugars could be used as a substitute to white sugar in traditionally sugar-rich foods such as jams, syrups, jellies or pastries, but they could also be used to formulate new antioxidant-enriched products by using matrix engineering techniques such as osmotic dehydration or vacuum impregnation.

2. Materials and methods

2.1. Non-refined sugarcane products

Supermarkets and specialized stores in Valencia (Spain) were visited so as to get a representative sample of the different non-refined cane sugars commercially available. Twelve products, including cane honey, several brown sugars, different jaggeries and white refined sugar, were selected for the study. Products were stored in dry conditions and at room temperature. The

twelve selected products were: white sugar (W), used as reference material, coated brown sugar (CB), light brown sugar (LB), raw brown sugar (RB), dark brown sugar (DB), wet brown sugar (WB), raw brown sugar with molasses (MB), light jaggery block (LJ), regular jaggery block (RJ), granulated jaggery (GJ), dark jaggery block (DJ) and sugarcane honey (CH).

2.2. Physiochemical characterization

Sugarcane products were analyzed in terms of moisture (x_w), water activity (a_w), total soluble solids (TSS), pH, colour and sugar profile (fructose, glucose, sucrose). Moisture was determined with an infrared scale (AD-4714A, Afora SA) and water activity was measured with a hygrometer (Aqualab 4TE). Total soluble solids and pH were measured on 1:10 and 1:4 (w/w) solutions of the non-refined sugars, respectively. Total soluble solids were determined by refractometry (Brix degrees). The ICUMSA method (De Whalley, 1964) was used for colour analysis. This method is considered the international official method for measuring crystallized white and brown sugars and consists of measuring the absorbance at 420 nm of a solution of the sugar, after filtering the samples through nylon mesh (0.45 μm). The method measures the purity of the sugar by determining yellowness. ICUMSA colour index (IC) is then calculated by the following equation, where Abs is the absorbance of the sample at 420 nm, b the light path length, and c the total solids content, obtained by refractometry (Brix degrees) and expressed in g/cm^3 by using density at 20 ºC.

$$IC = \frac{Abs}{b \cdot c} \cdot 1000$$

Fructose, glucose and sucrose content was determined by ion exchange chromatography (716 Compact IC Metrohm), with a Metrosep Carb column, using 0.1 M NaOH as the mobile phase. Measurements were performed on filtered solutions (0.45 μm) prepared at different concentrations (from 1.5:1,000 to 1:10,000 v/v) in deionized water. Fructose, glucose and sucrose standards (>99.5% purity) were purchased from Sigma-Aldrich.

2.3. Determination of total phenols

The total phenolic content of the non-refined sugar samples was measured using a modified colorimetric Folin-Ciocalteu method (Singleton et al., 1999; Wolfe et al., 2003). 0.125 mL of a 1:4 (w/w) dilution of the samples were mixed with 0.5 mL of deionized water. Folin-Ciocalteu reagent (0.125 mL) was added to the solution and allowed to react for 6 min. Then, 1.25 mL of a 7% sodium carbonate solution was added. Finally, the mixture was diluted to 3 mL with deionized water and colour was allowed to develop. After 90 min, the absorbance of the mixture was read at 760 nm using a Helios Zeta UV/Vis (Thermo Scientific) spectrophotometer. Absorbance measurements were compared to a standard curve of gallic acid (purity ≥ 98%, Sigma-Aldrich) and expressed as mg of gallic acid equivalents (GAE) per gram of product.

It is known that sugars, mainly the reducing sugars glucose and fructose, may interfere in the Folin-Ciocalteu method by overestimating the amount of total phenolic compounds present in the sample (Slinkard & Singleton, 1977; Singleton et al., 1999). In order to evaluate and eliminate this interference, several

standard gallic acid curves were also prepared with different proportions of sugars (sucrose, glucose and fructose). The total amount of sugars in the calibrating curves was 25 g/100 mL, and the amount of reducing sugars ranged from 0% to 8% over the total sugars. Five different calibration curves were obtained and were further used as appropriate to calculate the overestimation due to sugar presence in the samples.

2.4. Determination of flavonoid content

The flavonoid content of the non-refined sugars samples was measured using the colorimetric method of aluminum chloride (Luximon-Ramma et al., 2002). 1.5 mL of a diluted sample (1:25 w/w) were mixed with 1.5 mL of aluminum chloride solution (2% w/v in methanol), the mixture was vigorously shaken and allowed to react for 10 min. The absorbance at 368 nm of the mixture was then measured and compared to a standard curve of quercetin (purity ≥ 95%, Sigma-Aldrich). The flavonoid content was expressed in mg of quercetin equivalents (QE) per gram of product.

2.5. Quantification of antioxidant activity

Antioxidant activity (AO) was assessed by determining the radical scavenging abilities of non-refined cane sugars using 1,1-diphenyl-2-picryl hydrazyl (DPPH•) and 2,20-azobis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS) methods.

The DPPH• method was based on the proposed by Brand-William et al. (1995) and consisted of diluting 30 μL of the sample (i.e. different sugar solutions at concentrations from 1:4 to 1:30

w/v) in 970 µL of methanol, and adding the mixture to 2 mL of a DPPH•-methanol solution (0.1 mM). Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Percent radical scavenging activity or percentage of inhibition was determined using the following equation where I represents the inhibition of DPPH•, in percentage, and A the absorbance at 517 nm of the blank and sample.

$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

The ABTS or TEAC (Trolox Equivalent Antioxidant capacity) was determined according to Re et al., 1999. This method is based on the ability of an antioxidant to scavenge the preformed radical cation ABTS+ relative to that of the standard antioxidant Trolox. ABTS (7 mM) was made to react with potassium persulfate (2.45 mM) during 16 hours at room temperature, so as to obtain the ABTS+ radical. Then, the solution was diluted in phosphate buffer (pH 7) to an absorbance of 0.700 ± 0.020 at 734 nm. 90 µL of the sample or control were then added to 2910 µL of the ABTS+ in phosphate buffer solution and absorbance at 734 nm was then read at 1, 2, 3 and 6 min of reaction. In controls, deionized water was used. TEAC values were expressed in µmol Trolox/g of sample.

2.6. Thermal treatments.

The products having higher antioxidant capacity underwent thermal treatment in order to evaluate the effect of thermal processing of the food on AO capacity. Solutions of the sugars

were prepared and treated at 60, 80 and 100 ºC during 10, 30 and 60 min in a thermostatic bath (P.Selecta, Precisdig). After that, AO properties were evaluated by the DPPH• and TEAC methods.

2.7. Statistical analysis

All analytical determinations were performed on at least three different samples of each product. Depending on the analysis, one to three replicates of each repetition were performed in order to obtain the sample value further subjected to statistical analysis. Results were statistically analyzed using Statgraphics Centurion XVI.

3. Results

3.1. Physicochemical characteristics of sugarcane products

Physicochemical attributes of white and non-refined sugarcane sugars are given in table 1. Although water activity was relatively similar for all samples, water content was significantly higher for cane honey than for jaggeries and crystal sugars. Jaggeries water content was in the range of the reported by other authors (Mujica et al., 2008). TSS content was close to 1 for all sugars except for CH, which lower TSS content suggests a significant amount of other compounds different from sugars. A relationship between refining and pH is identified, since crystal sugars have higher pH than jaggeries and cane honey. Carbonatation takes place in the sugar manufacturing process which facilitates the precipitation of undesired impurities and increases the pH of the syrup. Other compounds such as organic

acids can also be responsible for a difference in pH. In addition, CH contains citric acid as a preservative. In the case of CH, however, it could also have affected the fact that CH contains citric acid as a preservative.

Concerning the colour of the samples according to the ICUMSA method, a correlation between the purity and the IU. is observed. As expected, white sugar presented very low IU., whereas crystallized brown sugar had higher indexes. Brown sugar containing molasses had a colour similar to jaggeries and cane honey had the highest IU. The values obtained in the present work for brown sugars (CB, DB, LB and RB) were in the range of the reported by Wojtczak et al. (2013) who analyzed brown (1,368 to 3,256 IU.) and raw cane sugars (3,702 ± 1,509 IU). Other authors (Saska et al, 2010) have reported values for sugarcane juice between 10,000 and 20,000 IU. and higher than 38,000 for molasses (Saska & Chou, 2002).

The sugar profile of the products is also presented in table 1. Three were the sugars identified by ion exchange chromatography: sucrose, glucose and fructose. In white sugar, only sucrose was identified and reducing sugars were not detected, whereas a rather negligible amount of glucose and fructose was identified in coated and dark brown sugars. Other brown sugars had less sucrose, and it was even lower for jaggeries and, particularly, for cane honey. Although fructose and glucose are present in the raw material, reducing sugars may also come from sucrose inversion during post-harvesting and processing.

Table 1. Physicochemical characteristics of sugarcane products. Means ± standard deviations from triplicates.

	Water content (%)	Water activity (a_w)	TSS (x_s)	pH (1:4)	ICUMSA COLOUR	Glucose ($g/g_{product}$)	Fructose ($g/g_{product}$)	Sucrose ($g/g_{product}$)
W	1.3±0.1^{a}	0.68±0.07^{e}	1.00±0.05^{e}	6.3±0.2^{h}	31±33^{a}	nd	nd	0.97±0.09^{f}
CB	1.6±0.3^{a}	0.63±0.011^{b,c,d,e}	1.00±0.05^{e}	6.34±0.02^{h}	2,740±41^{b}	0.09±0.0009^{a}	0.001±0.0011^{a}	0.94±0.08^{f}
DB	1.2±0.1^{a}	0.62±0.05^{a,b,c,d}	1.00±0.06^{d,e}	5.72±0.09^{e,f}	4,783±384^{c}	0.0004±0.0008^{a}	0.000±0.0000^{a}	0.9±0.2^{e,f}
LB	1.2±0.1^{a}	0.61±0.04^{a,b,c}	0.99±0.08^{c,d,e}	6.25±0.12^{g,h}	3,692±172^{b,c}	0.002±0.0012^{a}	0.0016±0.0008^{a}	0.882±0.006^{d,e,f}
RB	1.4±0.1^{a}	0.59±0.02^{a,b}	1.0±0.06^{d,e}	6.1±0.2^{g}	4,598±138^{c}	0.0010±0.0005^{a}	0.0015±0.0002^{a}	0.86±0.09^{d,e,f}
WB	1.2±0.2^{a}	0.57±0.02^{a}	1.0±0.06^{d,e}	5.21±0.14^{b}	9,504±578^{d}	0.010±0.005^{a,b}	0.01±0.011^{a}	0.9±0.10^{d,e,f}
MB	1.3±0.2^{a}	0.572±0.008^{a}	1.0±0.12^{c,d,e}	5.6±0.12^{d,e}	17,002±156^{h}	0.008±0.005^{a,b}	0.006±0.002^{a}	0.83±0.06^{c,d,e,f}
GJ	3.6±0.1^{b}	0.6083±0.0004^{a,b,c}	1.0±0.13^{c,d}	5.26±0.02^{b}	16,606±306^{g,h}	0.030±0.010^{b}	0.021±0.002^{a}	0.75±0.06^{a}
LJ	4.5±0.3^{c}	0.62±0.02^{a,b,c,d}	0.94±0.16^{b}	5.460±0.013^{c,d}	15,142±1,421^{f}	0.1±0.13^{c}	0.053±0.007^{b}	0.7±0.10^{b}
RJ	7.0±0.7^{d}	0.67±0.010^{d,e}	0.9±0.2^{b}	5.35±0.03^{b,c}	15,546±1,270^{f,g}	0.08±0.02^{d}	0.07±0.010^{b}	0.6±0.12^{b}
DJ	3.1±0.5^{b}	0.60±0.03^{a,b,c}	1.0±0.3^{c}	5.9±0.02^{f}	11,552±1,543^{e}	0.019±0.002^{a,b}	0.019±0.006^{a}	0.80±0.05^{c,d,e}
CH	25.9±0.6^{e}	0.656±0.003^{c,d,e}	0.8±0.3^{a}	4.55±0.04^{a}	18,715±201^{i}	0.23±0.03^{e}	0.23±0.06^{c}	0.25±0.06^{c}

$^{a,b,c...}$Values with different superscript letters within the same column are significantly different ($p < 0.05$).

W, white sugar; CB, coated brown sugar; LB, light brown sugar; RB, raw brown sugar; DB, dark brown sugar; WB, wet brown sugar; MB, raw brown sugar with molasses; LJ, light jaggery block; RJ, regular jaggery block; GJ, granulated jaggery; DJ, dark jaggery block (DJ); CH, sugarcane honey.

3.2. Total phenolic content.

According to Slinkard & Singleton (1977) and Singleton et al. (1999) reducing sugars may react with the Folin-Ciocalteau reagent apparently increasing the total phenol content of the samples. In order to eliminate this interference, sugars (sucrose, fructose and glucose up to 25% w/w) were added to standard gallic acid curves. Results confirmed that there was an interference of the three sugars, including sucrose, and that this interference was more significant when reducing sugars were added to the standard solution.

Apparent and corrected phenol contents of the non-refined cane sugars are shown in table 2. Phenols were not found in white sugar, whereas all non-refined alternatives presented certain phenolic content. Cane honey, regular jaggery and light jaggery have the highest phenol content, closely followed by granulated jaggery; whereas phenolic content in brown sugars is significantly lower. Harish-Nayaka et al. (2009) reported a similar phenolic content in brown sugar (0.37), whereas Payet et al. reported a wider interval (0.1-0.41). In the case of jaggery, Harish-Nayaka et al. (2009) obtained a higher value (3.83). Differences in processing, as well as in the origin and sugarcane cultivar (Kadam et al., 2008) could be responsible for these discrepancies. In addition, most of the values available in the literature do not consider the interference of sugars in the analysis for which some data might be slightly overestimated. Nevertheless, by comparing apparent and corrected values it is deduced that the presence of sugars in the samples has only a significant impact on the results

in the case of sugarcane honey, which contains around 65% of inverted sugar, moderately modifies the real value in light and regular jaggeries (15-20% of inverted sugars), and has very small impact in the rest of cases, it being totally negligible in brown sugars which inverted sugar content is below 3%.

Table 2. Total phenol (App.: apparent and Corr.: corrected) and flavonoid content of the 12 sugarcane products studied. Phenol content is given in mg Gallic Acid Equivalents (GAE) and total flavonoids in mg Quercetin Equivalents (QE), per gram of product. Means ± standard deviations from triplicates.

	App. PHENOL CONTENT mg GAE/$g_{product}$	Corr. PHENOL CONTENT mg GAE/$g_{product}$	FLAVONOID CONTENT mg QE/$g_{product}$
W	0.004 ± 0.001^{a}	0.0 ± 0.0^{a}	0.022 ± 0.016^{a}
CB	0.372 ± 0.009^{b}	0.371 ± 0.009^{b}	0.69 ± 0.09^{b}
DB	0.42 ± 0.02^{b}	0.42 ± 0.02^{b}	1.225 ± 0.015^{c}
LB	0.38 ± 0.02^{b}	0.38 ± 0.02^{b}	0.85 ± 0.03^{b}
RB	0.58 ± 0.06^{c}	0.58 ± 0.06^{c}	1.25 ± 0.11^{c}
WB	0.560 ± 0.016^{c}	0.546 ± 0.015^{c}	1.893 ± 0.008^{d}
MB	0.81 ± 0.03^{d}	0.80 ± 0.03^{d}	2.15 ± 0.03^{d}
GJ	1.76 ± 0.11^{f}	1.71 ± 0.10^{f}	2.72 ± 0.10^{e}
LJ	2.29 ± 0.13^{g}	2.18 ± 0.12^{g}	3.78 ± 0.08^{f}
RJ	2.50 ± 0.12^{h}	2.33 ± 0.11^{h}	3.75 ± 0.10^{f}
DJ	1.02 ± 0.04^{e}	1.00 ± 0.04^{e}	1.59 ± 0.13^{d}
CH	3.26 ± 0.16^{i}	2.62 ± 0.13^{i}	6.15 ± 0.14^{g}

$^{a,b,c...}$Values with different superscript letters within the same column are significantly different ($p < 0.05$).

W, white sugar; CB, coated brown sugar; LB, light brown sugar; RB, raw brown sugar; DB, dark brown sugar; WB, wet brown sugar; MB, raw brown sugar with molasses; LJ, light jaggery block; RJ, regular jaggery block; GJ, granulated jaggery; DJ, dark jaggery block (DJ); CH, sugarcane honey.

3.3. Total flavonoid content.

In table 2, total flavonoids in the sugarcane products are given in mg of Quercetin Equivalents (QE) per gram of product and per gram of sugar. Flavonoids were present in all the non-refined sugarcane products analyzed, these being significantly abundant in sugarcane honey, as well as in jaggeries, especially light and regular jaggery blocks. The presence of sugars may also have interfered in this analysis, as some flavonoid content was obtained for white sugar; however, taking into account the previous assay, it was considered that this influence could only have significantly affected the cane honey value. Comparison with similar values reported by other authors was not possible since similar data were not found in the literature.

Although the aluminium chloride colorimetric method using quercetin as a standard has been widely used to determine the total flavonoid content (Dowd, 1959; Chang et al., 2002; Luximon-Ramma et al., 2002; Ahn, et al., 2004; Bahorun et al., 2004; Kosalec et al., 2004; Kumazawa et al., 2004; Meda et al., 2005; Lin & Tang, 2007), the generalized use of this method to determine total flavonoid content has been recently questioned (Denni & Mammen, 2012). According to these authors, different flavonoids have picks of absorbance at different wave lengths and, when reacted with $AlCl_3$ flavonols have absorption maxima around 440 nm, whereas most flavones exhibit their maxima below 400 nm. In particular, quercetin exhibits absorption maxima at 445 nm, whereas some of the flavones abundant in sugarcane (tricin and apigenin) have absorption maxima below 400 nm when reacted

with $AlCl_3$. Consequently, measuring the absorbance of the reaction at 368 nm using quercetin as a standard may overestimate the total flavonoid content, since quercetin shows low absorption at this wave length and flavones have high absorption; whereas measuring the absorbance above 400 nm, as it has also been suggested, may underestimate the flavonoid content, since flavones reacted with $AlCl_3$ have very low absorbance at this wave length and quercetin exhibits its absorption maxima. However, this colorimetric method is commonly used in the literature to estimate the total flavonoid content without taking these considerations into account. In our particular case, it was noticed that values for total flavonoids were higher than values for total phenols. In any case, results indicate that, as an average, jaggeries have 5 times more flavonoids than coated brown sugar.

3.4. Antioxidant properties of sugarcane products.

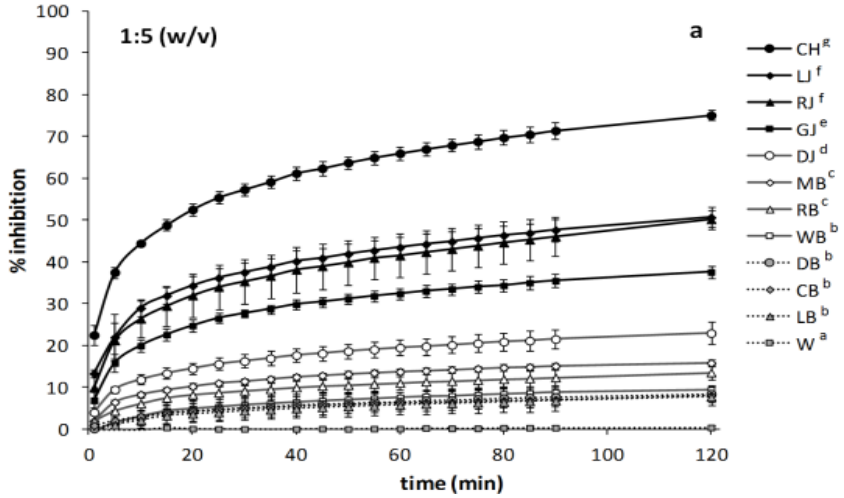
In Figure 1 the evolution of DPPH• inhibition (in percentage) during two hours of reaction is shown for the twelve products studied at one concentration (1:5 w/v), and at different concentrations for a single product (RJ). In all cases, kinetics of the DPPH• reaction corresponded to a hyperbolic curve, which is characteristic of components that react slowly with DPPH• (Brand-Williams, 1995). In fact, for some products, the steady state value was not reached in the two hours registered at the highest concentrations assayed. According to these results, the components present in the sugarcane derived products have a slow-kinetics response to the DPPH• assay; nevertheless, a

combination of fast + slow response was also identified in jaggeries and cane honey. According to Sendra et al. (2006), the antioxidants that are capable of both fast and slow hydrogen atom transfer are components which normally have a free or mono-substituted catechol group; whereas the slow-kinetics group would consist of components having exclusively slow-acting antiradical groups, such as those which lack the catechol group in the B-ring. Considering the phenolic compounds identified by other authors in sugarcane juice and sugarcane derivatives (Duarte-Almeida et al., 2006; Harish-Nayaka et al., 2009) components such as luteolin and some phenolic acids would contribute to the fast + slow response, whereas tricin and apigenin (most abundant flavones in sugarcane) would be responsible for the slow response observed in the non-refined sugars.

According to percentage inhibition values of the DPPH• after two hours of reaction (Fig. 1a), significant differences were found between white sugar and the non-refined ones, indicating that all the non-refined alternatives analyzed exhibit in vitro antiradical activity. Among non-refined sugars, brown sugars together with wet brown sugar and sugar with molasses presented the lowest antiradical activity, raw brown sugar and molassed sugar being classified in a different group with slightly higher antiradical activity. On the opposite side, jaggeries and cane honey presented the highest capacity to inhibit DPPH• radical, cane honey showing the highest percentage of inhibition and dark jaggery the lowest. However, it must be taken into account that cane honey contains citric acid as a preservative, which could have affected

the DPPH· scavenging measurement (Dawidowicz *et al.*, 2012). Results evidence that degree of refining or the process undergone highly determines the amount of antioxidant components present in the sugarcane products. It also may be observed that although all the non-refined alternatives to white sugar have certain antiradical activity, jaggeries and cane honey have, as an average, six times more antiradical activity than brown sugars.

For those products which showed the highest antiradical activity (CH, RJ, LJ and GJ), the concentration providing 50% inhibition (IC_{50}) was calculated by plotting the sample concentration (final concentration in cuvette) against the corresponding scavenging effect. Results indicated that cane honey has an IC_{50} equal to 1.31 $g_{product}$/mL (0.92 g_{sugar}/mL), RJ and LJ have an IC_{50} of 2.00 $g_{product}$/mL (1.45 and 1.57 g_{sugar}/mL, respectively), and granulated jaggery 2.5 $g_{product}$/mL (2.02 g_{sugar}/mL).



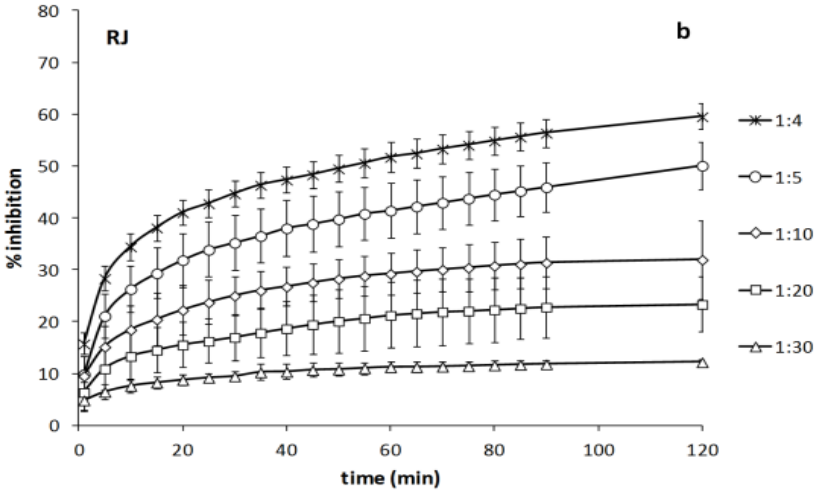


Figure 1. (a) Evolution of the DPPH reaction during 2 hours, for the twelve sugarcane products analyzed, at concentration 1:5 (w/v). (b) Evolution of the DPPH reaction during 2 hours, for regular jaggery (RJ), at different concentrations (1:4, 1:5, 1:10, 1:20, 1:30) (w/v). W, white sugar; CB, coated brown sugar; LB, light brown sugar; RB, raw brown sugar; DB, dark brown sugar; WB, wet brown sugar; MB, raw brown sugar with molasses; LJ, light jaggery block; RJ, regular jaggery block; GJ, granulated jaggery; DJ, dark jaggery block (DJ); CH, sugarcane honey.

It is recommended the use of more than one single method to estimate the antioxidant activity of complex samples (Ozgen et al., 2006). In this case, the ABTS free radical method, which has been reported to be more sensitive to hydrophilic antiradicals (Del Caro et al., 2004), was used in addition to DPPH• radical scavenging capacity. TEAC (Trolox Equivalent Antioxidant Capacity) values were obtained by the ABTS method at 1, 3 and 6 min of reaction (Fig. 2). Reaction continued for the 6 minutes analyzed in all cases, although differences were more significant in samples that showed higher AO capacity. TEAC values at 6 min of reaction were taken as definitive. Except for the white sugar, which showed a negligible AO activity, all the non-refined sugars

exhibited certain capacity to scavenge the ABTS+ free radical. In general, results were similar to the obtained with the DPPH• analysis, since brown sugars presented the lowest AO capacity and light jaggeries and cane honey had the highest ones. However, these two groups were more differentiated than before, dark jaggery belonging to the same group than all brown sugars, and granulated jaggery being closer to the other light jaggeries and cane honey. Differences among the results obtained by the DPPH• and TEAC-ABTS methods were probably due to their different sensitivity to the antiradical compounds that may be present in the sugarcane products. Time of reactions could also have produced some differences; nevertheless, ABTS reaction is usually faster than DPPH• inhibition reaction (Ozgen et al., 2006).

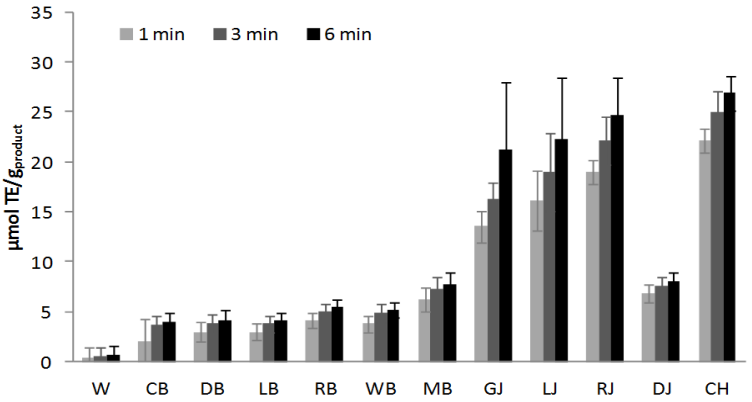


Figure 2. Evolution of the ABTS-TEAC reaction during 1, 3 and 6 minutes, for the twelve sugarcane products analyzed. Values of free radical ABTS+ scavenging ability are given in µmol TE/g product. W, white sugar; CB, coated brown sugar; LB, light brown sugar; RB, raw brown sugar; DB, dark brown sugar; WB, wet brown sugar; MB, raw brown sugar with molasses; LJ, light jaggery block; RJ, regular jaggery block; GJ, granulated jaggery; DJ, dark jaggery block (DJ); CH, sugarcane honey.

Although values reported in the literature are not easily comparable since, apart from the method used, extract type, sample concentration or reaction times originate differences in the results, it is interesting to compare the AO capacity of sugarcane products with the AO properties of other foods. TEAC values obtained for jaggeries and cane honey were in the range or even higher than fruits with a considerable AO capacity such as blackberry, raspberry, strawberry, pineapple or orange (9-21 μmol TE/g as reported by Ozgen et al., (2006) and Pellegrini et al., (2003), significantly higher to other fruits such as apple, banana, mango, apricot, pear or plum (1.3-5 μmol TE/g as reported by Pellegrini et al., 2003 and Vijaya et al., 2010) and also higher to vegetables such as onion, broccoli, tomato, carrot, pepper or spinach (0.4-8 μmol TE/g as reported by Baourun et al., (2004) and Pellegrini et al., (2003). Brown sugars and dark jaggery also presented values in the range of fruits such as apple, orange or mango and higher to most vegetables. However, it is important to point out that sugarcane products are the result of sugarcane juice concentration and have very small amounts of water, whereas values provided for fruits and vegetables are usually expressed per gram of fresh weight. Yet, results confirm that 1 gram of non-refined sugar may provide from 1 to 6 times more TEAC than one gram of fruits such as apples, grape, pear or pomegranate.

In any case, in order to estimate the real contribution of a food to the AO intake through the diet, the frequency of consumption must be considered. Direct intake of sugar in Spain, i.e. direct consumption of sugar at home, is around 340 g/month,

and may be even higher in other industrialized countries. Taking into account this value, replacement of white refined sugar for a non-refined alternative would have an impact on the diet from 1.4 to 9.1 mmol TE/month, depending on the non-refined sugar used as a substitutive. This is of considerable importance since, for example, the TE intake due to apple consumption is 4.9 mmol TE/month (assuming Spanish consumption *per capita*) and that the TE intake due to fruits known for having a high AO capacity such as blackberry is almost negligible for being rarely consumed (assuming Spanish consumption *per capita*). If going further and considering non-refined sugarcane products as functional ingredients for the food industry, replacing refined sugar in some sugar-rich products such as jams, jellies, pastries or desserts, may substantially increase this AO intake.

3.5. Correlation between phenolic and flavonoid content and free radical scavenging assays.

The correlation coefficients (Pearson's) between the total phenolic content (apparent and corrected) measured by the Folin-Ciocalteu assay, the flavonoid content measured by the aluminum chloride colorimetric method, and the antioxidant properties of the samples as indicated by the DPPH and ABTS methods were calculated (table 3). All the combinations calculated were significantly correlated (0.01 significance level), which would indicate that the antioxidant properties of the sugarcane derived products are due to their phenolic content. Although all correlation coefficients were significant, certain differences were observed when comparing total apparent or corrected phenol

content. Flavonoid content and DPPH assay correlated better with total apparent content, which could indicate that both flavonoid and DPPH methods are also influenced by the reducing sugars present in the samples; on the contrary, ABTS correlation coefficient was higher when the corrected values were used, which could suggest sugars are not significantly interfering in this assay.

Table 3. Correlation coefficients between antioxidant tests (DDPH and ABTS-TEAC), total phenolic content (apparent, app.; and corrected, corr.) and flavonoid content.

	Phenol app.	Phenol corr.	Flavonoid	DPPH	ABTS
Phenol app.	1				
Phenol corr.	0.993	1			
Flavonoid	0.942	0.901	1		
DPPH	0.996	0.983	0.952	1	
ABTS	0.980	0.990	0.876	0.968	1

In sugar-derived samples it needs to be taken into account that Maillard reaction compounds, which are involved in the color and flavor of the sugar products, may also exhibit antioxidant activity (Payet et al., 2005, Dittrich et al., 2003). Considering that these products also react with the Folin-Cicolteau reagent (Harish-Nayaka et al., 2009), other authors have attributed low correlation coefficients between total phenol content and AO activity to Maillard reaction products (Payet et al., 2005). In the present work, however, AO activity and phenolic content do correlate significantly. In an attempt to elucidate if Maillard reaction products were significantly contributing to the measured

AO capacity or the total phenols, correlation coefficients of total phenols and AO capacity with the ICUMSA color were included in the analysis. In all cases, although still highly correlated, lower correlation coefficients were obtained (<0.9). High correlation coefficients were expected since both flavonoids and Maillard reaction products determine the color of the products.

3.6. Effect of thermal treatment on the AO capacity of sugarcane products.

The results corresponding to the AO activity of sugar samples after thermal treatments is summarized in table 4. The effect of thermal treatment was heterogeneous and results were dependent not only on the particular sugarcane product being analyzed, but also on the assay method used. Depending on the combination of time and temperature used, antioxidant activity of samples decreased, did not change or even increased. In line with this, cooking and other thermal treatments have been reported to increase the AO capacity of some vegetables or, in other cases, decrease it (Yamaguchi et al., 2001). According to the results obtained, short times and low to medium temperatures would be promoting the destruction of antioxidants naturally present in the samples, whereas an intensification of the treatment would originate the creation of other compounds or more active ones. In the case of sugarcane products, Maillard reactions products could have appeared and, therefore, produced an increase in the AO capacity. An increase in antioxidants bioavailability or an improved activity of naturally occurring antioxidants may also

occur due to treatments involving heating (Chan et al., 2009; Yamaguchi et al., 2001). On the other hand, differences due to the assay method used could be explained by their different sensitivity to the different antioxidant compounds present in the sample.

Although statistically significant differences were observed for some products such as brown sugar and cane honey, the thermal treatment did not produce a severe change in the AO capacity. AO properties of jaggeries were particularly not affected. As said, food processing can improve the properties of naturally occurring antioxidants or induce the formation of new compounds with antioxidant capacity, so that the overall antioxidant activity may increase or remain unchanged (Chan et al., 2009). Therefore, in the particular case of non-refined sugarcane products, it may be deduced that processing involving temperatures lower than 100 ºC and times shorter than 60 min, would not significantly affect their antioxidant activity.

Table 4. DPPH and ABTS-TEAC results of samples subjected to thermal treatments. Values ± standard deviation are given for the six products that exhibited higher AO capacity.

DPPH - % inhibition (1:5 w/v)							
Temperature (ºC)	Time (min)	MB	GJ	LJ	RJ	DJ	CH
-	-	15.8±1.2^{a}	38±7^{b}	51±6^{a}	50±4^{a}	22.9±0.7^{ab}	75.0±1.7^{a}
60	10	13.8±0.3^{abc}	44±3^{a}	49.7±1.9^{ab}	46.0±1.1^{a}	17.9±0.4^{b}	62.6±1.1^{ab}
60	30	13.9±0.5^{abc}	35.9±1.5^{b}	48.8±1.7^{ab}	47.1±1.2^{a}	17.3±0.6^{b}	62.9±1.5^{ab}
60	60	14.7±0.6^{ab}	35.25±1.5^{b}	48.5±1.7^{ab}	44±3^{a}	18.9±0.6^{ab}	65.1±1.6^{ab}
80	10	13.1±0.5^{bc}	36.1±1.1^{b}	48.4±1.0^{ab}	46±0.9^{a}	23.4±0.4^{ab}	64.3±1.1^{ab}
80	30	13.5±0.4^{abc}	36.1±1.0^{b}	49.4±1.1^{ab}	46.1±1.0^{a}	21.7±0.3^{ab}	54.5±0.7^{b}
80	60	14.0±0.3^{abc}	36±2^{b}	48.2±0.6^{ab}	45.2±0.7^{a}	25.9±0.2^{a}	63.3±1.6^{ab}
100	10	10.0±0.2^{d}	25.1±0.7^{c}	43.4±0.7^{c}	44.0±0.5^{a}	18.7±0.3^{ab}	62.0±0.5^{ab}
100	30	12.0±0.3^{cd}	33.9±0.9^{b}	49.2±0.8^{ab}	45.5±0.8^{a}	20.9±0.3^{ab}	68.1±0.3^{ab}
100	60	12.5±0.3^{bcd}	39.0±1.0^{ab}	47.0±1.0^{b}	48.6±0.2^{a}	22.2±0.3^{ab}	55.3±0.7^{b}
ABTS – µmol TEAC							
Temperature (ºC)	Time (min)	MB	GJ	LJ	RJ	DJ	CH
-	-	7.71±1.2^{a}	21±7^{a}	22±6^{a}	24.8±3.6^{ab}	7.6±0.7^{bcd}	26.9±1.7^{ab}
60	10	6.2±0.3^{c}	18±3^{a}	21.0±1.9^{a}	21.8±1.1^{c}	7.2±0.4^{cd}	20.6±1.0^{d}
60	30	6.6±0.5^{bc}	18.2±1.5^{a}	21.7±1.7^{a}	24.2±1.2^{bc}	7.2±0.6^{d}	24.7±1.5^{c}
60	60	6.9±0.6^{abc}	19.1±1.5^{a}	22.2±1.7^{a}	27±3^{ab}	8.0±0.6^{b}	26.6±1.6^{abc}
80	10	5.2±0.5^{d}	18.0±1.1^{a}	20.3±1.0^{a}	24.2±0.9^{bc}	8.0±0.4^{b}	25.3±1.1^{bc}
80	30	6.5±0.4^{bc}	18.8±1.0^{a}	21.5±1.1^{a}	24.9±1.0^{ab}	8.0±0.3^{b}	24.7±0.7^{c}
80	60	7.1±0.3^{ab}	21±2^{a}	22.5±0.6^{a}	25.8±0.7^{ab}	7.9±0.2^{bc}	25.9±1.6^{bc}
100	10	6.5±0.2^{bc}	17.6±0.7^{a}	21.2±0.7^{a}	24.6±0.5^{ab}	8.0±0.3^{b}	25.6±0.5^{bc}
100	30	6.8±0.3^{bc}	18.5±0.9^{a}	21.9±0.8^{a}	24.9±0.8^{ab}	8.3±0.3^{ab}	26.3±0.3^{bc}
100	60	7.2±0.3^{ab}	20.0±1.0^{a}	23.9±1.0^{a}	27.1±0.2^{a}	8.9±0.3^{a}	28.5±0.7^{a}

MB, raw brown sugar with molasses; GJ, granulated jaggery; LJ, light jaggery block; RJ, regular jaggery block; DJ, dark jaggery block; CH, sugarcane honey.

$^{a,b,c...}$Values with different superscript letters within the same column are significantly different ($p < 0.05$).

4. Conclusions

Results of the present work confirm that the non-refined sugarcane products studied exhibit *in vitro* antioxidant activity and that degree of refining determines the phenolic content and AO capacity of the products. Therefore, these sugarcane derivatives which are available in supermarkets for direct consumption are a promising source of natural antioxidants. Contribution of AO to the diet as a result of replacing white refined sugar for these alternatives has been discussed, and results indicate that the potential AO intake due to the sugarcane products can be considerable, especially if jaggeries or cane honey are used as a substitute. Full replacement of white sugar for the non refined alternatives may not be realistic since, among other reasons, significant changes in the food characteristics such as color or flavor may be expected. In relation to this, and according to the physicochemical characteristics of the products studied, changes expected in the formulated product would be indirectly related to the AO properties of the sugar. In any case, acceptance by a consumer panel should finally confirm or reject the new formulated products which, on the other hand, would have the added value of providing health benefits. Taking into account that sugar and sugar-rich products are widely consumed all over the world, the results of the present work should encourage the use of non-refined sugars in reformulation of traditional foods or in the design of new sugar-rich products with increased nutritional properties.

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Revalorization of blueberry juice waste: Obtaining functional powders

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Pending submission

Abstract

Blueberry pomace is considered as an important source of phenolic compounds and fibre with interesting applications in foods. Producing food powders from blueberry pomace allows reducing its environmental impact and gives value to this waste material. This work aims to evaluate the effect of air drying and grinding operation on functional and physicochemical properties of a blueberry pomace powder. First, fresh blueberry pomace was dried at 60 and 70 ºC and the effect of air temperature on physicochemical properties (water activity, moisture, soluble solids and colour) and functional properties, (total phenol content, mononeric anthocyanins content and antioxidant activity) was determined. After that, the pomace dried at 70 ºC was grinded to obtain powder with two granulometries (fine and coarse). The effect of granulometry on particle size, fibre content and main physicochemical properties, including antiradical capacity, total phenols and mononeric anthocyanins content, hydration and emulsifying properties was evaluated. Because of the high fibre powders content, the prebiotic effect of coarse and fine powders on *Lactobacillus salivarius* spp. s*alivarius* growth was tested. Finally, the evolution of physicochemical and functional properties during 20 weeks storage was determined. Results suggest that the increase in temperature does not have a

significant deterioration effect on antioxidant activity. However, the particle size distribution and fibre content were highly conditioned by grinding step, affecting significantly water and oil interactions properties. Combination of drying at 70 ºC followed by a grinding operation results in a blueberry pomace powder quite stable during 20 weeks storage.

Keywords: blueberry pomce, drying, antioxidant activity, functional powders.

1. Introduction

Agro-food industry, and specially fruit and vegetables industrialization, produces a huge amount of waste which has an important impact on the environment. Agro-food industry is responsible for around 40% of all the waste of the food chain (Mirabella et al., 2014). In fruit producing regions, juice processing, low processed products and canned vegetables produce a large quantity of waste characterized by having very different properties and composition. Most of this waste is used as animal feed or fertilizer, both considered of low economic value. However, those by-products are a potential source of bioactive compounds (Ayala-Zavala, et al., 2011), as well as carbohydrate, pectin, fibre, proteins, vitamins and minerals (Mirabella et al., 2014).

Waste management implies a significant environmental and economic cost. Recently there is a growing interest in looking for other alternative uses of these materials. In a more environmentally friendly system, it would be necessary to

consider industrial agro-food waste materials or by-products as edible source for new ingredients (Khanal et al., 2010; Su & Silva, 2006). In addition, dietary fibre contained in many fruit by-products confer functional properties associated with their water retention and water holding capacity properties, which may also influence the emulsifying capacity. In this context, obtaining functional powders combines the advantage of transforming a waste material into a value-added product and reducing managing costs and environmental impact (Goula & Lazarides, 2015).

In particular, blueberries world production has increase 70% between 2010 and 2016 with a production in 2016 of 552505 Tonnes (FAOSTAT 2018). Blueberry consumption is known to provide several health benefits such as anti-inflammatory and antioxidant properties that help to prevent health conditions such as obesity, hyperglicemia, urinary tract infections, heart disease, cancer, visual and age-related brain functions (Roopchand et al., 2013; Tsuda, 2012; Howell, 2008). They are known as a rich source of phenolic compounds such as anthocyanins and other flavonoids (Cho et al., 2004; Routray & Orsat, 2011; Zielinska & Markowski, 2016). Due to the fruit seasonality and the production increase, industrialization of blueberries into juice, dried fruit or powders has increase in recent years. Specifically, blueberry pomace, the by-product of juice processing, which is comprised of skins, seeds and some pulp is rich in fibre and has higher concentration of anthocyanins and phenolic compounds than the fruit itself (Oszmiański, et al., 2016; Mirabella et al., 2014; Ayala-

Zavala, et al., 2011) since the anthocyanins are mainly in the blueberry skins (Yousef et al., 2013). Moreover, according to Aura et al. (2015), dietary fibre contained in blueberry waste is higher than that of the entire blueberry fruit; in particular, insoluble non-carbohydrate dietary fibre, which may also have distinct physiological effects on health compared to other sources such as cardiovascular conditions and metabolic syndrome. Dietary fibre in many of its forms has recently raised interest with regard to its prebiotic effect. Accordingly, many waste materials have been evaluated as prebiotic ingredients, this is the case of cereals (Vasiljevic et al., 2007), banana, passion fruit or apple industrial wastes (do Espírito Santo et al., 2012). This fibre can be used as an ingredient and therefore added to a food in order to enhance its nutritional properties and sensorial attributes (Cassani et al., 2016).Fruit powders have become an emerging way of consuming fruit and vegetables and they have attracted a growing interest in recent years (Karam et al., 2016, Neacsu et al., 2015). Food powders are very versatile, since they are easy to storage and distribute (Tao et al., 2018) and they have a wide variety of applications as ready to add ingredients (dressing) or as food ingredients to be used as colorant, flavouring, natural preservatives, antioxidants, antimicrobials, flavourings, dyes and texturizers for the formulation of certain products (Ayala-Zavala, et al., 2011). In this context, obtaining fruit powders from the fruit peel or pomace, by using the industrial residues generated as a result of fruit processing, appears as an attractive solution. Powdering usually consists of drying and milling the resulting

dried material; occasionally, milling is used prior to drying in order to facilitate further processing (Fitzpatrick & Ahrné, 2005; Struck et al., 2016). To ensure a satisfactory shelf life is necessary to reduce the moisture content and water activity to microbiologically safe levels (Quek et al., 2007; Rawson et al., 2011; Saifullah et al., 2016). Among the different drying methods, air drying is one of the most cost-effective technologies, since there are other technologies such as freeze drying that preserve better the original healthy properties of the fruit but is much more energetically expensive. During the drying process takes place numerous physical and biochemical changes that can be detrimental for some health promoting compounds of the blueberry pomace that originate abundant breakdown products (Hamamma & Nawar, 1991).

The aim of this research article is to evaluate the effect of air drying temperature and grinding process on the functional properties of blueberry pomace powders and its stability during 20 weeks of storage time.

2. Material and methods

2.1. Obtaining a stable powder from the bagasse

Blueberry juice was obtained as described by Castagnini et al. (2015). Frozen organic blueberries (*Vaccinium corymbosum* var. *duke*) were supplied by Samanes S.L (Navarra, Spain). First of all blueberries were thawed and grinded at 4,000 rpm for 20 s

followed by 10 s at 5,000 rpm in a Thermomix® processor (Vorwerk, Spain). Processed blueberries were depectinized (Rodríguez- Durán et al., 2007) by adding 1 mL of a commercial enzymatic preparation (Viscozyme® L) per liter of grinded blueberries. Enzymatic treatment was performed under agitation in a thermostated bath (PSELECTA, PRECISTERM S-141, Barcelona, Spain) at 50 ºC for 150 min. After the enzymatic treatment, the juice was separated by double filtration through a 1.2 mm pore size sieve, and then through a second sieve of 0.7 mm.

Blueberry pomace (20% of the raw fruit weight, in accordance with the value reported by Skrede et al., 2008) was then dried with hot air at 60 ºC or 70 ºC in a convective drier (POL-EKO model CLW400 TOP, Controltecnica Instrumentación Científica, S.L, Madrid) for 3.5 and 2.5 hours, respectively, so as to reach a final water activity around 0.2. From the results obtained, drying at 70 ºC was selected as the best condition for the stabilization of blueberry pomace. Therefore, the effect of particle size after grinding was only evaluated on blueberry pomace dried at 70 ºC. Grinding was performed in a Thermomix® device (Vorwerk, Spain) at two different conditions: 10,000 rpm for 10 s to obtain a coarse powder (CP) and at 10,000 rpm for 2 min with stops every 30 s to obtain a fine powder (FP). Finally, powders were stored for 6 months at room temperature in glass jars covered with aluminium foil inside a chamber with controlled relative humidity (24%). Every 4 weeks each powder was analysed in terms of moisture content (x_w), water activity (a_w), colour and antioxidant properties,

including total phenol content, monomeric anthocyanins content and antioxidant activity by both DPPH and ABTS methods in order to evaluate the stability of these properties during storage.

2.2. Analytical determinations

2.2.1. Water activity, moisture, soluble solids, pH and specific volume

Water activity was measured with a dewpoint hygrometer (Aqualab 4TE, Decagon devices Inc., Pullman WA, USA). **Moisture** was calculated from the weight loss undergone by a certain amount of sample after drying in a vacuum chamber (Vaciotem, P-SELECTA, Barcelona, Spain) at -0.8 bar and 60 ºC until reaching constant weight (AOAC, 2000). **Total soluble solids** (x_{ss}) content of wet samples was calculated from their moisture content and the Brix measurement, obtained with a thermostatic refractometer (ABBE ATAGO, 3-T, Japan) at 20 ºC. In the case of dried samples, Brix measurement was obtained from 1:10 (w/v) aqueous solutions. **Specific volume**, defined as the inverse of the apparent density, was obtained by measuring the volume of a known amount of sample in a test tube. To minimize the air trapped, samples were gently hit until there was no decrease of the total volume (Chau et al., 2007). All determinations were performed in triplicate.

2.2.2. Hydration properties

Hygroscopicity of blueberry powders was measured according to the method described by Cai and Corke (2000), which consisted of

placing about 2 g of sample in a hermetic vessel with a saturated solution of Na_2SO_4 (81% RH) for one week. Hygroscopicity was calculated from the amount of water gained by the sample in such conditions and expressed in g of water per 100 g of sample (g/100 g). Powder **solubility** was determined as explained by Mimouni et al. (2009) as the ratio between total soluble solids and total solids (TSS:TS) contained in a certain amount of sample. TSS was obtained by preparing an aqueous dilution of the sample (1:20 w/v), then drying in a vacuum oven (at 63 ºC until constant weight) and weighting TS was obtained by centrifuging that same dilution at 4,400 x g for 5 min at room temperature, filtering the supernatant through a filter Whatman nº 1 and further drying it in a vacuum oven (at 63 ºC, until constant weight) before weighting. Following the principles of Freuding et al. (1999), **wettability** of CP and FP were defined as the time elapsed until 2 g of sample are completely moistened in a laboratory glass with 20 mL of water.

The **swelling capacity** (SC, in mL/g) was calculated, according to the method described by Raghavendra et al. (2004) and Robertson et al. (2000), as the inverse of the ratio between the initial weight of powder prepared in a 1:10 (w/v) aqueous solution and the volume reached by that mixture after 18 h at 25 ºC. Determinations were performed in triplicate.

Water holding capacity (WHC, in g/g) is defined as the amount of water retained inside de powder without any application of external force, just the gravity force and the atmospheric

pressure. In this study, WHC was calculated, as indicated in equation 1, from the weight of the mixture resulting from adding 10 mL of water to 1 g of powder (HP, in g) and the weight of the same mixture that, after 18 h at 25 ºC, was frozen at -40 ºC for 24 h and freeze-dried (TELSTAR LIOALFA 6-80) at -45 ºC and 0,1 mbar for 24 h (DR, in g). Determinations were performed in triplicate and results were expressed in g of water per g of powder.

$$WHC=\frac{HP\text{-}DR}{DR} \quad (1)$$

The **water retention capacity** (WRC), which is the amount of water retained inside de powder after the application of a centrifugation force, was determined as reported by Raghavendra et al., (2004) and Escalada Pla et al., (2012). Approximately 1 g of blueberry powder was weight in a conical tube and hydrated with 10 mL of water during 18 h at 25ºC. After, samples were centrifuged at 514 x g for 30 minutes (Medifriger BL, P-Selecta, Spain). Supernatant was discarded and the sample was weighed (P+W). After, residue was freeze dried during 24 h and weighed (DR). WRC was calculated with equation (2). Determinations were performed in triplicate and results were expressed in g of water per g of powder.

$$WRC=\frac{W}{DR} \quad (2)$$

2.2.3. Oil holding capacity

Oil holding capacity (OHC), defined as the capacity to retain oil, was obtained from the weight gain undergone by 0.2 g of blueberry powder when mixed with 1.5 g of commercial sunflower oil (Garau et at., 2007). After 12 h at room temperature the

mixtures were centrifuged at 1500 x g for 5 min (Medifriger BL, P-Selecta, Spain). Supernatant was discarded and the pellet was weighed. Determinations were performed in triplicate and results were expressed in g of oil per g of powder.

2.2.4. Emulsifying properties

Emulsifying activity (EA) was measured as described by Yasumatsu et al. (1972). For this, 7 mL of a 2 % (w/v) solution of blueberry powder in water was mixed with 7 mL of sunflower oil and vortexed at 2400 rpm for 5 min (Reax top, Heidolph, Germany). The mixture was then centrifuged at 12857 x g for 5 min (Medifriger BL, P-Selecta, Spain) in graduated conical tubes. The emulsifying activity was calculated according equation 3, from the volume of the emulsified layer (V_{EL}, in mL) and the total volume of the mixture (V, in mL).

$$\%EA = \frac{V_{EL}}{V} \cdot 100 \quad (3)$$

Emulsion stability (ES) was evaluated using the method described by Yasumatsu et al., (1972). Briefly, 7 mL of a 2% (w/v) aqueous solution of each powder was mixed with 7 mL of commercial sunflower oil and vortexed for 5 min at 2400 rpm (Reax top, Heidolph, Germany). The emulsion obtained was heated to 80 ºC for 30 min and then cooled with running water. Once at room temperature, the mixture was centrifuged at 514 x g for 5 min (Medifriger BL, P-Selecta, Spain). The emulsion stability was calculated according equation 4, from the volume of the remaining emulsified layer (V_{REL}, in mL) and the total volume of the mixture (V, in mL).

$$\% \text{ ES} = \frac{V_{REL}}{V} \cdot 100 \quad (4)$$

2.2.5. Particle size distribution

Particle size distribution of powders was determined with a Mastersizer 2000 equipment (Malvern Instruments, Worcestershire, UK) by diffraction laser. For the dried method, a dispersion unit Sirocco 2000 with air as dispersant at a pressure of 2.5 bar and speed of 60% was employed. Particle size distribution in wet conditions was also characterized. The refraction indexes were 1.52 for the sample and 1.33 for the dispersed phase (water) and the particle absortion index was 0.1. Particle size was characterized in terms of equivalent volume diameter D [4,3] (De Brouckere mean Diameter), surface area mean diameter D [3,2] (Sauter mean diameter), and the distribution percentiles d_{10}, d_{50} and d_{90}, defined as the particle size whose 10%, 50% and 90% of the distribution is below this size respectively (Instruments, M., 2007). Results are given as the average of five replicates.

2.2.6. Colour characterization

Colour of samples was measured with a spectrocolorimeter (MINOLTA model CM-1000R) using an illuminant D65 a 10º angle of vision and including the shining component. Results were provided in the CIE L*a*b* system, were L* is the brightness, a* is the red-green component and b* is the yellow-blue component. Attributes like h_{ab} (hue, a qualitative attribute of colour related to

the differences in absorbance at different wavelengths) and C_{ab} (chrome, quantitative attribute of colourfulness) were also obtained. Results are provided as the average of three triplicates.

2.2.7. Moisture sorption isotherms

Sorption isotherms of powders were obtained at 20 ºC using the static gravimetric method (Wolf et al., 1985), in which saturated solutions of different salts were employed to ensure a given relative humidity inside a closed chamber. Mass transfer between the saturated salt solution and the air inside the chamber and from it to the blueberry pomace powder took place by natural diffusion, thus varying the water activity of the powder during this exchange. At the equilibrium, when the water activity of the powder and the relative humidity of air inside the chamber were equal, the moisture content of the powder sample was determined. Saturated solutions of the following salts LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, Mg $(NO_3)_2$, NaCl, KCl and $BaCl_2$ resulting in the corresponding relative humidity (%) values at equilibrium 11.31, 23.11, 33.97, 43.16, 54.38, 75.47, 85.11 and 90.85 respectively (Greenspan, 1977), were used. A saturated solution of thymol (Panreac Química S.A., Barcelona, Spain) was also placed inside the chambers to avoid microbial growth. Experiment was performed in triplicate.

Collected data were adjusted to BET and GAB models. BET model (Brunauer et al., 1938) relates moisture at equilibrium (w_e) and water activity (a_w) through the parameters w_0 and C (equation 5), where w_0 is the monolayer moisture content layer of adsorbed

water and C is a characteristic constant of the material related to the heat released in the sorption process. The BET model has certain limitations since it only adjusts to a_w between 0 and 0.55; however, the BET monolayer value concept is accepted as a reference for the moisture content of higher stability for dry foods (Labuza and Atunakar, 2007).

$$w_e = \frac{W_0 \cdot C \cdot a_w}{(1-a_w)\cdot(1+(C-1)\cdot a_w)} \quad (5)$$

The Guggenheim-Anderson-De Boer (GAB) model was used as well. Equation (6) relates the equilibrium moisture content as function of water activity and C and K constants, which are related with the sorption heat of the multilayer.

$$w_e = \frac{W_0 \cdot C \cdot K \cdot a_w}{(1-K \cdot a_w)\cdot(1+(C-1)\cdot K \cdot a_w)} \quad (6)$$

2.2.8. Fibre content

In order to evaluate the fibre content of blueberry powders, the Van Soest method was used (AOAC, 2000; Mertens, 2002). This method provides information about the Neutral Detergent Fibre (NDF), the Acid Detergent Fibre (ADF), and the Lignin Detergent Fibre (LDF) contents. The NDF includes lignin, cellulose and hemicellulose; the ADF includes the non-soluble fibre (lignin and cellulose); LDF refers to lignin content, obtained after acid digestion with 72% of sulphuric acid. These values were used to calculate the concentration of hemicellulose (soluble fibre) cellulose, lignin (brut fibre or non-soluble) and total fibre. Fibre content was determined in duplicate and results were expressed as percentage in dried basis.

2.2.9. Antioxidant properties

Total phenols content, monomeric anthocyanins content and antioxidant activity, both by DPPH and ABTS methods, were analysed in all kind of samples and only in the case of blueberry powders, every four weeks during their storage for 6 months.

Extraction of antioxidant compounds involved diluting blueberry samples with 80:20 (v/v) methanol: water solvent in different proportions (1:20, w/v) for the fresh pomace and 1:100 (w/v) for the dried pomace and the powders), agitating for 1 h and centrifuging at 10,000 rpm for 5 min (Medifriger BL, P-Selecta, Spain). Determinations were made in triplicate on the supernatant of 3 different extracts.

2.2.9.1. Total phenols content

Total phenols content of samples was obtained following the Folin-Ciocalteau method (Singleton et al., 1999). An aliquot of 0.125 mL of the previously prepared extract was mixed with 0.5 mL of distilled water and 0.125 of the Folin-Ciocalteau reagent (Sigma Aldrich). The mixture was allowed to react for 7 min in darkness before adding 1.25 mL of a 7% sodium carbonate solution to stop the reaction and 1 mL of distilled water until completing a volume of 3 mL. The mixture was left in darkness for 90 min and absorbance was measured at 760 nm in a spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, England). Results were expressed in mg of Gallic Acid Equivalents (GAE) per 100 g of sample in dry basis.

2.2.9.2. Monomeric anthocyanins content

Total monomeric anthocyanin content was measured according to the pH differential spectrophotometric method described by Giusti & Wrolstad (2001) and Lee et al. (2005). Anthocyanin content was quantified using the molar extinction coefficient for cyanidin-3-O-glucoside (2,690 m^2/mol). Buffer solutions were prepared with potassium chlorine at 0.025 M and sodium acetate at 0.4 M were adjusted with chloric acid at pH 1 and at pH 4.5, respectively. A volume of 100 µL of samples dilution was mixed with each buffer and after 30 minutes of reaction, absorbance (Helios Zeta UV/Vis, Thermo scientific, England) was measured at 510 nm and 700 nm. In order to obtain the monomeric anthocyanin content, the following equations (equations 7 and 8) were applied:

$$ABS=(Abs_{510}-Abs_{700})_{pH\ 1.0}-(Abs_{510}-Abs_{700})_{pH\ 4.5} \quad (7)$$

where,

Abs_{510}: absorbance at 510 nm

Abs_{700}: absorbance at 700 nm

$$\text{Total monomeric anthocyanin}=\frac{ABS \cdot Mw \cdot f \cdot 1000}{\varepsilon \cdot l} \quad (8)$$

where,

ABS: absorbance of equation 7.

Mw: molecular weight of glucosid-3-cyanidin (449.2 g/mol)

f: dilution factor

Ɛ: molar extinction coefficient (26900 L/mol cm)

l: cuvette width (1 cm)

Results were expressed as milligrams of *cyanidin-3-O-glucoside* equivalents per 100 g of gram of sample in dry basis.

2.2.9.3. Antioxidant activity: DPPH and ABTS methods

The antioxidant activity of the fresh and dried pomace at 60 and at 70 ºC and powders grinded at two conditions was evaluated with DPPH and ABTS methods.

The DPPH (1,1-diphenyl-2-picryl hydrazyl) method (Brand-Williams et al., 1995) evaluates the scavenging capacity of the sample by measuring the change of colour of the DPPH in methanol when reacts with the sample. An aliquot of 30 μL of the extract was mixed with 970 μL of a 0.1 mM solution of DPPH in methanol and 2 mL of methanol. After 60 min in darkness, absorbance was measured at 517 nm in a spectrophotometer (Helios Zeta UV/Vis, Thermo scientific, England). Results were expressed in mg of Trolox Equivalent (TE) per gram of sample in dry basis.

The ABTS method was perform as described in Re et al. (1999). This method asses the capacity of the sample to scavenge the $ABTS^{+}$ cation. The radical ABTS+ was released by reacting 7 mM of ABTS with potassium persulfate (2.45 mM) during 16 h at room temperature in the dark. ABTS+ was mixed with phosphate buffer (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm (Helios Zeta UV/Vis, Thermo scientific England). An aliquot of 90 μL of the sample was added to 2910 μL of the solution ABTS+ in phosphate buffer with an absorbance of 0.700± 0.02 and the absorbance of samples was read at 6 min of reaction time. Distilled water was used as a reference. Presented results are the average of three repetitions and those were expressed in mg of Trolox Equivalent (TE) per gram of sample in dry basis.

2.3. Prebiotic effect

The prebiotic effect was evaluated by adapting the method from Moreno-Vilet et al. (2014), using *Lactobacillus salivarius* spp. *salivarius* (CECT 4063) as the probiotic strain. First, freeze-dried microorganisms were revived in MRS broth (Sharlau Chemie®, Barcelona, Spain) and incubated for 24 h at 37 ºC, thus obtaining the starting inoculum. Then, specific amounts of coarse or fine powder were added to flasks containing MRS broth in order to reach the following fibre equivalent percentages: 0.5%, 1% and 2% in w/v. Flasks were then inoculated with 1 mL of MRS broth containing 10^9 CFU of *L. salivarius* spp. In order to correct the possible effect of adding additional carbon source with the powders due to their sugar content, an equivalent amount of glucose (Sharlau Chemie®, Barcelona, Spain) was added to the non-containing powder media. Accordingly, sugar content of powders was estimated from the corresponding mass fraction of soluble solids (x_{ss}). After the growing of *Lactobacillus salivarius* spp. *salivarius* in the broth media, the amount of *L. salivarius* was measured by serial dilution in peptone water (Sharlau Chemie®, Barcelona, Spain) and sowed in plates with MRS Agar (Sharlau Chemie®, Barcelona, Spain). Due to the microaerophilic characteristics of the microorganism, sowing was performed in double agar layer. Subsequently, plates were incubated for 24 h at 37 ºC. Results given are the average and standar deviation of 3 replicates, and those were expressed in CFU/mL.

2.4. Statistical analysis

Results were analyzed statistically with the software Statgraphics (Centurion XVI.I, Statpoint Technologies, Inc.) with a confidence level of 95% (p-value ≤ 0.05). Data were processed by performing simple or multifactorial ANOVA, checking previously the normality of data.

3. Results and discussion

3.1. Effect of drying temperature on dried blueberry pomace

Water activity (a_w), **moisture content** (x_w, in g_{water}/g) **and total soluble solids** (x_{ss} in $g_{soluble\ solids}/g$) of blueberry pomace (P) and dried blueberry pomace at 60 and 70 ºC (DP60 and DP70, respectively) are shown in table 1.

Table 1. Water activity (a_w), moisture (x_w (g_w/g)) and soluble solids content (x_{ss} ($g_{soluble\ solids}/g$) and x_{ss} dm ($g_{soluble\ solids}/g_{dry\ matter}$)) of fresh and dried pomace at 60 ºC (DP60) and 70 ºC (DP70). Mean ± standard deviation of three replicates.

	a_w	x_w	x_{ss}	x_{ss} dm
P	0.989± 0.003^c	0.722± 0.003^c	0.079±0.002^a	0.283±0.006^a
DP60	0.236± 0.004^b	0.032± 0.002^b	0.280±0.012^b	0.289±0.012^b
DP70	0.189± 0.004^a	0.017± 0.002^a	0.276±0.011^b	0.281±0.011^b

Values with different superscript letters within the same column are significantly different (p-value ≤ 0.05).

As expected, drying of blueberry pomace significantly decreased both, its moisture content and its water activity, thus increasing its stability. Soluble solids content increases as a consequence of water removal, as it is easily deduced from the given in dry basis values.

Because drying is known to affect the colour of the samples, the **optical properties** of the obtained powders and the differences between powders and the original waste material are given in table 2. In table (table 2), the characteristic colour parameters CIEL*a*b* are shown for fresh pomace (P) and the pomace dried at 60 ºC (DP60) and at 70 ºC (DP70). The results indicated that colour of samples was significantly affected by drying. Regardless the drying temperature, the lightness (L*) of the samples increased significantly. Drying also implied a rise of coordinates a* and specially b*, indicating that the colour of dried samples moved a bit more towards red and yellow colours, respectively. Water removal and subsequent coloured pigments concentration, together with enzymatic browning and also any other chemical reaction of oxidation that may occur during drying, may have contributed to this coordinates increase. Statistically significant differences in coordinate a* were observed between samples dried at 60 and 70 ºC, so that DP60 showed a more intense red colour than DP70.

Table 2. CIEL*a*b* coordinates of fresh pomace (P) and dried pomace at 60 ºC (DP60) and 70 ºC (DP70). Mean ± standard deviation of three replicates.

	L*	a*	b*	ΔE	C	h
P	26.5± 1.0^{a}	3.0± 0.3^{a}	0.19± 0.3^{a}	-	3.0± 0.3^{a}	3.7± 0.7^{b}
DP60	37.52± 0.15^{b}	4.3± 0.5^{c}	0.70± 0.10^{b}	11.1± 0.8^{a}	4.3± 0.5^{c}	9.2± 0.6^{a}
DP70	37.5± 0.3^{b}	3.80± 0.10^{b}	0.65± 0.09^{b}	11.0± 0.7^{a}	3.86± 0.08^{b}	9.8± 1.5^{a}

Values with different superscript letters within the same column are significantly different (p-value ≤ 0.05).

Significant statistical differences between both drying processes applied were also found with respect to the chroma, C_{ab}. DP60 showed the highest chroma, whereas tone (h_{ab}) increased as water was removed from

the samples but without significant differences between both drying temperatures. Colour differences (ΔE) between dried pomace and the fresh one evidenced statistically similar colour changes under both temperatures.

Drying operation can have a positive or negative effect on the **antioxidant properties** of fruits products depending on the product type, the drying time and temperature (Nemzer et al., 2018). In a study carried out with the entire blueberry the convective drying at 80 ºC was reported to degrade antioxidant compounds (Zielinska & Michalska, 2016), whereas in a research performed by Bustos and colleagues (2018) with different berries dried at 65 ºC, an increase of total polyphenol content and also of the antioxidant activity was observed. In this work, the effect of air drying temperature was evaluated on antioxidant properties, total phenols and anthocyanins content of dried pomace.

As it can be observed in table 3, total phenol content is similar in the pomace and in the dried pomaces. Nemzer et al. (2018) obtained 1.92 mg GAE/100 $g_{dry\ matter}$ in dried blueberry at 70 ºC. Since the bioactive compounds of the blueberry are mainly in the skin (Yousef et al., 2013), our result of 4.48 ± 0.12 mg GAE/100 $g_{dry\ matter}$ obtained for the dried pomace at 70 ºC is consistent with that. Total phenols content seems to be more affected by temperature when it is dried at 70 ºC, being slightly higher the content in total phenols when fresh pomace is dried at 60 ºC. It could be that some components are degraded at 70 ºC into a phenol compounds (Sadilova et al., 2007). In cranberry pomace

White et al., (2011) obtained an increase of flavonol aglicones as a result of deglycosilation by drying at 60 and 80 ºC.

Table 3. Total phenols content (mg GAE/100 $g_{dry\ matter}$) and monomeric anthocyanins content (mg glucosid-3-cyanidin/100 $g_{dry\ matter}$) of fresh pomace (P) and dried pomace at 60 ºC (DP60) and 70 ºC (DP70). Mean ± standard deviation of three replicates.

	Total phenols mg GAE/100 g db	**Monomeric anthocyanins** mg glucosid-3-cianidin/100g db
P	4.4 ± 0.2^{b}	74.5 ± 0.4^{c}
DP60	3.94 ± 0.10^{a}	55.5 ± 0.4^{b}
DP70	4.48 ± 0.12^{b}	48.9 ± 0.7^{a}

Values with different superscript letters within the same column are significantly different (p-value ≤ 0.05).

Anthocyanidins are quite sensitive to the thermal treatment as reported by Patras et al. (2010). Thermal treatment causes anthocyanins and their conjugate sugars to break up into small chains of aldehydes and derivate benzoic acids. This was also evidenced in the present study (table 3), in which samples dried at 70 ºC suffered a more significant reduction in the anthocyanins content (Zoric et al., 2013; Khanal et al., 2010). The reduction of the content of monomeric anthocyanins as temperature increases is in accordance with Howard et al. (2012) in which a lineal relationship between the temperature of the treatment and the anthocyanins content was obtained. As reported by Sadilova et al. (2007), the thermal degradation of anthocyanins involves the formation of chalcone which is degraded into phenolic acids.

The **antioxidant activity**, analyzed by both DPPH and ABTS methods (figure 1) revealed that drying implied a statistically significant decrease in the antioxidant properties of blueberry

pomace. This result is in accordance to that reported by Arancibia-Avila et al. (2012) in which a thermal treatment at 100 ºC was applied to blueberry for one hour. In the case of DPPH antioxidant activity, with the higher drying temperature was a more remarkable reduction in the antioxidant properties; however, no significant differences between both dried pomaces were evidenced by the ABTS method. Bustos et al. (2018) evaluated the antioxidant activity with ABTS assay of blackcurrant berry after drying at 50 ºC, 65 ºC and 100 ºC and obtained a small difference of the antioxidant activity between drying at 50 ºC and 65 ºC; on the other hand, when berry was dried at 130 ºC antioxidant activity decreases.

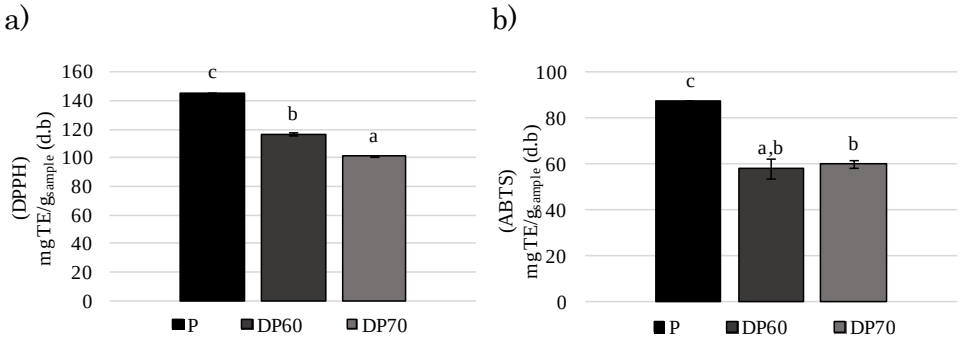

Figure 1. Antioxidant activity (mg Trolox Equivalents/ $g_{dry\ matter}$) determined by DPPH (a) and ABTS (b) methods of fresh pomace (P) and dried pomace at 60 ºC (DP60) and 70 ºC (DP70). Mean ± standard deviation of three replicates. Average results with different letters are significantly different (p-value ≤ 0.05).

Several studies have found a correlation (Wu et al., 2004; Rodriguez et al., 2016) between the anthocyanins content and the antioxidant activity measured by DPPH assay. Our results suggest a positive relationship due to a similar tendency between the antioxidant capacity and the anthocyanin content of powders, considering results of DPPH assay. The differences between

methods may be due to the different sensitivity that those have to the different antiradical compounds depending on their chemical structures (Zhao et al., 2008). Time of reaction is also very different between reagents, with ABTS reacting faster than the DPPH (Ozgen et al., 2006).

3.2. Effect of grinding on particle size distribution and fibre content.

Particle size distribution clearly influences physicochemical properties and the uses of powder products. Dried pomaces were grinded to obtain a coarse powder (CP), with a bigger particle mean size, and a fine powder (FP) with a smaller particle size (table 4).

Table 4. Characteristic parameters of particle size distribution (D [4,3], D [3,2], and d_{10}, d_{50} and d_{90}) for coarse (CP) and fine powder (FP) obtained from dried pomace at 70 ºC. Mean ± standard deviation of six replicates.

		D [4,3]	D [3,2]	d_{10}	d_{50}	d_{90}
Dry method	CP	659± 10^{d}	239± 8^{d}	129± 5^{d}	606± 10^{c}	1247± 17^{d}
	FP	211± 2^{a}	81± 4^{a}	36± 1^{a}	170.1± 0.7^{a}	446± 7^{a}
Wet method	CP	437± 106^{c}	177± 32^{c}	71± 13^{c}	398± 79^{b}	873± 236^{c}
	FP	293± 66^{b}	100± 48^{b}	52± 6^{b}	209± 51^{a}	680± 206^{b}

Values with different superscript letters within the same parameter are significantly different (p-value ≤ 0.05).

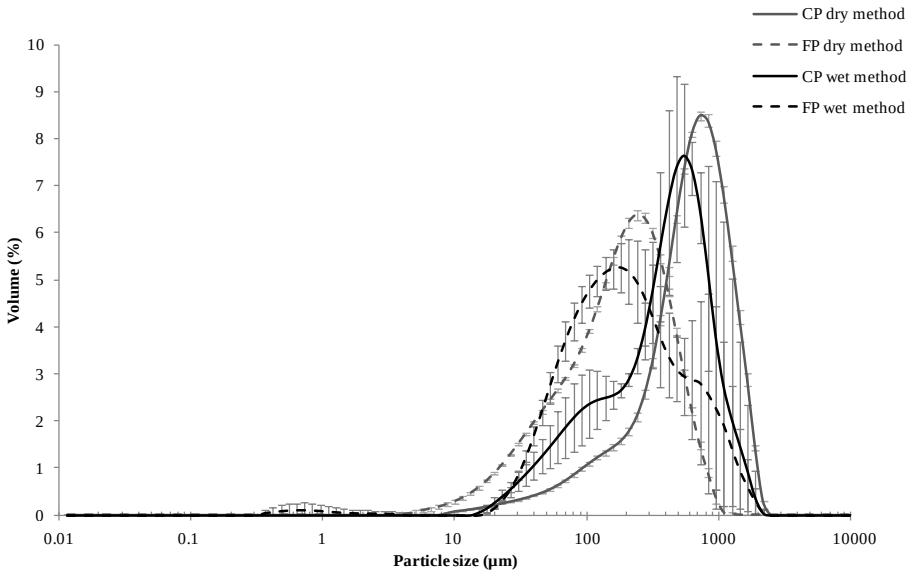

Figure 2. Particle size distribution of coarse (CP) and fine (FP) powder by dry and wet method. Mean ± standard deviation of six replicates.

Particle size affects other properties such as water interaction properties. In some cases, a smaller particle size increases the specific surface favouring surface adsorption phenomena (Raghavendra et al., 2004). In other cases, especially when the fibre is the main component, the mechanical grinding processes can modify the porous structure and affect the hydration properties (Lario et al., 2004). In the present study, particle size was measured by dry and wet methods in order to evaluate it for different applications; in the formulation of liquid foods (beverages, juices…) and solid foods (cookies, muffins…). According to results shown in table 4, the lower size obtained for CP in liquid media than in dry media, could indicate that the soluble compounds of the particles would have solubilized (Lario et al., 2004). However, these differences could also be due to the

way in which particles are aggregated, since results of the FP do not follow the same trend (figure 2).

Water activity (a_w), **moisture content** (x_w) and **total soluble solids content** (x_{ss}) of coarse powder (CP) and fine powder (FP) are shown in table 5. After drying at 70 ºC, water activity of powders was higher than that of the dried pomace DP70 (table 5). Soluble solids content is higher in FP than in CP, since mechanical disruption favors the release of soluble solids to the liquid phase (White et al., 2011). The increase in the particle size would have contributed to the increase in both the effective surface for the water exchange and the release of water from the structures containing it, thus increasing both x_w and a_w values. Tissue disruption has been reported to notably reduce the water holding capacity and therefore an increase in unbound water activity (Rodriguez et al., 2016).

Table 5. Water activity (a_w), moisture (x_w (g_w/g)) and soluble solids content (x_{ss} ($g_{soluble\ solids}/\ g$) and x_{ss} dm ($g_{\ soluble\ solids}/\ g_{dry\ matter}$)) of coarse (CP) and fine powder (FP) obtained from dried pomace at 70 ºC. Mean ± standard deviation of three replicates.

	a_w	x_w	x_{ss}	x_{ss} dm
CP	0.236 ± 0.004^{b}	0.017 ± 0.002^{a}	0.35 ± 0.011^{a}	0.34 ± 0.013^{a}
FP	$0.20\pm 0.06^{a,b}$	0.019 ± 0.0006^{a}	0.46 ± 0.012^{b}	0.45 ± 0.012^{b}

Values with different superscript letters within the same column are significantly different (p-value ≤ 0.05).

In table 6, **fibre content** for coarse (CP) and fine (FP) powders are shown. As it can be observed, CP had significant higher content for all kinds of fibre analysed. This fact could be explained by the stronger grinding applied to obtain the fine powder, which

affected the structure of the polysaccharide chains, releasing small chains sugars with higher solubility and/or higher digestibility. Since insoluble fibre, that is lignin and cellulose, has higher porosity and lower density, water retention capacity and viscosity, it would contribute to improve the intestinal tract (Licona, 2013). In contrast, hemicellulose chains are better solubilized since they are shorter and so they slow the transit and improve the absorption of macronutrients; moreover, they also lower cholesterol levels and favour the integrity of the intestinal tract (Martínez and García, 2006). In both cases, the total fibre content exceeded 30% of the samples weight and the majority was insoluble fibre. Comparing with the dietary fibre composition of blackcurrant pomace (Nawirska and Kwasniewska, 2005), blueberry pomace had a different fibre profile. Blackcurrant fibre contained 59% lignin, 25% hemicellulose, 12% cellulose and 3% pectin. However, blueberry pomace obtained in this study did not contained pectin due to the depectinization treatment applied during the juice manufacture. Blueberry powders also had a lower percentage of all the other fibre compounds. Compared to mango fruit (Vergara-Valencia et al., 2007), blueberry powders had lower total fibre content and a higher soluble fibre content. These differences might be due to the greater content of fibre in the pomace compared with the entire fruit.

Table 6. Content (g/ $g_{dry\ matter}$) of cellulose, hemicelluloses, lignin, insoluble fibre and total fibre of coarse (CP) and fine (FP) powder. Values in brackets are referred to the total fibre. Mean ± standard deviation of two replicates.

	Cellulose	Hemicellulose	Lignin	Insoluble fibre	Total fibre
CP	18.0± 0.4[b] (46.8%)	12.846± 0.018[b] (33.4%)	7.6± 0.2[b] (19.8%)	25.6± 0.8[b] (66.6%)	38.5± 0.8[b] (100%)
FP	16.69± 0.14[a] (49.6%)	10.444± 0.003[a] (31%)	6.6± 0.2[a] (19.4%)	23.24± 0.06[a] (69%)	33.69± 0.06[a] (100%)

Values with different superscript letters within the same column are significantly different (p-value ≤ 0.05).

3.3. Water and oil interaction properties of powders.

Particle size distribution, fibre content and type may affect the powders behaviour when mixed with water or oil (Elleuch et al., 2011), and determine their hydration and emulsifying properties. A decreased particle size usually presents higher water binding capacity (Struck et al., 2016). Results of solubility, specific volume, hydration and water retention properties as well as emulsifying properties for fine (FP) and coarse (CP) powder are shown in table 7.

The **specific volume** of the powders, is in the range of the reported by other authors (Martinez-Las Heras et al., 2017). Significantly higher values of the specific volume obtained for CP could be due to the higher porosity of CP compared to FP. In fact, materials made of small particles tend to compact, whereas coarse particles are characterized by facilitating pores creation within particles. This was evidenced by Forni et al. (2011), who demonstrated that increasing the particle size implied an increase in the amount of pores present in the microstructure. As stated before, the higher insoluble fibre content of the CP powder may also cause an

increase in the specific volume since insoluble fibre is more porous and has lower density than the soluble one (Elleuch et al., 2011).

Table 7. Specific volume, solubility, hydration and water retention properties and emulsifying properties of coarse (CP) and fine (FP) powder. Mean ± standard deviation of three replicates.

	CP	FP
Specific volume (mL/g)	9.60± 0.12^{b}	7.57± 0.06^{a}
Solubility (%)	31.6± 1.5^{a}	33.1± 0.7^{a}
Water interaction properties		
Higroscopicity (g/100 g)	61± 3^{a}	62.7± 1.8^{a}
Wettability (s)	175± 21^{b}	77± 6^{a}
Swelling capacity (mL/g)	2.88± 0.13^{b}	2.56± 0.06^{a}
Water holding capacity (WHC) (g/g)	5.1± 0.2^{b}	4.63± 0.16^{a}
Water retention capacity (WRC) (g/g)	3.4± 0.3^{a}	3.08± 0.18^{a}
Emulsifying properties		
Oil holding capacity (OHC) (g/g)	2.7± 0.6^{a}	2.9± 0.5^{a}
Emulsifying activity (%)	0.4± 0.1^{a}	0.53± 0.12^{a}
Emulsion stability (%)	3± 2^{b}	1.5± 0.7^{a}

Values with different superscript letters within the same row are significantly different (p-value ≤ 0.05).

Solubility of fruit powders depends on their microstructure (Aznar, 2014), particle size distribution, chemical composition and soluble fibre content. Blueberry pomace powders solubility was relatively low, but in the range of that reported by other authors. In particular, values were similar to those reported by Fuentes-Alventosa et al. (2009) for asparagus fibre (22.6 - 34.7%), but notably lower to that of raspberry powder (45.3%) (Si et al., 2016). Results showed no statistically significant differences between coarse and fine powders.

Properties of **hydration** and **water retention** of blueberry pomace powder are interesting attributes to be considered if they are incorporated to formulate food. As mentioned before, these properties depend on the physicochemical composition of the product, on the soluble and non soluble fibre content, as well as on porosity and particle size (Ramírez & Pacheco, 2009). The mechanical treatment as well as, the agitation required for some determinations, opens fibre structure leaving the hydroxyl groups of the cellulose free, so that they are available to link to water (Sangnark y Noomhorm, 2004). Despite the existing differences on fibre content between CP and FP, they had no impact on **hygroscopicity** values. Higroscopicity is the ability of a product to absorb water from the environment; it would determine the stability of the product during the storage. As lower hygroscopicity of powders, better for the packaging and storage conditions. Results of the present work were similar to those reported for freeze dried and atomized kiwi, 65 g/100 $g_{dry\ matter}$ and 54 g/100 $g_{dry\ matter}$, respectively (Wu-Ng et al., 2013), but higher to the reported for raspberry powder (Si et al., 2016), between 13 g/100 g and 18 g/100 g. The **wettability** is closely related to solubility and is very important for the industry in order to develop new products. The fine powder had significantly higher wettability than the coarse powder, probably due to the higher insoluble fibre content of the CP. As stated before, the insoluble fibre is more porous and has a lower density, so it remains more time on the surface increasing the time to get completely wet. The higher porosity of CP facilitates the swelling process (Kethireddipalli et al., 2002); this

could be the reason why CP showed a significantly higher **swelling capacity** than FP. The swelling capacity is related to the satiating effect perceived during the digestive process. Comparing with other commercial fruit fibres (such as lemon, peach, orange and apple), whose swelling capacity exceeds 7 mL/g (Martínez-Las Heras et al., 2017), the blueberry powders had a quite low swelling capacity. As for the swelling capacity, the grinding process changes the structural matrix and reduces **water holding capacity** (WHC) and the **water retention capacity** (WRC). Both WHC and WRC values were below those obtained by Martinez-Las Heras (2017) in lemon, orange, peach and apple. On the contrary, blueberry powders showed higher WHC and WRC values than those obtained by Figuerola et al. (2005) in grape, lemon, orange and apple which were below 2.26 g of water per g.

Regarding the emulsifying properties, ingredients with high fibre content tend to catch the fats and are generally used to prepare emulsions and stabilize foods with high fat content (Chantaro et al., 2007, Thebaudin et al., 1997). This parameter is affected by the type, size, shape and superficial area of the fibre constituents, and also by its chemical composition (Lopez et al., 1996). The **oil holding capacity** (OHC) of the blueberry powders was similar to that obtained in the commercial fibres of lemon, peach, orange and apple, as they were in the range from 2.5 to 2.9 g of oil/g (Martínez-Las Heras et al., 2017). On the contrary, the blueberry powders have slightly lower OHC than the powders of grapes, lemon, orange and apple reported by Figuerola et al. (2005), which does not reach to retain 2 g of oil per g. However, the **emulsifying**

activity of blueberry powders was very low for both powders, being much lower than the values obtained in other dehydrated powders (Martínez-Las Heras et al., 2017; Ramírez y Pacheco, 2009). **Emulsion stability** was higher for the CP than the FP, probably due to its higher fibre content; however they were lower than in other powders.

Stability of a product is mainly determined by the relationship between the equilibrium moisture content of the product and its water activity at a given temperature (Myhara et al., 1998, Temple & van Boxtel, 1999). One of the factors that affect the shape of water sorption isotherms are the composition and physical state of the product (Leung, 1986). Moisture sorption isotherms are crucial to predict the stability, storage conditions (packaging material) as soon as the equilibrium moisture content of the food products (Gal, 1983; Labuza, 1984).

Figure 3 shows the experimental results of the **sorption isotherms** of CP and FP at room temperature, which can be used to predict the moisture adsorption and desorption of the powders under storage conditions (Labuza et al., 1984). The sorption isotherm curve obtained had a sigmoidal shaped curve, classified as a type II isotherm according to Brunauer's classification (Brunauer et al., 1940). It was similar to that obtained by Martinez- Las Heras et al., (2014) for dried persimmon leaves and by Sormoli & Langrish (2015) for orange juice powder. There were no significant differences between the isotherms of coarse and fine powders. From the shape of the curves obtained, it can be stated that small

changes of moisture at low moisture level noticeably affect the water activity of the product, so it is very important to preserve properly the powders. In any case, storage conditions cannot be above a HR of 30%, otherwise the water activity will rise to 0.75, thus promoting the development of moulds and bacteria.

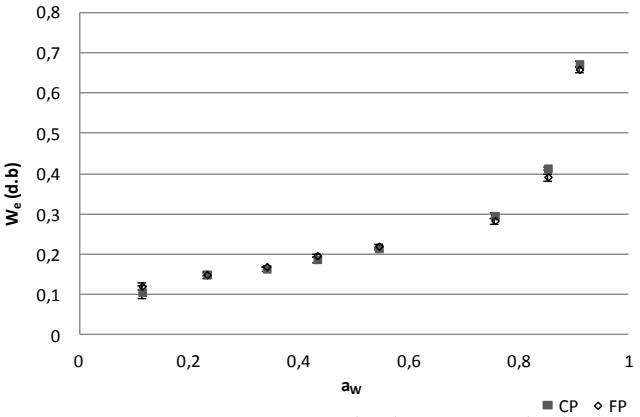

Figure 3. Sorption isotherms of coarse (CP) and fine (FP) powder at 20ºC. Mean ± standard deviation of three replicates.

Parameters of BET and GAB models in the range of a_w between 0 and 0.75 are shown in table 8 and goodness of the fit is shown in figure 4. According to the goodness of the fit, the model which better described the behaviour of the isotherm curve for both powders was the GAB model. This model could be used to describe both CP and FP behaviour during their storage at room temperature. In general, characteristic parameters of the GAB model obtained in the present study were of the order but not exactly identical to those reported by other authors for similar products. Tao et al. (2018), who modelled the sorption isotherms of freeze dried blueberry powder from juice, fruit and pomace using the GAB model obtained lower values of W_0 (between 0.041 and 0.045), higher values of K (between 1 and 1.039) and lower values

of C (between 15.3 and 18.66). W_0 values slightly higher for FP than for CP may be due to their different structure and composition, so that the monolayer water adsorbed increased with the reduction of the particle size during the grinding step.

Table 8. Characteristic parameters of BET and GAB models and goodness of fit for coarse (CP) and fine (FP) powder. Mean ± standard deviation of three replicates.

Model	Parameter	CP	FP
BET	Wo	0.099	0.124
	C	96.95	41.11
	R^2	0.9884	0.9813
GAB	Wo	0.141	0.149
	C	29.095	36.219
	K	0.712	0.653
	R^2	0.9952	0.9976

W_o: Monolayer moisture content (g_{water}/ $g_{dry\ matter}$), C: characteristic constant related to the heat released in the sorption process (Brunauer et al., 1938, Iglesias & Chirife, 1976) and K: correction factor related to the heat of sorption of the multilayer.

(a) (b)

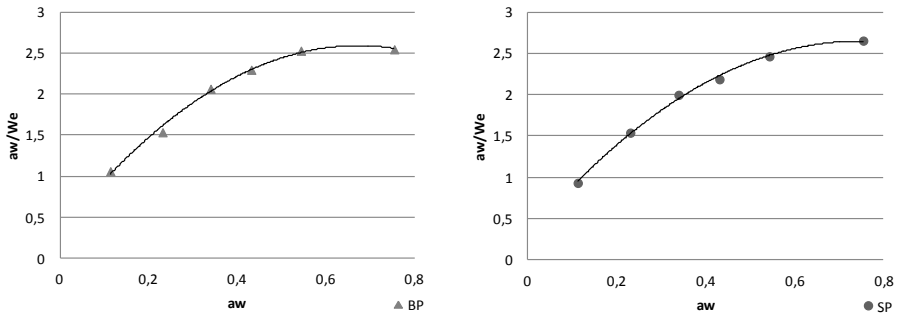

Figure 4. Fit to the GAB model for coarse (CP) (a) and fine (FP) (b) powder of the experimental data (in grey dots).

As it can be checked in table 9, grinding operation had not an important effect on the **colour** parameters. There were not

statistical differences between powders in terms of L* and a* parameters. As regards b* coordinate, it took significantly higher values in FP samples than in CP ones, thus indicating that the smaller size reached in FP powders might favour their chemical oxidation and their browning. Colour differences (ΔE) also reflect this effect.

Table 9. CIEL*a*b* coordinates of coarse (CP) and fine (FP) powders. Mean ± standard deviation of three replicates.

	L*	a*	b*	ΔE	C	h
DP70	37.5± 0.3^{b}	3.80± 0.10^{b}	0.65± 0.09^{b}	-	3.86± 0.08^{b}	9.8± 1.5^{a}
CP	37.5± 0.2^{a}	3.8± 0.08^{b}	0.68± 0.08^{b}	0.06± 0.12	3.9± 0.07^{b}	10.1± 1.3^{b}
FP	37.74± 0.13^{a}	3.37± 0.05^{a}	0.99± 0.05^{a}	0.46± 0.11	3.93± 0.5^{a}	14.6± 0.9^{a}

Values with different superscript letters within the same column are significantly different (p-value≤ 0.05).

3.3.1. Antioxidant properties

As it can be seen in table 10, a reduction of the particle size resulted in a higher **total phenol content**, probably because the more intense grinding involved an increase in both the membrane disruption and the specific surface that might favour the extraction of phenolic compounds (White et al., 2011). As it can be deduced from the higher total phenol content of DP70 samples, grinding also involved an increase in the oxygen exposure and so in the antioxidants degradation. For the same reason, the content of particularly sensitive to oxidation compounds, such **monomeric anthocyanins,** was lower in FP samples than in CP ones. In comparison to other results found in the literature, the amount of anthocyanins that remained in the product after drying and

grinding the blueberry pomace was quite high and in the range of 41.61% and 65.77% for FP and CP powders, respectively. Analyzing the amount of anthocyanins in blackberries before and after drying at 70 ºC for 9 h (Si et al., 2016) reported that only 20.67% of the initial content were found in the dried product.

Table 10. Total phenols content (mg GAE/100 $g_{dry\ matter}$) and monomeric anthocyanins (mg glucosid-3-cyanidin/100 $g_{dry\ matter}$) of coarse (CP) and fine (FP) powder. Mean ± standard deviation of three replicates.

	Total phenols mg GAE/100 $g_{dry\ matter}$	**Monomeric anthocyanins** mg glucosid-3-cyanidin/100 $g_{dry\ matter}$
CP	2.92 ± 0.11^{a}	49.0 ± 0.9^{b}
FP	3.35 ± 0.10^{b}	31 ± 4^{a}

Values with different superscript letters within the same column are significantly different (p- value ≤ 0.05).

Antioxidant activity of powders, measured both by DPPH and ABTS assays and expressed as mg of trolox equivalents per g of sample in dry basis, are shown in figure 5. It should be mentioned that antioxidant activity of blueberry pomace dried at 70 ºC was similar to that of CP samples, regardless on the methodology used. As it happened when evaluating the effect of drying conditions on the antioxidant activity of blueberry pomace, the effect of grinding conditions was more evident when antioxidant activity was measured by DPPH assay. In addition, the DPPH assay seemed to be more sensitive, since higher trolox equivalent values were obtained by using this reagent. Antioxidant activity of powders was very similar to the obtained for the dried pomace. Once again, antioxidant capacity seemed to be more related to anthocyanins content than to total phenols content, which was previously observed by Wu et al. (2004).

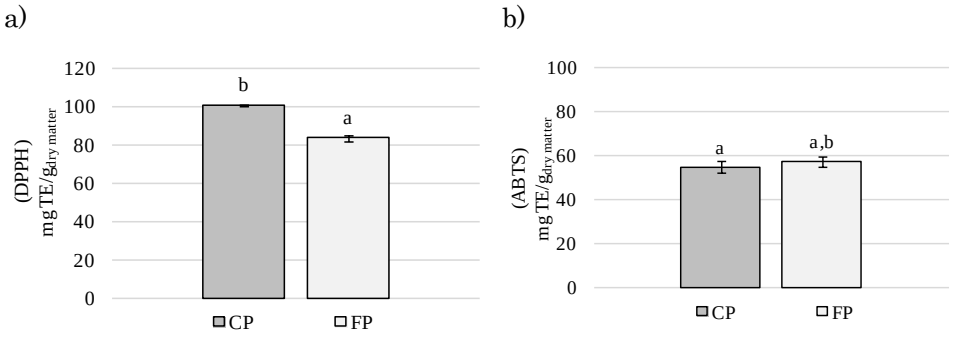

Figure 5. Antioxidant activity expressed in mg Trolox Equivalents/$g_{dry\ matter}$ in dry basis with DPPH (a) and ABTS (b) methods of powders. Mean ± standard deviation of three replicates. Average results with different letters are significantly different (p-value ≤ 0.05).

3.3.2. Prebiotic effect

Generally, the main components responsible of the prebiotic effect are fructoligosaccharides (FOS) included in the fibre fraction of the food. In this study, *Lactobacillus salivarius* spp. *salivarius* was used as a probiotic microorganism due to its probed effect against *Helicobacter pylori* infection (Aiba et al., 1998). Figure 6 shows the *Lactobacillus salivarius* spp. *salivarius* concentration (CFU/mL) in MRS broth and in MRS broth enriched with CP and FP powders at different concentrations after inoculation and incubation for 24 h at 37 ºC. Statistical analysis evidenced that both the granulometry of the powders and its concentration in the growth medium as well as their interaction significantly affected the microbial growth (p-value ≤ 0.05).

Regarding the addition of blueberry pomace powder to the MRS broth, it slightly improved the microbial growth, especially when adding 0.5% of fibre in the form of both CP and FP, but mainly in

the form of CP. Increasing the fibre content, especially in the form of FP, resulted in a decrease in the probiotic effect probably because other compounds, such as phenols with antimicrobial properties, were also present in the powders (Sauceda, 2011). As regards the particle size of the powders, not only because having a higher total phenol content, but also because phenolic compounds are more easily released to the culture media, *Lactobacillus salivarius* spp. *salivarius* growth in MRS broth containing FP was not as high as expected. Moreover, since having a lower density, the fine powdered sample was able to remain more suspended in the growing media, thus increasing the exposure of *Lactobacillus salivarius* spp. *salivarius* to the antimicrobial compounds, while coarse powdered samples tended to sediment to the bottom of the falsk. Similar experiments under agitation will be then required in order to realise the effect of the particle size on the microbial growth.

In short, it have been proven that the powder of blueberry waste could have prebiotic effect on the growth of *Lactobacillus salivarius* spp. *salivarius*, although this would be subject to the granulometry of the powder and the amount of powder added, since the balance between the prebiotic effect of the fibre and the inhibitory antioxidants present in the powder should be on the side of the prebiotic.

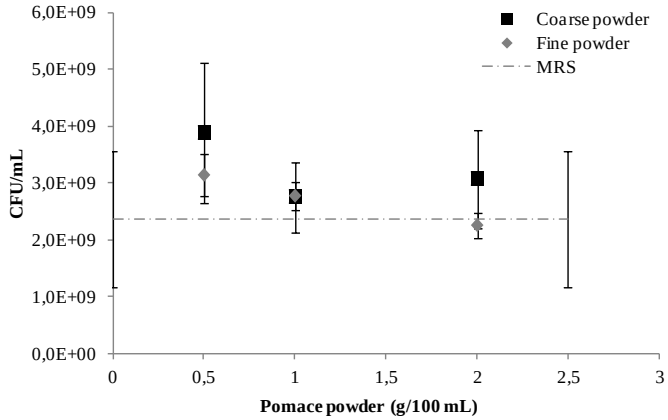

Figure 6. *Lactobacillus salivarius* spp. *salivarius* content (CFU/mL) in MRS broth (control) and in MRS broth enriched with CP or FP at different concentrations. Mean ± standard deviation of six replicates.

3.4. Storage evaluation

Table 11 shows the values of water activity and moisture content of CP and FP samples along their storage for 20 weeks. As it can be observed, both the water activity and the moisture content increased after 20 weeks of storage, especially in the case of FP samples that, as reported before, showed a greater ability to interact with water.

Table 11. Water activity (a_w) and moisture content (x_w (g_{water}/g)) of coarse (CP) and fine (FP) powder during 20 weeks of storage. Mean ± standard deviation of three replicates.

Storage (weeks)	a_w		x_w (g water/g)	
	CP	FP	CP	FP
0	0.236± 0.004^{b,c}	0.198± 0.06^{a}	0.017± 0.002^{a}	0.0190± 0.001^{a}
4	0.246± 0.003^{c}	0.218± 0.01^{a,b}	0.024± 0.005^{b}	0.023± 0.003^{a}
8	0.231± 0.003^{a,b}	0.254± 0.006^{b,c}	0.030± 0.005^{b,c}	0.028± 0.002^{b}
12	0.219± 0.004^{a}	0.305± 0.003^{c,d}	0.0264± 0.0013^{b,c}	0.0300± 0.001^{c}
16	0.276± 0.014^{d}	0.315± 0.003^{d}	0.029± 0.004^{b,c}	0.0343± 0.001^{d}
20	0.311± 0.008^{e}	0.293± 0.003^{d}	0.032± 0.001^{c}	0.039± 0.002^{e}

Values with different superscript letters within the same column are significantly different (p- value ≤ 0.05) for storage factor.

Regarding **total phenols** and **monomeric anthocyanins** (table 12) contents, they remained quite stable along the storage of the powders for 20 weeks. Only in the case of FP samples, the total phenols content was significantly reduced at the end of the storage, probably due to the fact that oxidation occurred more easily in those samples having a higher specific surface.

Table 12. Total phenols content (mg GAE/100 $g_{dry\ matter}$) and monomeric anthocyanins (mg glucosid-3-cyanidin/100 $g_{dry\ matter}$). Mean ± standard deviation of three replicates.

Storage (weeks)	**Total phenols** mg GAE/100 $g_{dry\ matter}$		**Monomeric anthocyanins** mg glucosid-3-cyanidin/100 $g_{dry\ matter}$	
	CP	FP	CP	FP
0	3.02± 0.12^{a}	3.36± 0.10^{b}	49± 3^{a}	40± 2^{a}
4	3.12± 0.15^{a}	3.27± 0.11^{a,b}	48± 5^{a}	44± 3^{a}
8	3.22± 0.03^{a}	3.05± 0.15^{a}	47± 2^{a}	40± 3^{a}
12	3.12± 0.2^{a}	3.00± 0.14^{a,b}	45.1±1.6^{a}	43± 2^{a}
16	3.3± 0.2^{a}	3.03± 0.18^{a,b}	47±3^{a}	40± 5^{a}
20	3.20± 0.13^{a}	3.01± 0.3^{a}	49±3^{a}	43± 3^{a}

Values with different superscript letters within the same column are significantly different (p- value ≤ 0.05) for storage time factor.

As regards the **antioxidant activity** (table 13), it remained constant when measured by ABTS assay, but slightly decreased when measured by DPPH assay, regardless of the granulometry.

Table 13. Antioxidant activity (mg Trolox Equivalents/ $g_{dry\ matter}$) determined by DPPH and ABTS of coarse (CP) and fine (FP) powder during the storage time. Mean ± standard deviation in mg of TE/100 $g_{dry\ matter}$ of three replicates.

Storage (weeks)	DPPH mg TE/100 $g_{dry\ matter}$		ABTS mg TE/100 $g_{dry\ matter}$	
	CP	FP	CP	FP
0	101.1± 0.7^{d}	84.5± 1.9 ^{b}	58.0± 1.2^{a}	62± 1.2 ^{a}
4	85.2± 0.9^{b}	87± 2^{b}	61± 3^{a}	62± 1.2^{a}
8	91.6± 0.6^{c}	86.9± 1.4^{b}	60.3± 1.8^{a}	63.1± 1.3^{a}
12	104.9± 0.8^{d,e}	85.5± 0.5^{b}	58± 0.4^{a}	62.6± 0.5^{a}
16	107± 3^{e}	86.4± 1.5^{b}	60.4± 1.9^{a}	63.37± 0.18^{a}
20	82.7± 1.5^{a}	76.3± 0.3^{a}	59± 3^{a}	61.8± 1.2^{a}

Values with different superscript letters within the same column are significantly different (p- value ≤ 0.05) for storage time factor.

CieL*a*b* coordinates of powders during their storage are shown in table 14. L* and a* remained during the storage constant and b* coordinate was the one that experimented an increase or a reduction depending on the granulometry. Regarding tone and chroma (table 15), this last one was stable, while the hue was more affected by the variation of the other coordinates.

Table 15. Chroma and hue of of fine (FP) and coarse (CP) powder during the storage time. Mean ± standard deviation of three replicates.

Storage (weeks)	C		h	
	CP	FP	CP	FP
0	3.86 ± 0.082^{b}	3.93 ± 0.13^{c}	14.6 ± 1.5^{b}	14.6 ± 0.2^{d}
4	3.61 ± 0.03^{a}	3.45 ± 0.12^{a}	6.99 ± 0.77^{a}	4.1 ± 2.9^{a}
8	3.47 ± 0.08^{a}	3.511 ± 0.014^{a}	7.6 ± 1.4^{a}	6.6 ± 1.3^{b}
12	3.81 ± 0.06^{b}	3.533 ± 0.012^{a}	11.67 ± 0.51^{b}	7.5 ± 0.5^{b,c}
16	3.78 ± 0.09^{b}	3.721 ± 0.011^{b}	14.00 ± 0.66^{d}	8.2 ± 0.3^{b,c}
20	3.86 ± 0.13^{b}	3.724 ± 0.013^{b}	14.63 ± 0.24^{d}	9.2± 0.5^{c}

Values with different superscript letters within the same column are significantly different (p- value ≤ 0.05) for storage time factor.

Table 14. CIEL*a*b* coordinates of fine (FP) and coarse (CP) powder during the storage. Mean ± standard deviation of three replicates.

Storage (weeks)	L*		a*		b*	
	CP	FP	CP	FP	CP	FP
0	37.51 ± 0.26^{a}	37.71 ± 0.12^{c}	3.80 ± 0.09^{c}	3.81 ± 0.12^{c}	0.65 ± 0.09^{b}	0.99 ± 0.05^{d}
4	38.04 ± 0.06^{b}	36.6 ± 0.2^{a}	$3.59 \pm 0.03^{a,b}$	3.49 ± 0.07^{a}	0.44 ± 0.05^{a}	0.25 ± 0.2^{a}
8	38.10 ± 0.22^{b}	$36.69 \pm 0.04^{a,b}$	3.44 ± 0.08^{a}	3.503 ± 0.012^{a}	0.46 ± 0.09^{a}	0.41 ± 0.4^{b}
12	37.72 ± 0.08^{a}	36.71 ± 0.12^{a}	$3.73 \pm 0.06^{b,c}$	3.502 ± 0.012^{a}	0.77 ± 0.03^{c}	$0.46 \pm 0.03^{b,c}$
16	37.49 ± 0.10^{a}	$36.78 \pm 0.03^{a,b}$	$3.67 \pm 0.10^{b,c}$	3.53 ± 0.04^{a}	0.91 ± 0.02^{d}	$0.501 \pm 0.012^{b,c}$
20	37.74 ± 0.12^{a}	36.85 ± 0.07^{b}	3.81 ± 0.12^{c}	3.67 ± 0.01^{b}	0.99 ± 0.05^{d}	0.59 ± 0.03^{c}

Values with different superscript letters within the same column are significantly different (p- value ≤ 0.05) for storage time factor.

4. Conclusion

Results of the work allow us to conclude that although air drying temperature affects negatively antioxidant capacity of blueberry pomace, the temperature increase from 60ºC to 70ºC does not produce a relevant decrease of antioxidant properties and other quality parameters such as colour.

Particle size distribution and fibre content of powders were highly influenced by grinding step, affecting significantly water and oil interactions properties and consequently the final uses of blueberry pomace powders.

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3.3.1. Conclusiones

El análisis de las propiedades fisicoquímicas y de la capacidad antioxidante puso de manifiesto que los azúcares no refinados derivados de la caña de azúcar son una alternativa más saludable que el azúcar blanco convencional. Su uso habitual como endulzante y en la fomulación de alimentos contribuiría al concepto de sostenibilidad global. Entre los productos evaluados, las panelas y la miel de caña fueron los productos con mejores propiedades antioxidantes, por lo que serían los más indicados para reemplazar al azúcar blanco en la formulación de alimentos, especialmente la panela granulada, que al ser un producto en polvo presenta mayor facilidad de uso a nivel industrial.

Por otro lado, se ha demostrado la viabilidad de la obtención de un polvo con propiedades funcionales a partir del bagazo de arándano. El polvo obtenido se vio afectado por la intensidad del triturado, afectando al contenido en fibra y a sus propiedades de interacción con el agua y el aceite. La capacidad antioxidante aumentó al disminuir el tamaño de partícula, y los fenoles y antocianinas monómericas se vieron afectados fundamentalmente por el aumento de la temperatura de secado. Durante las 20 semanas de almacenamiento el contenido en fenoles y antocianinas monómericas, así como las propiedades antioxidantes se mantuvieron estables.