UNIVERSIDAD DE BURGOS

DEPARTAMENTO DE BIOTECNOLOGÍA Y

CIENCIA DE LOS ALIMENTOS

REVALORACIÓN DE SUBPRODUCTOS DE LA INDUSTRIA CONSERVERA VEGETAL. OBTENCIÓN DE FIBRA DE ALCACHOFA Y ELABORACION DE GALLETAS TIPO *DIGESTIVE*

REVALUATION OF BY-PRODUCTS OF THE VEGETAL CANNING INDUSTRY. OBTAINING FIBRE OF ARTICHOKE AND DEVELOPMENT OF *DIGESTIVE* TYPE BISCUIT

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Como Directora de la Tesis Doctoral **"Revaloración de subproductos de la industria conservera vegetal. Obtención de fibra de alcachofa y elaboración de galletas tipo *digestive*"** / "**Revaluation of by-products of the vegetal canning industry. Obtaining fibre of artichoke and development of *digestive* type biscuit".** Desarrollada por FRANCISCO J. SAN JOSÉ BARRERO

INFORMA:

Favorablemente la presentación de dicha tesis, ya que reúne las condiciones necesarias para su defensa en cuanto a la realización de la fase experimental y la elaboración de la memoria.

Y para que así conste y a los efectos oportunos, firma el presente informe,

En Burgos, a 12 de Abril 2018

Fdo: Dra. Mª de Montserrat Collado Fernández

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“Y vi a Sísifo, que soportaba pesados dolores, llevando una enorme piedra entre sus brazos. Hacía fuerza apoyándose con manos y pies y empujaba la piedra hacia arriba, hacia la cumbre, pero cuando iba a trasponer la cresta, una poderosa fuerza le hacía volver una y otra vez y rodaba hacia la llanura la desvergonzada piedra. Sin embargo, él la empujaba de nuevo con los músculos en tensión y el sudor se deslizaba por sus miembros y el polvo caía de su cabeza”.

Homero. LA ODISEA

“Omnia vincit amor, et nos cedamus amori”

Publius Vergilius Maro

“De lo que te den. Da siempre….el doble”.

Néstor J. San José Barrero.

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SUMMARY

The artichoke by-products offer a great potential as source of functional substances, fibre (soluble and insoluble) and polyphenols, and could be of important technological and nutraceutical use, and if used in the design of functional foods. In general, the fibres extracted from vegetal by-products, have a higher capacity to retain water or oil, etc. than the fibre of cereals like wheat or oat. Thus, the utilisation of these by-products represents a good alternative to cereal fibres in the preparation of bakery and pastry products.

Previous used of fibre-rich powders (FRPA) from artichoke by-products are not yet being used in commercial products, it is necessary to understand how their functional properties and antioxidant content are modified by the extraction method and subsequently how developing novel food with Fibre-Rich Powders of artichoke can modify the sensorial and quality attributes, along functional and quality in this fibre-enriched biscuits.

In the chapter 1 of this thesis is explained how the fibre-rich powders of artichoke were obtained employing the two more common methods of extraction, direct dry and wet gridding with different extraction solvents (distilled water [W], 1 %$CaCl_2$*$5H_20$ [CA], ethanol 97 % [A]). Furthemorethe study of their different functional (total polyphenol content, antioxidant capacity, etc.) and technological (water and oil holding capacity, fibre content, etc.) qualities depending on the method of extraction was evaluated.

The most suitable texturometric technical was evaluated in the chapter number 2 and themost applicable fibre-rich powders of artichoke (CA and W) for their functional qualities for the study “digestive” biscuit.

Along the chapter number 3 was studied their industrial application, performing shelf life studies of biscuits enriched with fibre-rich powders of artichoke through colour, texture, chemical (polyphenol and antioxidant) and sensory analysis was performed in the chapter number 4.

The advantage of the artichoke fibre-rich powders (W, CA), compared to the reference fibre (P, Pea fibre), is that the dough of biscuits formulated with artichoke fibre has a higher capacity to retain moisture. This retention of moisture helps to slow down the evaporation of water during baking in the oven, which reduces the non-enzymatic reactions during baking and therefore yields a less dark-toasted colour.

From a functional point of view initially and during storage, the biscuits formulated with fibre-rich powders, W and CA, showed a higher polyphenol content and antioxidant activity compared to both the control biscuits without fibre (B) and the biscuits formulated with reference fibre (P, Pea fibre).

Finally, the utilization of fibre-rich powders from artichoke by-products increases the texture variables (hardness, maximum force of deformation and rigidity) in a similar way as commercial reference vegetal fibres (P) and the change of this sensorial attribute was determined as negligible by an expert sensorial panel.

In conclusion, it is possible to customize the functional (polyphenol and antioxidant content, etc.) and technological (Water and Oil holding capacity, fibre content, etc.) qualities of the fibre-rich powders of artichoke applying different methods of extraction, and if compared with the fibre of reference (P), all the fibre-rich powder of artichoke obtained in our study may offer better functional and technological qualities such as higher water and oil holding capacity, GDRI (glucose retardation index) and far more polyphenol and antioxidant content. All these qualities increase the chances of industrial revaluation, being a plausible alternative for the fibre enrichment of oven-baked products like biscuits.

RESUMEN

Los subproductos de la alcachofa ofrecen un gran potencial como fuente de sustancias funcionales, fibra (soluble e insoluble) y polifenoles, y podrían tener un importante uso tecnológico y nutracéutico, si se utilizan en el diseño de alimentos funcionales. En general, las fibras extraídas de subproductos vegetales tienen una mayor capacidad de retención de agua o aceite, etc. que la fibra de cereales como trigo o avena. Por lo que, la utilización de estos subproductos representa una buena alternativa a las fibras de cereales en la elaboración de productos panadería y pastelería.

Antes de la utilización comercial de extractos ricos en fibra (fibre-rich powders, FRPA) de subproductos de alcachofa, se debe estudiar si los posibles métodos de extracción industrial modifican sus propiedades funcionales, composición y concentración antioxidante. Una vez seleccionado los métodos más óptimos de extracción de los FRPA, se debe evaluar su impacto en los atributos sensoriales, funcionales y de calidad en las galletas enriquecidas en dicha fibra.

En el capítulo 1 de esta tesis se expone cómo se obtuvieron los "fibre-rich powders" de alcachofa aplicando los dos métodos más comunes de extracción, secado directo y molienda húmeda con diferentes disolventes de extracción (destilada agua [W], 1 % $CaCl_2$ * $5H_20$ [CA], etanol 97 % [A]). Así mismo se estudió sus diferentes cualidades funcionales (contenidos en polifenoles, capacidad antioxidantes-, etc.) y tecnológicas (capacidad de retención de agua y de aceite, contenido de fibra, etc.) en función del método de extracción aplicado.

La optimización y la selección de la técnica texturométrica más adecuada para el estudio de galletas "digestive" se realizó en el capítulo 2. . En el capítulos3, se ha estudiado la selección de los extractos ricos en fibra de alcachofa más aplicables (CA y W) por sus cualidades funcionales y su aplicación industrial realizando un estudio de vida útil de galletas enriquecidas con extractos de alcachofa ricos en fibra; determinando: un análisis colorimétrico, texturométrico y químico (determinación del contenido en polifenoles y de la capacidad antioxidante) y el análisis sensorial fue realizado en el capítulo 4.

La ventaja utilizar los extractos de alcachofa ricos en fibra (W, CA), en comparación con la fibra de referencia (P), es que la masa de galletas formulado con fibra de alcachofa tiene una mayor capacidad de retención de humedad. Esta retención de la humedad ayuda a ralentizar la

evaporación del agua durante el horneado, y por lo tanto se reduce las reacciones no enzimáticas durante el horneado y en consecuencia produce un color tostado menos oscuro.

Desde un punto de vista funcional, al principio y durante el almacenamiento, las galletas formuladas con los extractos de alcachofa ricos en fibra, W y CA, mostraron una mayor contenido de polifenoles y capacidad antioxidante en comparación con las galletas de control sin fibra (B) y las galletas formuladas con fibra de referencia (P, fibra de guisante).

Finalmente, la utilización de los extractos de alcachofa ricos en fibra incrementa las variables de textura (dureza, fuerza máxima de deformación y rigidez) de una manera similar a las fibras vegetal de referencia comercial (P), pero el panel sensorial experto determinó que el cambio de estos atributos sensoriales era despreciable.

En conclusión, es posible personalizar las propiedades funcionales (contenido en polifenoles y capacidad antioxidante, etc.) y tecnológicas (capacidad de retención de agua y de aceite, contenido de fibra, etc.) de los FRPA ricos en fibra aplicando diferentes métodos de extracción. Al comparar con la fibra de referencia (P), todos los extractos de alcachofa ricos en fibra obtenidos en este estudio ofrecen mejores cualidades funcionales y tecnológicas, tales: como mayor capacidad de retención de agua y de aceite, mejor GDRI (índice de retraso de glucosa) y mayor concentración de polifenoles y antioxidantes. Todas estas cualidades aumentan las posibilidades de revalorización industrial, y son una alternativa plausible para el enriquecimiento con fibra de productos horneados como las galletas.

INTRODUCCIÓN

1.- REVALORIZACIÓN DE LOS SUBPRODUCTOS DE LA INDUSTRIA

La revalorización de los subproductos de la industria conservera se puede plantear desde distintos puntos de vista:

- *Perspectiva medioambiental:* La revalorización subproductos vegetales es vital, ya que aumenta la viabilidad económica de los procesos de minimización de residuos orgánicos contemplados en la Directiva 1999/31/CE del Consejo europeo relativa a los residuos de vertedero. En la que se requería que antes del 2016, los Estados miembros reduzcan los residuos orgánicos biodegradables en los vertederos en un 65 % (en comparación con los niveles de 1995) (Unión Europea, 2012)[1]. Lamentablemente a día de hoy en la transformación y conservación de productos vegetales por las empresas conserveras y afines aún se producen una enorme cantidad de subproductos vegetales, que se revalorizan mínimamente como alimentación animal. No obstante esta tendencia se está revirtiendo, ya que en recientes estudios de revalorización de subproductos vegetales en la industria conservera han determinado que pueden presentar un gran potencial como fuente de compuestos funcionales (polifenoles, compuestos antioxidantes) y de fibra (soluble e insoluble) de gran utilidad tecnológica. Por ejemplo, hay diferentes publicaciones que indican que fibras extraídas de subproductos vegetales como espárragos, cardo, alubias verdes, etc. poseen cualidades funcionales, que podrían ser utilizadas en la elaboración de productos enriquecidos como: galletas, productos cárnicos y lácteos, etc. debido a que extractos ricos en fibra extraídos de residuos vegetales tienen mayor capacidad de retención de agua, o de aceite, etc. (Fuentes-Alventosa *et al.*, 2009[a2], 2009[b3]. Rodríguez *et al.*, 2006[4]) que la fibra de cereales como el trigo o avena u otras fibras vegetales que se comercializan extraídas de tubérculos o raíces como (Fibrex® (remolacha), Raftilosa® (achicoria)).

- *Perspectiva funcional y nutricional* : En los últimos años, la preocupación por llevar una vida sana, mediante una alimentación más equilibrada, así como un mayor conocimiento de la nutrición, por parte de los consumidores, ha derivado en un incremento notable del consumo de productos dietéticos, funcionales, enriquecidos, etc. y concretamente el mercado de las fibras alimentarías, para la formulación de nuevos productos con un alto contenido de fibra alimentaria, se ha visto especialmente favorecido.

Las propiedades de la fibra dietética son muy variadas en función de su composición y solubilidad. La fracción insoluble de la fibra, presente en mayor porcentaje en los cereales, está

relacionada con su acción laxante; mientras que la fibra soluble, presente en mayor porcentaje en frutas y verduras, ejerce acciones de disminución del colesterol y de los niveles de glucosa en sangre, además de tener un importante poder saciante y protector del intestino. Por tanto, están especialmente indicadas para personas que sufren de hipercolesterolemia, diabetes, o personas que quieran controlar su peso. Las recomendaciones de ingesta diaria es 22-25 g/día en mujeres y 30-35 g/día en hombres (Arnaceta & Serra, 2011)[5].En general, esta recomendación diaria de fibra no se llega a alcanzar mediante las dietas occidentales, ricas en proteínas, en harinas refinadas, etc. (Fundación Española de la Nutrición, 2016)[6]. Se debería de consumir productos que tuvieran una cantidad de fibra mayor a la habitual para los mismos "*enriquecidos en fibra*" o "*integrales*". A este respecto la simple incorporación de fibra dietética a la formulación de las galletas es un método sencillo que aumenta la funcionalidad de las galletas, con un mínimo coste. La utilización de fibra de diferentes subproductos vegetales puede ser una buena alternativa para enriquecer alimentos Giuntini *et al.,* (2003)[7], no sólo con fibra sino con compuestos antioxidantes Bilgicli *et al.,* (2005)[8], que a su vez puedan reducir procesos de rancidez u oxidación durante el almacenamiento (Talbot, 2010[9], Sakač *et al.,* 2016[10]).

Por ello la utilización de extractos ricos en fibra de diferentes subproductos vegetales (O'Shea *et al.,* 2012[11], Giuntini *et al.,* 2003[7]) puede ser una buena alternativa al aumento y/o fluctuación de los precios de las fibras tradicionales, como los de salvado de trigo o de otros cereales (FAO, 2002)[12], no sólo por su alto contenido en fibra sino también por su rica composición en compuestos antioxidantes (Bilgicli *et al.,* 2005)[8].

Distintos autores han realizado investigaciones sobre la revalorización de subproductos vegetales entre ellos se podría destacar los estudios de extracción de FRPA con alto poder antioxidante de: piel de zanahoria y tomate (Chantaro *et al.,* 2008[13], Navarro-González *et al.,* 2011) [14], hojas externas del repollo (Nilnakara *et al.,* 2009)[15], subproductos de la producción de cacao (Lecumberri *et al.,* 2007)[16], semillas de uva (Pérez Jiménez *et al.,* 2008[17], Zhu *et al.,* 2015)[18], subproductos de berenjena (Boulekbache-Makhlouf *et al.,* 2013)[19], cascara de semillas de sésamo (Nandi & Gosh, 2015)[20] y subproductos de esparrago (Fuentes-Alventosa *et al.,* 2009[a2,b3], Nindo *et al.,* 2003[21]) y alcachofa (Ruiz-Cano *et al.,* 2014)[22].

Con respecto a la aplicación de compuestos con un alto poder antioxidante, en productos horneados como galletas (Manley, 2011)[23] en los últimos años se han utilizado diferentes productos de extractos de plantas silvestres (Reddy *et al.,* 2005)[24] y hojas de té (Sharma & Zhou, 2012)[25], hasta polen de abeja (Krystyjan *et al.,* 2015)[26]. Sin embargo, debido a que los extractos

de plantas tienen que ser inmovilizados para aumentar su funcionalidad otros autores utilizaron directamente fibras vegetales con efecto antioxidante para facilitar su incorporación y mezcla en la masa de las galletas. Estas fibras se obtuvieron de subproductos vegetales como: manzana y avena (Vitali *et al.*, 2009)[27], mango (Ajila *et al.*, 2007)[28], vainas de guisante (Raymundo *et al.*, 2014)[29] y de piel de naranja (Larrea *et al.*, 2005)[30].

Actualmente, el proceso industrial de la alcachofa como: elaboración de conservas, congelados o para la formulación de menestras, etc., conlleva aproximadamente un aprovechamiento del 25 % en peso de la alcachofa. El subproducto generado por el trío, aproximadamente un 75 % en peso, se destina habitualmente a alimentación animal. Produciéndose una mínima revalorización económica de este subproducto. Diferentes estudios científicos han demostrado que de diferentes partes de la alcachofa (*Cynara scolymus* L.) se pueden obtener compuestos de valor añadido, como las fibras dietéticas, enzimas coagulantes, polifenoles, etc. (Ruiz-Cano *et al.*, 2014)[26].

Como se ha comentado en la actualidad, se comercializan fibras vegetales para el enriquecimiento de galletas con alto contenido de fibra, entre las que encontramos; de remolacha (Fibrex®), achicoria (Raftilosa®), guisante (Peafibf®), etc. y aunque la utilización de subproductos de alcachofa se han empleado en la elaboración de productos horneados como pan (Boubaker *et al.*, 2016)[31a,32b] no se han encontrado referencias bibliográficas de galletas formuladas con FRPA (Fibre Rich Powder of Artichoke) extraídos de subproductos. Suponiendo que la fibra extraída delos subproductos de alcachofa tenga una composición similar a la parte comestible de la alcachofa, este subproducto representa una fuente potencial de sustancias de alto valor añadido como fitoquímicos y fibra (soluble e insoluble) y podría ser la forma sencilla de enriquecer y agregar valor a la galleta, a la vez que se revaloriza un subproducto sin valor.

2.- LA ALCACHOFA: DESCRIPCIÓN YMORFOLOGÍA

Según la real academia de la lengua española la alcachofa es: Planta hortense, de la familia de las compuestas; con raíz fusiforme, tallo estriado y ramoso, de hasta de medio metro de altura, y hojas semi-espinosas, con cabezuela comestible.

Su nombre científico es *Cynara scolymus* L., deriva de las palabras "*Kynara*", posiblemente debido al nombre de isla del mar Egeo llamada "*kytnaros*" donde se conoce su cultivo, y *skolymos*, que en griego significa espinoso. Aunque existe un mito griego que explica que una joven llamada Cynara, habitante de la isla de Kynaros fue seducida por Zeus y para que pudiera habitar en el olimpo fue ascendida al grado de diosa. Cansada de su papel de amante, al estar Zeus casado con la diosa Hera, descendió sin permiso a Kynaros. Al enterarse de su huida, como venganza Zeus envolvió su cuerpo de escamas coriáceas hasta dejar preso su corazón desdeñado, creando así la primera alcachofa (Sonnante *et al.*, 2007)[33]. Otras fuentes atribuyen la palabra Cynara a la recomendación del escritor romano Lucius Junius Moderatus Columella de fertilizar este cultivo con cenizas, siendo cineres su palabra en latín (Chevallier 1996)[34]. El nombre común de alcachofa en castellano proviene del árabe "*Al-Quarshuff*" o "*Al-qabsíl*". En realidad, la alcachofa (*Cynara scolymus* L.) es originaria del Noreste de África, y por sus cualidades nutricionales y terapéuticas se extendió por todo el mediterráneo durante la Grecia y Roma clásicas, desde el antiguo Egipto (Hepper, 2009[35], Van der ven & Tabinor, 2007[36]), como anécdota está documentado que ya el rey egipcio Ptolomeo III Euergetes (246–222 a.C.) ya recomendaba a su ejército comer alcachofas porque eran considerados una fuente de fortaleza y valentía. Entre el siglo IX y XVI debido a su cultivo en diferentes monasterios en (Sicilia, España, Florencia y Venecia) la alcachofas se extienden por toda Europa. Posteriormente debido al descubrimiento de América, los inmigrantes europeos extienden su cultivo por el continente Americano durante el siglo XVI al XVIII.

Actualmente la producción mundial de alcachofa asciende a 1.793.015 taño, siendo el 60 % producida en Europa (FAOSTAT, 2013)[37], donde Los tres principales líderes productores en Europa son Italia (547.799 t), España (199.900 t) y Grecia (28.600 t), pero otros países como China y Perú están aumentando su producción anualmente.

La alcachofa es considerada una delicatesen y es habitualmente consumida fresca aunque también se consume mínimamente procesada, en conserva o congeladas después del

procesamiento mínimo consistente en un ligero escaldado que evita el pardeamiento enzimático producido por la enzima polifenoloxidasa (PPO) (Padino *et al.,* 2017[38], Guida *et al., 20*09[39]).

2.1. Morfología, ecología y empleo de la planta de alcachofa

Las partes comestibles de la planta son grandes inflorescencias inmaduras, llamadas cabezas, compuestas de hojas carnosas (brácteas) internas y la parte superior del receptáculo llamado pedúnculo. La inflorescencia de la alcachofa, consiste en un pedúnculo muy largo (hasta 180 cm), donde se insertan flores y brácteas externas. Por cada planta se produce una cabeza principal (*capitulum*) y de entre 4 a 20 cabezas secundarias y terciarias. Durante la cosecha únicamente se recolecta las cabezas en las primeras etapas de su desarrollo (30-40 % de peso fresco de la alcachofa) (Rottenberg& Zohary, 1996)[40]. Teniendo en cuenta que únicamente se consume la parte interna de la cabeza (corazón de la alcachofa), las hojas, brácteas externas y tallos desechados por la industria de procesamiento de alcachofa, representan una gran cantidad de material desechado (cerca de 80 – 85 % de la biomasa total de la planta), que podría ser utilizado como una fuente de compuestos funcionales y nutracéuticos (Llorach *et al.,* 2002[41], Lopez-Molina *et al.,* 2005[42]) o como materia prima en la industria de la química verde como producción de papel, celulosa, colorantes de biocombustibles (De Falco *et al.*,2015)[43] y sería comercialmente viable dada la alta producción de biomasa de la planta, hasta 33 t/ha (Archontoulis *et al.,* 2010)[44].

Después de la recolección, la planta se acondiciona para la próxima campaña; se dejan las hojas momentáneamente en la planta, para reponer la raíz de fotosintatos y de componentes nutritivos y posteriormente se corta el tallo a la altura de la base. Después de las primeras lluvias de otoño, las yemas basales empiezan su crecimiento utilizando las reservas acumuladas en la raíz, cuya composición en azúcar es del 25 %, de los cuales 89.4 % es la inulina (Raccuia *et al.,* 2004)[45].

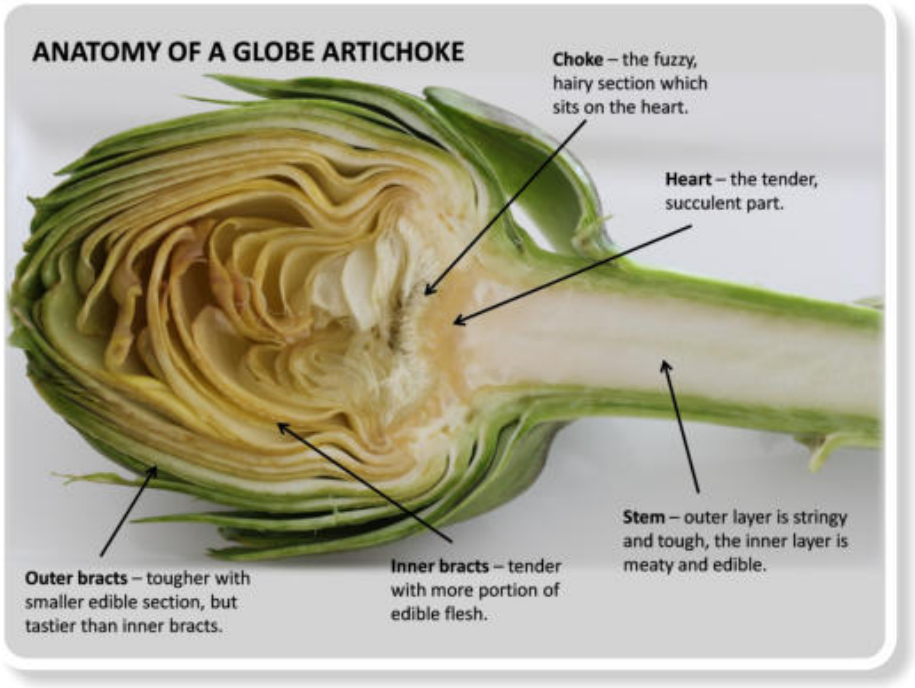

Imagen 1. Anatomía de la cabeza de alcachofa. Cortesía del Sidney living museum. (http: sydneylivingmuseums.com.au) Pagina Visitada el 7/12/2017.

2.2. Composición y valor nutricional de la alcachofa

Las propiedades nutricionales y farmacológicas de las cabezas de alcachofa y las hojas son bien conocidas desde la antigüedad (Ceccarelli *et al.*, 2010[46], Garbetta *et al.*, 2014[47]). La alcachofa es una de las plantas comestibles con mayor contenido de polifenoles totales (Brat *et al.*, 2006)[48] , tales como: ácido fenólico, cinarina (Ácido 1,5-dicafeoylquinico), Ácido clorogénico (Acido 5-cafeoilquinico) y fibra (principalmente inulina). También cabe destacar su alto poder antioxidante (Ajila *et al.*, 2008[32], Peschel *et al.*, 2006[49]), que es en parte el resultado de su composición polifenólica (Kammerer *et al.*, 2014[50], Fuentes-Alventosa *et al.*, 2009[a3]). Otros autores han demostrado que la inulina, aumenta la absorción gastrointestinal de iones como calcio, magnesio y hierro (Azorín-Ortuño *et al.*, 2009)[51], afecta a la difusión de glucosa en el torrente sanguíneo y reduce los niveles colesterol en el suero sanguíneo (Coudray *et al.*, 1997[52], Niness 1999[53]). Desde un punto de vista nutricional (Lutz *et al.*, 2011)[54] las alcachofas tienen un 3 % de proteínas y menos del 0,3 % de lípidos, 1,65 %cenizas y 16 % de carbohidratos y un alto contenido de fibra (5,5 %) y macrominerales [K, (360mg 100 g^{-1} p.f) y Ca (50 mg 100 g^{-1} p.f) (Romani *et al.*, 2006)[55]], y microminerales [Fe (1,5) y Zn y 26.2 mg kg−1P.S., Pandino *et*

al. 2011)[56]] y vitamina C (10 mg/100 g p.f (Gil-Izquierdo *et al.*, 2001)[57]. Dentro de su composición, los compuestos funcionales que destacarían por su alta composición serían la inulina y los polifenoles.

Tabla 1. Valores nutricionales de la cabeza de alcachofa. (Petropoulos *et al.*, 2017)[58]

Valores Nutricionales de la alcachofa.	
Humedad (g/100 g peso fresco)	84-75
Grasa (g/100 g peso fresco)	0.57-0.26
Ceniza(g/100 g peso fresco)	1.67-1.01
Proteína (g/100 g peso fresco)	4.25-1.69
Carbohidratos (g/100 g peso fresco)	11.8-19.09
Fructosa (g/100 g peso fresco)	0.18-0.07
Glucosa (g/100 g peso fresco)	0.21-0.03
Sacarosa (g/100 g peso fresco)	0.66-0.21
K (mg per 100 g peso fresco)	579-276
Na(mg per 100 g peso fresco)	104-17
Ca(mg per 100 g peso fresco)	861-158
Mg (mg per 100 g peso fresco)	91-31
Mn(mg per 100 g peso fresco)	1.17-0.57
Fe(mg per 100 g peso fresco)	2.9-1.7
Zn(mg per 100 g peso fresco)	1.17-0.48
Energía (kcal/100 g peso fresco)	0.83-058
Composición de los ácidos graso (%)	
SFA	69.7-39.28
MUFA	10.3-2.26
PUFA	57.36-23.02

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated, fatty acids; n6/n3: ratio of omega 6/omega 3 fatty acids.

3.- CONCEPTO DE ALIMENTO FUNCIONAL

Debido al constante incremento de las expectativas de vida en los países desarrollados y su impacto en el coste de la atención sanitaria, unido a la pérdida de los modos de vida y dietas tradicionales, han producido que consumidores, gobiernos e industria alimentaria impulsaran el desarrollo de nuevos alimentos (Mollet & Rowland, 2002[59], Young, 2000[60]), que además de su valor nutritivo propio, aporten un beneficio para la salud evitando o previniendo distintas deficiencias nutricionales o enfermedades (Kotilainen *et al.*, 2006[61], Roberfoid, 2000[a62], 2000[b63])

El término "alimento funcional" se acuñó por primera vez en Japón en 1984 (Roberfroid, 2000[64], Kubomara, 1998[65]). Debido al incremento de la población geriátrica, el gobierno Japonés quiso mejorar la calidad de vida de la población a través de intervenciones en la alimentación, subvencionando la investigación de alimentos funcionales o "Foods for Specific Health Uses (FOSHU): "*Productos alimenticios enriquecidos con componentes especiales que poseen efectos fisiológicos positivos*". Por tanto, los alimentos funcionales pueden *mejorar las condiciones generales del cuerpo* (prebióticos y probióticos, etc.), *disminuir el riesgo* de algunas enfermedades (por ejemplo, reduciendo el colesterol o la tensión arterial) y podría incluso ser utilizado para *tratar algunas enfermedades* (por ejemplo, tratamiento de la menopausia, etc.) (Hardy, 2000[66], Stanton *et al.*, 2005[67]). Por lo que respecta a su origen, los compuestos que hacen funcional a un alimento han estado siempre presentes en la naturaleza, pero los avances en nutrición han caracterizado a aquellos alimentos que tiene un efecto funcional intrínseco con sus beneficios para la salud, y el conocimiento tecnológico ha conseguido aislar nutrientes funcionales con los que enriquecer el mismo u otros alimentos. Como resultado se obtienen, alimentos fortificados (zumos de fruta fortificados con vitamina C), alimentos enriquecidos (margarina con estroles vegetales para reducir el LDL colesterol) y alimentos mejorados (huevos con mayor contenido de omega-3 mediante la alimentación alterada). También se ha avanzado en los procedimientos tecnológicos o biotecnológicos de eliminación de algunos componentes potencialmente dañinos para cierto espectro de consumidores (leche sin lactosa, alimentos sin gluten).

En Japón, al "alimento funcional" se le dio una categoría legislativa propia y debe satisfacer tres requisitos nutricionales: *eficacia en estudios clínicos y nutricionales* (evidencia científica de la dosis sugerida), *seguridad en estudios clínicos y no clínicos,* finalmente *descripción de los*

alimentos cualidades fisicoquímica (determinación y cuantificación de los componentes del activo) (Shimizu, 2003[68], Lee & Foo, 2014[69], Ringel *et al.*, 2015[70]).

Tabla 2: Lista de productos FOSHU aprobados

Specified Health Uses	**Principal Ingredients (ingredients exhibiting health functions)**
Foods to modify gastrointestinal conditions	Oligosaccharides, lactose, bifidobacteria, lactic acid bacteria, dietary fibre 8 ingestible dextrin, polydextrol, guar gum, psyllium seed coat, etc.)
Foods related to blood cholesterol level	Chitosan, soybean protein, degraded sodium alginate
Foods related to blood sugar levels	Indigestible dextrin, wheat albumin, guava tea polyphenol, L-arabiose, etc.
Foods related to blood pressure	Lactotripeptide, casein dodecaneptide, tochu leaf glycoside (geniposidic acid), sardine peptide, etc.
Foods related to dental hygiene	Paratinose, maltitiose, erythrytol, etc.
Cholesterol plus gastrointestinal conditions, triacylglycerol plus cholesterol	Degraded sodium alginate, dietary fibre from psyllium seed husk, etc.
Foods related to mineral absorption	Calcium citrated malate, casein phosphopeptide, hem iron, fracuto-oligosaccharide, etc.
Foods related to osteogenesis	Soybean isoflavone, MBP (Milk basic protein), etc.
Foods related to triacylglycerol	Middle chain fatty acid, etc.

Ministry of Health, Labour and Welfare of Japan (2017)[71].

Posteriormente, el interés por los alimentos funcionales se extendió por Europa y los Estados Unidos, donde además del objetivo principal de mejorar la salud de la población los alimentos funcionales, también podrían tener un potencial comercial en la industria alimentaria o "*function Claim*", que los podría distinguir de los alimentos existentes. Esto produce una divergencia considerablemente con respecto a la naturaleza de los alimentos funcionales en los países orientales. En Japón, por ejemplo, al alimento funcional se le considera como una clase distinta de producto, en el que su funcionalidad es preferida sobre el gusto. Mientras que en los países occidentales, para poder distinguir productos funcionales de los convencionales, la funcionalidad del producto se debe integrar en las características sensoriales propias del alimento (Fern, 2007[72], Kotilainen *et al.*, 2006[63]).

El Instituto Internacional de Ciencias de la Salud el International Life Science Institute (ILSI) la Comisión Europea define al alimento funcional como:

" *Un producto alimenticio sólo puede ser considerado funcional, si junto con el impacto nutricional básico, tiene efectos beneficiosos sobre una o varias funciones del organismo humano. Ya sea mejorando las condiciones generales y físicas o / y disminuyendo el riesgo de la evolución de enfermedades. La cantidad y forma de ingesta de los alimentos funcionales debe ser la esperada en una dieta equilibrada. Por lo tanto, no se considera alimento funcional al suministrado en forma de píldora o cápsula"* (Diplock *et al.*, 1999)[73].

Tabla 3: Nutrientes establecidos que se utilizan como ingredientes de alimentos funcionales y la opinión de los autores de la eficacia de los ingredientes. Katan & de Ross (2004)[74].

Ingredient	Product examples	Health claim	Strength of evidence in humans
Folic acid	Cereals	Protects against neural tube defects	++ (From the Centers for Disease Control and Prevention, 1993)
Dietary fibre	Drinks	Relieves constipation	++ (Marlett et al., 2002; Cummings and Macfarlane 2002)
Low in sodium	Drinks, soups	Reduces blood pressure	++ (Sacks et al., 2001)
Unsaturated fatty acids	Spreads, cookies	Reduces risk of heart disease	++ (Truswell, 1994; Sacks and Katan, 2002)
Sugar alcohols	Chewing gum	Reduce caries risk	++ (Hayes, 2001)
Soluble fibre from whole oats or psyllium husk	Cereals, cookies	Reduces cholesterol and risk of heart disease	++ for cholesterol lowering (Truswell, 2002)
Soy protein	Drinks, bars	Reduce cholesterol and risk of heart disease	+ for cholesterol lowering (Lichtenstein, 1998)
Calcium	Cereals, fruit juices, milk products, spreads	Protects against osteoporosis/help maintain bone density	+ for consumers with a low calcium intake (Heaney, 2000)
Folic acid, vitamin B6 (pyridoxine)	Cereals	Decreases homocysteine and risk of cardiovascular disease	++ for homocysteine, but no hard evidence yet for cardiovascular disease (Schnyder et al., 2002)
Vitamin E	Supplements	Antioxidant; prevents cardiovascular disease	+ for observational studies but − for clinical trials (Asplund, 2002)
Zinc	Sweets, lozenges	Prevention/cure of common cold	+/− (Marshall, 2000)
Vitamin C	Drinks, sweets	Protects against cardiovascular disease	+/− in observational studies, −− in clinical trials (Asplund, 2002)

(++, Probada eficacia, efecto consistente en múltiples estudios; + razonable evidencia de eficacia, efecto en un número limitado de estudios, o de alguna inconsistencia entre los estudios; 0, no hay datos sólidos; −, evidencia de ningún efecto, la ausencia de un efecto evidente a partir de un número limitado de estudios; – –, no se muestra eficaz, ausencia de un efecto evidente en varios estudios.

En el marco Europeo, la Comisión Europea concluyó que para otorgar el reconocimiento de alimentos funcional, sus efectos funcionales deben tener una base científica sólida basada en estudios epidemiológicos y experimentales, evaluados por la Autoridad Europea de Seguridad Alimentaria (EFSA) y estableció un marco normativo que aportara certeza legal a las empresas agroalimentarias, que evita la competencia desleal, estimula y protege las innovaciones en el

sector de la alimentación. Por tanto, las iniciativas legislativas de la UE se dirigen principalmente a restringir el uso de afirmaciones de salud, tanto en el etiquetado como en la presentación o publicidad (EC, 2006[75], Niva, 2007[76]). Según el Reglamento de la UE sobre nutrición y salud las alegaciones sobre alimentos (EC nº 1924/2006), se establecen un Registro comunitario las declaraciones autorizadas y los perfiles nutrientes de los alimentos que pueden contener afirmaciones de salud. Los reclamos de salud pueden ser " *function claims* o atributo funcional" y " *reduction of disease risk claims* o reducción del riesgo de enfermedad ".

Tabla 4. Atributo funcionales y sus definiciones en la Comunidad Europea SANCO D4. (EC, 2006)

Nutrition claims	Example
Claims describing the presence, absence or level of a nutrient contained in a foodstuff	"Low in saturated fat"
[functional] health claims	**Example**
Health claims that describe the roles of nutrients or other substances in growth, development and normal physiological functions of the body, based on long-established and non-controversial science	"High in protein. Protein helps build and repair body tissues."
Reduction of disease risk claim	**Example**
Any health claim that states, suggests or implies that the consumption of a food category, a food or one of its constituents significantly reduces a risk factor in the development of a human disease	"Sufficient calcium intake may reduce the risk of osteoporosis in later life. Food A is rich in calcium."

Los alimentos, contienen compuestos fitoquímicos o bioactivos que aun no teniendo una función nutricional definida y no siendo nutrientes esenciales, pueden paliar o prevenir una enfermedad (protegiendo el sistema circulatorio, la glucemia y la colesterinemia, reduciendo del riesgo de cáncer y mejorando la respuesta inmunitaria) e influyen en la actividad celular y en los mecanismos fisiológicos (Biesalski *et al.*, 2009)[77].

Las sustancias bioactivas o fitoquímicos son parte del metabolismo secundario de los vegetales, y tienen diferentes funciones en la planta, como: pigmentos, reguladores del crecimiento, aromas, protectores naturales frente a parásitos, insectos, o condiciones climáticas y por tanto éstos se encuentran en alta concentración en frutas y verduras.

Tabla 5. Componentes bioactivos de los alimentos de origen vegetal, principales fuentes dietéticas e ingesta en España (Martinez Roldan & Carbajal Azcona, 2012)[78].

Componente	Fuente dietética	Ingesta en España[a]
Terpenoides (varios miles)		
– **Carotenoides** (de los más de 700 identificados, solo unos 50 están en la dieta y de estos los siguientes representan el 95% de los carotenoides en sangre): • Carotenos: – α-caroteno, ß-caroteno (precursores de vitamina A). – Licopeno. • Xantofilas: – ß-criptoxantina (provitamina A). – Luteína, zeaxantina	**ß-caroteno:** hortalizas y frutas de color naranja (por ejemplo, zanahoria, mango, albaricoque, melón, melocotón, fruta de la pasión, ciruela); verduras de hoja verde oscuro (por ejemplo, espinacas); tomate y derivados. **Licopeno:** tomates, sandía, pimiento rojo, pomelo rosado. **ß-criptoxantina:** naranjas, papaya. **Luteína y zeaxantina:** verduras de hoja verde (por ejemplo, acelgas, espinacas, lechuga, apio), naranjas, patatas, tomates, pimientos rojos, maíz, aguacate, melón.	**Carotenoides, total:** 3-4,3 mg/día. Licopeno: 1,3 mg/día. ß-caroteno: 1 mg/día. Luteína: 0,5 mg/día. Zeaxantina: 0,1 mg/día. Europa: ≈ 12 mg/día.
– **Fitoesteroles** (> 250): • Esteroles y estanoles (2 g/día para reducir LDL-c)[b]: ß-sitosterol, estigmasterol, campesterol, sitostanol, campestanol.	Aceites vegetales (maíz, girasol, soja, oliva), cereales, legumbres, frutos secos, hortalizas. Alimentos enriquecidos.	**Dietas occidentales:** 150-555 mg/día. **Oriental y vegetariana:** 300 mg-1 g/día.
Compuestos fenólicos (> 8.000)		
– **Alcoholes y ácidos fenólicos simples** (tirosol, hidroxitirosol, ácidos hidroxibenzoicos e hidroxicinámicos [elágico, gálico, vanilínico, capsaicina, cumárico, cafeico, ferúlico, clorogénico, etc.]).	Cítricos, aceitunas, aceite de oliva virgen, otras frutas, hortalizas, avena, soja, frutos secos, vino, cerveza, té, etc.	
– **Polifenoles:** • Flavonoides (> 5.000): – Flavonoles (quercetina, kamferol, miricetina, rutina, etc.). – Flavanoles (flaván-3-oles): – Catequinas (catequina, epicatequina, etc.). – Pro(anto)cianidinas o taninos condensados. – Flavanonas (naringenina, hesperitina, naringina, hesperidina, etc.). – Antocianinas (con azúcar en posición 3) y antocianidinas (antocianinas sin azúcar) (cianidina, etc.). – Flavonas (apigenina, luteolina, etc.). – Isoflavonas (genisteína, daidzeína, etc. [fitoestrógenos]). • Estilbenos (resveratrol). • Curcuminoides (curcumina). • Lignanos (principal fuente de fitoestrógenos en occidente).	**Quercetina, kamferol, miricetina y antocianidinas:** cebollas (30-40 mg/100 g), puerros, lechuga, brécol, tomates, uvas, naranjas (pulpa), manzanas, cerezas, moras, frambuesas, arándanos, aceitunas, vino tinto, té, orégano y otras hierbas aromáticas. **Catequinas y proantocianidinas:** manzanas, peras, cerezas, uvas, albaricoque, melocotón, frutos secos, legumbres, cacao, chocolate negro, vino, sidra, cerveza, té. **Hesperidina, naringenina:** cítricos, zumo de uva. **Apigenina, luteolina:** perejil, apio, pimiento, tomillo, aceitunas. **Resveratrol:** piel de la uva, vino, zumo de uva, arándanos. **Fitoestrógenos:** isoflavonas (genisteína, daidzeína) y lignanos: soja y derivados, otras legumbres, cereales integrales, frutos secos, frutos del bosque, brécol, ajo, zanahorias, etc.	**Polifenoles, total:** 2.500-3.000 mg/día. **Flavonoides:** Unos 25 mg/día. **Quercetina:** Unos 23 mg/día. **Fitoestrógenos:** España: < 1 mg/día. Asiáticos: 20-50 mg/día.
Compuestos azufrados		
– De aliáceas (alilína, alicina, ajoeno, dialilsulfuro, etc.). – Glucosinolatos (> 120) (isotiocinato, sulforafano, Indol-3-carbinol).	**Alilína, dialilsulfuro:** cebolla, cebollino, cebolleta, puerro, ajo. **Isotiocinato, sulforafano, I3C:** repollo, coliflor, brécol, berza, coles de Bruselas, lombarda, ajo, cebollas, nabo, mostaza.	**Glucosinolatos, total:** 6,5 mg/día. Norte/Sur: 7,3/5,4 mg/día.
		Total: 3,5 g/día

a)Saura-Calixto & Goñi (2009)[79].b)National Cholesterol Education Program (NCEP), Adult Treatment Panel III (ATP III) (2002)[80].

3.1.- FIBRA DIETÉTICA

La fibra dietética es un nutriente crucial para una nutrición sana al estar relacionada íntimamente con diferentes funciones corporales, como entre otras el aumento del bolo fecal, absorción de minerales, absorción de azúcares, etc. y debido a su heterogeneidad química, históricamente no se habla de un único tipo de fibra. Siendo la definición de Trowel *et al.*, (1976) [81]la más ampliamente aceptada al definir las fibras dietéticas como:

"*Los polisacáridos vegetales y la lignina, que son resistentes a la hidrólisis por los enzimas digestivos del ser humano*".

Posteriormente debido a la evaluación de técnicas analíticas y al descubrimiento de sus efectos fisiológicos, la definición de fibra es ampliada (American Association of Cereal Chemist, 2001)[82]:

"*La fibra dietética es la parte comestible de las plantas o hidratos de carbono análogos que son resistentes a la digestión y absorción en el intestino delgado, con fermentación completa o parcial en el intestino grueso. La fibra dietética incluye polisacáridos, oligosacáridos, lignina y sustancias asociadas de la planta. Las fibras dietéticas promueven efectos beneficiosos fisiológicos como el laxante, y/o atenúa los niveles de colesterol en sangre y/o atenúa la glucosa en sangre*".

Posteriormente esta definición se completa con otros hidratos de carbono absorbibles como: el almidón resistente, la inulina, diversos oligosacáridos y disacáridos como la lactulosa y se incluye el concepto de fibra funcional. Por tanto, en la actualidad la fibra total es la suma de fibra dietética per se, más la fibra funcional.

En resumen, las fibras son principalmente hidratos de carbono o sus derivados, que resisten la hidrólisis por los enzimas digestivos humanos y llegan intactos al colon donde algunos pueden ser hidrolizados y fermentados por la flora intestinal y son de origen vegetal.

Desde un punto de vista clínico, la definición de fibra se basaría únicamente en sus efectos fisiológicos o biológicos.

3.1.1. Clasificación de la fibra

Ha *et al.,* (2000)[83] hacen una ordenación conceptual de los diferentes compuestos con las cualidades que definen el concepto de fibra (figura. 1), siendo los principales componentes polisacáridos no amiláceos (NSP). Son los polímeros de carbohidratos que contienen al menos veinte unidades de monosacáridos. Siendo el almidón un polisacárido digerido y absorbido en el intestino delgado, se utiliza el término "polisacáridos no amiláceos (NSP)" para aquellos que llegan intactos al colon manteniendo el efecto fisiológico de fibra.

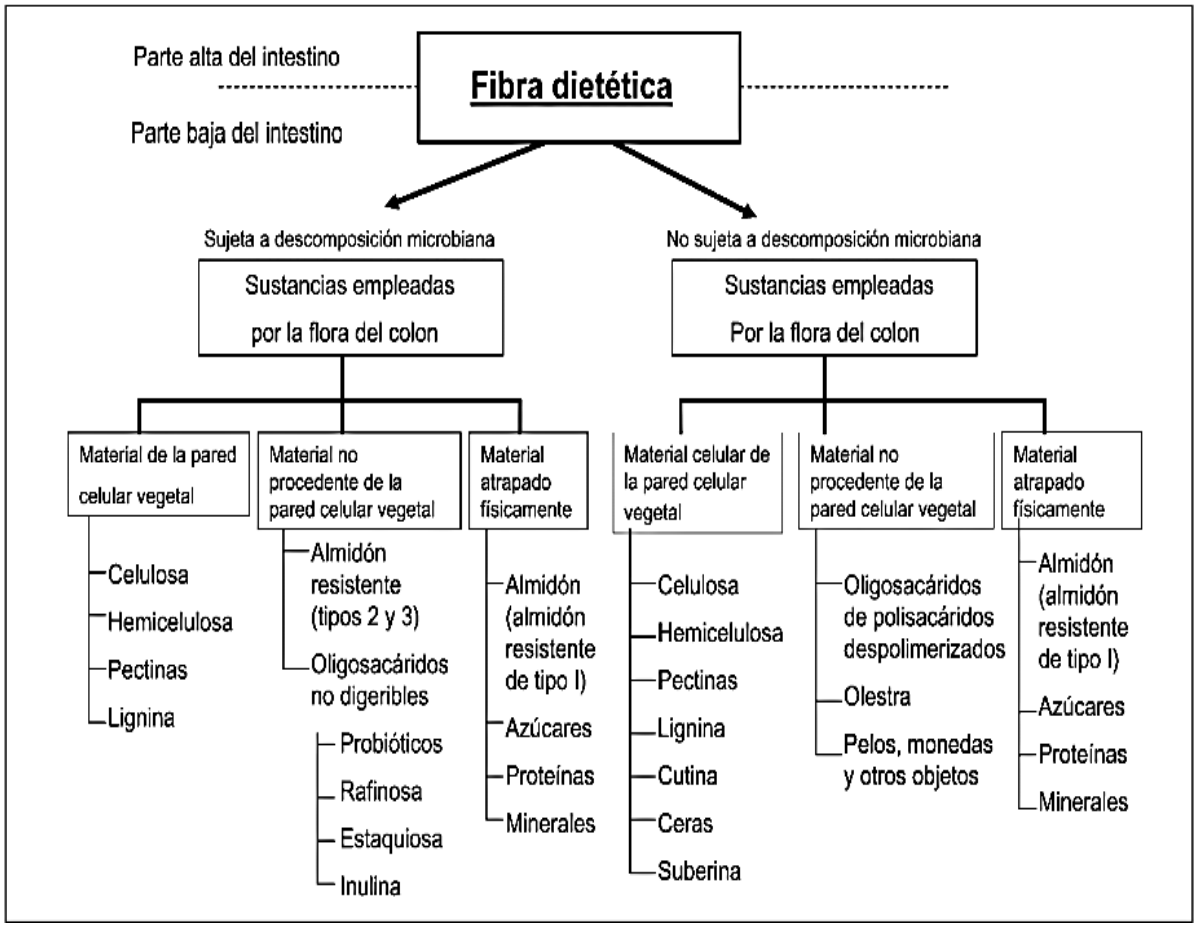

Figura1. Clasificación de la fibra dietética vegetal Ha *et al.,* (2000)[90],Escudero Alvarez & González Sánchez **(**2006)[84].

Clasificación en: celulosa (compuesto más abundante de las paredes vegetales), β-glucanos, hemicelulosas (asociados a la celulosa como constituyente de las paredes celulares), pectinas y sus análogos (laminilla media de la pared de las células vegetales), mucílagos (constituyentes celulares normales y con capacidad de retención hídrica) y gomas (provienen de la transformación de polisacáridos de la pared celular):

- Oligosacáridos resistentes

Son los polímeros de carbohidratos con un nivel de polimerización menor, tienen de tres a diez unidades de monosacáridos.
Clasificados en: fructooligosacáridos (FOS), galactooligosacáridos (GOS), xilooligosacáridos (XOS), isomaltooligosacáridos (IMOS)

- Ligninas

Son los polímeros que resultan de la unión de varios alcoholes fenilpropílicos; y que incrementan la resistencia a impactos y al ataque microbiano de la pared celular vegetal. La lignina ni se digiere ni se absorbe y tampoco es fermentada por la microflora bacteriana intestinal.

- Sustancias asociadas a polisacáridos no amiláceos (NSP)

Son los poliésteres de ácidos grasos e hidroxiácidos de cadena larga y fenoles, como: la suberina y la cutina. Presentan una cubierta hidrófoba y se localizan junto con las ceras en la parte externa de las estructuras vegetales

- Almidones resistentes

La suma del almidón y de sus productos de degradación no absorbidos en el intestino delgado de los individuos sanos (Englyst & Cummings, 1990)[85]

Tipos:

- Tipo 1 o AR1 (atrapado): granos de cereales y en las legumbres.
- Tipo 2 o AR2 (cristalizado): no hidrolizable enzimáticamente si antes no se gelatiniza. Patatas crudas, plátano verde y la harina de maíz
- Tipo 3 o AR3 (retrogradado): almidón que cambia su conformación ante fenómenos térmicos y en contacto de agua. Gelatinización- Retrogradación.
- Tipo 4 o AR4 (modificado): almidón modificado químicamente.

3.1.2. Propiedades de la fibra dietética

La fibra dietética presenta las propiedades de solubilidad, fermentabilidad y viscosidad que dependen de la composición de la fibra concreta y estarían relacionadas entre sí. Las fibras solubles en contacto con el agua forman un retículo donde queda atrapada, originándose soluciones de gran viscosidad, por tanto el grado de solubilidad en agua y viscosidad estarían relacionadas y sería muy variable para las distintas fibras. Los efectos derivados de la viscosidad de la fibra son responsables del metabolismo lipídico, hidrocarbonado y su potencial anticarcinogénico. Las fibras poco solubles o insolubles, en función del tamaño de partícula, son capaces de retener el agua en su matriz estructural formando mezclas de baja viscosidad; esto produce un aumento del bolo fecal que acelera el tránsito intestinal. Por otra parte también contribuye a disminuir la concentración y el tiempo de contacto de potenciales carcinogénicos con la mucosa del colon (Kin, 2000)[86].

De todas las propiedades la fermentabilidad es la más importante, ya que de ella derivan multitud de efectos como, por ejemplo, la reducción de la retención hídrica en el intestino grueso (Mataix & Gassull, 2002)[87]. La fermentabilidad es función de la solubilidad y alteración de cada fibra a la llegada al intestino grueso. Donde en condiciones anaeróbicas las bacterias fermentativas del colon pueden digerir enzimáticamente las fibras en función su estructura (Zarzuelo & Gálvez, 2005)[88]. En el colon se dan fundamentalmente dos tipos de fermentación: fermentación proteolítica y fermentación sacarolítica. La fermentación proteolítica produce derivados nitrogenados como aminas, amonio y compuestos fenólicos algunos de los cuales son carcinogénicos.

Los principales productos de la fermentación de la fibra son: ácidos grasos de cadena corta (AGCC), gases (hidrógeno, anhídrido carbónico y metano) y energía (Figura 2).

El metabolismo bacteriano utiliza la glucosa por la vía metabólica de Embdem-Meyerhoff hasta la obtención de piruvato y éste a su vez es convertido en ácidos grasos de cadena corta (AGCC): acetato, propionato y butirato. A parte de piruvato también se producen: valerato, hexanoato, isobutirato e isovalerato.

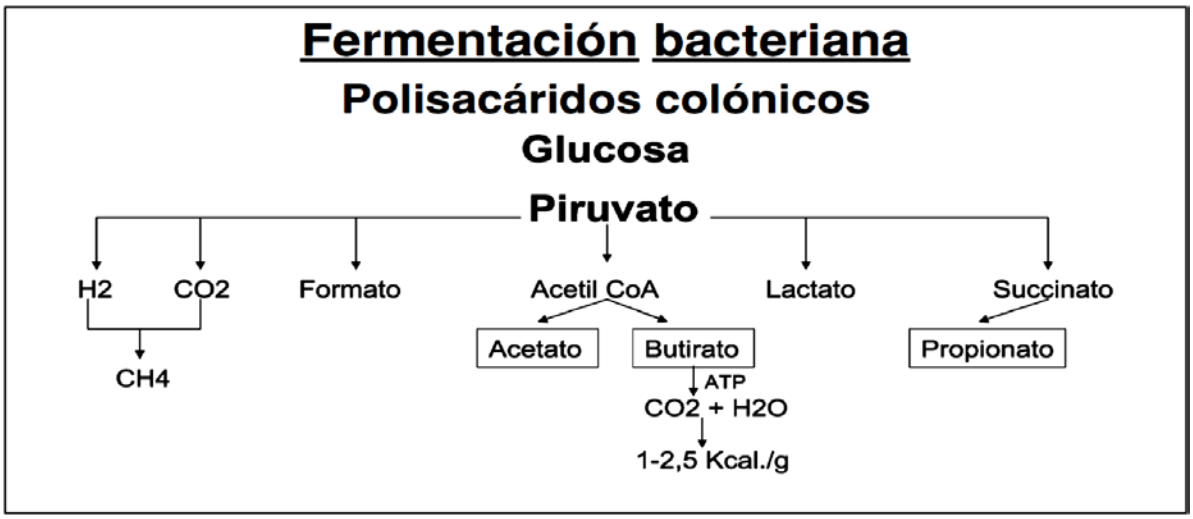

Figura 2. Fermentación bacteriana. García Peris*et al.*, 2002[89], Escudero Alvarez & González Sánchez **(**2006)[84].

Salvo la lignina, todos los tipos de fibra son fermentables en diferente proporción, siendo las fibras solubles las más fácilmente fermentables. Por ejemplo: mientras la fibra guar, el almidón resistente y los fructooligosacáridos tienen una fermentabilidad del 100 %, la celulosa presenta una fermentabilidad entre 20-80 %; y la hemicelulosa entre 60 -90 %. Por tanto más del 50 % de la fibra ingerida es degradada en el colon, el resto (mayoritariamente fibra insoluble y no fermentable) es eliminado con las heces.

Cuando llegan suficientes carbohidratos al colon, la fermentación proteica y de aminoácidos y sus subproductos de fermentación proteica (tóxicos como: amonio, compuestos fenólicos, etc.) se reducen. Los AGCC se absorben rápidamente en más del 90 % por el colonocito, por lo que también se produce una importante absorción de sodio y agua (Musch *et al.,* 2001[90]. El orden de absorción de los AGCC por el colonocito es butirato > acetato > propionato (Roediger, 1982)[91].

-El butirato es la principal fuente de energía del colonocito, que lo metaboliza hasta CO_2, cuerpos cetónicos y agua, también ejerce acciones antiinflamatorias especificas en el colon. En los estudios realizados in vitro por Inan *et al.,* (2000)[92], determinaron que la acción antiinflamatoria se debía a la disminución en la producción de algunas citoquinas proinflamatorias (TNF), modulando la actividad del factor de trascripción NF-êB en células colónicas.

-El propionato no metabolizado por la mucosa colónica, junto con el acetato, llegan al hígado a través del sistema porta, donde es metabolizado y actúa como precursor en la gluconeogénesis y la lipogénesis.

-El acetato es metabolizado en cuerpos cetónicos y glutamina (acetoacetato y α-hidroxibutirato). Una vez en el intestino delgado la glutamina es su principal factor respiratorio (Souba *et al.*, 1985)[93].

Cabe destacar, la estimulación del crecimiento de microorganismos en el colon como resultado de la generación de subproductos de fermentación y de la propia fibra. La ingestión de fructooligosacáridos (fibra funcional) puede multiplicar por diez la concentración bifidobacterias

(Beatnik *et al.*, 1996)[94]. A este efecto de crecimiento selectivo y/o la actividad y/o de una o un número limitado de microrganismos beneficiosos en el colon del huésped, se ha denominado efecto prebiótico (Gibson& Roberforid, 1995[95], Gibson, 2004[96]).

Los Bifidobacterium y Lactobacillus se han asociado con los efectos beneficiosos para la salud (Hartemink *et al.*, 1997)[97], como producción de vitaminas del grupo B o reducción de bacterias patógenas y sus toxina (*Escherichia colli, Klebsiella, Fusobacterium, Bacterioides* y *Clostridium*) (Nelson *et al.*, 1994)[98] y la eliminación del amonio tóxico por disminución del pH colónico al generar grandes cantidades de ácido láctico. En los estudios llevados a cabo por Gibson *et al.*, 1995[99] determinaron en individuos sanos, que la suplementación durante dos semanas con 15 g/día de inulina o fructooligosacáridos (FOS), produjo un incremento significativo de bifidobacterias en heces, mientras que disminuyó la producción de *Bacterioides*, *Clostridium* y Fusobacterias.

3.1.3. Efectos fisiológicos de la fibra

La fibra juega un rol decisivo en todos los estadios del sistema digestivo, desde la masticación del alimento hasta su defecación. Las dietas con un contenido en fibra elevado requieren más tiempo de masticación, enlenteciendo la velocidad de deglución e incrementado la salivación.

A nivel estomacal y del intestino delgado, la mayor viscosidad las fibras solubles prolongan la sensación de saciedad al enlentecer el tiempo de tránsito. También aumentan el espesor de la capa de agua que los solutos necesitan traspasar para alcanzar la membrana del enterocito, este efecto provoca una disminución en la absorción de glucosa, lípidos y aminoácidos (Cherbut, 1998)[100]. La absorción de determinados minerales como el calcio, hierro, cobre y zinc pueden disminuir si se ingieren dietas muy ricas en fibra. Algunos minerales pueden formar compuestos insolubles, como los fitatos, tanatos u oxalatos, pero éstos pueden ser liberados y absorbidos en

cantidades importantes en el colon, por el metabolismo bacteriano. La absorción del calcio atrapado y transportado hasta el colon se libera al hidrolizarse la fibra por efecto de las bacterias colónicas. Los AGCC producidos facilitan su absorción a través de las paredes del colon e incluso de las del recto ((Kritcheusky *et al.*, 1990)[101].

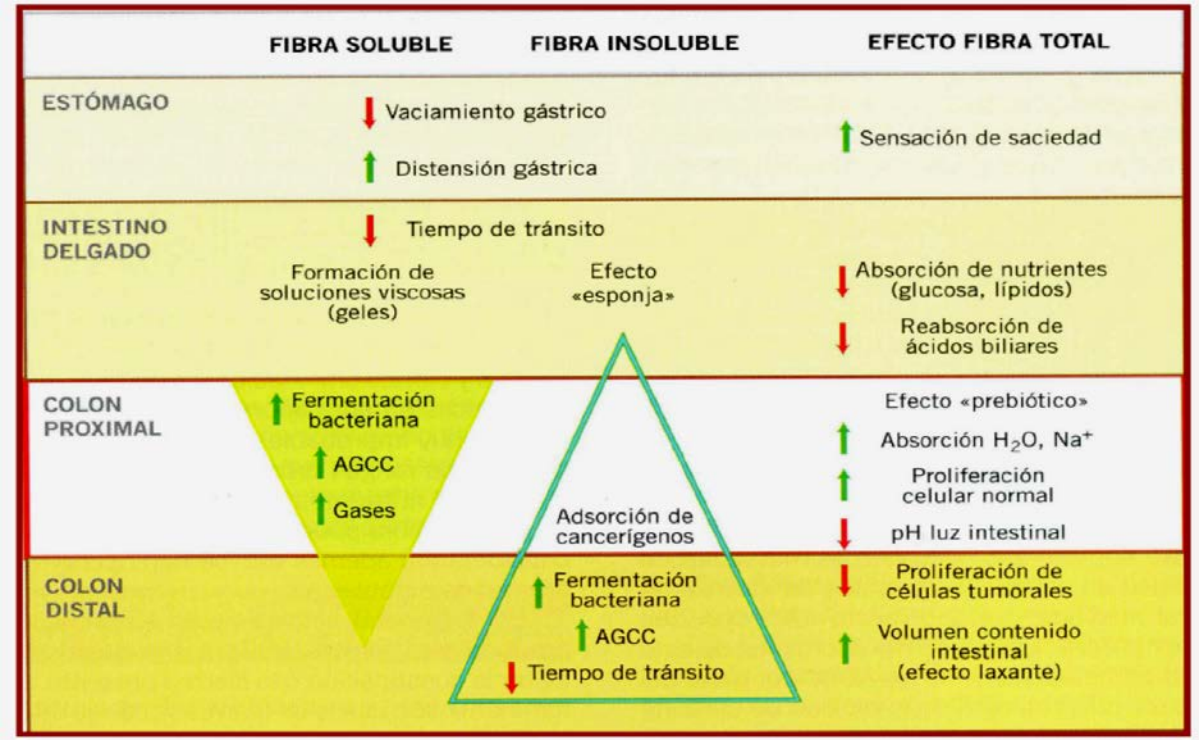

Figura 3. Resumen conceptual de los efectos fisiológicos de los tipos de fibra (Gil, 2010)[102].

3.1.4. Inulina como principal compuestos de la fibra de la alcachofa

Un componente importante en la composición de la fibra soluble de la alcachofa es la inulina.

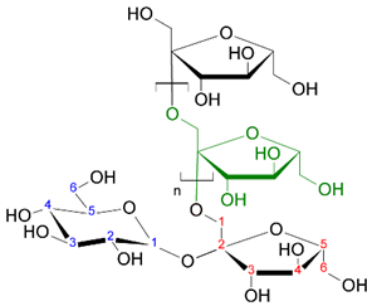

Figura 4. Estructura química de la inulina. Cortesía de acta química Mexicana (http://www.posgradoeinvestigacion.uadec.mx/AQM/No.%207/6.html)

El 75 % del contenido total de azúcar de las partes comestibles de la alcachofa se atribuye a la inulina (Lattanzio *et al*., 2009)[44]. Su concentración estaría influenciada por el estado de madurez

de la planta, y al actuar a modo de reserva de energía de la planta (Muzzarelli *et al.*, 2012)[103]su concentración es máxima antes de la recolección.

El nombre de inulina procede de la primera planta en que se aisló en 1804, el helenio (*Inula helenium*) y designa a una familia de glúcidos complejos (polisacáridos), compuesta de cadenas complejas y ramificadas de moléculas de fructosa (fructano polidisperso en enlaces β-2.1) (Phelps, 1965)[104], que se almacena como sustancia de reserva en las raíces, tubérculos y rizomas de ciertas plantas fanerógamas (bardana, achicoria, diente de león, yacón, alcachofa, etc.). Por tanto dependiendo del tipo de planta del que se extrae su grado de polimerización y sus atributos funcionales serán diferentes (Leroy *et al.*, 2010)[105].

La peculiaridad de la inulina con respecto a otros polisacáridos complejos es que las unidades de fructosa (F), de la mezcla de polímeros lineales y oligómeros, están ligadas por enlaces β (1→2). En consecuencia, mientras que la molécula de glucosa (G), es fácil de digerir por las enzimas digestivas al encontrarse ligada al extremo de cada cadena de fructosa por un enlace α-1, 2, como en la sacarosa; durante la digestión, los enlaces β (1→2) de la inulina resisten la acción la enzimas amilasa (saliva) o ptialina (secreción pancreática) (López-Molina *et al.*, 2005)[46]. Como resultado, la inulina sólo sufre un grado bajo de hidrólisis ácida en el estómago, atravesando la mayor parte del tracto digestivo prácticamente sin cambios. Sólo en la primera porción del intestino grueso (colon), las bifidobacterias degradan y metabolizan la inulina en ácidos grasos de cadena corta (especialmente ácido butírico), dióxido de carbono, hidrógeno y metano (Robenfroid, 1999[106], Costabile *et al.*, 2010[107]). Por tanto, el efecto prebiótico de la inulina fomenta el crecimiento de bacterias beneficiosas (bifidobacterias, y lactobacilos), inhibe a su vez el crecimiento de microbiota patogénica, productora de toxinas y carcinógenos, estimula la producción de componentes beneficiosos para el sistema inmune y ayuda a la absorción de ciertos iones (calcio, magnesio, fosforo) y la síntesis de vitaminas del grupo B; y aumentarían la sensación de saciedad (Morris & Morris, 2012)[108].

3.2.- COMPUESTOS ANTIOXIDANTES

3.2.1 Definición de antioxidante

La definición de antioxidante, según Halliwell & Guttedrige (1989)[109], es cualquier sustancia que presente a bajas concentraciones, comparado con el sustrato oxidado, es capaz de prevenir significativamente la oxidación de un sustrato.

Los antioxidantes detienen el deterioro oxidativo de los alimentos (Pastene, 2009)[110] por lo que son adicionados en la industria alimentaria para prevenir la rancidez oxidativa de las grasas y mantener sus cualidades nutritivas y sensoriales a lo largo de toda la vida útil del alimento (Llancari & Matos, 2011)[111].

3.2.2. Polifenoles: Origen, estructura y distribución de los polifenoles

Los polifenoles son fitoquímicos de bajo peso molecular que presentan una estructura molecular caracterizada por la presencia de uno o varios anillos fenólicos, con más de 800 estructuras conocidas (Kähkönen *et al.*, 1999)[112], de distinta complejidad (desde moléculas simples como el ácido fenólico, hidroxitirosol, fenilpropanoides, flavonoides), hasta compuestos altamente polimerizados (ligninas, taninos) (Manach *et al.*, 2004[113], Manach *et al.*,2005[114]). Se originan principalmente en las plantas, como producto de metabolismo secundario, siendo indispensables para las funciones fisiológicas vegetales y de defensa ante situaciones de estrés (hídrico, luminoso, etc.).

Generalmente, los alimentos contienen una mezcla compleja de polifenoles y se encuentran distribuidos en un gran número de plantas diferentes (frutas, verduras, cereales, leguminosas, té, vid, etc.), pero hay polifenoles que son específicos de determinados alimentos (flavanonas en cítricos, isoflavonas en soja). Su concentración es función de factores medioambientales como la horas de luz, el grado de madurez, clima (exposición al sol, precipitaciones, etc.) o factores agronómicos (tipos de cultivos, rentabilidad del árbol, etc.). En particular, la exposición a la luz y el grado de conservación es uno de los principales condicionantes para determinar el contenido de la mayoría de los polifenoles, permitiendo la formación de sustancias polimerizadas que afectan al color y a las características organolépticas de los alimentos.

El contenido de polifenoles está también determinado por los métodos culinarios de preparación de los alimentos; por ejemplo el consumo de alimentos cocidos puede disminuir el contenido

inicial de polifenoles hasta un 75 %, por efecto de la dilución en el agua de cocción, (Van der Sluis *et al.,* 2001)[115].

El contenido cualitativo y cuantitativo de polifenoles es diferente en cada especie vegetal. Entre las plantas con alto contenido en polifenoles se encuentran el cacao *(Theobroma cacao),* la uva *(Vitis vinifera),* el té *(Camelia sinensis),* la manzana *(Malus domestica)* y diversas frutos salvajes. Así pues, las fuentes mayoritarias de polifenoles en la dieta humana son principalmente las frutas, el té, el vino y el chocolate. En el cacao los flavanoles, están principalmente en forma de epicatequinas, catequinas y procianidinas. El vino es rico en catequinas y procianidinas, y en el té los flavanoles se encuentran fundamentalmente como derivados de galatos (Unachukwu *et al.,* 2010[116], Guadalupe *et al.,* 2006[117]).

3.2.3. Clasificación de los polifenoles

Las clases y subclases de polifenoles se definen en función del número de anillos fenólicos que posean y de sus elementos estructurales. Los principales grupos de polifenoles son: ácidos fenólicos (derivados del ácido hidroxibenzoico o del ácido hidroxicinámico), estilbenos, lignanos, y flavonoides (Figura 5). La biosíntesis de los polifenoles tiene lugar a través de dos importantes rutas primarias (Bravo, 1998)[118]:

La ruta del ácido siquímico proporciona la síntesis de los aminoácidos aromáticos (fenilalanina o tirosina), ácidos cinámicos y sus derivados (fenoles sencillos, ácidos fenólicos, cumarinas, lignanos y derivados del fenilpropano). Esta ruta dependiente de la luz y se inicia en los cloroplastos por condensación de dos productos típicamente fotosintéticos, la eritrosa-4-fostato, procedente de la vía de las pentosas fosfato, y el fosfoenolpiruvato, originario de la glucólisis. Tras diversas modificaciones, se obtiene el ácido siquímico, del que derivan directamente algunos fenoles. La vía del ácido siquímico puede continuar con la adhesión de una segunda molécula de fosfoenolpiruvato, dando lugar a la fenilalanina. La cual entra a formar parte del metabolismo secundario por acción de la enzima fenilalanina amonioliasa, que cataliza la eliminación de un grupo amonio, transformando la fenilalanina en el ácido trans-cinámico. Posteriormente, el ácido trans-cinámico se transforma en ácido r-cumárico por incorporación de un grupo hidroxilo a nivel del anillo aromático. La acción de una Coenzima A (CoA), la CoA-ligasa, transforma el ácido ρ-cumárico en ρ-cumaroilCoA, que es el precursor activo de la mayoría de los fenoles de origen vegetal. La ruta de los poliacetatos proporciona quinonas y xantonas; y comienza a partir de una molécula inicial de acetilCoA, y a través de una serie de

condensaciones se originan los poliacetatos. Por reducción de los poliacetatos se forman los ácidos grasos, y por ciclación posterior se forman una gran variedad de compuestos aromáticos, como las quinonas y otros metabolitos que se generan a través de rutas mixtas. Las rutas mixtas combinan precursores tanto de la vía del ácido siquímico como de la ruta de los poliacetatos. Este es el caso de un importante grupo de moléculas biológicamente activas, denominadas genéricamente flavonoides.

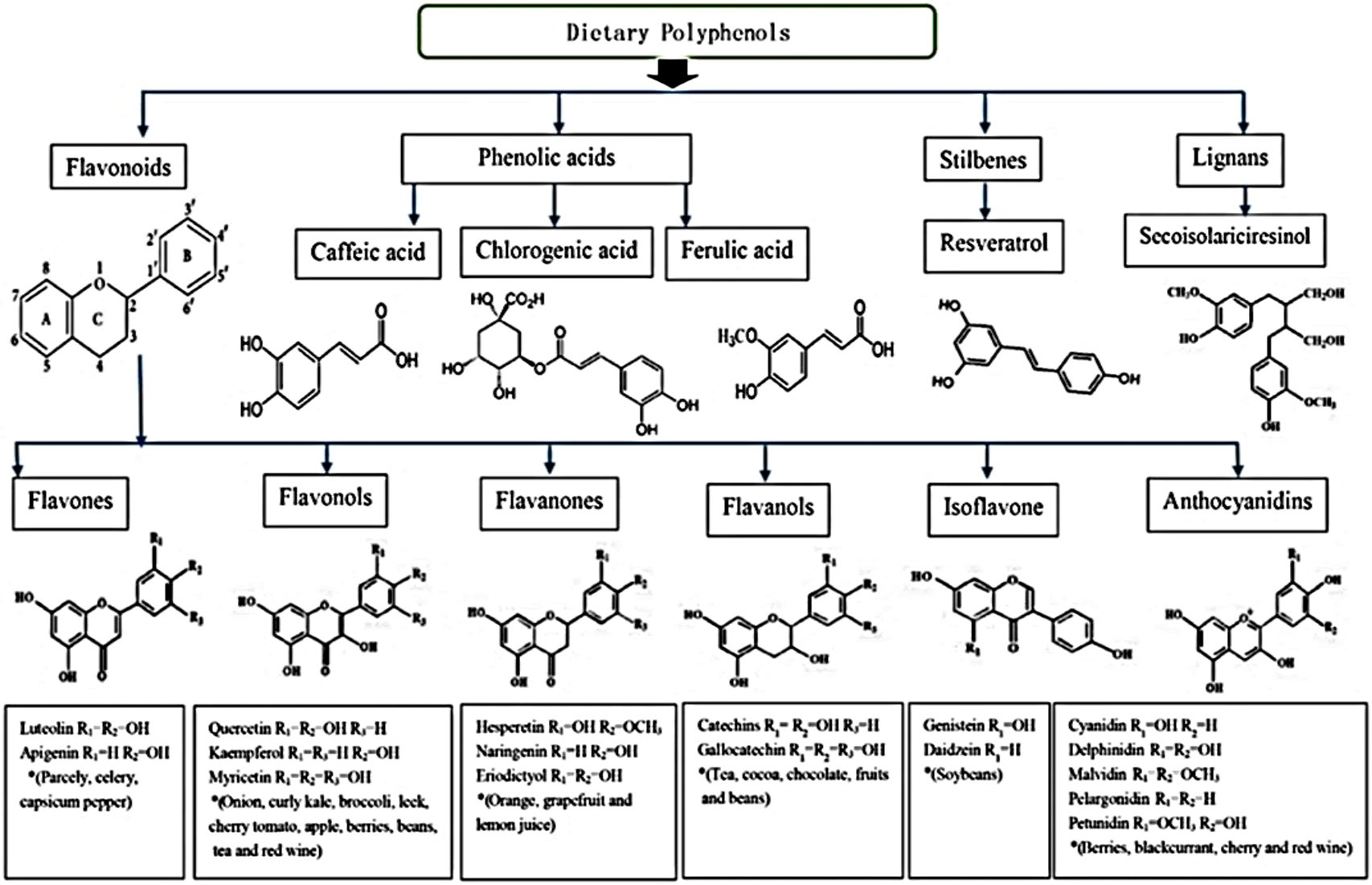

Figura 5. Principales grupos de polifenoles. (Quiñones *et al*., 2012)[119] Figura extraída de (Kim *et al*., 2016)[120].

3.2.3.1. Flavonoides

Los flavonoides, constituyen la subclase más abundante de polifenoles, son compuestos de bajo peso molecular que comparten un esqueleto común difenilpirano (C_6-C_3-C_6), compuesto por dos anillos fenilo (A y B) ligados a través de un anillo C de pirano heterocíclico. Por tanto son estructuras hidroxiladas en sus anillos aromáticos. De los tres anillos, el A se biosintetiza a través de la ruta de los poliacetatos, y los anillos B y C proceden de la ruta del ácido siquímico (Figura.6).Los flavonoides se encuentran como: glucósidos, agliconas flavonoide (flavonoides libres), sulfatos, dímeros o polímeros. Existen dos formas de glucósidos: O-glucósidos con los carbohidratos ligados por átomos de oxígeno (enlace hemiacetal), y C-glucósidos con los carbohidratos ligados por enlaces carbono. Existen varios subgrupos de flavonoides.

En la figura 6 se muestra la clasificación y estructura química de los flavonoides.

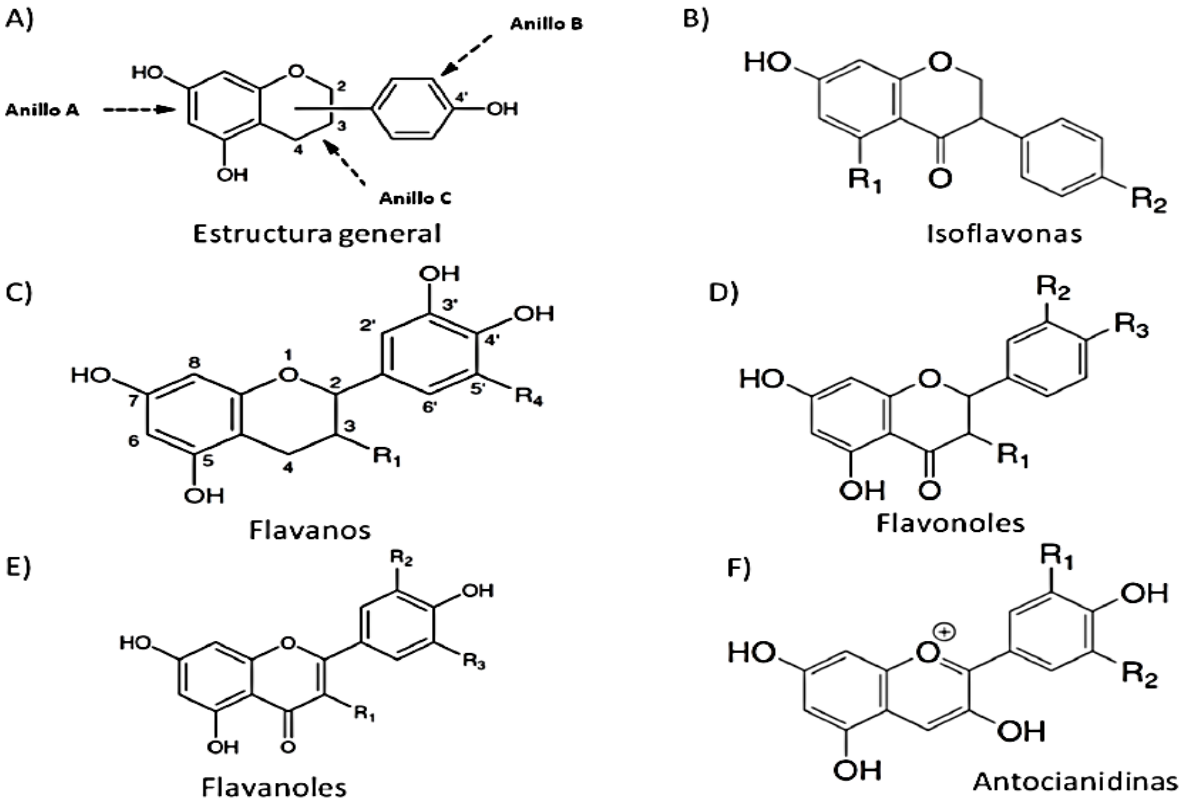

Figura 6. Clasificación y estructura química de los flavonoides (Quiñones *et al.*, 2012)[130].

Los flavonoides se localizan en la membrana del tilacoide de los cloroplastos de las partes aéreas de las plantas angiospermas, aunque también se encuentran en hongos y algas. Son necesarios para

el desarrollo fisiológico de los vegetales, participan en la vía de expresión de dos enzimas multigénicas, la fenilalanina amonio liasa y la chalcona sintasa, todos ellas relacionadas con el proceso de pigmentación de los vegetales. Además, los flavonoides colaboran en los sistemas de defensa, actúan como marcadores químicos y actúan directa o indirectamente sobre enzimas relacionados con la fisiología y el metabolismo de las plantas.

Por tanto, debido a su diferente funcionalidad hay ciertos alimentos que contienen una mayor concentración en ciertos flavonoides. Se comentan algunos ejemplos delos estudios realizados por diferentes investigadores, como los desarrollados por Escamilla *et al.,* (2009)[121]en fresas y frutos rojos(antocianinas), en el té y cacao (catequinas) y los realizados por Perea *et al.*, (2009)[122], en cacao y chocolate (procianidinas y flavononas), en naranjas y mandarinas(citro-flavonoides), en semillas de uva, mosto y vino tinto (protoantocianidinas) y finalmente en la soja y la cascara de las semillas de linaza y centeno. (Isoflavonoides [genisteína y daidzaína]).

3.2.3.2. Flavonoles

Son el grupo de polifenoles más extendido en los alimentos (verduras, frutas, té y el vino) siéndola quercetina el flavanol más representativo. Al ser su biosíntesis un proceso fotosintético, se localizan en el tejido externo y aéreo de la planta, siendo su distribución y la concentración mayor en los frutos expuestos a la luz.

3.2.3.3. Flavonas

Poseen un grupo cetona en el carbono C_4 y una instauración entre los carbonos C_2 y C_3. Las flavonas son los flavonoides menos abundantes en los alimentos. Están presentes en perejil, apio y la piel de las frutas.

3.2.3.4. Flavanonas

Análogos de las flavonas, constituyen un grupo minoritario en los alimentos, se localizan mayoritariamente en las partes sólidas de la fruta y en particular en su albedo. Están presentes en cítricos, tomates y en plantas aromáticas como la menta.

3.2.3.5. Isoflavonas

Poseen un anillo bencénico lateral en posición C_3, similar a la estructura de los estrógenos (hormona estriol y la estrona). Tanta es su similitud que las isoflavonas que se pueden unir a receptores de estrógenos, y por ello se clasifican como "fitoestrógenos". Están presentes casi exclusivamente en plantas leguminosas, siendo la soja la que posee la mayor concentración. La isoflavonas de las leguminosas incluyen GEN y Daitseína, los cuales pueden existir como glucosilados (tres veces más potentes que el estradiol) o como aglicones (los más fácilmente transportados y absorbidos a través del epitelio intestinal) (Kuiper *et al.*, 1998[123], Bonilla, 2004)[124].

3.2.3.6. Antocianidinas

Son heterópsidos con los tres anillos conjugados en su estructura, que conforman uno de los grupos de pigmentos vegetales más importantes. Su glucosilación ocurre principalmente en la posición 3 del anillo C o en las posiciones 5 y 7 del anillo A. También es posible la glucosilación de las posiciones 3´, 4´ y 5´ del anillo B, aunque esta glucosilación aparece con menos frecuencia. Las antocianidinas están ampliamente distribuidas en la dieta humana. Mayoritariamente están presentes en frutas, aunque se encuentran también en distintas variedades de cereales, vegetales y en el vino tinto.

3.2.3.7. Flavanoles

Los flavanoles más representativos son de tipo flavan-3-ol, y estos pueden aparecer como catequinas (monómeros), procianidinas (dímeros condensados y oligómeros), o proantocianidinas o taninos condensados (polímeros). La epicatequina y catequina son los flavanoles mayoritarios en frutas. Las catequinas también se encuentran en alta concentración en vino y chocolate. En cambio, las mayores concentraciones de galocatequina, epigalocatequina y epigalocatequina galato se encuentran principalmente en el té (Arts *et al.*, 2000)[125].

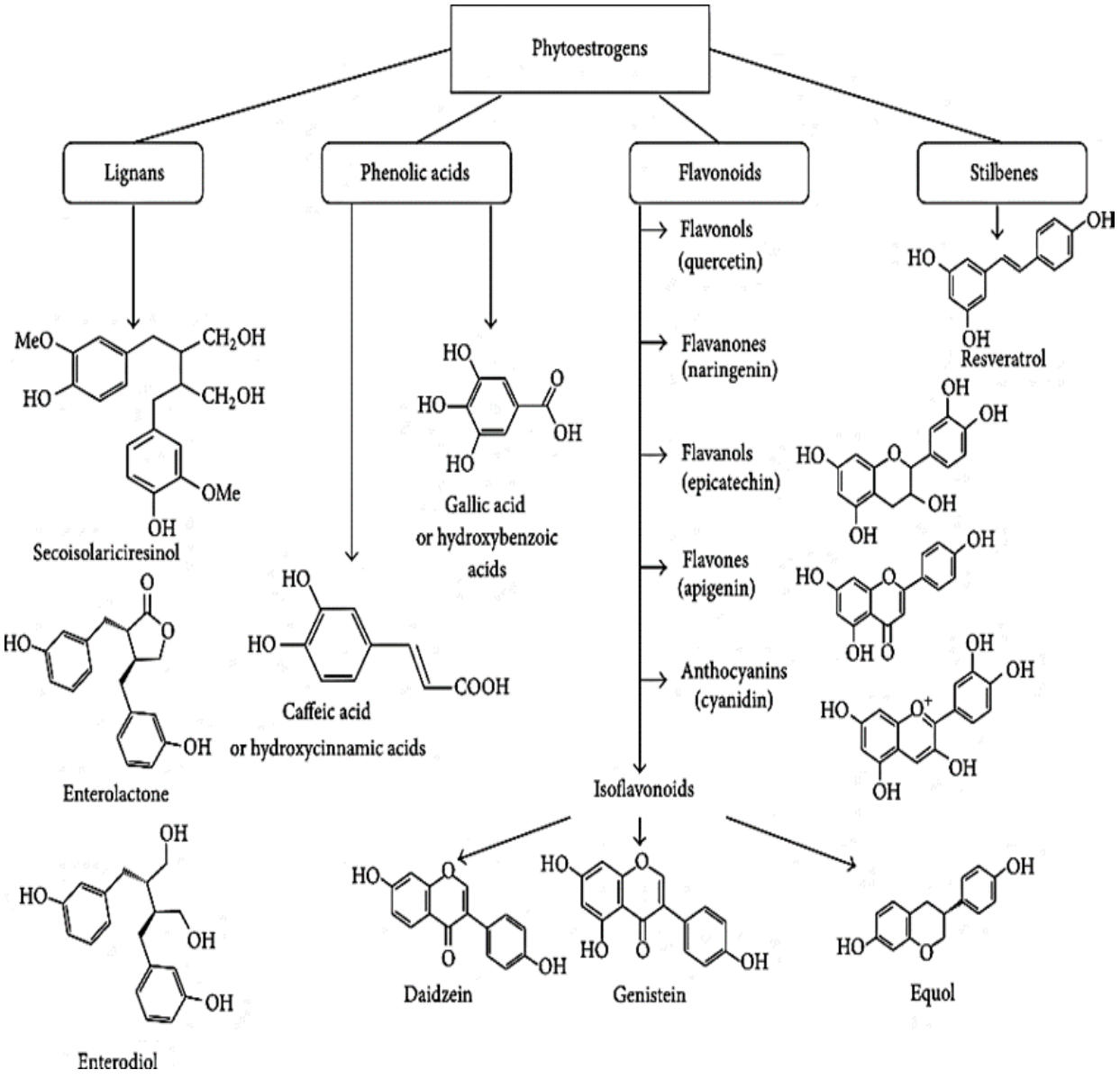

Figura 7. Estructura química de los principales compuestos fitoestrogénicos. (Lephart, 2015)[126].

Debido a su amplio rango estructural y pesos moleculares muy variables es difícil valorar el contenido de proantocianidinas en los alimentos (Rasmussen *et al.*, 2005)[127]. Los datos mayoritarios disponibles, en cuanto a la caracterización de estos compuestos, hacen referencia principalmente a dímeros y trímeros de catequinas, que representan las formas mayoritarias (De Pascual-Teresa, 2000[128], Valls *et al.*, 2009[129]).

Con respecto a la actividad antioxidante, habría que tener en cuenta que su biodisponibilidad puede ser modificada por el modo de ingesta (Lopez *et al.,* 2012)[130], y si son acompañados de otros alimentos. Las moléculas fenólicas tienen amplia afinidad por las proteínas, por lo que su capacidad antioxidante puede disminuir in vivo, si se ingieren con alimentos muy proteicos, como los lácteos (Ramírez *et al.,* 2009)[131].

3.2.3.8. Taninos

Los taninos son compuestos polifenólicos de origen vegetal, de masa molecular elevada, y de estructura más o menos compleja, sabor astringente, conocidos desde la antigüedad por su capacidad de curtir pieles. Los taninos se encuentran en las vacuolas celulares, combinados con alcaloides o proteínas.
Se clasifican en:

Taninos hidrolizables.- Estos taninos se hidrolizan con facilidad por ácidos, álcalis y por vía enzimática. Se localizan en algunas dicotiledóneas como: *Fagaceae*, *Anacardiaceae* y *Leguminosae*. En este grupo se encontrarían los taninos gálicos (compuestos por polímeros del ácido gálico, ésteres de un poliol, generalmente de la glucosa con varias moléculas de ácido gálico) y los taninos elágicos o elagitaninos ésteres del ácido hexahidroxidifénico y sus derivados.

Taninos no hidrolizables.- Taninos condensados o proantocianidinas. Este tipo de taninos se producen durante el metabolismo fisiológico vegetal y se encuentran ampliamente distribuidos en el reino vegetal. Químicamente se originan por condensación de catequinas o catecoles (flavanoles) con uniones directas C-C entre las moléculas, en 4- 8 o en 4-6.

De las actividades farmacológicas de los taninos, cabe destacar sus propiedades astringentes. Por vía interna se emplean para tratar diarreas infecciosas al precipitar los enzimas extracelulares secretados por microorganismos infeccioso y también presentan propiedades antioxidantes, alcomportarse como captadores de radicales libres (Olivas-Aguirre *et al.,* 2014)[132].

3.2.4. Biodisponibilidad de los polifenoles

Funcional y tecnológicamente, la cantidad total de polifenoles presente en un alimento o ingrediente es importante, pero nutricionalmente es más importante conocer el porcentaje de nutrientes que continúan activos después de ser digeridos, absorbidos y metabolizados mediante las distintas rutas metabólicas o "Biodisponibilidad" (Srinivasan, 2001)[133]. Por tanto, en el caso de los polifenoles conocer su biodisponibilidad cobra más importancia que en otros nutrientes, ya que los polifenoles más activos y más absorbidos en el intestino y metabolizados en el organismo no son siempre los de mayor concentración en los alimentos o los que producen una mayor concentración de metabolitos activos de tejidos diana (D´Archivio *et al.,* 2010)[134].

El efecto beneficioso de los polifenoles es función de su estabilidad durante la digestión gastrointestinal, del porcentaje de liberación de la matriz sólida, de la biodisponibilidad y de su destino metabólico. El intestino está sometido a diferentes factores de estrés oxidativos por alimentos, como: peróxidos lipídicos o hierro, los cuales incrementan la generación de radicales libres en el colon y agravan condiciones clínicas prexistentes, como inflamación del intestino, síndrome del colon irritable o celiaquía (Verspohl *et al.,* 2008[135], Emendörfer *et al.,* 2005[136]).

Los polifenoles deben ser liberados de la matriz alimentaria para poder contactar con las vellosidades intestinales del intestino delgado y ser absorbidos por difusión pasiva paracelular, a través del enterocito. Probablemente esto no sería su vía de entrada (Stahl *et al.,* 2002)[137], porque la mayoría los polifenoles son demasiado hidrófilos para penetrar la pared intestinal por difusión pasiva. Durante la digestión del alimento y debido la complejidad de las formas glucosiladas de la mayoría de estructuras de los polifenoles, esteres, glucósidos y polímeros resisten la hidrolisis acida del estómago y llegan intactos al intestino, donde no son absorbibles directamente por el organismo hasta que son hidrolizados, por enzimas intestinales como la β-glucosidasa, o degradados por la microflora intestinal (Németh *et al.,* 2003)[138] hidrolizando los glucósidos en agliconas y metabolizando las agliconas en ácidos aromáticos, hasta generar ácido benzoico (Setchell *et al.,* 2002)[139]. La absorción de los compuestos fenólicos se considera baja cuándo no es superior a las concentraciones plasmáticas de 10 μm, (Manach *et al.,* 2005)[140]. Ocasionalmente, la microflora del colon genera metabolitos activos específicos como el equol, enterolactona y enterodiol. Hay que destacar que el proceso de bioasimilación de algunos polifenoles puede

incrementar el efecto biológico de su compuesto original; por ejemplo las propiedades fitoestrogénicas del equol son aún mayores que los de la isoflavona de la que procede (Mousavi& Adlercreutz, 1992)[141].

Posteriormente a su absorción, los polifenoles se metabolizan a través de una secuencia de reacciones que incrementan su capacidad hidrófila, que permiten su eliminación urinaria o biliar. Éstas reacciones serían similares a las empleadas en la eliminación metabólica de muchos xenobióticos (Manach *et al.,* 2004)[115] y en consecuencia los metabolitos presentes en el plasma y en los tejidos son muy distintos a los presentes en los alimentos dificultando su evaluación de actividad biológica (Day & Williamson, 2001[142], Natsume *et al.,* 2003[143]). Principalmente, los tejidos donde se encuentran es donde se han metabolizado (tejido hepático, gástrico, intestinal, colónico y nefrítico (Clifford, 2004[144], Graf *et al.*, 2006[145]), o en tejidos dianas específicos (tejido pulmonar, pancreático, cerebral, cardiaco (Hong *et al.*, 2002[146], Parkar *et al.,* 2008[147]).

4.- COMPUESTOSBIOACTIVOSEN LA COMPOSICIÓN DE LAS ALCACHOFAS

La alcachofa es una rica fuente de compuestos bioactivos, que contribuyen a sus efectos beneficiosos para la salud (De Falco *et al.,* 2015)[47].Las principales sustancias bioactivas de la alcachofa (cabeza y hojas), son los compuestos polifenólicos, destacando los ácidos fenólicos derivados de luteolina y apigenina como la apigenina-7-O-glucurónidoluteolina, (Alarcón-Flores *et al.*, 2014)[148],y derivados delos ácidos mono- y di-cafeoilquinico (cinarina, ácido 1, 3-O-dicafeoilquínicos) (Lutz *et al.,* 2011)[56]de los cuales el 5-O-cafeoilquinico (ácido clorogénico) es el compuesto más abundante (Wittemer *et al.,* 2005[149], D'Antuono *et al.,* 2015[150]), flavonoides y antocianinas (Romani *et al.,* 2006)[151] y cinarina (ácido 1, 3-O-dicafeoilquínicos) (Lutz *et al.,* 2011)[56]. Aparte de éstos, Abu-Reidah *et al.,* (2013)[152] han identificado otros ácidos hidroxibenzoicos, flavanoles, flavononas, lignanos y compuestos polares, hasta un total de 61 compuestos bioactivos, sugiriendo la gran importancia terapéutica de la cabeza de alcachofa.

La bioactividad potencial de la alcachofa está relacionada principalmente con las propiedades antioxidantes de los polifenoles. En experimentos realizados in vitro con polifenoles, se ha demostrado que son capaces de reaccionar con una amplia gama de especies de oxígeno reactivo (ROS) como los radicales superóxido, hidroxilo y piróxilo y también son capaces de quelar iones metálicos (Petropoulos *et al.,* 2008)[153], también en algunos de los compuestos polifenólicos se ha comprobado su actividad hepatoprotectora directa, reduciendo peroxidación lipídica, en hepatocitos primarios de rata expuestos al estrés oxidativo (Adzet *et al.*,1987[154], Colak *et al.,* 2016[155], Ben Salem *et al.,* 2015[156], Speroni *et al.,* 2003[157]).

Una vez en vez ingeridos, el organismo, por solubilidad de los polifenoles pueden localizarse sobre la superficie de la partícula de LDL, disminuyendo el consumo de los antioxidantes propios de las LDL como vitamina E y carotenoides (vitamina A), y en algunos casos regenerando vitamina E oxidada en la partícula de LDL. Por su capacidad inhibitoria, activan o protegen enzimas específicas en el organismo. Se ha demostrado que el consumo de los flavonoides presentes en el vino, como: catequina, quercetina, preservan la actividad de la paraoxonasa (Fuhrman & Aviram, 2001)[158]. Esta enzima, está asociada a las HDL o colesterol "bueno", puede hidrolizar y regenerar lípidos oxidados en las LDL. Otros polifenoles inhiben oxigenasas celulares y por tanto, impide la producción de especies oxidantes del oxígeno y del nitrógeno dentro del cuerpo humano. La

quercetina y sus glucósidos inhiben la oxidación del colesterol LDL inducida por lipoxigenasa. La catequina, la epicatequina, la epigalocatequina, la epicatequin-galato y la epigalocatequin-galato inhiben la producción de radicales libres por inhibición de la xantino oxidasa hepática.

En la alcachofa, los polifenoles están involucrados en numerosos procesos fisiológicos y bioquímicos de la planta, como: el crecimiento y la reproducción, la defensa contra patógenos y parásitos, la protección de la contaminación atmosférica, las temperaturas extremas y la reparación de tejidos. Por lo que la cantidad de compuestos bioactivos son extremadamente variables ya que depende de la época del año, factores genéticos, fisiológicos y ambientales, y también de la fechas de cosecha; así como el modo de cocción (Azzini *et al.,* 2007)[159] y/o el tipo de proceso realizado (Lombardo *et al.,* 2012)[160], y dela parte de la alcachofa analizada (pedúnculo, cabeza, brácteas internas o externas y hojas).Según Pandino *et al.,*(2011[a161],2011[b162], 2012[163]) la concentración de compuestos fenólicos es mayor en las parte interior de las cabezas de alcachofa (brácteas exteriores < brácteas internas < receptáculo), mientras que para los ácidos hidroxicinámicos la tendencia es la opuesta, ya que durante el proceso de maduración éstos compuestos están involucrados en el proceso de lignificación de las brácteas externas.

De los polifenoles presente en la alcachofa, el más estudiado es la quercetina. Algunos estudios experimentales en ratas, han demostrado que mientras la quercetina es absorbida a nivel gástrico sus glucósidos no lo son (Crespy *et al.,* 2002)[164]. Sin embargo, la glucosilación de la quercetina facilita su absorción intestinal, debido a su propia aglicona (Morand *et al.,* 2000)[165] esto sugería que los glucósidos deberían ser transportados al interior del enterocito mediante un transportador de glucosa sodio dependiente (Gee *et al.,* 1998)[166] e hidrolizado posteriormente por una β-glucosidasa citosolica (Day *et al.,* 1998)[167]. En humanos se ha caracterizado la absorción de quercetina mediante el análisis de muestras de plasma sanguíneo, encontrándose formas conjugadas de quercetina distintas a las administradas por vía oral (Sesink *et al.,* 2001)[168].Otros estudios posteriores han demostrado la absorción gástrica de las antocianinas (Talavéra *et al.,* 2003)[169], encontrándose que su absorción en el intestino delgado es muy limitada debido a su alto peso molecular y estructura polimérica, mientras que para Prasain *et al.,* 2009)[170] los trímeros serían menos absorbibles.

En síntesis cuando los polifenoles superan la digestión y entran en el torrente sanguíneo, estos pueden actuar como potentes inhibidores de la oxidación del colesterol LDL por varios mecanismos:

- Como antioxidantes propiamente: comportándose como captadores de radicales libres. Los distintos polifenoles tienen distinta especificidad por las distintas especies oxidantes que se generan en el organismo. (D'Archivio *et al.*, 2010)[135].
- Como agentes quelantes de iones de metales de transición: uniéndose a estos iones y reduciendo la capacidad de estos metales pesados de generar radicales libres.

Por tanto, suponiendo que los subproductos de alcachofa tienen una composición similar a la parte comestible de las alcachofas, la extracción de extractos ricos en fibra de sus subproductos representaría un modo simple de incorporar compuestos bioactivos, polifenoles, antioxidantes y fibra, tanto soluble como insoluble, en el desarrollo de nuevos productos.

5.- ELABORACIÓN DEGALLETAS

“Las galletas" son los productos alimenticios elaborados por una mezcla de harina, grasas comestibles y agua, adicionada o no de azúcares y de otros productos alimenticios o alimentarios (aditivos, aromas, condimentos, especias, etc.) sometidos a un proceso de amasado y al posterior tratamiento térmico, dando lugar a un producto de presentación muy variada, caracterizado por su bajo contenido en agua (<5 %) (B.O.E. 4 de Junio 1982. Real Decreto 1124/1982)[171].

5.1. Introducción

5.1.1. Introducción histórica

La galleta surge de la necesidad de aprovisionar a la tripulación de los barcos con alimentos nutritivos y de alta densidad energética, fáciles de transportar y con una larga vida útil. Por lo que en la antigüedad, ya los marineros egipcios elaboraban un pan plano y quebradizo de mijo llamado *dhourra*, mientras que los romanos tenían una galleta llamada *buccellum* (Edwards, 1984)[172]. El libro de cocina romano Apicius la describe como: "una pasta espesa de harina de trigo fina que se hierve y se extiende en una placa. Cuando se ha secado y endurecido, se corta y luego se fríe hasta que esté crujiente y luego se sirve con miel y pimienta".

Los romanos observaron cómo debido a la captación de la humedad ambiente durante el almacenamiento y transporte, las galletas se reblandecían aumentando las reacciones de rancidez y el deterioro por microorganismo (mohos). Para resolver este problema, los panaderos romanos intentaron crear una galleta lo más dura posible, por lo que sometían a las galletas a dos procesos de horneado primero en un horno caliente y posteriormente secadas en el horno frío. El origen de la palabra Biscuit provendría de esta técnica al ser la combinación de dos palabras en latín, *bis coctus*, que significa dos veces cocida. Debido a esta gran dureza su modo de consumo era diferente al actual, ya que para suavizar la galleta a menudo se sumergía en salmuera, vino o algún otro líquido o eran cocidas en una sartén con la comida.

El origen de la palabra castellana galleta deriva de la palabra francesa *galette* que a su vez sería el diminutivo de la palabra galleta, que significa literalmente guijarro y tal vez este término esté relacionado con la dureza que originariamente tenían las primeras galletas.

Como anécdota, en 1588 la cantidad diaria a bordo de un buque de la Marina Real Británica, era una libra de galletas más un galón de cerveza. Las galletas continuaron siendo una parte importante de la dieta de los marineros de la Marina Real hasta la introducción oficial de los alimentos enlatados en 1847.

5.1.2. Clasificación de las galletas

Según la legislación española (Real Decreto 1124/1982), las galletas se pueden clasificar como:

-"Cracker" y de aperitivo. Se fabrican a partir de harina de trigo y grasas comestibles, generalmente saladas o sin azúcar. Sus masas se pueden fermentar para conseguir su tradicional ligereza e incrementar su sabor.

-Barquillos con o sin relleno. Formuladas base harina de trigo, féculas, glucosa y sal. La alta concentración de azúcares produce que las masas adquieran una consistencia líquida-viscosa. La alta elasticidad y viscosidad produce que los barquillos puedan adquirir diferentes formas: rectangulares, cilíndricas, abanicos, etc. en función de la forma de las planchas metálicas en las que se forman, Pueden elaborarse solos o con rellenos a base de azúcar, dextrosa, grasa, aromas o chocolate.

-Bizcochos secos y blandos. Elaborados a base de harina de trigo, azúcar y huevos. Las masas son batidas a gran velocidad. Son horneados en moldes o en chapas planas en función de su volumen deseado. Se clasifican en secos y blandos según porcentaje de humedad que contienen a la salida del horno.

-Galletas tipo sándwiches. Son dos galletas, a las que se adiciona un relleno consistente en una mezcla de azúcar, grasa o chocolate.

-Pastas blandas y duras. Se clasifican en este grupo las galletas obtenidas a partir de masas batidas (azúcar, grasa y otros productos alimenticios) a las que posteriormente se adiciona la harina para impedir el desarrollo del gluten.

-Bañadas con aceite vegetal. Después de horneadas las galletas son sometidas a una dispersión o baño de aceite vegetal en superficie.

-Recubiertas de chocolate: Después de horneadas las galletas son recubiertas de chocolate, pasta de cacao o mezcla de azúcar, gelatina y agua.

- Surtidas: Conjunto de galletas de diferentes especialidades agrupadas en un solo envase.

Sin embargo la clasificación clásica de Wade (1988)[173] únicamente distingue dos tipos fundamentales de galletas en función de su tipo de masa: "masas duras" ("*hard dough*") y "masas blandas" ("*short dough*"), la diferencia fundamental entre ambos tipos de galleta es su extensibilidad por la existencia de largas cadenas de gluten (Manley, 1991)[174]. En las masas duras el gluten está desarrollado, proporcionando cualidades visco-elásticas a la masa, mientras que en las masas blandas la mayor cantidad de azúcar y grasa en su formulación evita que el gluten se pueda desarrollar completamente, esto provoca que estas galletas aumentan su tamaño durante las primeros etapas del proceso de horneado. En el mercado Español, las galletas de "masa blanda" (*"Short dough")* son conocidas como "pastas de té".

Según esta definición clásica: Una galleta Digestive, se describe como una galleta semidulce de masa blanda (*"shortdough"*), originaria del Reino Unido, desarrollado en 1839 por dos médicos escoceses para ayudar a la digestión (McVitei's, 2016)[175] debido a la creencia de que uso de bicarbonato de sodio con el que se formulaban las primeras galletas tenían propiedades antiácidas, pero posteriormente debido a la incorporación de los procesos de malteado de las harinas se patentaron diferentes formulaciones de galletas digestive, asegurando que sus ingredientes fueran más fáciles de digerir que las galletas convencionales.

Imagen 2.Extraídadel libro “Biscuit Baking Technology (Second Edition) Processing and Engineering Manual 2016”, Pages 1–34.

5.1.3. Sector galletero en España

Según el informe de Producción Industria Distribución, Consumo Alimentación en España del año 2016[176]. El mercado total de galletas durante el último ejercicio creció un 1,3 % en volumen, rozando las 567.400 t, mientas que el mercado nacional se redujo en un 3,9 % y quedando en 336.710 t. El citado ejercicio mostró unas ventas de 1.090 millones de euros, un 1,7 % inferior al año anterior, de los cuales las ventas nacionales rondaron los 699 millones de euros, suponiendo un descenso del 4,9 %. Después del aumento dela demanda interna durante los años 2011 y 2013, en los tres últimos años se ha producido una caída en las ventas. Ante esta fluctuación de la demanda, los principales operadores del sector han apostado por la internacionalización, la innovación, la oferta de mayor valor añadido y la modernización sus procesos productivos con el objetivo de reducción de costes. Según los datos dela Asociación Española del Dulce (Produlce, 2016)[177], el principal porcentaje en valor del mercado nacional se comercializa como galletas de meriendas (25 % del total), seguido delas de desayuno infantil (22,1 %) y familiar (22 %), las galletas saludables (15,4 %), las especialidades para adultos (7,4 %), las galletas saladas (5,4 %) y finalmente los surtidos (2,7 %).

Durante el año2016, las ventas de galletas en la distribución organizada alcanzaron un volumen 252.980 t, por un valor de 894,3 millones de euros, indicando unas reducciones interanuales del 0,5 % en volumen y del 1 % en valor. Las galletas dulces acaparan el 74,5 % de todas las ventas en volumen y el 71,1 % en valor, mientras que las dietéticas e integrales suponen 22,3 % y 24,5 % respectivamente. Aparecen, por último, las galletas saladas, cuyas cuotas son del 3,2 %en volumen y del 4,4 % en valor. Dentro de las galletas dulces, las de mayor demanda son las desayuno siendo el 59,2 % de todas las ventas en volumen y el 38,1 % en valor, seguidas por las especialidades (34,4 % y 51,6 %), los surtidos de galletas (4,8 % y 7,9 %) y las galletas con cereales (1,6 % y 2,4 %).

5.1.3.1. Estructura empresarial del sector galletero en España

Según el informe del2016 de Producción Industria Distribución, Consumo Alimentación en España. El mercado de galletas en España está concentrado en un número reducido de grupos empresariales. Las marcas de las cinco empresas más importantes se reparten el mercado nacional, al acaparar un 42,6 % del volumen total del mercado y un 55 % en valor. Excluyendo a las marcas

blancas, los demás fabricantes tienen una cuota conjunta del 5,3 % en volumen y el 10,5 % en valor. Atendiendo a sus ventas, el operador más importante supera los 294,3 millones de euros, con una producción de 114.000 t. La segunda empresa en importancia tiene una producción superior (121.000 t) y unas ventas de 216,8 millones de euros, mientras que la tercera se sitúa en 72.000 t y 195 millones de euros, la cuarta ronda las 80.000 t y 147 millones de euros y la quinta alcanza las 16.500 t y 37,8 millones de euros. En el libre servicio, las marcas de distribución acaparan el 52,1 % de todas las ventas en volumen y el 34,6 % en valor, mientras que la primera marca presenta unas cuotas del 19,2 % y del 22,1 %, la segunda se queda en 14,8 % y 22,9 % y la tercera alcanza el 6,4 % y el 6,8 %.

Tabla 6.Principales empresas por volumen de negocio en el sector galletero español

EMPRESA	**VENTAS (Millones Euros)**
Mondelez International – Grupo. *	600,00
Unilever España, S.A. – Grupo. *	600,00
Galletas Siro, S.A. – Grupo. *	562,90
Nutrexpa, S.L. *	542,00
Grupo Bimbo.*	340,00
Grupo Dulcesol*	292,00
Galletas Gullón, S.A.*	286,60
Kellogg's España,S.L. *	150,00
Galletas Artiach, S.A.*	84,00
Sanchís Mira.*	80,00
* *Sus datos incluyen actividades en otros sectores. FUENTE: Informe Anual de ALIMARKET/2015*	

5.1.4. Comercio exterior del sector galletero español

Las exportaciones son clave para explicar el sector galletero español. En el último ejercicio se exportaron 195.220 t, un 16,1 % superior el ejercicio anterior, por un valor total de 368,3 millones de euros, con un incremento interanual del 15,9 % en valor. Las exportaciones suponen el 35,7 % del mercado total de galletas de nuestro país en volumen y el 34,2 % en valor, representando ya entre el 33 % y el 40 % de todo su volumen de negocio. Estos resultados obtenidos son el claro ejemplo de cómo los fabricantes españoles de galletas están apostando en sus estrategias comerciales por la internacionalización. Los principales mercados de destino son otros países de

la Unión Europea (un 80 % de todo este comercio exterior).Pero recientemente se han añadido otros nuevos destinos: en ambas Américas, Asia-Pacifico y el Magreb. Las importaciones, comparadas con las exportaciones, son mucho menos importantes al situarse en torno a 71.700 t, por un valor de 184,6 millones de euros. Estas cifras suponen unos significativos incrementos interanuales del 19,4 % en volumen y del 8,9 % en valor. Siendo Francia y Alemania las principales proveedoras de galletas del mercado español.

5.2. Proceso de elaboración de las galletas de masa blanda ("*Short dough*")

5.2.1. Formulación

La gran mayoría de galletas consumidas por todo el mundo están hechas de masa blanda o "short dough", y consecuentemente la gama de formas, tamaños, sabores e ingredientes y fórmulas es enorme, pero hay unos requisitos imprescindibles como son:

-La harina es generalmente débil, con entre 8-9 % de proteína.

-No hay reglas para las proporciones de grasa y azúcar, que puede variar respectivamente hasta el 1:1 y 1:2 del peso de la harina. Pero habitualmente para mantener su consistencia fluida la proporción de grasas es el 65-76 % del peso de la harina, y esta se puede completar con un 15 – 25 % del peso de harina en claras de huevo batidas.

-El azúcar es alrededor 35-40 % del peso de harina. Distintas vitaminas y minerales pueden añadirse a la masa para satisfacer los requerimientos regulatorios locales.

Tabla 7. Ingredientes de las galletas digestive. (Davidson, 2016)[178]

Ingrediente	Peso (g)
Harina (débil)	100.00 g
Azúcar en polvo	29.50g
Aceite vegetal	19.50g
Lecitina	0.62g
Glucosa	2.50 g
Fructosa	1.25g
Leche desnatada en polvo	2.35g
Sal	1.10g
Aluminio fosfato de sodio	0.30g
Bicarbonato Amónico	0.58g
Carbonato cálcico	0.49g
Vitaminas	mix 0.11g
Aromas	0.21g
Agua	13.33g

5.2.2. Materia prima e ingredientes en la elaboración de las galletas

- *Ingredientes Principales*

5.2.2.1. Harina

El ingrediente principal de las galletas es la harina de trigo. El grano de trigo se compone de salvado (12 %), de endospermo, (85.5 %) y del germen (2,5 %). La harina para la elaboración de galletas se moltura con una extracción del 70-75 % (El porcentaje de extracción de la harina integral es del 75-100 % y el de la harina blanca es el 70-75 %).

La harina se compone de una mezcla de fragmentos de endospermo junto con gránulos de almidón y fragmentos de proteína (Wade, 1988)[188] producido por la molturación de los granos del trigo. En particular para la producción de galletas se emplea harinas débiles que son una mezcla de almidón (70-75 %), proteínas (8-11 %), lípidos, varios polisacáridos no amiláceos (pentosanas, etc.) (Fustier *et al.*, 2008)[179] y con una humedad de entre 13 – 15 %. El contenido de proteína (gluten)

es bajo (7-9 %) (Hadi Nezhan & Butler, 2009)[180]. En el caso de las galletas de masa corta, con poca cantidad de agua y con alta cantidad de sustancias interferentes como grasa y azúcar, impiden que el gluten no pueda hidratarse completamente (Gaines, 1990)[181]. El contenido en gluten juega un rol importante en la expansión de la galleta y en su diámetro final, siendo menor conforme aumenta el contenido de gluten (Pareyt & Delcour, 2008[182], Kaldy *et al.*, 1993[183]).

Tabla 8: Especificaciones típicas de la harina para elaboración de galletas short dough (Calaveras, 2004)[184]

Parámetros	**Valores**
P: tencidad (resistencia a la rotura de la masa)	30/35
L:extensibilidad (capacidad elastica de la masa)	130/150
W: Fuerza (trabajo de deformacion de una lámina de masa hasta su rotura.)	105/90
P/L: equilibrio (relación entre la tenacidad y la extensibilidad.)	0,10/0,30
Degradación (pérdida de las cualidades plásticas)	<10%

5.2.2.1.1. Salvado

Es uno de los productos obtenidos por la molienda de los granos de cereales y serían las capas protectoras de la semilla. Debido a la alta composición de fibra del salvado y su alta capacidad de absorción de agua el salvado reduce la elasticidad de la masa (Sudha *et al.*, 2007[185], Gujral *et al.*, 2003[186]).

5.2.2.1.2. Contenido en Gluten

Las Galletas de masa blanda o *"Short dough"* generalmente son elaborados con harina de baja proteína (7-9 %). La harina con bajo contenido en proteína hace que la masa tenga una red de gluten mucho más débil. Además, estas masas presentan un alto contenido en grasa. La grasa cubre las partículas de harina e inhibe la hidratación de las proteínas y la formación de la red de gluten.

Los tiempos de mezclado son más cortos y se produce un menor desarrollo de las cadenas de gluten y por lo tanto, las galletas tienen una textura compacta.

5.2.2.1.3. Contenido en Almidón

El almidón es el principal componente de la harina de trigo. Representa casi todo el contenido de hidratos de carbono y alrededor del 80 % del contenido total de energía de la harina de trigo. El almidón es un polisacárido conformado por unidades de glucosa unidas entre sí en cadenas de amilosa (cadena lineal) y amilopectina (cadena ramificada). Siendo la amilosa la molécula principal del almidón en la harina de trigo, y normalmente comprende el 28 % de la cantidad total de almidón. Las moléculas de amilosa contribuyen a la formación de gel. Aunque el almidón es insoluble en agua, sin embargo, los gránulos de almidón absorben una cantidad limitada de agua en la masa (un tercio de su peso). Por encima de los 60-70 °C comienza la gelatinización y puede continuar hasta que los gránulos de almidón se hinchan completamente, pero en las galletas es normal que gelatinización sea parcial. En masas short dough, la gelatinización del almidón es inhibida por su alto contenido de grasa y azúcar. Su alta concentración de azúcares retrasa la gelatinización del almidón, al competir con el agua de la formulación.

La gelatinización del almidón es importante en la elaboración de las galletas ya que contribuye a su rigidez y la textura característica. Si se calienta en exceso la masa de la galleta, se produce la dextrinización del almidón y la reacción de Maillard e incrementa la coloración de la galleta.

5.2.2.2. Grasa o "Shortening"

La grasa es segundo componente en peso y se emplea para facilitar el amasado, ligando todos los ingredientes ya que cantidad de agua de formulación no es suficiente (Sai Manohar & Haridas Rao, 1999b[187], Parety *et al.*, 2008[188]). Por tanto, la grasa actúa como lubricante y anti-aglutínate, rodeando la superficie de la harina e inhibiendo la formación de la red cohesiva y extensible del gluten; y debilitando la estructura proteína-almidón al rodear a los gránulos de almidón, (Ghotra *et al.,* 2002)[189]. Estos fenómenos afectan a la textura de la masa, haciendo que sea menos elástica para que no encoja tras el proceso de laminación (Baltsavias *et al.,* 1997[a])[190], pero a su vez hace que la galleta sea más frágil (Maage-Rezzoug *et al.,* 1998)[191] y por tanto si se disminuye el

contenido de grasa en la formulación la textura de las galletas "*short dough*" se endurece (Burt & Thacker, 1981)[192]. El tipo de grasa y su origen también influye en las características físicas de las grasas, y repercuten en la textura final de las galletas "*short dough*" (Hornstein *et al.,* 1943)[193]. Por ejemplo, si se emplea en la elaboración de galletas "*short dough*" grasa extraída de la leche la textura de las galletas se endure más que las galletas formuladas con manteca Wade (1968)[194]

Se utilizan mezclas de grasas vegetales que son sólidas a temperatura ambiente y se funden en un amplio rango de temperaturas. La mayoría de grasas y aceites empleados en la elaboración de galletas se funden por debajo de temperatura corporal (36,9 °C), evitando que la sensación residual en boca sea cerosa.

Las grasas se especifican con un índice de solidificación (Solid Fat Index o SFI), que indica el porcentaje de grasa sólida en la grasa total. Las grasas empleadas en la elaboración de galletas tienen que tener un SFI alrededor de 18 % a 25 °C y de 12 % a 30 °C. El grado de fusión de las grasas es importante para controlar la extensión de la masa de galletas sobre la banda de cocción acero al entrar en el horno, donde la temperatura de la masa aumenta por encima de 35 °C.

5.2.2.3. Azúcar

El azúcar mayoritariamente empleado es la sacarosa. En el proceso de amasado el azúcar, por su higroscopicidad, compite por el agua con la harina (almidón y gluten) inhibiendo la formación de gluten (Gallagher *et al.,* 2003)[195] y modificando la consistencia de la masa. Durante el horneado, el azúcar influye en la gelatinización del almidón y movilidad del gluten (Spies & Hoseney, 1982[196], Pareyt & Delcour, 2009[197]), en las reacciones de Maillard, y en la expansión de la galleta (Kulp *et al.,* 1991)[205]. Por tanto, la adición y la cantidad de azúcar afecta durante todo el proceso: a las dimensiones, color, gusto y textura de la masa y de las galletas (Gallagher, 2003)[203].

El jarabe de azúcar invertido es una mezcla de glucosa y fructosa. Al ser el jarabe invertido es más dulce que el azúcar se utiliza en menor cantidad y mejora la textura tierna de las galletas.

- *Ingredientes minoritarios*

5.2.2.4. Agua

A pesar de ser un ingrediente minoritario (14 % en peso) en el proceso de fabricación, el agua es un ingrediente clave del proceso de fabricación de las galletas al actuar como disolvente y plastificante de la masa (Pareyt & Delcour, 2008)[197]. En la primera fase del amasado, el agua disuelve algunos de los ingredientes (azúcar, sal y agentes leudantes) y gracias a la lecitina (fosfolípidos) se emulsiona con la grasa, también interviene en la creación de la red de gluten, considerada como red proteica tridimensional, viscoelástica. La hidratación de gluten y carbohidratos provoca que sus proteínas se orienten, alineen y desplieguen parcialmente). Por tanto, determina el comportamiento reológico de las masas de galleta. Debido a la sobrehidratación de la red de proteínas que modifica sus uniones y deshace sus extractos proteicos, cuanta más agua de formulación haya, reduciendo la proporción de grasa, las masas de galletas son más cohesivas, extensivas y adhesivas, disminuyéndola fuerza de la masa(Sai Manohar & Haridas Rao,1999)[190].La cantidad final de agua influye en la consistencia de la galleta, de forma que, las galletas de baja humedad son más frágiles, y a medida que se aumenta la cantidad de humedad el punto de estrés de fractura de la galleta aumenta; debido a su mayor elasticidad y deformabilidad. (Baltsavias *et al.,* 1999[b])[197].

5.2.2.5. Leche desnatada en polvo

La leche desnatada en polvo contribuye a la textura, gusto y color superficial de la galleta (sus aminoácidos favorece las reacciones de Maillard durante el horneado, (Wade 1988)[188]).

5.2.2.6. Sal

El cloruro sódico o sal común se utiliza como potenciador de sabor. Su concentración más eficaz, sin variar atributos sensoriales de la galletas varía entre el 1-1,5 % del peso de la harina (Manley, 1998)[27].

5.2.2.7. Agentes leudantes o gasificantes

5.2.2.7.1. Bicarbonato sódico (CO_3HNa)

Al calentarse, el bicarbonato en medio ácido, se descompone dando anhídrido carbónico y agua, esponjando la masa. Sin embargo, en un medio poco ácido el bicarbonato libera algo de dióxido de carbono y permanece como carbonato sódico, Es importante controlar el pH ya que puede afectar a la expansión de la masa y al color final de la galleta (Manley, 1998) [27].

5.2.2.7.2. Carbonato amónico ($CO_3 (NH_4)_2$)

Se disuelve rápidamente en el agua de formulación aumentado el pH de la masa y ablandándola. Al igual que el bicarbonato sódico, al descomponerse por el calor del horneado el carbonato amónico desprende gases (anhídrido carbónico, amoniaco gaseoso) que esponjan la masa (Manley, 1998) [27]. Al ser una reacción básica se debe disminuir el contenido en agua en la formulación. La utilización del carbonato amónico se aconseja en productos horneados con bajo contenido en humedad, para que se pueda eliminar el amoniaco.

5.3. Amasado

Una vez pesado los ingredientes se procede a la mezcla de los mismos. Al ser las masas "short dough" masas suaves y de consistencia fluida, aparte de usar harina débil, para evitar que el gluten en la masa se desarrolle, y endurezca la galleta, los ingredientes se deben mezclar en un proceso de dos etapas. Siendo la primera etapa un batido de la grasa y el azúcar (Cauvain & Young, 2006)[198], posteriormente se añaden la harina y otros ingredientes seco. Las masas "short dough" seguirán el modelo bifásico de distribución de los ingredientes propuesto por Pareyt & Delcour (2008)[197], donde la fase grasa rodearía a las partículas de la fase no grasa. Es importante mantener la temperatura de la masa baja y el mezclador debe estar refrigerado mediante un sistema de doble camisa. Posteriormente se le adiciona el resto de ingredientes (harina y otros ingredientes secos como: el gasificante) con un mínimo tiempo de mezcla mínimo (1 minuto a velocidad lenta y 1-2 minutos a alta velocidad), para evitar que la masa se endurezca (Baltsaviase*t al.*, 1999[b]) [205]. La

temperatura de la masa es un factor importante para su correcta dosificación y moldeado en cada tipo específico de galleta, oscila generalmente entre 10 y 22 °C.

En las técnicas modernas, se reducen los tiempos de mezcla, y se tiende a mezclar todos los ingredientes a la vez. Las masas son cohesivas y plásticas, con poca extensibilidad: su consistencia variará según los requerimientos de la maquinaria utilizada para formar y moldear las galletas.

5.4. Reposo

Debido a la poca duración de la segunda mezcla, la harina no está hidratada totalmente y la masa es aún muy pegajosa para poder ser moldeable. Por ello la masa debe reposar, en un lugar fresco, durante 30 minutos antes de formar. El endurecimiento de la masa es debido absorción de agua libre por los componentes hidrófilos de la harina (gluten y almidón de la harina) (Wade 1988[188], Pareyt & Delcour, 2008[197]).

5.5. Laminado y Moldeado

Los dos principales procesos para formar galletas "short dough" son el moldeado rotatorio y corte por alambre ("*wire cutting*"):

- El moldeado rotatorio es originario de los moldes de madera utilizados en las cocinas monacales para troquelar las superficies de las galletas. Industrialmente, la masa almacenada en una tolva es continuamente forzada a fluir hacia el rodillo giratorio, a través de un dosificador de metal rectangular. Después del horneado las galletas "short dough", obtenidas por moldeado rotatorio, deben ser finas y lisas (sin grietas superficiales o irregularidades), de textura suave, delicada y una sensación de "derretimiento en la boca".

  A diferencia de las masas duras, que tienden a encoger durante el horneado, las galletas "short dough" se expanden debido tipo de harina empleado (débil), y a los altos niveles de azúcar y grasa. Por lo cual, durante el laminado es recomendable que la masa se gire 90º en cada paso por la laminadora, de lo contrario el gluten se alinea únicamente en la dirección de laminación. Dando lugar, tras el horneado, a galletas irregulares (Fustier *et al.*, 2008[194], Thacker, 1993[199]), de logotipo borroso, quebradizas y difíciles de empaquetar.

- El Corte de alambre o "*Wire-cutting*": La masa es extorsionada través de una boquilla y cortada por un alambre a intervalos de tiempo determinados, en función de longitud o espesor deseada. Posteriormente, la masa de galletas cae en la banda de cocción móvil, en dirección al horno. Esta técnica se aplica generalmente para formulaciones de masa más suaves que las empleadas en moldeado rotatorio y al no aplastar la masa, permite la incorporación de ingredientes sólidos, como: trozos de chocolate, frutos secos o uvas pasas.

5.6. Horneado

Existen tres tipos de horno en función del modo en el que el calor es transmitido a las galletas: modo directo, indirecto y mixto (mezcla de los dos anteriores). Para mayor eficiencia, el vapor se reutiliza recirculándolo a la entrada del horno, consiguiendo recuperar parte de la energía calorífica, y que la humedad del aire a la entrada del horno sea alta. Esto permitirá que la galleta en la primera zona del horneado pueda conseguir un buen volumen, evitándose acortamientos por choque térmico.

Existen distintos tipos de transporte en función del tipo de producto: sólido de banda, piedra, malla, etc.

Tabla 9.Variables del horneado para galletas digestive. (Davidson, 2016)[193]

Tiempo de horneado	5.0–5.5 minutos
Temperaturas	180/200- 200/220 °C
Humedad final	<3.0 %

5.6.1. Características del horno

Al comienzo del horneado se debe proporcionar una transferencia de calor rápida pero que mantenga flexible y húmeda la parte externa de la masa de las galletas, permitiendo su posterior la expansión y elevación. Para su consecución, y reducción del coste energético, parte del aire húmedo eliminado en la parte media y final del horno es venteado hacia al comienzo del horno.

En la parte media del horno, la humedad debe ser eliminada eficientemente de la masa, por lo que la potencia de extracción de la cámara de cocción es la más elevada del horno.

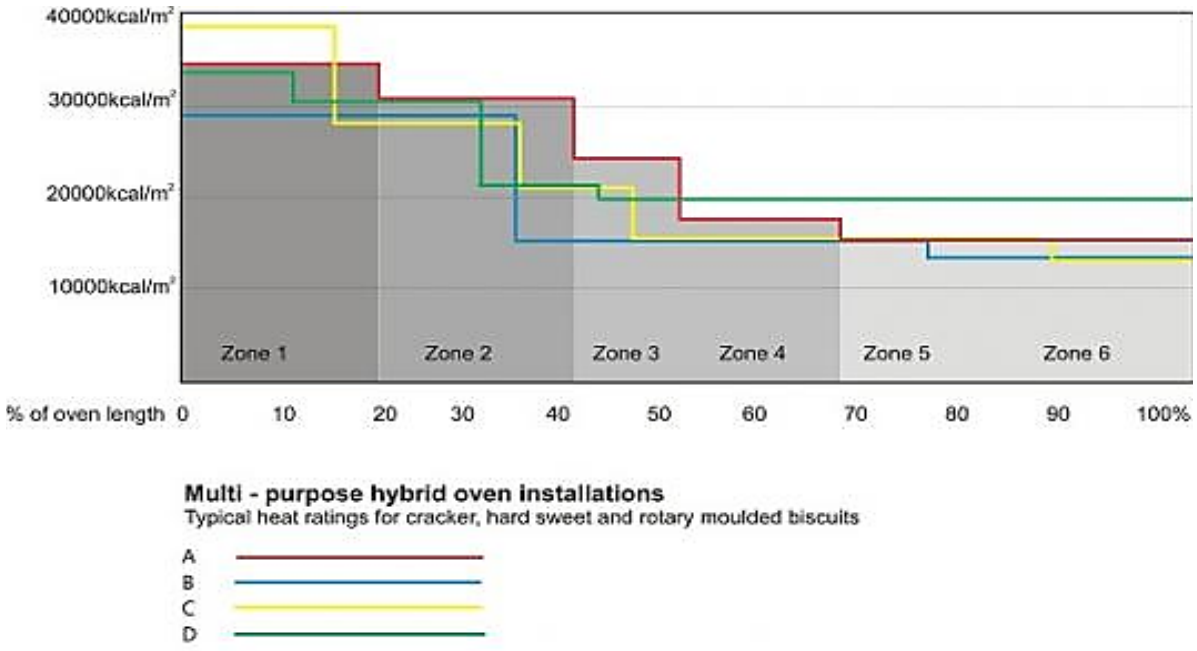

Figura8. Clasificación calorífica (entrada de energía calorífica por m2 de la banda de horno) para varios hornos de galletas.(Cortesía de "Biscuit people," Página visitada el 13/3/2018). https://www.biscuitpeople.com/magazine/post/biscuit-baking-process.

En las zonas finales, es donde la superficie de la galleta adquiere su color y humedad característica. Por lo que un control de transferencia de calor lateral es necesario para mantener un contenido humedad y color similar entre las galletas que se desplazan por la cinta de transporte del horno.

-Transferencia de calor:

La especificación del sistema de horneado debe basarse principalmente en el tipo de productos a realizar, sus requerimientos de estructura, textura, densidad, sabor, color, y el tipo de transferencia de calor que es apropiado en cada etapa/zona del proceso de cocción(radiación, conducción y convección). (Figura 9)

Los tres modos de transferencia de calor (radiación, conducción y convección) ocurren durante el horneado de las galletas y su aplicación depende del tipo de horno.

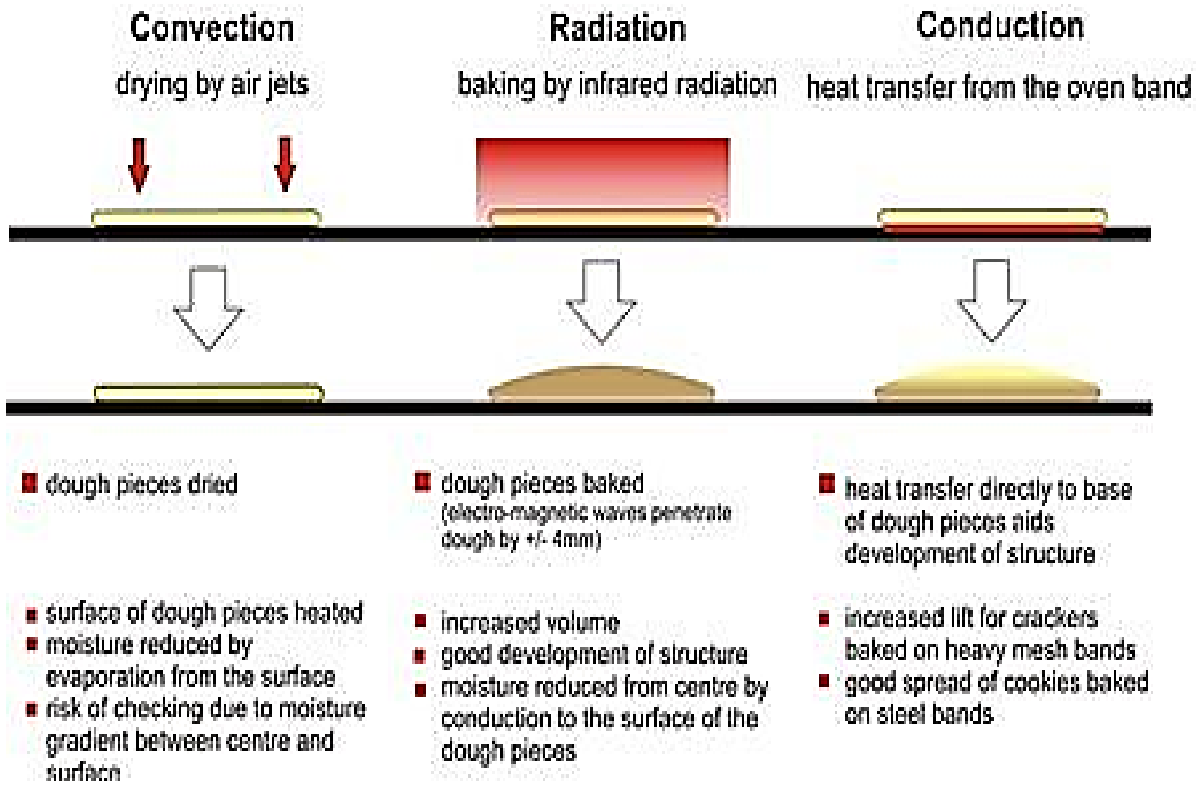

Figura 9. Modos de trasmisión de calor durante el horneado de galletas

(Cortesía de "Biscuit people," Pagina visitada el 13/3/2018.

https://www.biscuitpeople.com/magazine/post/heat-transfer-for-biscuit-baking

Como se indica en la figura 9los modos de trasmisión de calor, difieren en importancia y efecto sobre el producto.

- *Radiación*

La radiación es el método más importante de transferencia de calor durante el horneado de galleta. Se produce principalmente por radiación electromagnética de longitudes de onda infrarrojas de quemadores de gas directos, las superficies calientes del horno cámara y tubos o conductos que transporten gases calientes de los quemadores. Este calor radiante es penétrate y eficiente y no

produce efectos secundarios adversos, tales como el rápido secado y formación de ampollas en la superficie de la masa de galletas.

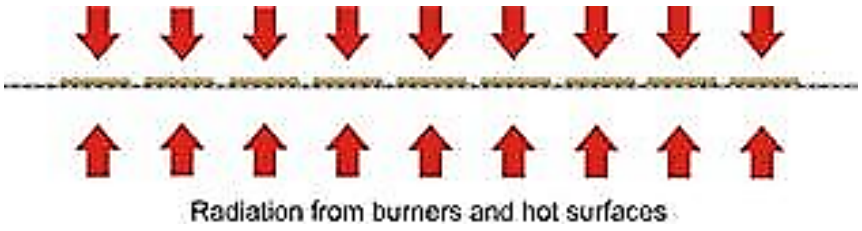

Figura 10: Diagrama de transmisión de calor por radiación.
(Cortesía de "Biscuit people," Pagina visitada el 13/3/2018
https://www.biscuitpeople.com/magazine/post/heat-transfer-for-biscuit-baking))

La radiación (transferencia de energía térmica) es dependiente de varios factores:

1. *La naturaleza y emisión de la superficie radiante.*
2. *La distancia entre la superficie de radiación y la masa de las galletas.*

   La energía térmica transferida es inversamente proporcional al cuadrado de la distancia (*Intensidad* $= I/d^2$).Por tanto una pequeña disminución en la distancia provoca un gran aumento en la transferencia de calor a las piezas de masa.

3. *La diferencia de temperatura entre la superficie de radiación y la masa de galletas.*

   La transferencia de calor es proporcional a la temperatura de la superficie de radiación a la potencia de cuatro o lo que es lo mismo un pequeño aumento en la temperatura de la superficie de radiación causa un aumento muy grande en la transferencia de calor.

   ($P = e * \theta * T^4$).

   Donde P es el poder de radiación, la e es emisividad del objeto, θ es la constante de Stefan-Boltzmann ($\theta = 5.67 * 10^{-8} W / m^2K^4$) y la T la temperatura del emisor.

- *Conducción*

La conducción es la transferencia de energía térmica entre moléculas próximas dentro de un objeto o entre objetos en contacto entre sí. Transfiriéndose de una zona de mayor temperatura a una zona de menor temperatura y actúa hasta igualarse la diferencia de temperatura. La ley de conducción de calor se conoce como ley de Fourier, según la siguiente ecuación.

$(Q = -k * T)$

El calor por conducción (Q) es proporcional a la conductividad térmica (k) multiplicada por el gradiente negativo de temperatura (T). La conductividad térmica es la propiedad de un material que indica su capacidad para conducir el calor. La conductividad térmica predice la pérdida de potencia en vatios a través de un pedazo de material. Esto es importante para calcular el aislamiento óptimo requerido para el horno.

Figura 11. Diagrama de transmisión de calor por conducción.
(Cortesía de "Biscuit people," Pagina visitada el 13/3/2018
https://www.biscuitpeople.com/magazine/post/biscuit-baking-process)

La conducción térmica es dependiente de varios factores:

1. *Se transfiere el calor directamente de la banda de horno a la base de la masa de las galletas. La transferencia de calor por conducción depende de la temperatura, superficie en contacto y la masa de la banda de transporte del horno.*

Por tanto, en la elaboración de galletas "soft dough" es particularmente importante que para desarrollar la estructura y textura de las galletas, el calor se pueda transferir rápidamente a la base de masa de galletas. Por ello, las bandas de transporte de las galletas se deben precalentar antes de introducirse en el horno.

2. Del *aislamiento del horno:* El calor de la cámara de cocción es transferido a través de cubiertas exteriores del horno. Para evitar pérdidas caloríficas al exterior del horno, éste debe de asilarse con un material de aislamiento con una baja conductividad térmica.

- *Convección*

La convección es el movimiento debido a la diferencia de temperatura que se produce en un medio fluido, por ejemplo el aire en la cámara de cocción. El diseño de los sistemas de control de temperatura se debe tener encuentra que el aire caliente, al ser menos denso, tendera a desplazarse por las partes superiores del horno, por lo tanto un cambio de temperatura en la parte inferior del horno afectará la temperatura en la parte superior.

La Convección se describe por ley de enfriamiento de Newton, que establece que "la tasa de transferencia de calor es proporcional a las diferencias de temperaturas entre el cuerpo y su entorno".

$$\frac{dQ}{dt} = h \cdot A \cdot (T(t) - T_{\text{env}}) = h \cdot A\Delta T(t)$$

Siendo: Q es la energía termia en julios, h es el coeficiente de transmisión (W/ (m^2 K)), A es la superficie de transferencia (m^2), ΔT (t) es el gradiente térmico dependiente del tiempo entre medio y objeto.

La convección se utiliza en la industria de la galleta para describir un sistema de transferencia de calor empleando chorros de aire caliente directamente sobre la superficie de la pieza de masa y la banda de horno desde arriba y desde abajo. Este aire caliente rápidamente elimina la humedad de la superficie de las piezas de masa y aumenta la temperatura de la superficie de las piezas de masa

Figura 12.Diagrama de transmisión de calor por convención.
(Cortesía de “Biscuit people,” Pagina visitada el 13/3/2018.
https://www.biscuitpeople.com/magazine/post/biscuit-baking-process.

El sistema de convección es muy eficiente en la eliminación de humedad en la parte media del proceso de cocción y es muy poco penetrante. El flujo de aire caliente incide en la superficie de la masa de galletas y evapora la humedad superficial rápidamente, posteriormente el aire de la cámara de cocción es eliminado a través del sistema de extracción. El rápido secado de la superficie provoca una fina corteza, que previene de la expansión y la "elevación" de las masas en la zona media del horno, previniendo posibles deformaciones y unificando el color de las galletas en todas las secciones de la línea de transporte del horno.
El calentamiento convectivo debe ser minimizado en las secciones del primer tercio del horno, para posibilitar que la masa desarrolle su textura característica.

La convección térmica depende de varios factores:

1. De la temperatura y del caudal de aire en contacto con la masa de galletas. La mayoría de los hornos de convección tienen una velocidad fija de circulación de aire caliente, y ajustan el porcentaje de caudal utilizando deflectores de área regulable.

2. De controlar independientemente las temperaturas superior e inferior del horno. Como se ha comentado con anterioridad el aire caliente tendera a desplazarse a la partes superiores del horno, y por tanto cambios en la temperatura inferior de la cámara del horneado afectaran el flujo de aire caliente en la cámara de horneado.

5.6.2. Cambios durante la cocción

En el horneado se producen distintos cambios estructurales de la galleta, como: desnaturalización proteica, fusión de la grasa, reacciones de Maillard, expansión de gases leudantes (Chevallier *et al.*, 2002)[200]. La combinación y solapamiento de estos cambios hace que el producto desarrolle una textura abierta y porosa, disminuya su densidad, cambie su coloración y por evaporación del agua de formulación se reduzca su humedad hasta el 1-4 % final. Durante el horneado, el primer cambio estructural seria debido a la fusión de la grasa, dando a la masa su carácter plástico (Pareyt & Delcour, 2008)[197.] A continuación se produce la elevación, por expansión de los gases de los agentes leudantes, y posterior colapso de la galleta (Chevallier *et al.*, 2000)[211]. En la última etapa del horneado se finaliza evaporación el agua de formulación y la galletas adquieren su diámetro, estructura (masa no coagulada, de matriz azucarada) y color final.

Por tanto la duración de esta última etapa de horneado se determina mediante un examen visual, función del color y el contenido en humedad final deseados (Wade, 1988)[188].

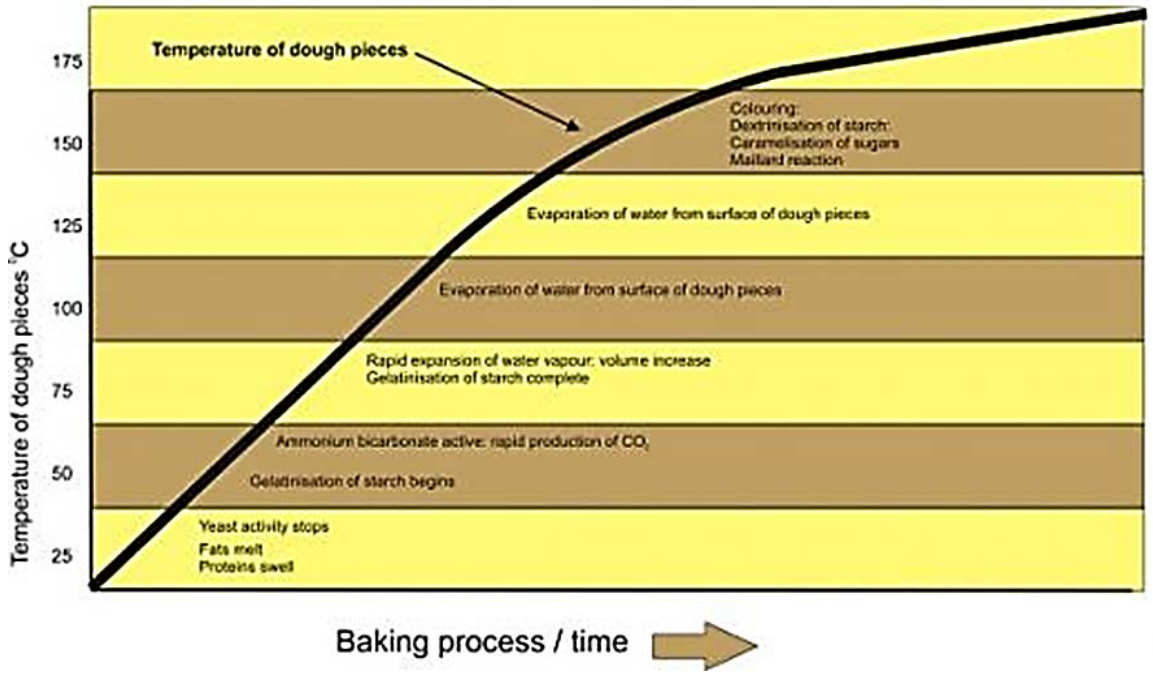

Figura 13: Diagrama resumen de la formación de la estructura y textura de la galleta durante el horneado.(Cortesía de "Biscuit people," Pagina visitada el 13/3/2018. https://www.biscuitpeople.com/magazine/post/biscuit-baking-process

5.7. Enfriamiento

Durante el enfriamiento, las galletas terminaran de perder parte de su humedad y de conseguir su característica crujiente (Manley, 1998)[27]. Para evitar el choque térmico, las galletas se deben enfriar suavemente a la salida del horno, Generalmente el tiempo requerido para el enfriamiento es proporcional al tiempo de horneado 1:1.5.

El enfriamiento de las galletas es especialmente necesario en las fábricas de reducido tamaño, en las que el empaquetado se realiza de manera inmediata a la producción de las galletas. Ya que si las galletas se empaquetaran aun calientes parte de su humedad podría condensar en el empaquetado plástico, causando el reblandeciendo la galleta, aparición de aromas extraños o causar la proliferación de microrganismos, como: mohos y levaduras.

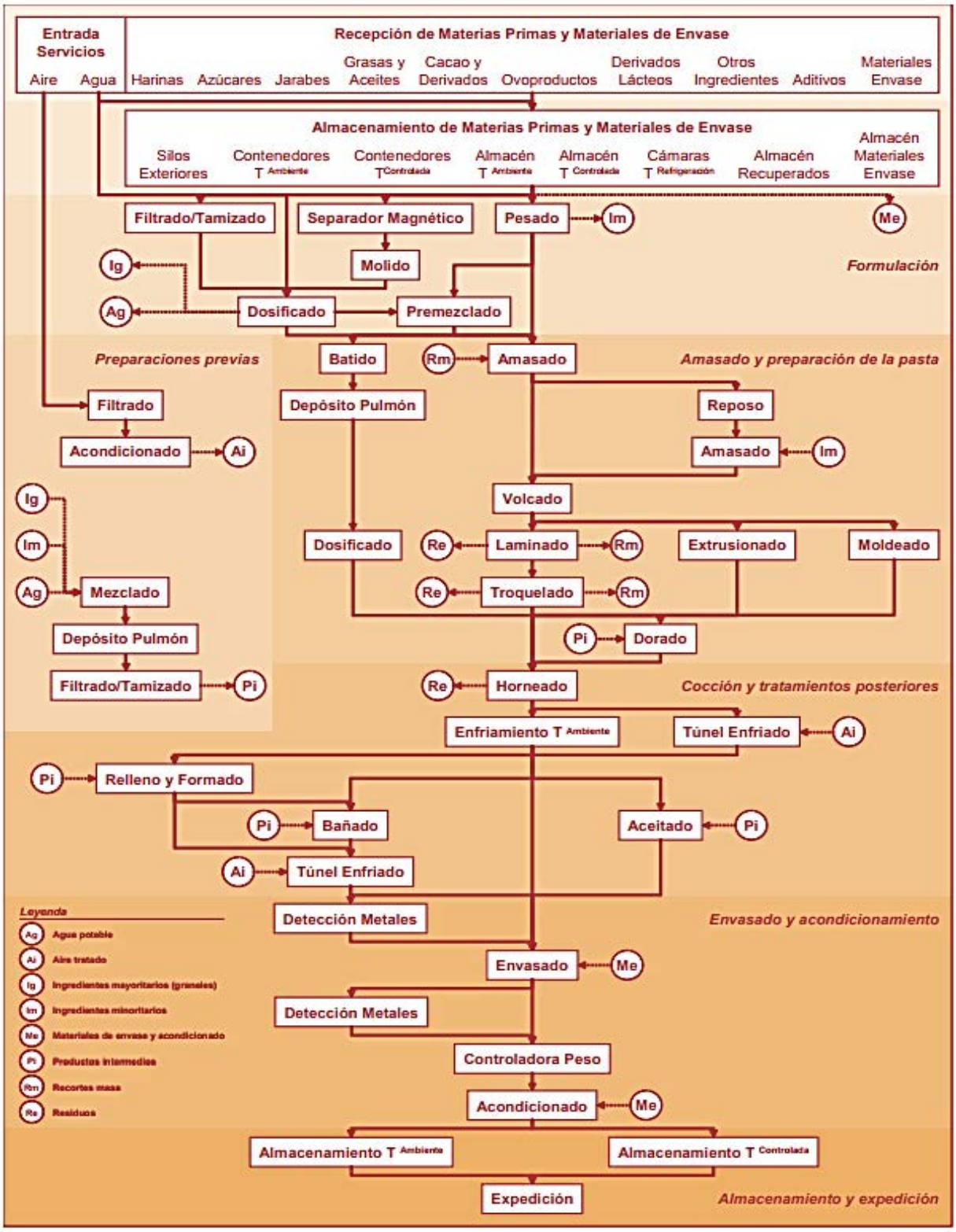

Figura 14: Diagrama de Flujo del Proceso de Fabricación Industrial de Galletas (MINISTERIO DE MEDIO AMBIENTE Y MEDIO RURAL Y MARINO, 2009[201]).

5.8. Características nutricionales y contribución a la dieta de las galletas

Los ingredientes básicos de las galletas (harina, grasa, azúcar) son los que determinan su alta densidad calórica.

Si se laboran de forma artesana o tradicional, las galletas podrían situarse en la base de la pirámide alimentaria, en una alimentación equilibrada, por su alta composición en hidratos de carbono. Pero en muchas de las pirámides alimentarias las galletas se suelen posicionar en el vértice de la pirámide aconsejando su consumo ocasional. Esto es debido a que en su fabricación industrial se emplea gran cantidad de azúcares simples y grasas saturadas (mantequilla, aceite de palma o de coco) o aceites parcialmente hidrogenadas (grasas trans), para mantener su vida útil, evitar la peroxidación lipídica y facilitar el procesado.

Respecto a la contribución de estos alimentos en la dieta diaria, su consumo está muy extendido entre la población infantil y juvenil, siendo en el desayuno y en la merienda los momentos principales de ingesta. El estudio sobre las fuentes alimentarias de factores de riesgo de enfermedades no trasmisibles, realizado por la AESAN en 2004 (Llavina, 2004)[202] para identificar la ingesta de energía y de diferentes principios inmediatos en la población adulta y escolar, corrobora que galletas como la cuarta fuente energética para la población escolar española (4,97 % de la ingesta calórica diaria total) sólo tras aceites, pan y leche. Entre la población adulta, las galletas se situarían en el séptimo lugar con el 2,98 % Debido a su menor tamaño y conveniencia (bajo precio, fácil consumo y larga vida útil) las galletas son productos ideales para consumir durante el desayuno, merienda y para picar entre horas. Pero su abuso puede ser un factor de riesgo en el incremento calórico de la dieta, y sobre peso en población infantil.

Tabla 10: Tabla de composición nutricional de las Galletas.

	Por 100 g de porción comestible	Por ración (150 g)	Recomendaciones día-hombres	Recomendaciones día-mujeres
Energía (Kcal)	440	132	3.000	2.300
Proteínas (g)	7	2,1	54	41
Lípidos totales (g)	14	4,2	< 100	< 77
AG saturados (g)	2,62	0,79	< 23	< 18
AG monoinsaturados (g)	6,37	1,91	> 57	> 43
AG poliinsaturados (g)	10	3	10-20	8-15
ω-3 (g)	0,106	0,032	0,33-3,3	0,25-2,6
ω-6 (g)	1,439	0,432	1,3-16,5	1,2-10,4
Colesterol (mg)	Tr	Tr	< 300	< 230
Hidratos de carbono (g)	71,5	21,45	375-450	288-345
Fibra (g)	1,5	0,45	38	29
Agua (g)	2,5	0,75	1.000-2.000	1.000-2.000
Calcio (mg)	115	34,5	800	800
Hierro (mg)	2	0,6	10	18
Yodo (µg)	0	0	140	110
Magnesio (mg)	32	9,6	350	330
Zinc (mg)	0,6	0,18	15	15
Sodio (mg)	410	123	< 2.400	< 2.400
Potasio (mg)	140	42	3.500	3.500
Fósforo (mg)	190	57	700	700
Selenio (µg)	7,3	2,19	70	55
Tiamina (mg)	0,13	0,039	1,2	0,9
Riboflavina (mg)	0,08	0,024	1,8	1,4
Equivalentes niacina (mg)	2	0,6	20	15
Vitamina B_6 (mg)	0,06	0,018	1,8	1,6
Ácido Fólico (µg)	0	0	400	400
Vitamina B_{12} (µg)	0	0	2	2
Vitamina C (mg)	0	0	60	60
Vitamina A: Eq. Retinol (µg)	—	—	1.000	800
Vitamina D (µg)	0	0	5	5
Vitamina E (mg)	—	—	12	12

Extraído de Fundación española de Nutrición (2017)[203]. Recomendaciones: Ingestas Recomendadas/día para hombres y mujeres de 20 a 39 años con una actividad física moderada. Recomendaciones: Objetivos Nutricionales/día. Índice de símbolos. —: No determinado. Tr: Trazas. (Moreiras *et al.*, 2013)[204].

5.9. Control de calidad de las galletas

Los parámetros de calidad de las galletas terminadas están relacionados directamente con sus variables de formulación y de producción.

5.9.1. Control de dimensiones

Control de grosor: Después del horneado, se mide la altura de las galletas con un pie de rey. El objetivo de esta medición es la de comprobar que la masa tiene las dimensiones correctas y que los pasos de producción anteriores como el laminado han conseguido la extensibilidad de la masa deseada. Uno de los defectos de producción que intenta detectar este control es la detección del acortamiento de las galletas debido a la formación de la red gluten y no un insuficiente reposo tras el laminado. Uno de los defectos de producción que intenta detectar este control es la detección de formulaciones deficientes (por incorrecta adición de agentes leudantes) o deficiencias en la extensibilidad de las masas por incorrecto orden de mezcla de los ingredientes o empleo de harinas no apropiadas o por falta de reposo de la masa laminada.

5.9.2. Control de calidad durante la producción

El personal de control de calidad analiza los parámetros sensoriales de calidad de las galletas. El objetivo es determinar que la galleta cumple con las especificaciones sensoriales, como:

Apariencia: Las galletas deben ser simétricas, con el estampado del logotipo comercial claro, sin arrugas ni burbujas superficiales. La apariencia puede detectar errores de fabricación, como: formulaciones incorrectas, inadecuada calidad de ingredientes, incorrecto orden de ingredientes o tiempos de amasado incorrectos. Aparte de la inspección visual por parte del personal del departamento de calidad, se está investigando la implantación industrial de análisis morfológico de las galletas por análisis digital de la imagen (Naranjo *et al.*, 2014)[205].

Color: Las galletas tienen que tener un color homogéneo, y característico. El color se utilizar para determinar si la velocidad de cocción y la temperatura del horno han sido correctas. La medida de este parámetro se puede realizar ayudándose de tarjetas normalizadas de color o mediante equipos de análisis de color (colorímetros y escáner).

Textura: Las galletas tienen que tener una textura homogénea, crujiente y característica. La textura se utiliza para determinar si diferentes parámetros de producción, como: formulación, amasado, moldeado y horneado, se han realizado correctamente. A parte de la medida sensorial del responsable de calidad, la medida de este parámetro se puede realizar mediante equipos de análisis de textura (texturómetro).

Sabor: Las galletas tienen que tener un sabor característico. El análisis de sabor se utiliza para determinar si diferentes parámetros de producción, como: formulación, y horneado, se han realizado correctamente.

Humedad: Las galletas tienen que tener un valor máximo de humedad que es función de que el tiempo de horneado y de la formulación de la masa ha sido los correctos. Este análisis pretende evitar el crecimiento de microorganismos durante el almacenamiento y el reblandecimiento de la textura por condensación y resorción de la humedad en el empaquetado. El análisis de humedad se lleva a cabo mediante una balanza de medición de humedad, al estar termostatada la muestra se deseca hasta peso constante calculándose la humedad por diferencia entre peso inicial y final.

Las características técnicas de la galleta digestive, de masa “short dough” y moldeada mediante un rotor giratorio, se describen a continuación en la tabla 11.

Tabla 11.Características técnicas de las galletas digestive (Davidson, 2016)

Dimensiones	56-58 mm × 35-37 mm
Grosor	6.0-7mm
Peso	5.0-6.0 g
Apariencia	Compacta
Color	Dorado-marrón claro
Textura	Suave
Sabor	Dulce
Humedad	1.2–1.4 %

5.9.3. Determinación de la Textura de las galletas

Los parámetros texturométricos se pueden definir como el conjunto de características físicas, ligadas a los elementos estructurales del alimento, que son perceptibles por el sentido del tacto, que están relacionadas con la deformación, desintegración y flujo del alimento, cuando éste es

sometido a un esfuerzo y que pueden ser medidos objetivamente, en términos de masa, tiempo y distancia (Bourne, 1982)[206].

En los productos horneados en general y en especial en las galletas, los consumidores consideran que la textura es la característica más importante. Al consumir una galleta se espera que no se desmenuce y que sea crujiente. Las galletas se diferencian de otros productos horneados por su contenido en humedad que es inferior a 5 %, mientras que los bizcochos pueden tener entre un 15-30 % y el pan entre un 30-45 %. En consecuencia, un factor muy importante que influye en la textura es la humedad de la galleta, ya que se trata de un producto higroscópico (además las galletas tipo "digestive" presentan un alto contenido en fibra) por lo que va a tender a captar agua una vez abierto el envase y también durante el almacenamiento, por tanto cambiará su textura a lo largo del almacenamiento y puede resultar desagradables para el consumidor. Consecuentemente, para evaluar la calidad de las galletas es necesario caracterizar su textura y estudiar su evolución durante el almacenamiento. La dureza de la muestra es un valor indicador de su frescura, mientras que la crujencia (Crispiness) define su estructura interna y características de horneado. El rechazo de una galleta por parte del consumidor se debe principalmente a una textura no esperada. Por lo tanto, el estudio de la evolución texturométrica de las galletas durante el almacenamiento es un factor importante para determinar la aceptabilidad y la vida útil de la galleta.

La determinación de parámetros texturométricos en galletas, es particularmente difícil debido a su composición y a su estructura heterogénea. La estructura tridimensional de las galletas varía en función del dibujo de impresión propio de cada marca y de las perforaciones de evaporación. En general, no fluyen frente a esfuerzos de presión, pero son frágiles y quebradizas (Saleem *et al.*, 2005)[207]. Para determinar parámetros texturométricos de galletas tipo "Digestive" de forma instrumental, se emplean distintas técnicas como "Prueba de penetración" o por Punción por compresión vertical (Mandala *et al.*, 2005[208], Tyagi *et al.*, 2007[209], Singh *et al.*, 2005[210]) y "Prueba de quiebre de tres puntos" o TPB (Singh *et al.*, 1993[211], Kaur *et al.*, 2015[212]). Ambas pruebas son de carácter destructivo y se basan en la aplicación de fuerzas a las muestras para obtener parámetros texturométricos deducibles de gráficos fuerza vs deformación que se obtienen con un equipo universal para prueba de materiales. La prueba de penetración se basa en la medición de la fuerza de cizalla máxima, requerida para atravesar completamente con sonda de penetración una sección del producto. Por tanto hay una relación entre la fuerza de cizalla que hay que aplicar y la

resistencia del producto. La Prueba de quiebre de tres puntos es una prueba ruptura por flexión, consistente en evaluar la fuerza máxima necesaria para producir un quiebre total de la estructura del producto (Gaines, 1994)[196] y es utilizada para evaluar la dureza y Rigidez de galletas, tabletas de chocolate, entre otros productos.

5.9.4. Determinación del color de galletas

Durante el horneado se producen varios cambios físicos y químicos en la superficie de la galleta que contribuyen a su coloración. Estos cambios de coloración se producen por la casi total evaporación del agua superficial y al aumento de la temperatura de las galletas (150 °C).

Hay tres procesos que contribuyen a al oscurecimiento de las galletas:

–Caramelización: Reacción producida por la descomposición de azúcares a altas temperaturas. En función de su estructura química de los azúcares, la caramelización se produce a diferentes temperaturas: [fructosa110° C, glucosa y sacarosa a 160° C].

– Dextrinación: Reacción de ruptura de las moléculas del almidón por acción de calor del horneado. Esto produce piro-dextrinas, de color marrón. La dextrinización del almidón se produce a temperaturas de entre 100-200° C.

– Reacción de Maillard: Reacción química compleja (pardeamiento no enzimático) generada por la reacción de las proteínas y aminoácidos (por ejemplo entre la leche y los azúcares reductores. Los compuestos finales de la reacción de Maillard son de color oscuro y contribuirán al color final de las galletas.

Todos los procesos de dorado requieren altas temperaturas, y por eso se producen en la etapa de final del proceso de horneado, cuando no se produce más evaporación de agua y la superficie de la galletas seca.

En la actualidad se aplican dos métodos para medir el color:

- *Método Sensorial*: La medición sensorial se realiza mediante un panel de evaluadores entrenados, siguiendo la Norma ISO 11037:2011 (2011)[213], utilizando como patrón de

referéncialas Guías Pantone (2018)[214] olas 300 imágenes patrón correspondiente a la norma española de colores UNE 48-103-94 (UNE, 1994)[215].

- *Método Instrumental:* En la actualidad existen diferentes equipos de análisis de color como los colorímetros, escáneres o cámaras digitales [Sistema Digi Eye (Song & Luo, 2000)[216]] que permiten medir y analizar el color de las superficies de los alimentos utilizando espacios cromáticos uniformes (CIELAB) y no uniformes (CIEXYZ), y representar colores en dos y tres dimensiones (Trussel *et al.,* 2005)[217].

La mayoría equipos digitales utilizan el modelo RGB, en el que la luz reflejada por el objeto se detecta mediante tres sensores, que capturan la intensidad de la luz reflejada en los componentes rojo (R=*Red*), verde (G=*Green*) y azul (B=*Blue*) posteriormente la imagen se analiza digitalmente y transformar las coordenadas RGB mediante modelos matemáticos, obteniéndose sus coordenadas correspondientes de los espacios cromáticos CIELa* b*.Este sistema describir cuantitativamente el color usando tres variables: L*, a* and b* (CIE, 2004[218], HunterLab, 2001[219]). Coordenadas L * describe la luminosidad de una muestra, ubicación en el eje central desde el negro al blanco (0-100 respectivamente) y el color definidos por las coordenadas a * (valores negativos verde y positivos rojo) y b * (valores negativos azul y positivos amarillo) azul a amarillo, respectivamente (HunterLab, 2001). El Chroma, C *, de las muestras puede ser expresado como una raíz cuadrada de los cuadrados de las coordenadas a * y b * ($=\sqrt{a^{*2}+b^{*2}}$) (CIE, 2004)[266] Croma (C *) es el atributo que permite cuantificar el grado de diferencia en comparación con un color gris de la misma ligereza que se determinará para cada tono, por lo que se considera el atributo cuantitativo de colorido.

Entre los modelos matemáticos empleados, los de mayor precisión son el “método de redes neuronales” y el “modelo cuadrático”, mientras que los “modelos gamma y directo” resultan más rápidos para el cálculo (León *et al.,* 2006)[220].

5.9.5. Análisis Sensorial

5.9.5.1. Contexto histórico del análisis sensorial

Desde las sociedades cazadoras-recolectoras hasta la actualidad, a la largo de la evolución humana nos hemos servido de los sentidos para juzgar la calidad y seguridad de los alimentos de las que nos nutríamos, evitando aquellos que por su sabor nos alertan de su salubridad. Pero ineludiblemente siendo la única especie que elabora y cocina sus alimentos hemos adaptado nuestras tecnología culinarias a nuestro percepción sensorial de los mimos, aparte que el tratamiento culinario de los alimentos nos permitió aumentar su seguridad (eliminando bacterias, y compuestos tóxicos) y valor nutritivo (por ejemplo los almidones del arroz no son digeribles si no cocinan).

A lo largo de la historia ha quedado constancia escrita de que cada sociedad ha dejado su impronta en el gusto en función de los alimentos a los que tenía acceso, pero como la humanidad la gastronomía y el gusto que la rige han cambiado, por lo que platos delicioso hace siglos hoy no serían aceptados, ni aceptables en la sociedad actual. Un ejemplo seria el *garum* romano preparada a base de vísceras fermentadas de pescado) que era tan exclusivo que solo las clases altas de la sociedad podían consumirlo. La función gastronómica del *garum* era la de aumentar el sabor de los alimentos sin tener añadir sal a los platos, y lo que hoy sería un potenciador de sabor como el glutamato mono sódico (Gonzzini Giacosa, 2004)[221].

Pero hacia el siglo XIV, aparecerían las primeras asociaciones profesionales de catadores de vino en Francia, haciéndose ya la referencia del término “degustador” como la profesional dedicado a catar vino para definir su calidad organoléptica y fijar un precio justo al producto. Pero se tuvo que esperar hasta el año 1825, cuando se concibe la primera obra relacionada con el análisis sensorial por parte del gastrónomo francés Jean-Anthelme Brillât-Savarin[222] “*La fisiología del gusto o meditaciones de la gastronomía*”.

Finalmente, el análisis sensorial de los alimentos, como lo conocemos en la actualidad, surge durante la Segunda Guerra Mundial 1939-1945 (Stone & Sidel, 2004)[223] cuando la industrialización de los procesos productivos de las raciones de las fuerzas armadas americanas requiere, que aparte de los parámetros físico-químico, microbiológico de los alimentos también se

controlen sus aspectos organolépticos, como su similitud a la receta original y su calidad organoléptica se mantenga durante toda su vida útil (Jones *et al.,* 1955)[224].

Pero habrá que esperar hasta finales de la década de los cuarenta cuando de la mano de los expertos de cada especialidad: vinos (Amerine, 1948)[225]; sodas y cerveza (Beattie, 1949)[226]; dulces (Cameron, 1947)[227]; whisky (Pereyan, 1947)[228] desarrollaron las primeras normas y técnicas para la medida y control de la calidad sensorial de los productos, siendo lo atributos primarios como aspecto, sabor y textura los que definirán la calidad sensorial del producto. Durante el periodo postguerra es cuando se publican las primeras técnicas de pruebas sensoriales, pruebas de diferenciación (Peryam & Swartz, 1950)[229], como el test triangular (Byer &Abrhams, 1953[230], Rosseler *et al.,* 1948[231]), test consumidores (Ishler *et al.,* 1955[232]) y panel sensorial (Krum, 1955[233], Nair, 1950[234]). También se sentaron las bases del tratamiento estadístico de los datos obtenidos para la obtención de resultados significativos (Wood, 1948[235], Rao, 1950[236], Bradley *et al.,* 1953[237], Fisher & Yates, 1953)[238].

Finalmente en el año 1971, Tilgner[239]en su retrospectiva del análisis sensorial lo define como "el conjunto de técnicas de evaluación, que a través de uno o más sentidos permiten determinar, clasificar y cuantificar las propiedades de los alimentos".

En la actualidad el análisis sensorial tendría un carácter científico y estaría regulado y normalizado por normas de estandarización específicas (UNE-ISO, 1986)[240]. Haciendo del análisis sensorial un instrumento muy útil y de amplio campo de aplicación (desde productos de alimentación, productos de higiene personal (cuánto dura en aclararse un champú del pelo), hasta la automoción (olor a nuevo de los coches)).

5.10. Productos saludables y funcionales en el sector de las galletas

El Reglamento 1924/2006, sobre declaraciones nutricionales y propiedades saludables en los alimentos en vigor desde el 1 de julio de 2007, establece que podrá declararse un alimento como "fuente de fibra" sólo cuando tenga un aporte mínimo de 3 gramos de fibra cada 100 gramos (o, en otra unidad de medida, 1,5 gramos cada 100 Kcal). Podrá decirse que el alimento posee "Alto contenido en fibra" cuando aporte un mínimo de 6 gramos de fibra cada 100 gramos, o de 3 gramos cada 100 Kcal. Los alimentos no podrán atribuir propiedades de salud, a un alimento enriquecido

con fibra, a menos que sus beneficios estén demostrados mediante estudios científicos y sean aceptados por las autoridades pertinentes.

La industria galletera consciente de las connotaciones perjudiciales para la salud inherentes a sus productos (elevada densidad energética, concentración de azúcares simples y grasas trans), los últimos años ha intentado mejorar el perfil nutricional de sus formulaciones siguiendo las directrices de la estrategia NAOS (AESAN, 2005)[241], e incluir ingredientes funcionales que aporten beneficios "extra" para la salud. El enriquecimiento o incremento de funcionalidad de los alimentos debe ir acompañado de mejoras generales en contenido calórico total, reducción de azúcares simples, grasa saturadas y grasas trans e incluir lo delimitado a efectos normativos por el establecimiento de los llamados "perfiles nutricionales" recogidos en el Reglamento 1924/2006[242] sobre declaraciones nutricionales y de propiedades saludables en los alimentos. Por lo que estos ingredientes deben encontrarse en las proporciones requeridas para aportar una cantidad significativa para poder ejercer su efecto beneficioso. Por tanto, es importante verificar que el enriquecimiento sea significativo y el ingrediente biodisponible, porque de lo contrario el alimento no ofrecería un ninguna ventaja al consumidor, comparado con el original e induciría a un error de elección en el consumidor. Pero también se debe evitar posibles sobredosificaciones de determinados nutrientes (como las vitaminas liposolubles perjudiciales para la salud en altas dosis, como la vitamina A), teniendo en cuenta otras fuentes de la dieta para el mismo oligoelemento y el estado nutricional de la población objeto. Los aspectos de seguridad en enriquecimiento con vitaminas, minerales y otras sustancias han sido regulados por el Reglamento 1925/2006 del Parlamento Europeo y el Consejo[243]en paralelo con el Reglamento de declaraciones nutricionales y de propiedades saludables en los alimentos.

En los últimos años las galletas con alto contenido en fibra se han constituido como uno de los uno de los sectores de la alimentación funcional más estable, debida a que la población occidental trata de consumir productos que le aporten la fibra, que su ritmo de vida y alimentación actual no le aporta (Glanz & Kolonel, 1998[244], Mongeau *et al.,* 1989[245], Bes-Rastrollo *et al.,* 2006[246], Slavin, 2005[247]). Por lo que enriquecer productos alimenticios, como galletas, con nuevas fuente de fibra alimentaria obtenidas de subproductos vegetales puede ser una buena alternativa para completar las necesidades de fibra de la población mediante el consumo de un producto cotidiano (Guillon *et al.,* 2012)[248]. Además de rentabilizar la gestión ambiental de estos subproducto y reducir

agravamiento de las enfermedades que sufren poblaciones de países industrializados por falta de consumo de fibra como: cáncer gástrico, diabetes, estreñimiento, obesidad, síndrome de colon irritable etc. (Zhang *et al.,* 2013[249], McEvoy & Woodside, 2015[250] , Brownlee, 2011[251], Jaakkola *et al.,* 2013[252] , Reicks *et al.,* 2014[253], Pezdirc *et al.,* 2015[254] , Aller *et al.,* 2004[255]). Tecnológicamente, el empleo de estas fibras ayuda a espesar formulaciones y reducir el contenido calórico por reducción o sustitución de grasa sin renunciar al sabor y la textura característicos (Laguna *et al.,* 2011)[256]. Por su simplicidad y bajo coste, la tendencia general de las maracas de galletas en España ha sido la sustitución de harinas refinadas por harinas integrales, procedentes de la molienda de granos enteros y el enriquecimiento con fibras solubles de efecto prebióticos

Fibras Insolubles:

La fibra de salvado de trigo de las harinas integrales es por antonomasia la fibra insoluble más utilizada, ya que industrialmente sólo hay que sustituir la harina refinada por harina integral. Popularmente el salvado de trigo se asocia a una prevención del estreñimiento, al disminuir el tiempo de tránsito intestinal, y a aumentar la sensación de saciedad.

Fibras Solubles:

Las fibras solubles se obtienen principalmente, como subproductos de procesos industriales destacando entre todas la Inulina y la oligofructosa. La funcionalidad de estas fibras consiste en aumentar le aumentar el volumen y consistencia del bolo fecal, previniendo el estreñimiento, por su alta fermentabilidad por flora intestinal beneficiosas.

Extractos vegetales

Recientemente se ha investigado con éxito aplicación de algunos antioxidantes naturales para productos de panadería, tanto por su potencial efecto beneficioso para la salud como por su efecto inhibidor delos procesos de oxidación durante el almacenamiento. Empleándose principalmente polifenoles procedentes de plantas (manzana, té verde, boniato, Moringa, frambuesa), especias (canela, cardamomo, clavos, jengibre, anís, romero, comino negro, curry, cúrcuma, cilantro, salvia) y cereales (amaranto, harina de avena, extractos de salvado de arroz, trigo sarraceno) [Bhanger *et al.,* 2008[257], Vergara-Valencia *et al.,* 2007[258], Bialek *et al.,* 2014[259] , Mildner-Szkudlarz *et al.,*2009[28], Abdel-Samie *et al.,* 2010[260], Huang & Li, 2011[261], Badei *et al.,* 2002[262],

Nanditha *et al.,* 2009[263], Basuny *et al.,* 2012[264], Ishida *et al.,* 2003[265] , Reddy *et al.,* 2005[28], Isobe *et al.,* 2004[266], Kozlowska *et al.,* 2014[267]].

También se han utilizado, extractos de plantas silvestres (Reddy *et al.,* 2005)[28], hojas de té (Sharma & Zhou, 2012)[29], hasta polen de abeja (Krystyjan *et al.,* 2015)[30]. Sin embargo, debido a que los extractos de plantas tienen que ser inmovilizados para aumentar su funcionalidad y facilitar su incorporación y mezcla en la masa de las galletas, otros autores utilizaron fibras vegetales con efecto antioxidante propio, obtenidas de subproductos vegetales. Por ejemplo, subproductos de: manzana y avena (Vitali *et al.,* 2009)[31], mango (Ajila *et al.,* 2007)[32], vainas de guisante (Raymundo *et al.,* 2014)[33] y naranja (Larrea *et al.,* 2005)[34].

Subproductos vegetales

Debido a que los subproductos vegetales tienen una rica composición en compuestos antioxidantes, los fibre rich powder obtenidos de los subproductos vegetales pueden ser una buena alternativa para enriquecer alimentos con compuestos antioxidantes, que a su vez puedan tanto reducir procesos de rancidez u oxidación de grasas durante el almacenamiento de las galletas (Talbot, 2010)[9], como ser un complemento a la dieta en estos nutrientes. En la bibliografía se encuentra distintos estudios de obtención de fibre rich powder empleando subproductos de distintos productos de origen vegetal (O'Shea *et al.,* 2012[11], Elleuch *et al.,* 2011[268]). Chantaro *et al.,* (2008)[15] extrajeron fibre rich powder con alta actividad antioxidante de piel de zanahorias, Nilnakara *et al.,* (2009) [17]de las hojas externas del repollo (cabbage), Lecumberri *et al.,*(2007)[18] extrajeron fibra de los subproductos de la elaboración del cacao Pérez Jiménez *et al.,*(2008)[17] y Zhu, *et al.,*(2015)[20] de semillas de uva, Nandi & Gosh (2015)[22]cascara de semillas de sésamo, Fuentes-Aleventosa *et al.,*(2009)[3] de subproductos de esparrago, López-Vargas *et al.,*(2013) [269] subproductos de la elaboración de zumos de frutas: fruta de la pasión, Amaya-Cruz *et al.,* (2015)[270], Navarro-González (2011)[16] de subproductos de tomate (piel) y Garau *et al.,* (2007)[271] de subproductos de naranja.

Pero con respecto al presente estudio de elaboración de galletas con fibre rich powder antioxidantes podemos destacar los estudios de Vitali *et al.,* (2009)[31] que utilizan fibra de subproductos de manzana y Ajila *et al.,* (2007)[32] que utilizan subproductos mango y Fernández *et al.,* (2016)[272] que utilizan hojas de remolacha.

JUSTIFICACIÓN Y OBJETIVOS

Los subproductos vegetales tienen una gran cantidad en compuestos funcionales y bioactivos, de gran utilidad nutracéutica para el desarrollo de alimentos funcionales. En la actualidad, tras la transformación industrial de productos vegetales, los subproductos vegetales generados (60-75 %) del producto procesado, (Fonollá & Boza, 1993)[273] se revalorizan únicamente como forraje y compost. Pero, existe una fracción importante que se dirige a vertedero, dificultado su gestión y sostenibilidad medioambiental. Para remediar el impacto ambiental y aumentar la rentabilidad económica de los procesos de minimización de residuos orgánicos, la Directiva 1999/31/EC del Consejo Europeo relativa a los residuos de vertedero requirió a los Estados miembros reducir al 65 % los niveles los residuos orgánicos biodegradables en vertederos generados en 1995 (EC, 1999) e impulsar medidas de optimización y revalorización subproductos vegetales. Por ejemplo: mediante la formulación de productos funcionales con fibra, antioxidantes u otros compuestos promotores de la salud para el consumidor.

En los últimos años, la preocupación de los consumidores por llevar una vida y alimentación más sana y equilibrada ha derivado en un incremento notable del consumo de productos dietéticos, funcionales, enriquecidos, etc. Concretamente, el mercado de las fibras alimentarías se ha visto especialmente favorecido con el desarrollo de nuevos productos, con un alto contenido de fibra alimentaria. Su éxito ha radicado en los múltiples beneficios que una dieta rica en fibra supone para la salud y a que estos productos “enriquecidos en fibra” o “integrales” ayudan a complementar fácilmente la ingesta diaria recomendada de fibra, de 25 y 30 gramos de fibra/día, en la que la dieta occidental es deficiente al ser ésta muy rica en proteínas, harinas refinadas, etc.

A parte de la fibra soluble e insoluble obtenida de los subproductos de alcachofa, diferentes estudios científicos, expuestos a lo largo de esta tesis, han demostrado que de diferentes partes de la alcachofa (*Cynara scolymus* L.) se pueden obtener compuestos funcionales de fácil aplicación industrial, como las polifenoles y compuestos antioxidantes que pueden jugar un rol importante en el enriquecimiento de alimentos.

Composición de interés de los subproductos que justifican esta investigación

En la actualidad existe una gran demanda por fuentes alternativas de antioxidantes debido a la preferencia de los consumidores y los problemas de salud asociados con el uso de antioxidantes sintéticos como BHT, BHA (Oyeneho & Hettiarachchy, 1993[274], Azizah *et al.,* 1999[275]) y los sub-productos y productos de rechazo dela alcachofa (*Cyrana scolymus* L.), podrían juagar un papel importante al ser fuentes de compuestos polifenólicos antioxidantes (Kukic *et al.,* 2008)[276].

Las variables influyentes en composición fenólica final y en el límite máximo de extracción de los subproductos están relacionados con:

- El contenido total de los compuestos polifenólicos de la plantas varía entre diferentes variedades, la edad, la madurez de la planta, las condiciones de cultivo, las condiciones de cosecha y post-cosecha y tipo de almacenamiento los procedimientos tecnológicos utilizados (Tomás-Barberán & Espín, 2001[277], Pandino, 2011[a175]). La intensidad de los procesos de fabricación puede producir un aumento del poder antioxidante de los distintos compuestos fenólicos durante el almacenamiento y procesamiento de alimentos (Titchenal & Dobbs, 2004)[278].Por ejemplo, durante el proceso de escaldado se eliminan enzimas que causan la oxidación de compuesto fenólicos (Smith *et al.,* 2005[279], Ferrance *et al.,* 2008[280]) como la polifenoloxida o PPO. Sin embargo, la degradación química aún puede ocurrir durante el almacenamiento, dependiendo el oxígeno disponible y la exposición a la luz.
- Los compuestos fenólicos también son solubles en agua, haciéndolos susceptibles a la lixiviación, produciendo mermas durante los procesos de escaldado. Dichas pérdidas son admisibles ya que disminuyen la acción de la PPO, elimina el aire ocluido, y por diferencias de presión se producen rotura de tejidos, ayudando a la acción de los líquidos de extracción. Debido a la hidrosolubilidad de los compuestos fenólicos varios investigadores han reportado erróneamente importantes descensos en contenido polifenoles totales (TP) causados por tratamientos térmicos, pero la evidencia sugiere que la disminución en su contenido se debe en gran parte a la lixiviación en el líquido de gobierno, en lugar de oxidación (Chaovanalikit & Wrolstad, 2004)[281]
- Tiempo de almacenamiento antes del procesado: TP de los vegetales disminuye con el tiempo de almacenamiento. Un estudio con brócoli (Vallejo *et al.,* 2003)[282] concluye que después de un periodo de 10 días de almacenamiento, simular al tiempo máximo en transporte y distribución (7

días) y el tiempo venta (3 días). se pierden grandes cantidades de compuestos fenólicos. Los autores informaron pérdidas de hasta el 78 % de derivados del ácido sinópico, flavonoides totales y derivados del ácido cafeíco.

Interés tecnológico-nutracéutico de los subproductos de alcachofa

La alcachofa presenta una composición nutritiva que se caracteriza por un elevado contenido en ciertos minerales como fósforo, sodio y sobre todo manganeso (20 mg/100 g de producto comestible), mayor que cualquier hortaliza o legumbre. Su contenido de vitaminas no es particularmente elevado; sin embargo, es un alimento de relativo valor por poseer una menor cantidad de agua y un mayor contenido de carbohidratos y proteínas que la mayoría de las hortalizas.

Como nutrientes, contiene proteínas, minerales, una baja cantidad de lípidos, fibra dietética y destaca por una alta proporción de compuestos fenólicos (Fratianni *et al.*, 2007)[283]. Los fenoles incluyen cinarina (ácido 1,3-di-O-caffeoilquinico), luteolina, cinarosido (luteolin-7-O-glucósido), (luteolin-7-rutinosido); ácidos fenólicos como: el cafeíco (Tomás-Barberán *et al.*, 2000)[284,] hidroxicinámico, hidroxicinámico, ferúlico, cafeoilquinico (Lattanzio *et al.*, 1994)[285].También se incluyen derivados del ácido; ácidos mono y di cafeoilquinico, siendo el ácido clorogénico (5-O cafeoilquinico) el más importante de estos derivados incluyendo ácido clorogénico; alcoholes ácidos; glucósidos (Aubert & Foury, 1981)[286], flavonoides, entre otros (glucósidos y rutinósidos) (Sánchez Rabaneda *et al.*, 2003)[287].

Su interés se basa en el uso de subproductos alcachofa como fuentes de polifenoles y compuestos antioxidantes y fibra:

1º El gran volumen de subproductos generados por las industrias conserveras y congeladoras en toda Europa.

2º El gran contenido en fibra, principalmente inulina, polifenoles y antioxidantes hacen a los subproductos de alcachofa un alimento funcional per se, desaprovechado industrialmente. Siendo junto a soja y arándanos la fuentes más ricas de antioxidantes dietéticos fenólicos, mostrando una capacidad antioxidante total de más 9.000 μmol de TEAC (capacidad antioxidante equivalente al

Trolox)/100 g de peso fresco (Pennington & Fisher, 2009)[288], aunque otras fuentes como el "Explorer polyphenol database" la determinaría en 400 mg Eq de ácido gálico/ 100 g de producto (Perez-Jimenez *et al.*, 2010)[289].Respecto a su composición de fibra la alcachofa tiene un alto contenido en fibra, principalmente inulina, (19-36 % del peso seco) Lattanzio *et al.*, (2009)[44], de alta funcionalidad tecnológica y nutricional.

3º Los compuestos polifenólicos presentes en la composición de la alcachofa toleran tratamientos térmicos altos. Por tanto tienen un potencial uso en la formulación funcional-tecnológica de sus productos tratados con calor ya que los polifenoles pueden mantener su poder antioxidantes después de tratamientos térmicos elevados, pudiendo sustituir o ayudar a la acción antioxidante de vitaminas termolábiles. Las principales vitaminas antioxidantes hidrosolubles C y B1, utilizadas para prevenir procesos de oxidación son especialmente sensibles a los tratamientos térmicos como pasteurización y esterilización. Por ejemplo el Ácido ascórbico (vitamina C) se utiliza generalmente como un marcador de degradación de nutrientes. Mientras que las vitaminas liposolubles como la Vitamina E y A, junto con otros carotenoides, están menos afectados por tratamientos tales como cocción, y pasteurización. También son susceptibles a la oxidación.

OBJETIVOS

El **objetivo principal** de esta Tesis Doctoral ha sido el estudio de la revalorización de los subproductos de la industria conservera de alcachofas como alternativa a la fibra comercial de referencia (fibra guisante) y su utilización en productos derivados de cereales como las galletas digestive. Para alcanzar este objetivo principal se plantearon los siguientes

Objetivos específicos

1º Extracción y caracterización de la fibra de alcachofa a partir de los subproductos del procesado de conservas de alcachofa.

2º Elaboración de galletas con fibras de alcachofas extraídas de los subproductos de alcachofas.

3º Determinar la textura para el control de calidad de galletas con alto contenido en fibra.

4º Determinar las características funcionales y sensoriales de las galletas elaboradas con fibras de alcachofas.

CAPÍTULO 1: EXTRACCIÓN Y CARACTERIZACIÓN DE LA FIBRA DE ALCACHOFA

CAPÍTULO 1: EXTRACCIÓN Y CARACTERIZACIÓN DE LA FIBRA DE ALCACHOFA

En este capítulo se presentan los estudios realizados para la obtención del primer objetivo de esta Tesis Doctoral: Obtención y Caracterización de subproductos de alcachofa.

- Obtención de subproductos de alcachofa empleando para su extracción distintos disolventes y estudiar el posible efecto que tienen sobre sus cualidades funcionales-tecnológicas de la fibra ya que puede repercutir en su posterior aplicación y viabilidad industrial. Para ello se comparará con los "fibre-rich powders" de guisante que son el más empleado en la actualidad.
- Para la caracterización de los "fibre-rich powders" obtenidos se determinaron: la composición en fibra (soluble e insoluble y total), la capacidad de retención de agua (WHC), la capacidad de retención de aceite (OHC), la solubilidad (SOL), la humedad (M), el Hinchado (SWI) y el color.
- Para la evaluación de las cualidades funcionales se determinaron: la capacidad de retardo del índice de la glucosa (GDRI), los polifenoles totales (PPT) y su capacidad antioxidante (por distintos métodos: ABTS.+, DPPH. y FRAP).

Se hallaron diferencias significativas ($p<0.05$) en los parámetros funcionales como: La WHC y OHC, de los "fibre-rich powders" obtenidos en función del disolvente del método extracción empleados. Los subproductos de alcachofa, en general presentan mejores cualidades funcionales: capacidad de retención de agua (WHC), capacidad de retención de aceite (OHC) e hinchado (SWI) que la fibra comercial de referencia (guisante).

Se observó que el método de extracción de fibra a partir de los subproductos de alcachofa influye considerablemente en las propiedades funcionales: índice de retardo de la glucosa, capacidad antioxidante y contenido en polifenoles. De todos ellos, el mejor método es el secado directo por su sencillez, además es el más viable económica e industrialmente, y es el que proporciona un mayor índice de retardo de la glucosa y tiene una mayor capacidad antioxidante y contenido en polifenoles. Todos los "fibre-rich powders" obtenidos de subproductos alcachofa independientemente del método utilizado poseen mayor GDRI, una mayor concentración de polifenoles totales y mayor capacidad antioxidante que la fibra comercial de guisante.

En conclusión, la obtención de “fibre-rich powders” a partir de subproductos de alcachofa supondría una revalorización de dichos subproductos, una reducción de contaminación medio ambiental y una buena alternativa al empleo de fibras vegetales tradicionales, como las fibras de guisante.

1.1.-Characterization of fibre-rich powders extracted from artichoke (*Cynara scolymus* L.) by-products

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ABSTRACT

Factory processing artichoke for food generates by-products that are approximately 70 % of the initial raw material. These by-products are primarily used as animal feed with minimal economic revaluation. Usually those by-products are only used as animal feed, being minimum its economic revaluation.

Artichoke composition is notable for high fibre and antioxidant compounds. If by-products from processed artichoke have a fibre content comparable to that of artichoke edible parts, then efficiently extracting fibre from by-products benefits formulation of fibre enriched novel food products.

The aim of this work is to obtain fibre-rich powders from artichoke by-products, using dry and wet extraction techniques, and select the most industrially efficient method. Functional and

technological properties including, fibre composition (soluble and insoluble and total), water holding capacity (WHC), oil holding capacity (OHC), water solubility Index (WSI).

1. **INTRODUCTION**

European population consumes more fibre enriched products, because their current diet does not fulfill the daily minimum intake (Bach Knudsen 2001, Martínez-González *et al.,* 2002). Numerous studies confirm that vegetable fibre is beneficial to health, preventing diseases such as: gastrointestinal disorders, duodenal ulcer, constipation, hemorrhoids, Type II diabetes, obesity and cardiovascular diseases (Afaghi *et al.,* 2015, Vitaglione *et al.,* 2008) kidney stones (Sorensena *et al.,* 2014).

Scientific studies conducted on artichoke fibre Lattanzio *et al.,* 2009, prove artichokes have a number of beneficial health properties such as: antioxidant, anticarcinogenic and antibacterial power, diuretic, bile segregation properties, and inhibition of both biosynthesis of cholesterol and LDL oxidation.

Other authors have shown that extracted inulin from artichoke increases the gastrointestinal absorption of calcium, magnesium and iron (Azorín-Ortuño *et al.,* 2009), effects the diffusion of glucose in human blood, and reduces the level of cholesterol and serum lipids (Coudray *et al.,* 1999, Niness, 1997).

The content of dietary fibre of the artichoke is high; singling out its high content of inulin, which can be considered the most important industrial and functional composite as a prebiotic compound. Inulin is within the artichoke's composition as a form of energy reserve by carbohydrates (Muzzarellim*et al.,* 2012). The unique aspect of the structure of the inulin is its links β -1, 2, which prevent the inulin to be digested as a typical carbohydrate, causing its prebiotic function, and reducing its caloric value (López-Molina *et al.,* 2005), compared with other carbohydrates, while controlling satiety (Morris & Morris, 2012). On the other hand, new investigations have found other applications for extracted inulin, such as: reagents for diagnosis, drug carriers and its use as an anti-cancer agent (Hughes & Rowland, 2001, Chourasia & Jain, 2003).

During the extraction of the hearts of artichokes, in the processing factories of canned artichoke hearts, the processed 70 % by weight are by-products of artichoke. Usually those by-products are only used as animal feed, being minimum its economic revaluation. Assuming that the by-products have a composition similar to the edible part of the artichokes, these by-products can be a

promising source of new value added compounds such as phytochemicals and fibre (both soluble and insoluble) (Ruiz-Cano *et al.*,2014).

Note that recent publications of revaluation indicate that vegetable by-products of the canning industry (asparagus (Nindo *et al.*, 2003, Fuentes-Alventosa *et al.*, 2009), chard, cardoon, green beans, etc. (Randhawa *et al.*,2015) have good functional qualities, such as Water or Oil holding capacity, and high content of antioxidant compounds (polyphenols, etc.) and fibre (soluble and insoluble); All of these qualities are in high demand by manufactures of enriched products (such as biscuits, meat, dairy products) who eventually could select vegetal fibre-rich-powder over other fibres extracted from grains (Rodríguez *et al*., 2006), rice (Gula *et al.*,2015) or tubers or roots as (Fibrex® (sugar beet), Raftilosa® (chicory) with less functional qualities.

There are no commercial products of food fibre-rich powders of artichoke, although it would be beneficial to study their possible industrial application. The aim of this work is to obtain food fibre-rich powders from artichoke by-products, using dry and wet extraction techniques and selecting one, based on the functional and technological properties, which is most feasible for a posterior industrial application.

From an environmental perspective the revaluation of vegetable by-products is critical because it increases the economic viability of waste minimization processes. In December 2014, the European Commission decided to withdraw a pending legislative proposal and annex to review recycling and other waste-related targets in the EU Waste Framework Directive 2008/98/EC. The proposal responded to the Member States' legal obligation to review their waste management targets of Directive 1999/31/EC on the landfill of waste requiring them to reduce the organic biodegradable waste in landfills (comparing with 1995 levels) by 65 % before 2016, ensuring "high quality recycling and the use of recycled waste as a major, reliable source of raw material for the Union".

2. MATERIALS AND METHODS

2.1. Artichokeby-products

Samples of artichoke by-products, (*Cynara Scolymu*s L.*)* variety (Blanca de Tudela), were obtained from the canning company GVTARRA S.A. (Villa Franca, Navarra, Spain). The vegetable by-product, mainly external bracts and peduncle obtained by the blanching process and core extraction, were sent to our laboratory between 2-4 hours after its production. The by-product was centrifuged once, removing the remains of blanching water, and stored in polypropylene bags by lots of the same weight (500g). By-product was stored frozen to -14 °C, until fibre extraction and chemical analysis were done. All analyses were carried out in triplicate.

2.2. Extraction methods

Prior to extraction, the frozen samples of artichoke by-products (500g) were kept at room temperature for 6 hours, and artichoke by-products were centrifuged again in order to eliminate the leachate caused by freezing.

The dry extraction was performed using conventional air drying process with a convection oven (Zanussi), at a constant temperature of 65 °C for 6 hours or till a constant weight. Then aliquots of 50 g of dry by-products were ground using a coffee grinder (Solac MC6250 110W) at maximum speed for 5 minutes.

The method employed by Fuentes-Alventosa *et al.,* (2009) for the extraction of dietary fibre from asparagus was used in the wet extraction trial with some modifications. In this study, three different extraction solvents were used: 98 % ethanol (AL), water distilled (W), and a dissolution of 1 % (w/w) $CaCl_2 * 5H_2O$ (pH: 6.5) in distilled water (CA); using a proportion of 1 liter of solvent per 1 Kg of frozen by-products of artichoke (-10 °C). Each solvent extraction method was performed in triplicate. The wet grinding was performed using a 2 L Blender (Hallde, Germany) by dividing the load into four batches of 5 minutes each at maximum speed, renewing the solvent in each batch. After extraction, the solute was wrapped in absorbent paper for degreasing, and part of the absorbed solvent removed by compression. Then, in order to compare the effect of those extraction solvents on the functional properties of the liquid extracted by-products of artichoke it was performed the same setting used for the direct dry extraction, sequentially the different solutes were dried in a convection oven (Zanussi), at a constant temperature of 65 °C for 6 hours or till

the dried solute reached a constant weight. Then aliquots of 50 g of dry by-products were ground using a coffee grinder (Solac MC6250 110W) at maximum speed for 5 minutes.

2.3. Physicochemical Analysis

The fibre-rich powders were characterized performing the following analysis: soluble, insoluble and total fibre, moisture determination, water holding capacity (WHC), oil holding capacity (OHC), swelling water capacity (SWC) and water solubility index (WSI). All analyses were made in triplicate.

2.4. Dietary fibre determination

Aliquots from dry and wet extraction of the fibre-rich powders samples were analysed using Lee *et al*., 1992 method, obtaining soluble, insoluble and total fibre composition. In triplicate, approximately 1 g of a sample was suspended in 40 mL MES–TRIS buffer, submitted for enzymatic hydrolysis sequence: 50 μL of thermos-resistant α-amylase in water bath for 35 min, and 100 μL of protease in water bath at 60 °C ± 1 °C for 30 min. The pH was corrected to a range of 4.0–4.7, and 300 μL amyloglucosidase was added and placed in water bath at 60 °C ± 1 °C for 30 min. After digestion, insoluble fibre (IF) was recovered by filtration on a sintered glass crucible (nº. 2), washed with distilled water and dried overnight at 100 ºC (ash and protein were corrected during this step). Four volumes of hot ethanol were added to filtrates and the suspensions of soluble fibre (SF) were left overnight at 4 ºC to allow SF to precipitate. Afterwards, SF was recovered by centrifugation (20 min and 2.500rpm), dissolved in distilled water, and freeze-dried.

2.5. Moisture Determination

The method of Nollet, 1996 was used with slight modifications to determine moisture. The different samples of fibre-rich artichoke (250 mg) powders were weighed on an analytical balance (Mettler Toledo. USA) and dried in a lab oven until constant weight (24 h. at 90 °C).

2.6. Water and Oil holding capacity (WHC, OHC) and water solubility index (WSI) Determination

The method described by Jiménez *et al.,* (2000) was used for WHC and WSI: 250 mg of different samples of fibre-rich artichoke by-products powders were suspended in 15ml of distilled water,

agitated (model Reax-Top, Selecta, Spain) 24 hours at 120 rpm at room temperature, then the suspension was centrifuged (model Magnus R, Ortho-Alresa, Spain) at 3500 rpm for 1 hour at room temperature. Once the supernatant was removed carefully, the solute **S1** (hydrated dietary fibre) was weighed to express the WHC as g water / g fibre. The supernatant was incubated in a water bath at 80 °C for 30 min. and centrifuged at 3500 rpm for 1 hour at room temperature, obtaining a second weighed solute (**S2**). The supernatant was collected carefully in a heavy dish and dried, using a muffle hoven at 103 ºC ± 2 °C, until total evaporation (24h). The obtained residue (S3) was weighed. WSI was calculated by applying the following formula: WSI (%) = (**S3**–**S2**) / **S1** × 100 %.

For the determination of OHC, 250 mg of different samples of fibre-rich artichoke by-products powders were suspended in 15 mL sunflower oil (ρ: 1.0053 g/mL), shaken, on a horizontal Shaker (model Reax-Top, Selecta, Spain) 24 hours at room temperature, the suspension was centrifuged (model Magnus R, Ortho-Alresa, Spain) at 3500 rpm for 1 hour at room temperature. The supernatant was carefully removed and weighed from hydrated dietary fibre. The OHC is expressed as mL oil/g fibre.

2.7. Swelling water capacity (SWC)

The SWC was determined using the method of Robertson *et al.,* (2000). Weighed accurately, 1 g of al extracted samples by moth extraction method was transferred to a calibrated cylinder and 10 mL of distilled water containing 0.02 % sodium azide was added. After mixing thoroughly, the cylinders were left to stand undisturbed for 18 h at room temperature. The volume of sample was recorded and expressed as final volume / initial volume, SWC = V_f / V_i.

2.8. Colour

Digital image analysis. Digital images were obtained with a scanner with light of cold cathode Epson Perfection V10 Photo (Epson America, USA) with a minimum resolution of 400 dpi (see Figure 1). From each sample a square, with 256 pixels of side, was extracted using Photoshop image processing program 9.0 cs2 (Adobe, USA). The colour values were calculated from the RGB coordinates, calibrating the system with 300 image pattern corresponding to Spanish colours UNITES 48-103-94 standard (UNE, 1994). Correlated the RGB values with the coordinates L *, a * and b * relevant according to the simple quadratic model used by León *et al.*,(2006), those

parameters were calculated by minimum quadratic using the Solver tool of Microsoft Excel 2003 (Microsoft, Excel®). To get fractal dimension, the same algorithm proposed by Valous *et al.*,(2009) was used based on the frequency of Fourier analysis. The realization of these transformations and calculations were carried out with an Application developed in Matlab 7.0 (Mathworks, USA).

2.9. Statistical analysis

Results were expressed as mean values ± standard deviations. The differences between the variables and samples were analysed using ANOVA, one-way and in the event of significant differences ($p < 0.05$) and the LSD test. Statistical analysis was performed using the software Statgraphics Centurion XVI 16.1.03 version (Statpoint Technologies Inc.).

3. RESULTS AND DISCUSSION

The revaluation of the by-products of the artichoke can be very beneficial from an environmental and technological point of view. Artichoke by-products are a good source of fibre; hence to prove their industrial application and production; it is necessary to understand what changes in composition and functional qualities are caused by the extraction solvents, drying and grinding.

Content of soluble and insoluble fibre has been studied comparatively for effect of dietary fibre content, from fibre-rich powder produced by direct drying of the by-products and its content after wet grinding, caused by the three studied solvents (distilled water (W), with 98 % Ethyl Alcohol (AL), and 1 % (w/w) $CaCl_2 * 5H_2O$ (CA)), and using a reference commercial (Pea fibre, P). Table 1 shows the content of dietary fibre total (TDF), insoluble fibre (IF), soluble fibre (SF) and its ratio (IF/SF) for fibre-rich powders obtained from different processes.

Table 1. Dietary fibre fractions of different fibre-rich powder from artichoke by-products (% dry matter).

Sample	IF % dry matter	SF% dry matter	TDF% dry matter	IF/SF
Direct Drying	$49{,}91 \pm 1{,}12^{b}$	$9{,}51 \pm 0{,}93^{c}$	$59{,}44 \pm 2{,}15^{b}$	$5{,}25 \pm 1{,}12^{a}$
W	$51{,}74 \pm 2{,}24^{b}$	$9{,}82 \pm 0{,}64^{c}$	$61{,}55 \pm 3{,}16^{b}$	$5{,}28 \pm 2{,}62^{a}$
AL	$36{,}90 \pm 3{,}14^{a}$	$4{,}14 \pm 0{,}65^{b}$	$41{,}07 \pm 2{,}95^{a}$	$9{,}00 \pm 2{,}54^{c}$
CAL	$51{,}45 \pm 3{,}24^{b}$	$9{,}05 \pm 0{,}72^{c}$	$60{,}43 \pm 2{,}95^{b}$	$5{,}71 \pm 1{,}64^{a}$
P	$76{,}42 \pm 3{,}21^{c}$	$3{,}61 \pm 0{,}83^{a}$	$80{,}06 \pm 3{,}83^{c}$	$21{,}20 \pm 2{,}47^{b}$

Values are mean ± SD (n= 3); figures in a column followed by different letters differ significantly ($p < 0.05$).IF (insoluble fibre), SF (soluble fibre), TDF (dietary fibre total),

As it can be seen in table 1, the concentration obtained from IF content is not significantly different between two aqueous solvents (51.74 % W and 51.45 % CA), but there are significant statistical differences between aqueous solvent extractions and the alcoholic solvent (36.90 % AL) extractions. Significant differences were found between the aqueous and alcoholic extraction method with the SF composition. Water soluble fibre precipitates coming in contact with an alcoholic solution, produce a significant decrease in the concentration of soluble fibre, increasing the ratio between insoluble and soluble fibre (IF/SF). These results are in agreement with studies of other vegetables (Almazán & Zhou, 1995).

Insoluble fibre concentration obtained by direct drying (49.91 %) is slightly lower than that obtained by aqueous methods (51.74 % W and 51.45 % CA) however, do not present significant differences between those methods. The same results are obtained by comparing the soluble fibre; there is a significant difference between the values in the methods of aqueous extraction W (9.82 %), CA (9.05 %) and direct drying (9.51 %).

The insoluble fibre composition obtained from different fibre-rich powders of artichoke by-products, employing extraction or direct drying, is minor compared to a commercial reference fibre (76.42 % P); and therefore their index insoluble/soluble fibre is significantly higher (21.20 %). This difference between insoluble and soluble fibre can affect the industrial application of this fibre-rich powders; it makes their functionality different for the fibre-rich powders obtained from artichoke by-products by wet extraction or direct drying.

The TDF contents are usually ranked as low (30 to 50 %); intermediate (50 to 70 %); and high (70 to 90 %)(Jiménez-Carballo & Cofrades, 2001, Figuerola *et al.,* 2005, Rodríguez *et al.,* 2006). The current artichoke by-product results correspond to the intermediate rank.

3.1. Functional properties of the fibre-rich powders extracted from artichoke by-products

The fibre-rich powders obtained by wet grinding (W: 6.51 %, AL: 4.66 %, CA: 6.06 %) have lower moisture % than the obtained by direct drying (9.61 %) (Table 2). Comparing the moisture result of the direct drying with the values obtained by wet extraction, it can be observed how using aqueous solvents (W, CA) decreased their initial humidity due to structural damages on the vegetal tissues and to particle size reduction caused by the wet grinding, causing an increase of the contact surface of the fibre rich powders with hot air during the air drying and speeding up water evaporation (Rosell *et al.,* 2009). On the other hand, even lower moisture was found for rich powders extracted using alcohol (AL).Due to fact that during wet grinding, alcohol dehydrates the by-products and during the oven drying, being alcohol more volatile than water, alcohol evaporates faster, making the oven drying more intense.

As it can be seen in Table 2, results obtained for the functional properties of WHC, OHC and WSI extraction methods influence quite differently and cause significant changes in the functional qualities of the fibre-rich powders. For the WHC, it is observed that aqueous extraction methods do not show significant differences among them W (11.07) and CA (10.67), but they differ significantly from the alcoholic extraction AL (15.36), and the direct drying of artichoke by-products (13.21), so there is be a clear relation between the type of extraction liquid used and the WHC values obtained. These WHC results are similar to those obtained for the fibre of asparagus by Fuentes-Alventosa *et al.,* (2006). It can be concluded that the alcoholic solvent extraction (AL) increases the water holding capacity (WHC), as a result of increasing the proportion of insoluble fibre (IF), which has more WHC than the SF. As mentioned in fibre composition for fibre-rich powders, the alcoholic solvent (AL) produces a severe precipitation of soluble fibre (SF) caused by low solubility in alcoholic solvents, increasing the ratio of IF/SF and total insoluble fibre composition.

Comparing the WHC values for all aqueous extraction (W 11.07, CA 10.67) with direct drying (13.2), the latter is significantly higher. There are no significant differences between the concentration of IF, SF, and their IF/SF ratio, and it seems that the use of an aqueous solvent

solution of 1 % (w/w) of $CaCl_2*5H_2O$, does not significantly modify the WHC of the artichoke fibre-rich powders. Changes of the structure in fibre by-products, during the extraction and subsequent drying process explain the differences found in the WHC values, as suggested by other authors (Almazán & Zhou, 1995).

Table 2. Functional properties of different fibre-rich powder from artichoke by-products.

Sample	MOISTURE	WHC	OHC	WSI	SWI
	(W/W)	g water/g powder	g oil/g powder	(% dissolution)	(Vf/Vi)
Direct drying	9,61 ± 0,13 [c]	13,21 ± 0,79 [c]	2,24 ± 0,13 [b]	24,30 ± 2,39 [c]	4,82 ± 0,66 [c]
W	6,51 ± 1,38 [b]	11,07 ± 1,74 [b]	3,92 ± 0,65 [d]	15,36 ± 1,24 [b]	1,94 ± 0,28 [a]
AL	4,66 ± 0,27 [a]	15,36 ± 1,59 [d]	2,19 ± 0,37 [b]	17,59 ± 1,57 [b]	5,34 ± 0,85 [d]
CAL	6,06 ± 1,61 [b]	10,67 ± 1,48 [b]	2,66 ± 0,28[c]	15,39 ± 2,20 [b]	3,15 ± 0,68 [b]
P	6,93 ± 1,49 [b]	4,00 ± 0,41 [a]	1,34 ± 0,16 [a]	13,91 ± 1,58 [a]	2,00 ± 0,16 [a]

Values are mean ± SD (n= 3); figures in a column followed by different letters differ significantly ($p< 0.05$).WHC (water holding capacity), OHC (oil holding capacity), WSI (water solubility index), SWC (swelling water capacity)

Commercial Pea fibre (P) (4.0) has lower WHC than all the artichoke fibre-rich powders, including the higher amount of IF, showing that the internal structure of the fibres has greater effect on WHC than on IF content. For food products which need greater water holding capacity to avoiding syneresis, such as yoghurts or restructured meat, the artichoke fibre-rich powders would be more convenient than the commercial Pea fibre (P).

Other agricultural by-products have lower values than those commented upon above, e.g. rice bran (Abdul-Hamid & Luan, 2000) and cocoa husks (Lecumberri *et al.*, 2007), both with a WHC value of 5 mL water/g fibre. On the basis of these values, the artichoke by-products could be promoted as a modifier of viscosity and texture of formulated products.

OHC is essential for the formulation of products rich in fats, such as confectionery and sauces, where needed to retain fat in the food matrix. Data suggests the fibre-rich powders with higher oil holding capacity (OHC) are obtained by wet grinding; especially using water W (3.92) as aqueous solvent, over other fibre-rich powders obtained by CA (2.66), direct drying (2.24) or employing an alcoholic solvent AL (2.19). CA most likely does not increase OHC as much as W, due to the union of ion Calcium to the structure of the insoluble fibre, reducing the fibre adherence to oil. (Harrington *et al.*, 2001)

Values similar or lower than those for fibre-rich powders of artichoke, e.g. 0.6–1.8 mL oil/g for apple pomace and citrus peel (Figuerola *et al.*, 2005) and around 2 mL oil/g for unripe banana flour (Rodríguez-Ambriz *et al.*, 2008) , along with the WHC, demonstrates that the extraction solvent also modifies the OHC. Compared with all the fibre-rich powders P (1.34) has the minimum oil holding capacity.

In contrast to the previous results, the solubility (WSI) of the artichoke fibre-rich powders obtained by wet grinding, AL (17.59), CA (15.39), and W (15.36), did not present significant differences, so in this case the extraction solvent does not affect the WSI of the artichoke fibre-rich powders obtained by wet grinding. However, there are significant differences between wet grinding extraction and direct drying (24.30), WSI values imply that the direct drying process does not dilute and remove soluble compounds of the by-products, as the wet grinding. Related to P (13.91), WSI is similar to the fibre-rich powders of artichoke obtained by wet grinding, AL (17.59), CA (15.39), and W (15.36). Based on the WSI of artichoke fibre-rich powder obtained from by-products could be a feasible alternative to replace the commercial reference fibre meeting its swelling capacity.

Regarding the SWC, the fibre-rich powders obtained by wet-grinding and alcohol as solvent of extraction has the highest SWC value (AL: 5.34) followed by those extracted with water W and dissolution CA (CA: 3.15, W: 1.94). As it was mentioned previously, the precipitation of the soluble fibre increases the proportion of insoluble fibre, which has more SWC due to its higher WHC. On the other hand, the difference between CA and W could be due to the formation of links between ion Calcium (Ca^{2+}) and the IF during the wet grinding extraction, creating a holding net which increases the final volume of the CA fibre rich powders.

In regard to the fibre-rich powders obtained by direct drying method, its SWC value (Direct drying 4.82) is significantly higher than the fibre-rich powder obtained by wet grinding using aqueous solvents (W: 1.94 and CA: 3.15) and slightly lower than using an alcoholic solvent (AL: 5.34). The soluble compounds diluted and removed during the aqueous wet grinding; enhance the SWC values of fibre-rich obtained by direct drying.

According to the results obtained, also the extraction solvent employed during the wet grinding modifies the swelling capacity of the artichoke fibre-rich powders and determinate the extraction design to obtain fibre-rich powders with high moisture retention capacity, being both swelling water index and water holding capacity related. Industrially the high water holding capacity and

SWC are required to reverse syneresis, in products such as cooked meat or dairy products (yoghurts, rice puddings, etc.). In conclusion, the commercial reference fibre has at least the same swelling water capacity (P: 2. 00) as the artichoke fibre-rich powder extracted by wet grinding using water as the solvent (W: 1.94), but comparing both extractions with the rest of the fibre-rich powders extracted by-products of artichoke with dry grinding (AL: 5.34, CA: 3.15, Direct drying: 4.82) these last fibre-rich powders have more swelling water capacity. Based on their water swelling capacity the artichoke fibre-rich powder obtained from by-products is a feasible alternative to replace the commercial reference fibre meeting its swelling capacity.

3.2. Colour analysis

During purchase and consumption of food products, colour is an important sensorial attribute for consumers' decision-making (Calvo *et al.*, 2001), for example: judging whether the production process is completed (Mamat *et al.*, 2010, Wade, 1988) providing information about shelf life (Kilcast, 2011) and influencing perception of other sensory attributes, for instance darker biscuits are perceived as harder (Spence, 2015) to bite.

Determining the industrial application (bakery, dairy, etc.) would be more suitable for each fibre-rich powder depending on their colour. It is important to determine how the method of extraction and the solvent employed modify the colour of the fibre-rich powder of artichoke obtained.

Figure 1. Fibre extracted with: 1) Direct Drying, 2) Distilled water (W), 3) Alcohol 98 %. (AL), 4) 1 % (w/w) $CaCl_2*5H_2O$ (pH: 6,5) (CA) and 5) Commercial Pea fibre (P).

In general, comparing direct drying with the results of wet grinding (figure 1 and table 3), it is observed after the wet grinding the colour of the different artichoke fibre-rich powders get lighter because of degradation and dilution of chlorophyll (Wasmund *et al.*, 2006). That is the reason why

the fibre-rich powder obtained by direct drying (a - 6.13, b 39.02, L 58.43) have the darker colour compared with those extracted by wet grinding. There are not significant differences in colour for those fibre-rich powders extracted by an aqueous solvent W (a - 4.39, b 22.41, L 50.96) and CA (a - 4.53, b 22.71, L 55.12), but due to the greater solubility of chlorophyll in organic solvents, the fibre-rich powders of artichoke extracted by alcohol as extraction solvent, the AL fibre-rich powders has a lighter colour (a - 0.68, b 20.14, L 69.52). With regard to the colour of the commercial reference fibre, it presents the lightest colour of all fibre-rich powders studied (a - 0.25, b 14.06, L87.55).

Table 3. Colour parameters (CIELAB Coordinates).

SAMPLE	a*	b*	L*
Direct drying	-6.13 ± 0.33^{d}	39.02 ± 0.43^{e}	58.43 ± 0.48^{b}
W	-4.39 ± 1.01^{c}	22.41 ± 0.65^{d}	50.96 ± 1.30^{a}
AL	-0.68 ± 0.26^{b}	20.14 ± 1.29^{c}	69.52 ± 0.90^{c}
CA	-4.53 ± 1.01^{c}	22.71 ± 1.03^{d}	57.12 ± 1.88^{b}
P	-0.25 ± 0.33^{a}	14.06 ± 0.38^{a}	87.55 ± 0.81^{d}

Values are mean ± SD (n= 3); figures in a column followed by different letters differ significantly ($p< 0.05$).

As most of fibre enriched products need to be light-colored (dairy, bakery, etc.) to avoid undesired sensorial colour changes, the darker colour of fibre-rich powders of artichoke may cause more application problems than the commercial reference fibre (P), much lighter in colour. In order to achieve the same functional effects (WHC, OHC, etc.) produced by the commercial reference fibre (Pea), add approximately less than half the amount of artichoke fibre-rich powders, not having their darker colour a dramatic impact on the final colour of the food product and making the application of fibre rich powders of artichokes feasible at an industrial level.

4. CONCLUSIONS

The values of the functional qualities of the fibre-rich powders extracted from by-products of artichoke obtained in this article have similar values to those described by Ruiz-Cano *et al.*, (2014) for other by-products of artichoke.

However, if the functional qualities of fibre-rich powders of artichoke are compared with other extracted from other vegetal by-products, such as: asparagus, cardoon, and pea; all fibre-rich

powders obtained from artichoke provide better functional qualities (WHC, OHC and SWC). This increases its industrial revalorization and application in food formulations with high fibre content, such as crackers, sauces, meat products, yogurts, replacing the most used vegetal fibre (P) and it can be concluded that the functional properties of the fibre-rich powders obtained from by-products of artichoke are influenced by the method and the solvent of extraction used. Due to the precipitation and solution effect of the soluble fibre caused by extraction solvents and to physical structural modifications of the fibre during wet grinding.

From a technological point of view, to carry out a wet grinding previous to drying, would decrease WHC, SWC, Colour and increase OHC comparing to direct drying. It could be possible to customize the method of extraction more suitable according to functional fibre-rich requirements. For example, for those products requiring greater WHC and SWC would be more suitable than wet extraction using alcohol as extraction solvent; on the other hand for products requiring a greater OHC would be preferable a wet extraction with water as extraction solvent.

In regard to the fibre content of the artichoke fibre-rich powders, FDT values are intermediate compared with the commercial reference fibre (P) located in the maximum range. It has been proven that this commercial fibre presents an imbalance between insoluble and soluble fibre that may affect its functional qualities and some industrial applications, as it is causing its low OWH and WHC.

It can be concluded that a simple method of extraction allows revaluing artichoke by-products produced in canning industries as fibre-rich powders, providing viable alternative to traditional vegetable fibre (P) used currently in the food industry.

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1.2. - Influence of the Extraction Method on the Functional Properties of Fibre-Rich Powders from By-Products of Artichoke (*Cynara Scolymus* L.)

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ABSTRACT

The by-products generated in the canning industry of artichokes make up approximately 70 % of the processed weight, while its economic revaluation is minimal and its environmental management very problematic.

The aim of this study is to extract functional fibre-rich powders from artichoke by-products by applying different methods and extraction solvents, to estimate how their functional and technological properties may be altered. Also, as their industrial applicability in novel fibre-rich products may depend on achieving the same results of the commercial reference (Pea fibre), this fibre was also analysed during the study as a control. For the fibre-rich powders extraction were used two simple techniques, wet gridding with different solvents (ethanol 90 %, water and 1 %$CaCl_2$*$5H_20$) or direct drying. The functional properties of the obtained fibre-rich powders were characterized as glucose dialysis retardation index (GDRI), the total content of polyphenols (PPT) and antioxidant capacity (by using various methods: $ABTS^{\cdot+}$, $DPPH^{\cdot}$ and FRAP). It was observed that the method employed had a considerable influence on the functional properties. However,

independently of the method used all of the fibre-rich powders obtained from artichoke by-products possess better functional qualities than the reference fibre (Pea fibre), such as higher GDRI, PPT and antioxidant capacity. Of the methods studied, the best results on functional properties were obtained by direct drying, and due to its simplicity, it may be the most industrially viable. In conclusion, getting fibre-rich powders from artichoke by-products would offer a revaluation alternative to traditional vegetal fibres, like Pea fibres, reducing environmental impact.

Keywords: Glucose retardation capacity, total polyphenols and antioxidant capacity.

1. INTRODUCTION

In the fruit and vegetable transformation industry, and especially in the canning industry of plants, a lot of residues are produced, between 20 % and 80 % depending on the product and their most frequent uses are for animal feed (EFSA 2008). This represents a considerable loss of raw materials and leads to environmental impact. In December 2014, the Commission decided to withdraw a pending legislative proposal and annex to review recycling and other waste-related targets in the EU Waste Framework Directive 2008/98/EC. The proposal responded to the Member States' legal obligation to review their waste management targets of Directive 1999/31/EC on the landfill of waste which required them to reduce the organic biodegradable waste in landfills (comparing with 1995 levels) by 65 % before 2016, by ensuring "high quality recycling and the use of recycled waste as a major, reliable source of raw material for the Union". From an environmental point of view, the revaluation of these by-products is vital as it may increase the economic viability of the minimization of vegetal waste; reutilize them in other industrial products, like functional foods.

The healthy and nutritional qualities of the artichoke are well-known from ancient times (Hepper, 2009, Van der Ven & Tabinor, 2007) and even nowadays it still being one of the most representative vegetables of the Mediterranean diet. The cultivation of artichoke is widely distributed, with a cropping area of 124.511 Ha with an annual production of 1.440.903 t, being its production mainly concentrated along Mediterranean region (Spain, Italy), with an annual production of 668.778 t (FAOSTAT, 2013), but recently Asia (China) and South-America (Peru) have raised as new global players.

The artichokes are one of the edible plants with the highest content of total polyphenols (Llorach *et al.,* 2002, Lombardo *et al.,* 2012, Peschel *et al.,* 2006), besides other substances are interesting for their functional properties, such as: phenolic acid, cynarine (1,5-dicaffeoylquinic acid), chlorogenic acid (5-caffeoylquinic acid) and fibre (mainly inulin) (López-Molina, 2005, Lattanzio *et al.,*2009) Consequently the artichokes have high antioxidant potential (Ruiz-Cano *et al.,*2014, Lutz *et al.,* 2011, Garbetta *et al.,*2014).Therefore, the artichoke by-products offer a great potential as a source of functional substances, fibre (soluble and insoluble) and polyphenols, which could have an important technological and nutraceutical application designing functional and novel foods.

Foodstuff with a high fibre content constitutes one of the growing sectors of the food industry in recent years since the western population wants to consume products that supply fibre, which the current diet does not provide it (Montagnese *et al.,*2015, Klimis-Zacas *et al.,*2007).Therefore, the use of alternative fibres, extracted from vegetable by-products like: cabbage, tomato, asparagus, eggplant or peas (O'Shea *et al.,* 2012, Nilnakara *et al.,* 2009, Navarro-González *et al.,* 2011, Nindo *et al.,* 2003, Fuentes-Alventosa *et al.,* 2009[a], Boulekbache-Makhlouf *et al.,* 2013) may be a good alternative to the increasing prices of traditional fibres, which are subjected to the global market price, depending on productions zones and annual yields, and climatology (FAO, 2002).

Due to their vegetable sources, these alternative fibres possess very exceptional functional qualities for the development of new products, which implies novel and functional bakery and pastry formulations (Raymundo *et al.,* 2014, Villemejane *et al.,* 2013). For instance, If compared with the cereal fibres like wheat or oat, the fibres extracted from vegetal by-products, in general, have a higher water and oil-holding capacity, etc. (Rodríguez *et al.,*2006). Currently, vegetal fibres are already marketed for the enrichment of biscuits, meat and dairy products, etc., being commercialized under brands like Unicell® Pea (pea), Fibrex® (beetroot), and Raftilosa® (chicory). Therefore, since fibre-rich powders from artichoke by-products are not yet being used in commercial products and assuming that the by-products have a similar composition as the edible part of the artichoke, fibre-rich powders of artichoke represent a potential source of novel high value-added compounds easily to extract and use industrially. Thus the objectives of this research were to study how the functional and technological properties of fibre-rich powders extracted from

artichoke by-products are affected by the method of extraction, using the Pea fibre as the commercial reference.

2. MATERIALS AND METHODS

2.1. Artichoke by-products

The by-products were outer bracts and peduncles of artichoke (*Cynara scolymus* L.) variety "Blanca de Tudela", which were obtained from the canning company GVTARRA S.A. (Villafranca, Navarra, Spain) after the blanching and hearts extraction processes. The by-products were sent to our laboratory within 2-4 hours after its production and once arrived the by-products were centrifuged, to eliminate the rests of the blanching water, and packed in polypropylene bags at an equal weight (500 g) and stored frozen at -14ºC, until fibre extraction or chemical analysis.

2.2. Extraction processes

To extract the fibre-rich powders of artichoke, there were applied the two simplest methods, dry and wet extraction. The dry extraction was performed as a conventional air drying process using a convection oven (Zanussi), setting a constant temperature of 65 °C for 6 hours or till reaching a constant weight. Then aliquots of 50 g of dry by-products were gridded using a coffee grinder (Solac MC6250 110W) at maximum speed for 5 minutes. For the wet extraction, it used the method employed by Fuentes-Alventosa *et al.*, 2009[b] for the extraction of dietary fibre from asparagus, with some modifications. In this study it used three different extraction solvents: 98 % ethanol (AL), water distilled (W) and a dissolution of 1 % (w/w) $CaCl_2 * 5H_2O$ (pH: 6.5) in distilled water (CA); using a proportion of 1 liter of solvent per 1 Kg of frozen by-products of artichoke (-10 °C). Each solvent extraction method was performed in triplicate. The wet gridding was performed using a 2 L Blender (Hallde, Germany) by dividing the load into four batches of 5 minutes each at maximum speed, renewing the solvent in each batch. After extraction, the solute is wrapped using absorbent paper, and part of the absorbed solvent is removed by compression. Then, one by one the different solutes were dried in a convection oven (Zanussi), using the same setting applied for dry extraction.

2.3. Analyses of functional properties

To study the effect of the extraction method on the functional properties, glucose dialysis retardation index, total polyphenol content and antioxidant capacity were analyzed. All analyses were performed performed nine times.

2.3.1. Glucose dialysis retardation index (GDRI)

The GDRI was determined according to the method of Lecumberri *et al.*, (2007), with some modifications. To compare the effect of the different extraction solvents on the GDRI of the fibre-rich powders, the dialysis processes were carried out without alcohol washing of the fibre-rich powders. Due to alcohol washing of the sugars proposed by Lecumberri before dialysis may cause precipitation of the soluble fibre and modifies the functional properties of the fibre-rich powders of the by-products, and consequently may alter their GDRI.

The obtained samples of fibre-rich powders were hydrated with 15 mL of distilled water in a screw container; sample control consisted of the same amount of solution but without fibre-rich powder. After agitation on a horizontal tray for 1 h at room temperature, 30 mg of glucose was added to each solution and immediately transferred to hydrated dialysis bags of 15 cm length (12.000 MWCO, Sigma Chemical Co.). All dialysis bags were submerged in independent containers containing 400 mL of distilled water and kept at 37 ºC in a thermostatic bath. The samples were continuously shaken during 1 hour. Until completion of 1 hour, 0.5 mL of the dialyzed liquid was extracted at 10 min. intervals. After the dialysis process, glucose concentration was measured in the collected dialysates using the anthrone method (Dische, 1965).

The GDRI of the samples was calculated as the percentage of glucose diffused from the dialysis bags over time, compared to the glucose diffused from the control dialysis bag.

GDRI=100-(100 x (mg glucose diffused, sample/mg glucose diffused, control))

2.3.2. Determination of total polyphenol content and antioxidant capacity of extracts of artichoke by-products

For the determination of total polyphenols and antioxidant capacity, the samples were prepared according to the method of Gao *et al.*, (2002) with the modifications employed by Vitali *et al.*, (2009). A two-step chemical extraction of functional compounds was performed on the high-

fibre powders obtained from artichoke by-products and on reference fibre (Pea fibre). The first step consisted of an extraction with a mixture of conc. HCl /methanol/water (1:80:10, v/v) during 24 h, at 20ºC, with constant agitation. After centrifugation (3500 rpm, 10 min) the supernatant (**Ext.1**) was stored frozen at -4ºC, until analysis.

To obtain the insoluble polyphenols retained in the solute of the first extraction, it was performed the second step of extraction, according to the method of Hartzfeld (2002) in acid hydrolysis of the solute. The solute samples were treated with a mixture of methanol and sulphuric acid (10:1, v/v) for 20 h, at 85 ºC. After centrifugation (3500 rpm, 10 min) the supernatant (**Ext.2**) was stored at -4ºC, until analysis.

2.3.2.1. Total polyphenols

The estimation of total polyphenols in the supernatants obtained from the extractions (**Ext.1** and **Ext.2**) was carried out using the spectrophotometric method of Singleton & Rossi (1965). Absorbance was measured at a wavelength of 765 nm. The results were compared with a calibration curve previously obtained using standard solutions of gallic acid with concentrations between 0 and 0.1 mg mL^{-1}. Results were expressed as mg gallic acid per mL of extract.

2.3.2.2. Determination of antioxidant capacity

For the estimation of antioxidant capacity **Ext.1** was used. Several methods of analysis were applied: $ABTS^{\cdot+}$, FRAP and $DPPH^{\cdot}$.

$ABTS^{\cdot+}$

$ABTS^{\cdot+}$ was determined according to the method of Re *et al.*,(1999).This test is based on the discoloration that occurs when the cation radical $ABTS^{\cdot+}$ is reduced to ABTS. To obtain $ABTS^{\bullet+}$ a solution of 5 mL of 7 mM of ABTS is prepared in distilled water and kept at room temperature in the dark for 16 h until transformation to the cation radical $ABTS^{\cdot+}$ is complete. This solution can be used until three days after its preparation.

Before the analysis the $ABTS^{\cdot+}$ solution was diluted with Phosphate Buffered Saline (PBS) 0.01 M (pH 7.4) until an absorbance of 0.700 ± 0.02 at 732 nm is achieved. For the estimation of antioxidant activity, 100 µL of extract samples and extraction solvent (blank) was added to1.900 µL of the $ABTS^{\cdot+}$ solution (when results fell outside the range of measurement the appropriate

dilutions were made) and they were incubated in the dark. Absorbance was measured at 732 nm after 7 and 30 minutes of incubation.

The calibration curve was prepared using Trolox as a standard. The antioxidant activity of the extracts was expressed as Trolox equivalent antioxidant capacity (TEAC), by calculating the percentage of reduction in absorbance and comparing it with the calibration curve. For the preparation of the calibration curve, a stock solution of Trolox of 4 mM (1 mg/mL) was diluted with 80 % methanol to concentrations between 25 and 800 µM.

DPPH•

The DPPH test was carried out according to the method of Brand-Williams *et al*., (1995), based on the reduction of the free radical DPPH• which leads to its discoloration, with some modifications. A DPPH• stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and was stored at - 20 ºC until the performance of the analyses. The DPPH• working solution was obtained by dilution of the stock solution until an absorbance of 0.70± 0.02 was obtained at a wavelength of 517 nm, using a spectrophotometer. Fibre extracts (100 µL) were left to react with 1900 µL of the DPPH working solution during 30 minutes in the dark and absorbance was measured at 517 nm. Extracts were diluted when measurements fell outside the range of the calibration curve. A calibration curve was performed using Trolox concentrations between 25 and 800 µM, obtained by various dilutions with 80 % methanol of a Trolox stock solution of 4 mM (1 mg/mL).Results were expressed in µM TEAC/g peso fresco.

FRAP

The FRAP test was carried out according to Benzie & Strain (1996) with some modifications. The stock solutions were 300 mM acetate buffer (3.1 g $C_2H_3NaO_2$ * $3H_2O$ and 16 mL $C_2H_4O_2$), at pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazina), 40 mM HCl and 20 mM $FeCl_3$*$6H_2O$ solutions. The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3$* $6H_2O$ solution and heating to 37 °C before use. Fibre extracts (100 µL) were allowed to react with 1900 µL of FRAP solution for 30 min in the dark and the colored product was measured spectrophotometrically at a wavelength of 593 nm. Results were expressed in µM TE/g fresh weight. When a measurement of the extract was higher than the values of the linear range of the standard curve, a proportional dilution was performed. For the preparation

of the calibration curve, a stock solution of Trolox of 4 mM (1 mg/mL) was diluted with 80 % methanol to concentrations between 25 and 800 µM.

2.3.4 Statistical analysis

The results were expressed as mean values ± standard deviation. Differences between variables and samples were analysedby one-way ANOVA and, in the case of significant differences ($p < 0.05$), by the LSD post-hoc test. Statistical analysis was performed using the software Statgraphics Centurion XVI 16.1.03 version (Statpoint Technologies Inc.).

3. RESULTS AND DISCUSSION

3.1 Glucose Dialysis Retardation Index

The success of the method of extraction of fibre-rich powders from artichoke by-products will depend on whether these fibre-rich powders are capable of reducing the postprandial glucose peak when they are ingested in a product formulated with glucose. This has been reported in several studies on diabetes control (Afaghi *et al.*, 2015).

Figure 1 presents the graphs of the percentages of glucose diffusion. The glucose diffusion curves of the control and the fibres tested were fitted to a quadratic function ($y = ax^2 + bx$) with $R > 0.95$. The fits for the different fibres and the control can be seen in this figure.

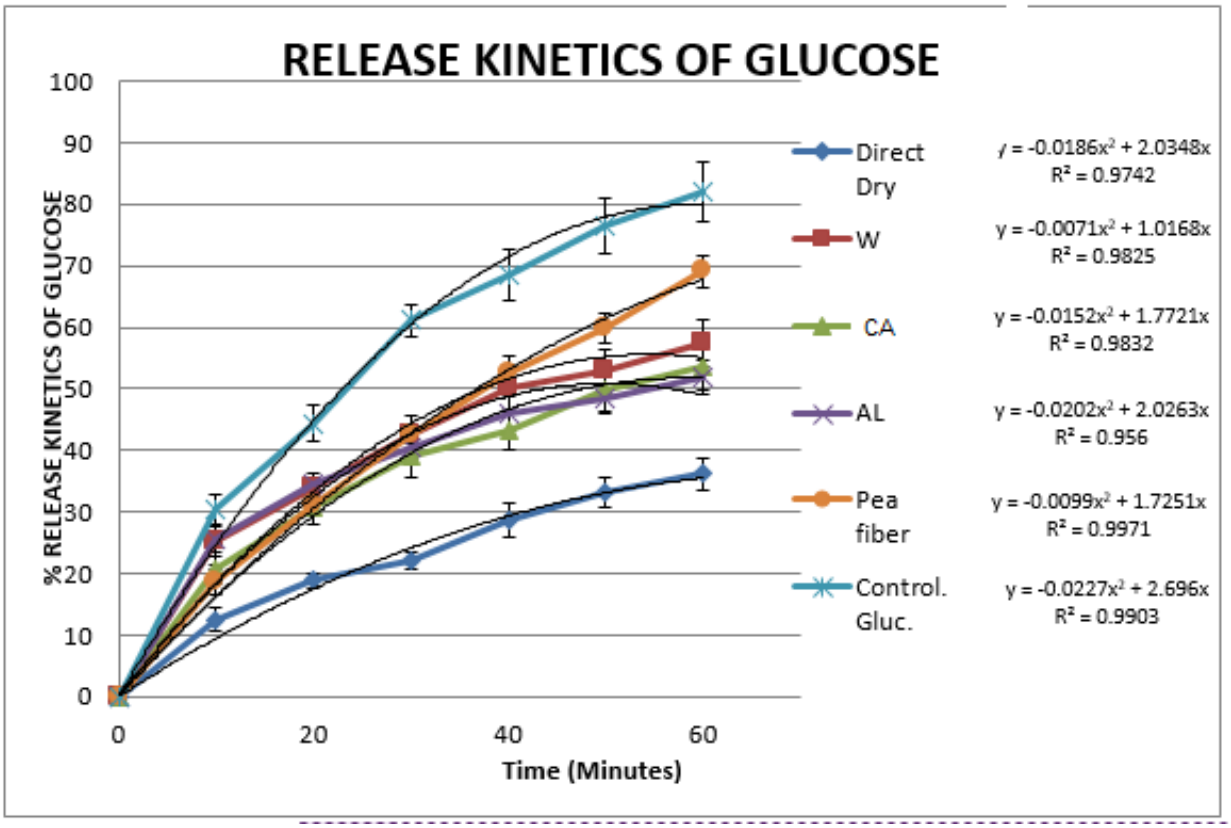

Fig1 Glucose diffusion kinetics. Initial index Fibre extracted with: 1) Direct Drying, 2) Distilled water (W), 3) Alcohol 98 %. (AL), 4) 1 % (w/w) $CaCl_2*5H_2O$ (pH: 6.5) (CA) and 5) Commercial Pea fibre (P).

From the regression equation of each sample in the course of 60 minutes the glucose dialysis retardation index(GDRI) was calculated for the control without fibre in the dialysis bag, the samples of fibre-rich powders of artichoke by-products and the commercial reference fibre (Pea fibre) and the results are presented in Table 1.

The results of the percentage of glucose diffusion retardation show that the fibre-rich powders of the artichoke by-products significantly reduced the diffusion of glucose in the dialysis bags compared to the commercial reference fibre (P), at each moment of the diffusion process. In particular, the percentage of retardation or GDRI of the fibre-rich powders of artichoke by-products obtained by the Direct Dry method was double that of the Pea fibre.

Among the results of the fibre-rich powders obtained by wet extraction only, significant differences existed in the final values of glucose diffusion depending on the extraction solvent used, since each solvent will modify the structure and composition of the fibre-rich powders in a different way. For example, when alcohol was used as extraction solvent the GDRI values were

somewhat lower than those for aqueous extracts, due to the modification of the structural properties, precipitation and loss of soluble fibre (Almazán& Zhou, 1995). With regards to aqueous solvents, it was shown that the addition of 1 % of $CaCl_2*5H_2O$ (CA) increased GDRI values compared to aqueous extraction with distilling water (W)due to the binding of calcium to the insoluble fibre (Selvendran *et al.,* 1987). The GDRI is an in vitro analysis that allows us to predict easily the effect that fibre has on the delay of glucose absorption in the gastrointestinal tract (López *et al.,* 1996). In the literature, there is a wide range of values available for different food ingredients, like wheat bran of 5.3 % or guar gum of 43 % (Larrauri *et al.,* 1996). The majority of plant by-products have intermediate values, like for example mango peel – 21 % (Larrauri *et al.*,1996), *Averrhoa carambola* – 25 % (Chau, *et al.*,2004)or artichoke fibre – 27 % (López *et al.*,1996). Therefore, the results obtained in this study are consistent with those in the literature, where it is explained that the GDRI is a function of the concentration of dietary soluble fibre and the uronic acid content of the insoluble fibre (Edwards & Blackburn, 1987, Wolever, 1990).However, other authors have pointed to the relationship between the internal structure and properties of the surface of the fibres and the diffusion of glucose (López *et al.,* 1996).

Table 1 Glucose Dialysis Retardation Index (GDRI).

Time (min.)	Direct Dry	W	AL	CA	P	Glucose Control
0	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
10	$12.50b \pm 1.82^{b1}$	$25.25b \pm 1.40^{b4}$	25.63 ± 2.14^{b4}	20.55 ± 1.77^{b3}	18.57 ± 1.99^{b2}	30.44 ± 2.29^{b5}
20	$18.80c \pm 2{,}15^{c1}$	33.80 ± 2.03^{c23}	34.49 ± 2.79^{c3}	30.73 ± 2.64^{c2}	31.09 ± 1.22^{c2}	44.26 ± 2.94^{c4}
30	$22.01d \pm 2{,}63^{d1}$	42.52 ± 2.64^{d4}	40.42 ± 3.46^{d3}	39.21 ± 3.13^{d3}	42.36 ± 1.30^{d4}	61.15 ± 2.65^{d5}
40	$28.50e \pm 3{,}22^{e1}$	50.21 ± 3.16^{e4}	46.03 ± 3.34^{e4}	43.37 ± 3.64^{e2}	52.59 ± 2.76^{e5}	68.53 ± 4.12^{e6}
50	$33.10f \pm 3{,}18^{f1}$	52.97 ± 3.40^{e3}	48.54 ± 3.60^{e2}	49.92 ± 3.31^{f2}	59.89 ± 2.41^{f4}	76.45 ± 4.44^{f5}
60	$36.10g \pm 3{,}53^{g1}$	57.45 ± 3.79^{f4}	51.88 ± 3.87^{f2}	53.53 ± 3.81^{g3}	69.08 ± 2.66^{g5}	81.97 ± 4.90^{g6}

Values are mean ± SD (n= 9); values in a column followed by different letters differ significantly ($p< 0.052$) Initial index Fibre extracted with: 1) Direct Drying, 2) Distilled water (W), 3) Alcohol 98 %. (AL), 4) 1 % (w/w) $CaCl_2*5H2O$ (pH: 6.5) (CA) and 5) Comercial Pea fibre (P).

3.2 Total polyphenols

The extraction of phenolic compounds from foodstuff is affected by their chemical nature, the extraction method, the type of solvent, the particle surface, the storage time and conditions of the food product, the presence of interfering substances, etc. (Naczk & Shahidi, 2004, Rivero-Perez *et al.*,2007). Consequently, there is no typical and standardised procedure for the phenolic extraction

of food matrices. As mentioned before the method of Vitali *et al.*, (2009) for products with low moisture content was chosen. The results obtained for the different extracts and fibres are shown in Table 2.As it can be seen in Table 2, the total polyphenol content shows significant differences depending on the extraction method used and on the fraction analyzed. The highest content of total polyphenols was obtained by direct drying, while it significantly decreased in all wet extraction processes. This reduction in concentration is due to the effect of dilution and loss of polyphenols in the wet extraction solvent after centrifugation and compression, while during the direct drying process the polyphenolic compounds are concentrated inside the by-product as the water evaporates. This dilution effect is confirmed when the soluble polyphenol concentration (**Ext.1**) of the fibre-rich powders obtained by direct drying or wet gridding are compared. Due to the extracted polyphenols in the **Ext.1** are less linked to the physiological structure of the by-product (Vitali, 2009), a decrease in the polyphenol content in Ext.1 would be related to a dilution and loss of polyphenols in the extraction solvent used for the wet grinding.

Table 2. Polyphenolic composition of various "fibre-rich powders" of artichoke by-products.

Sample.	Polyphenols **Ext.1** mg gallic acid/ g fibre	Polyphenols **Ext.2** mg gallic acid / g fibre	Total Polyphenols mg gallic acid / g fibre
DIRECT DRY	46.14 ± 7.23^{e}	33.59 ± 2.99^{b}	79.73 ± 7.47^{e}
W	19.98 ± 2.03^{b}	28.06 ± 1.84^{b}	48.04 ± 2.43^{b}
CA	36.31 ± 5.25^{d}	29.77 ± 3.43^{b}	66.07 ± 5.35^{d}
AL	26.0 ± 6.11^{c}	32.71 ± 1.56^{b}	58.71 ± 6.03^{c}
P	1.53 ± 0.25^{a}	3.22 ± 0.65^{a}	4.75 ± 0.46^{a}

Values are mean ± SD (n= 9); values in a column followed by different letters differ significantly ($p< 0.05$). The polyphenol content of the fibre-rich powders obtained by 1) Direct Drying, 2) Distilled water (W), 3) Alcohol 98 %. (AL), 4) 1 % (w/w) $CaCl_2*5H_2O$ (pH: 6,5) (CA) and 5) Comercial Pea fibre (P).

When the soluble polyphenol concentrations (**Ext.1**) are compared to the different extraction solvents used with wet grinding, it is observed that the fibre-rich powders CA retained a higher amount of soluble polyphenols than W. This may be caused by the action that Ca^{2+} has in plant structures that contain pectins. Regarding the content of insoluble polyphenols (**Ext.2**), no significant differences were observed in the polyphenol content among the different extraction

methods used. Hence, none of the extraction methods reduced the content of the polyphenolic compounds bounded to the physiological structures of the by-products.

Total polyphenol content was calculated by adding the polyphenol concentrations obtained by both extraction processes (**Ext.1**and**Ext.2),** is evident that the differences in total polyphenol content are due to differences in the concentration of soluble polyphenols. The polyphenol content of Pea fibre-rich powders was much lower than that of artichoke fibre-rich powders, independently of the extraction method used. This significant difference is due to the fact that the by-product from which the Pea fibre is derived has a different concentration of polyphenolic compounds and its production process (Ekvall *et al.,* 2007) does not apply a blanching process, which would inhibit the action of the polyphenol oxidase responsible for the degradation of polyphenolic compounds. On the contrary in the production process of canned artichokes, blanching is carried out to avoid the browning reaction resulting from the polyphenol oxidase. Therefore, in the by-products, the polyphenolic compounds would be preserved before the extraction and drying processes

3.3 Antioxidant capacity

For antioxidant capacity measurements in the fibre-rich powders of the artichoke by-products only **Ext.1**, was used. As the second extraction step (**Ext.2**) is done with sulphuric acid, which aggressively deteriorates antioxidant compounds, does not yield reliable results. Table 3 shows the results of the antioxidant capacity obtained by the different methods used.

In Table 3 it can be seen that the antioxidant capacity was affected by the method used to extract the fibre from the artichoke by-products. In general, no significant differences were observed in antioxidant capacity between the methods of wet extraction with an aqueous solvent (W and CA), independently of the method used ($ABTS^{\cdot+}$, DPPH•, FRAP).

On the contrary, significant differences ($p < 0.05$) were detected between antioxidant capacity in AL and the fractions W and CA. This is explained by the different solubility of the antioxidant compounds in the extraction liquids. The difference found between the various methods of analysis of antioxidant capacity is in agreement with fibre-rich powders obtained by direct drying of the artichoke by-products showed the highest antioxidant capacity.

Table 3.Antioxidant capacity of various “fibre-rich powders” of artichoke by-products (Ext.1).

Sample	$ABTS^{\cdot+}$ 7 min	$ABTS^{\cdot+}$ 30 min	$DPPH^{\cdot}$	FRAP
	mEqTrolox / g fibre	mEqTrolox / g fibre	mEqTrolox / g fibre	mEqTrolox /g fibre
DIRECT DRY	221.39 ± 19.24[d]	310.08 ± 25.25[c]	406.33 ± 16.86 [d]	253.87 ± 13.19[c]
W	169.95 ± 22.45 [b]	265.73 ± 32.85[b]	303.85 ± 18.82[b]	114.79 ± 5.03 [b]
AL	221.96 ± 14.61[d]	301.15 ± 19.65 [c]	348.47 ± 14.44 [c]	176.02 ± 7.76 [c]
CA	189.90 ± 18.70 [c]	253.08 ± 26.04[b]	296.18 ± 15.08[b]	127.66 ± 9.87[b]
P	7.82 ± 1.08 [a]	9.75 ± 1.23 [a]	7.17 ± 1.30[a]	4.45 ± 2.91 [a]

Values are mean ± SD (n= 9); values in a column followed by different letters differ significantly ($p< 0.05$). Antioxidant capacity ($ABTS^{\cdot+}$, $DPPH^{\cdot+}$,FRAP) content of the fibre-rich powders obtained by: 1) Direct Drying, 2) Distilled water (W), 3) Alcohol 98 %. (AL), 4) 1 % (w/w) $CaCl_2*5H_2O$ (pH: 6,5) (CA) and 5) Commercial Pea fibre (P).

These results agree with the results obtained by the determination of total polyphenols. We conclude that antioxidant compounds are lost with the extraction solvent when this is eliminated during compression and centrifugation of the wet extraction solute. All the fibre-rich powders of artichoke showed a higher antioxidant capacity compared to the reference fibre (Pea fibre, P), independently of the extraction method used or the method applied for its analysis. These results are similar to the ones obtained for the polyphenol content, due to the by-product of which the Pea fibre is extracted from not have the same concentration of polyphenolic compounds as the artichokes, and consequently its antioxidant power is much lower.

4. CONCLUSIONS

The method used to extract fibre-rich powders from artichoke by-products has a considerable influence on their functional properties: glucose dialysis retardation index, antioxidant capacity and polyphenol content. The direct drying shows higher glucose retardation values (GDRI) since the wet grinding of the artichoke by-product modifies its chemical composition and its structure. Concerning the aqueous extraction methods, the use of Ca^{2+}favors the formation of bonds with the insoluble fibre which leads to an increase in the GDRI values. The reduction in glucose index produced by the fibre-rich powders extracted from artichoke by-products is up to 30 % higher than the decrease by the commercial reference fibre (Pea fibre, P).

The direct drying method results in higher concentrations of polyphenolic and antioxidant compounds, due to the dilution effect caused by wet grinding of the by-products. Regarding the aqueous extraction methods, the use of Ca^{2+} favors the formation of bonds in the plant structure leading to a higher content of total polyphenols and a higher antioxidant capacity.

All the fibre-rich powders obtained from artichoke by-products have a higher total polyphenol concentration and a higher antioxidant capacity than the commercial Pea fibre, independently of the methodology used.

It is concluded that direct drying is the best extraction method to obtain highly antioxidant artichoke fibre–rich powders, which would be an excellent alternative to traditional vegetal fibres, like Pea fibres. Besides, due to its simplicity direct drying would be the most industrially feasible extraction method revaluating and reducing environmental impact of by-products of artichoke

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CAPÍTULO 2.- DETERMINACIÓN DE LA TEXTURA DE GALLETAS DIGESTIVE

CAPÍTULO 2.- DETERMINACIÓN DE LA TEXTURA DE GALLETAS DIGESTIVE

En este capítulo se presentan los estudios realizados para la obtención del segundo objetivo de esta Tesis Doctoral: Determinar cuál es la técnica más precisa de las dos técnicas texturométricas más comunes para el control de calidad de galletas con alto contenido en fibra, “Prueba de punción” y corte en tres puntos “TPB” y su optimización.

Se realizó el estudio en 4 marcas comerciales de galleta tipo digestive. Sealmacenaron durante cuatro meses a temperatura ambiente. Se determinó la evolución de la variables texturométricas de las dos técnicas de análisis más empleadas: Prueba de punción y Corte en tres puntos,

Se ha observado que durante el almacenamiento de las cuatro marcas comerciales de galletas se produce un aumento de la humedad que conlleva cambios en la textura, y que estos son mejor analizados en función de las condiciones de ensayo.

De este estudio se puede concluir que:

- Para la técnica de corte, la distancia de separador de 2 cm es la que mejor describe la evolución de la textura de cada marca individualmente y diferencia entre marcas comerciales.
- Respecto a la técnica de punción se ha observado que con la sonda cilíndrica de P/2 con una velocidad de ensayo a 3’75 mm/s (8 segundos de análisis) aplicada en la zona central y media de las galletas, es la técnica más eficaz para determinar la evolución de la textura de cada marca de galletas individualmente y entre galletas de diferentes marcas comerciales.

2. Determination of the most efficient texturometric technique to evaluate digestive-type biscuits

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ABSTRACT

Biscuits with a high fibre content, digestive-type, constitute one of the rising sectors of the food industry, since the European population wants to consume more products that supply fibre, which the current diet does not provide. As texture is the most important sensorial parameter in the perception of quality for oven-baked products like biscuits, and due to the high fibre content, digestive-type biscuits tend to easily capture ambient humidity, this product is very susceptible to changes in texture, which could be disagreeable for the consumer.

The aim of this study was to optimize the test settings of two different texturometric techniques (vertical puncture and Three-point bending), and determine which one would be better as a quality assurance technique, characterizing the texture of the digestive-type biscuits during the self-life.

Therefore four commercial brands of digestive-type biscuits, usually sold on the Spanish market, were stored at room temperature for four months and analysed monthly. It was observed how increasing moisture values modifies the texture of the biscuits and which technique and analysis settings keep measurements more precise. It was observed that puncture technique, applying a test speed of 3.75 mm/s with a cylindrical P/2 probe, within the central and intermediate area of the biscuits was the most accurate method to determine and compare the evolution of the texture of each brand and between different commercial brands of biscuit, respectively.

Practical Applications: This article determines between the two most employed texturometric technics for biscuits (three point bending TPB, Puncture test), which one is the most precise detecting texture changes, caused by moisture variation during self-life and stablishing which are their optimized settings of analysis (test speed, position, etc.).

Keywords: Moisture, Puncture analysis, Three-point bending analysis.

1. INTRODUCTION

Biscuits with a high fibre content, “Digestive-type”, constitute one of the rising sectors of the food industry in recent years. Since the European population wants to consume more fibre enriched products, due to the current diet does not provide enough (Bes-Rastrollo*et al.*, 2006, Garaulet *et al.*, 1998, Farajian & Zampelas, 2015).

For all oven-baked products, but biscuits in particular, texture is considered to be the most important sensorial attribute by consumers. When eating a biscuit it is expected that it will not break down and be crispy. Biscuits are distinguished from other oven-baked products by its moisture content which is lower than 5 %, while cakes may have a moisture content of between 15-30 % and sandwich bread of between 30-45 %. Consequently, the moisture of the biscuit is a very important factor influencing its texture. It is a hygroscopic product (moreover digestive-type biscuits have a high fibre content) and thus will tend to capture water once the packaging is opened and also during storage. Hence its texture may change during storage, becoming disagreeable for the consumer. Therefore, in order to evaluate the quality of the biscuits it will be necessary to

characterize instrumentally their texture and to study its evolution during storage. For instance, the hardness of the sample is a value indicative of its freshness, while the crispness defines its internal structure and the oven-baking characteristics.

The texturometric parameters can be defined as the set of physical features, related to the structural elements of the foodstuff, that are perceptible by the sense of touch, that are related to deformation, disintegration and flow of the foodstuff when it is subjected to a force, and that can be measured objectively in terms of mass, time and distance (Bourne, 1982).

The determination of the texturometric parameters of biscuits is particularly difficult due to their composition and heterogeneous structure, caused by their three-dimensional structure depending on the imprinted commercial logo, specific for each brand, and on the evaporation perforations. Thus all these factors make that the biscuits do not flow and react the same way when exposed to forces of pressure, making them fragile and crumbly (Saleem *et al.*, 2005).

For the instrumental determination of texturometric parameters of "Digestive-type" biscuits, the most employed techniques are "Vertical penetration test" or also knew as "Puncture by vertical compression" (Mandala *et al.*, 2006, Tyagi *et al.*, 2007, Singh *et al.*, 2005) and "Three-point bending test" or TPB (Singh *et al.*, 1993, Kaur *et al.*, 2015). Both tests have a destructive nature and are based on the application of vertical forces on the samples in order to obtain texturometric parameters deduced from force vs. deformation graphs that are achieved with a universal apparatus to test materials (Texturometer). Depending on the research question, one can use one or more of the above tests. Puncture has not been so often used in biscuits, but it can give useful information about the variability of the texture in the same biscuit by puncturing at different locations, something that is not possible with the three-point bending test, which breaks the biscuit in two symmetric halves.

The penetration test is based on the measurement of the maximum shear force necessary to completely pass through a section of the product with a penetration probe. Thus there is a relation between the shear force that has to be applied and the resistance of the product. The three-point bending test is a breaking test by bending, which consists in evaluating the maximum force

necessary to produce a complete breaking of the product structure (Gaines *et al.*, 1994) and is used mainly to estimate the hardness and rigidity of biscuits, chocolate bars, amongst other products.
The aim of this study was to optimize and determine the best method for measuring the texture of biscuits, in order to establish it as a parameter for quality control of digestive-type biscuits. Therefore, during 4 months of storage at room temperature the textural evolution of four commercial brands of digestive-type biscuits, usually sold on the Spanish market, were studied using two different techniques "Puncture test" and TPB, employing different setting of analysis.

2. MATERIAL AND METHODS

Four trademarks of biscuit analysed are commonly sold in any Spanish supermarket, and all of them had a 4 % (w/w) of fibre in their composition. For reasons of commercial privacy they were coded as follows: biscuit E, biscuit F, biscuit G, and biscuit H.
The purchased biscuits were stored during 4 months in their original packaging, in an incubation chamber (Incudigit Selecta, Spain). The conditions of relative humidity (HR 55 %) and temperature (25 ºC) were kept constant throughout the storage. The instrumental analyses were carried out every 28 days until the end of storage.

2.1.Determination of Moisture

The Moisture (M) of the biscuits was measured employing the method described by Nollet (1996) with slight modifications. Biscuit samples (250 mg) were weighed in triplicate with an analytical balance (Mettler Toledo, USA) and they were dried in a dehydration chamber at 90ºC for 24 hours. Moisture of the samples was calculated as the difference in weight (%M= (Initial weight-Final weight)*100 / Initial weight sample).

2.2.Instrumental Determination of the Texture

The instrumental texture was measured with a texturometer TA-XT-2 Texture Analyzer (Stable Micro Systems, Haslemere, England).

2.2.1. Three-point bending test (TPB)

For this test a three-point bending test, it was used a Heavy Duty Platform (HDP/3PB). Once the biscuit is placed on the two stands of the adaptor, separated by a certain distance, the cutting probe is moved vertically until it comes in contact with the biscuit. The probe acts as a third contact point, which exerts an increasing pressure until the structure of the product breaks. The experimental conditions were the following: Pre-Test speed: 1.0 mm/s, Post-Test speed: 15.0 mm/s, Distance: 5 cm, Test speed: 3.0 mm/s (James *et al*., 2011, Rojo & Vincent, 2009). To optimizing parameters, the width of the cutting stands studied was: 3 cm, 2 cm and 1 cm. By modifying the width between the stands, the distance is studied that yields the most accurate results, in order to compare among groups of biscuits.

Since each commercial brand moulds its own logo during the rotatory moulding of the biscuits, the results of the texturometric analysis of the cutting in halves may not be comparable between commercial brands. Therefore, as a complementary method, the texturometric analysis of the biscuit halves generated by the first by bending, was also carried out. It was applied in both cases the same distance between the separators and the same analysis settings

2.2.2. Puncture test

For the vertical puncture analysis, the biscuits were placed on a Heavy Duty Platform (HDP/90), and by means of a cylindrical puncture probe P/2various puncture measurements were performed at different points of the biscuit. In this optimization study, the puncture test was not done with probes with diameters bigger than 2 mm, because when larger diameters were used the biscuits completely fell apart which impeded the performance of several measurements on the same biscuit. Variables of the operation settings were modified in order to study their optimization effect on the precision of the measurements. So, three penetration areas were studied (centre, intermediate and radius) and two compression speeds: 3 mm/s (analysis time: 10 s), 3.75mm/s (analysis time: 8 s). Figure 2 shows the distribution of the points analysed

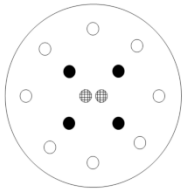

Figure 1: Outline of the distribution of the points analysed in each biscuit. ⊕ center, ○ radius, ● intermediate area between center and radius

2.3.Statistical analysis

The data obtained by the texturometric analysis were analysed with the statistical package STATGRAPHICS Centurion XVI, version 16.1.03 (Statpoint Technologies Inc.) for Windows. One-way ANOVA was performed with LSD post-hoc test. In the case of significant differences ($p < 5.05$), these were labelled with an increasing alphanumerical scale. Two complementary methods of multivariate comparison were also used: discriminant function analysis and analysis by neural networks.

3. RESULTS AND DISCUSSION

3.1.Moisture determination

During storage at room temperature the biscuits were maintained in their original packaging. Results obtained of moisture for the four types of biscuits studied throughout the storage period are shown in Table 1.

Month of storage	E	F	G	H
0	2.09 ± 5.06^{a1}	1.95 ± 5.18^{a1}	3.29 ± 5.21^{c2}	3.85 ± 5.08^{cd3}
1	2.60 ± 5.08^{b1}	2.55 ± 5.09^{b1}	3.37 ± 5.15^{c2}	4.01 ± 5.08^{cd3}
2	3.31 ± 5.07^{c1}	3.36 ± 5.23^{c1}	3.48 ± 5.07^{c1}	4.19 ± 5.07^{d2}
3	3.72 ± 5.05^{c1}	3.75 ± 5.17^{c1}	4.09 ± 5.13^{d2}	4.47 ± 5.07^{e23}
4	4.13 ± 5.11^{d1}	4.16 ± 5.13^{d1}	4.70 ± 5.19^{f2}	4.88 ± 5.11^{f2}

Table 1: Values are mean ± SD (n= 3); significant ($p< 0.05$) differences in storage time within biscuits (column) are indicated with different letters; significant ($p< 005$) differences in type of biscuit with the same storage time (row) are indicated by different numbers.

It was shown that even in their original packaging moisture (w/w) of the biscuits increased significantly during storage in all cases, while in some cases moisture content was doubled. These results are in agreement with what was found in the studies carried out by Robertson (2011) andManley & Clark (2011).

3.2. Texture

For the instrumental determination of texture, the two previously described methods were used. By modifying the operation settings, like puncture position and speed, or width of the cutting stands, etc. it was analysed what the most ideal settings and thus how these settings affected the precision of the measurements. The results will be presented according to the variables obtained in the corresponding analyses performed.

3.2.1. Three-point bending (TPB)

The three-point bending test is a test of breaking by deflection. It consists in evaluating the maximum force necessary to produce a complete breaking of the product structure (Saleem *et al.*, 2005) and it is used to assess the hardness and rigidity of biscuits and chocolate bars, amongst other products (Chena & Opara, 2013).

The precision of the classification of the groups formed by the different commercial brands of biscuits might vary according to the opening of the texturometer stands, applying the same speed during the analysis.

In order to obtain throughout the storage period, the average values of each texturometric variable, their coefficient of variation and the similarity of the formed groups, statistical analysis by one-way ANOVA was applied. For each time of analysis it is established as more precise measurements of a texturometric variable those with a lower coefficient of variation and more number of homogenous groups.

To obtain the percentages of classification of the groups of biscuit, all their texturometric variables are compared at the same time by two complementary statistical analyses (discriminant function analysis and analysis by neural networks). Thus, better differentiation of the texturometric qualities between the groups of biscuits would be related to a higher percentage of classification of the groups of biscuits.

3.2.1.1. Maximum breaking force

The data of the texturometric variables (“Maximum breaking force”, “Deformation”, “Rigidity”) generated by the TPB are shown in table 2 shows the values of the maximum forces obtained for each type of biscuit analysed, according to the distance applied (1 cm, 2 cm and 3 cm).

As can be seen in table 2, the maximum force necessary to break the biscuits was lower when the distance of separation between stands was increased, due to an increase in the moment of force when the distance between stands is increased. This variation of the force, due to changes in the moment of force, also affects the dispersion of the measurements which also varies according to the separation length of the stands on which the biscuits are placed, having the distance 2cm the lowest coefficient of dispersion.

Also it can be observed how all the brand of biscuits the average values of maximum breaking force and its coefficient of dispersions increased throughout the storage, due to the increase of the moisture.

On regard to the homogenous groups obtained for each brand of biscuit individually during storage, it observed that during the storage the distances 1 and 2 cm increased the number of homogenous groups, while the distance 3 cm detected less accurately the evolution of the variable maximum breaking force having less homogenous groups. Comparing all the biscuit brands with each other for each time of analysis, it is observed that for each single time of analysis the distance 1cm obtained as many number of homogenous groups as brand of biscuit, and how throughout the storage time its number of groups reminded constant. For each time of analysis, when the distance of the stand was increased (2 cm and 3cm) a similar number of homogenous groups was created, depending on the different moisture content of the biscuits. One group with the brand G, which had more moisture and higher values of maximum breaking force, and other with the brands E,F and H. It can be concluded that a distance of 2 and 3 cm between each stand produces the best results achieving a relatively low coefficient of dispersion throughout the storage period for each type of biscuit, and define accurately how the moisture modifies the variable maximum force during the storage.

Table 2: Texturometrical variables obtained by TPB

Distance	Storage (Months)	Maximum breaking force (g)				Deformation (mm)				Rigidity (g/mm)			
		Biscuits brands				Biscuits brands				Biscuits brands			
		E	F	G	H	E	F	G	H	E	F	G	H
1cm	0	3579.85 ±5.82 %^{a2}	3156.65 ±9.32 %^{b1}	4539.15 ±4.87 %^{a3}	3613.32 ±17.97 %^{a2}	36.63 ±5.27 %^{a2}	33.32 ± 4.58 %^{a1}	33.82 ± 5.36 %^{a1}	36.94 ± 5.71 %^{a2}	97.73 ± 6.01 %^{a2}	72.37 ±6.92 %^{a1}	134.19 ± 5.15 %^{a3}	98.51 ±9.47 %^{a2}
	1	3997.28 ±14.88 %^{ab2}	3284.83 ±7.14 %^{b1}	5045.57 ±11.62 %^{b4}	4305.64 ±12.42 %^{b3}	36.72 ±5.39 %^{a2}	35.93 ± 4.59 %^{b2}	34.34 ±5.59 %^{a1}	36.90 ± 5.81 %^{a2}	108.87 ± 15.25 %^{b2}	91.75 ± 11.42 %^{c1}	146.88 ± 11.36 %^{b3}	116.65 ± 11.01 %^{b2}
	2	4557.11 ±25.11 %^{bc2}	3452.74 ±23.03 %^{c1}	5506.72 ±11.87 %^{b3}	4273.98 ±17.68 %^{b2}	36.98 ± 5.44 %^{a12}	38.08 ± 2.88^{c2}	35.49 ±4.89 %^{b1}	36.41 ± 5.34 %^{a1}	124.43 ± 24.92 %^{c2}	86.04 ± 15.11 %^{b1}	155.11 ± 15.54 %^{c3}	116.37 ± 15.40 %^{b2}
	3	4974.59 ±17.42 %^{c2}	2928.83 ±35.63 %^{a1}	6013.19 ±25.94 %^{c3}	4395.64 ±26.76 %^{b2}	37.07 ±5.37 %^{b1}	45.52 ± 6.31 %^{e2}	36.01 ±4.73 %^{bc1}	36.91 ± 5.65 %^{a1}	135.33 ± 21.91 %^{d3}	95.78 ±5.73 %^{c1}	167.42 ± 17.11 %^{d4}	113.50 ± 16.18 %^{b2}
	4	5534.42 ±35.73 %^{d2}	3641.98 ±12.89 %^{cd1}	6474.34 ±25.71 %^{c4}	4183.97 ±24.08 %^{b3}	37.33 ± 10.59 %^{b1}	42.50 ± 6.95 %^{di2}	37.16 ± 9.34 %^{c1}	36.94 ± 5.71 %^{a1}	152.81 ± 39.95 %^{e3}	95.20 ± 24.34 %^{c1}	174.59 ± 19.00 %^{e4}	119.52 ± 25.40 %^{b2}
2cm	0	2411.56 ±12.95 %^{a1}	2081.90 ±9.26 %^{a1}	3256.45 ±5.24 %^{a3}	2775.58 ±3.69 %^{a2}	36.89 ±5.39 %^{a2}	34.09 ± 5.42 %^{a1}	33.89 ±5.26 %^{a1}	36.49 ± 5.71 %^{a3}	60.37 ± 13.21 %^{a12}	54.36 ± 15.34 %^{a1}	96.36 ± 11.43 %^{a3}	69.49 ±8.98 %^{a2}
	1	2672.79 ±15.86 %^{b12}	2334.50 ±6.44 %^{b1}	4125.94 ±4.57 %^{c3}	2872.25 ±1.91 %^{ab2}	36.83 ±5.29 %^{a2}	36.98 ± 5.48 %^{b2}	34.70 ±5.30 %^{a1}	36.45 ± 5.73 %^{a2}	62.95 ± 16.45 %^{a12}	58.42 ± 19.07 %^{a1}	96.58 ± 18.79 %^{a4}	72.10 ±3.82 %^{ab3}
	2	2466.30 ±16.21 %^{a12}	2116.91 ±15.49 %^{a1}	3507.69 ±11.35 %^{b3}	2725.59 ±5.17 %^{a2}	39.19 ±5.32 %^{b2}	38.93 ± 5.32 %^{c2}	36.40 ±5.50 %^{b1}	39.55 ± 6.53 %^{b2}	69.70 ±8.19 %^{b2}	63.11 ±6.37 %^{b1}	96.07 ±5.36 %^{a4}	73.30 ±3.68 %^{ab3}
	3	2727.54 ±18.29 %^{b12}	2841.18 ±24.48 %^{c1}	4372.18 ±15.69 %^{c2}	2822.25 ±3.88 %^{ab12}	39.12 ± 5.32 %^{b2}	41.20 ± 3.04 %^{d3}	37.21 ±5.60 %^{b1}	39.47 ± 5.34 %^{b2}	65.80 ± 11.13 %^{b1}	61.08 ±9.48 %^{b1}	117.45 ± 15.48 %^{b3}	75.80 ±5.11 %^{b2}
	4	2687.71 ±25.28 %^{b2}	2465.94 ±25.52 %^{b1}	3758.92 ±18.92 %^{b3}	2675.59 ±9.10 %^{a2}	41.48 ±5.52 %^{c2}	42.52 ± 5.70 %^{d2}	38.91 ±5.83 %^{c1}	45.48 ± 6.62 %^{c3}	72.55 ± 15.86 %^{b12}	69.40 ± 17.64 %^{c1}	118.73 ± 4.71 %^{b3}	74.61 ±1.95 %a^{b12}
3cm	0	1649.74 ±11.82 %^{a1}	1632.80 ±5.81 %^{a1}	2351.62 ±14.95 %^{a3}	2051.54 ±23.32 %^{a2}	37.06 ±5.25 %^{a2}	34.33 ± 5.17 %^{a1}	34.17 ±5.34 %^{a1}	35.49 ± 5.72 %^{a1}	37.56 ± 33.05 %^{a1}	41.81 ± 35.55 %^{a2}	62.81 ± 24.74 %^{a4}	53.18 ±23.33 %^{a3}
	1	1963.56 ±11.32 %^{ab2}	1711.54 ±7.30 %^{a1}	2475.12 ±12.56 %^{a3}	2185.20 ±2.56 %^{a2}	36.94 ±5.30 %^{a2}	36.52 ± 5.42 %^{b2}	34.87 ±5.56 %^{a1}	35.55 ± 5.33 %^{a1}	44.50 ± 11.81 %^{b1}	44.12 ± 22.74 %^{ab1}	65.56 ± 18.21 %^{ab3}	53.62 ± 12.08 %^{a2}
	2	1601.41 ±15.75 %^{a1}	1634.21 ±5.44 %^{a1}	2385.97 ±7.82 %^{a3}	2168.37 ±12.55 %^{a2}	39.23 ± 5.42 %^{b2}	39.08 ± 5.36 %^{c2}	36.32 ±5.79 %^{b1}	39.23 ± 5.55 %^{b2}	45.83 ± 15.99 %^{b1}	45.10 ± 19.34 %^{b1}	67.66 ± 17.16 %^{b3}	56.21 ±12.58 %^{ab2}
	3	1915.22 ±18.35 %^{ab1}	1857.71 ±19.27 %^{b1}	2499.46 ±26.24 %^{ab3}	2068.37 ±12.08 %^{a2}	39.10 ±5.52 %^{b2}	41.19 ± 5.45 %^{c2}	37.02 ±1.30 %^{b1}	39.54 ± 5.75 %^{b2}	48.97 ± 18.36 %^{bc1}	46.86 ±7.48 %^{b1}	68.81 ± 15.10 %^{b3}	56.65 ±8.23 %^{ab2}
	4	1719.74 ±23.91 %^{a1}	1925.14 ±32.36 %^{b1}	2415.31 ±23.64 %^{b2}	2285.20 ±8.16 %^{ab2}	41.39 ±5.75 %^{c2}	43.66 ± 5.53 %^{d2}	38.47 ±1.70 %^{c1}	45.45 ± 5.51 %^{c3}	53.14 ± 11.28 %^{c12}	47.55 ±5.83 %^{b1}	75.85 ± 12.82 %^{c3}	59.24 ± 8.23 %^{b2}

Note: Texturometrical variables obtained by TPB form the biscuit brands studied (E, F, G, H) during storage (months) according to three-point bending: three breaking distances 1 cm, 2 cm, 3 cm. Values are mean ± Standard Deviation (SD). (n= 3); significant (p< 5.05) differences between storage time within biscuit types (columns) are indicated with different letters, significant (p< 5.05) differences between biscuit types (rows) are indicated with different numbers

3.2.1.2. Deformation

On the table 2, It is seen how for each brand of biscuit individually, the separations 2 cm and 3cm can describe better the evolution of the variable deformation throughout the storage of the biscuit, due to the significant increase of the average values of the variable deformation. Also, It can be observed that the coefficient of dispersion, for breaking each distance and time of analysis, were significantly low for all biscuit types, as a result of the biscuits being fairly similar and homogeneous in their properties of composition and height.

On regard the homogenous groups created by each brand of biscuit throughout the storage, it was observed that for all the distances the number of homogenous groups increased during the storage. Being the distances 2 cm and 3 cm, with more number of homogenous groups, which could describe better the evolution of each brand of biscuit due to the moisture increase.

When it is studied the evolution of the number of homogenous groups comparing all the brands of biscuits with each other, it was observed that for each time of analysis the number of homogenous groups was very similar, for the separation distances 2 cm y 3cm; and how for these distances was observed how the number of homogenous groups increased during the storage.

It can be concluded that all the distances employed obtained very low dispersion throughout the storage period for each type of biscuit, and that the distances 2cm and 3 cm defined more accurately how the moisture modifies the variable deformation during the storage.

3.2.1.3. Rigidity of compression

Similar to what was observed for the maximum force, upon increasing the separation distance of the stands, on the table 2 the moment of force increases and hence the biscuits have less resistance to breaking or “rigidity”. As a consequence, the values of rigidity of the biscuits decreased with increasing separator distance. Also during the storage, it was observed that for all biscuit brands and stand separations the mean values of the variable rigidity increased with the time of analysis.

It was observed that at a distance of 1 cm, the values of rigidity and the coefficient of variation of all groups of biscuits increased with storage time. On the contrary, for the distances of 2cm and

3cm the coefficient of variation remained relatively during the storage time. Being the distance 2cm which obtained the lowest coefficient of variation thus this distance allows a better determination of the variable rigidity of each type of biscuit throughout the storage period.

On regard the homogenous groups of each brand of biscuit obtained throughout the storage, it was observed that all the separation distances increased the number of homogenous group throughout the storage, being the distance 1cm which created more number of groups for the brands E and G. As it was observed for the variable maximum force for distances (2 cm and 3cm) a similar number of homogenous groups was created for each time of analysis, depending on the different moisture content of the biscuits, one group with the brand G, which had more moisture and higher values of rigidity and other with the brands E, F and H.

It can be concluded that a distance of 2 and 3 cm between each stand produces the best results achieving a relatively low coefficient of dispersion throughout the storage period for each type of biscuit, and define accurately how the moisture modifies the variable maximum force during the storage.

For the comparative study of all the texturometric variables obtained by TPB, It was performed two different type of statistical analysis: discriminant function analysis and by neural networks.

3.2.1.4. Discriminant function analysis of three-point bending of the biscuits

Throughout the storage period, a discriminant function analysis was applied to the three texturometric variables (Maximum breaking force, Deformation, Rigidity), obtained by TPB the biscuit at the different holding distances (1cm, 2 cm, 3 cm). Individually, for each commercial brand of biscuits the results of the discriminant function analysis are shown in Table 3.

Table 3: Analysis multivariable of the texturometric variables generated by TPB					
Discriminant function analysis			Neural networks analysis		
Distance	Storage	Biscuit brands	Distance	Storage	Biscuit brands

	(Months)	E	F	G	H		(months)	E	F	G	H
1cm	0	83.33 %	100.00 %	83.33 %	33,33 %	1cm	0	83.33 %	100.00 %	83.33 %	66,67 %
	1	33.33 %	66.67 %	33.33 %	66,67 %		1	33.33 %	83.33 %	66.67 %	33,33 %
	2	55.00 %	83.33 %	33.33 %	16,67 %		2	55.00 %	83.33 %	55.00 %	16,17 %
	3	66.67 %	33.33 %	66.67 %	16,67 %		3	66.67 %	33.33 %	5.00 %	33,33 %
	4	55.00 %	66.67 %	83.33 %	66,67 %		4	66.67 %	5.00 %	83.33 %	0,00 %
total		58.67 %	70.00 %	60.00 %	40.00 %	total		60,0 %	65.00 %	56.70 %	35.00 %
2cm	0	55.00 %	66.67 %	100.00 %	16,67 %	2cm	0	66.67 %	100.00 %	100.00 %	33,33 %
	1	55.00 %	66.67 %	100.00 %	66,67 %		1	66.67 %	100.00 %	100.00 %	50,00 %
	2	66.67 %	100.00 %	100.00 %	50,00 %		2	55.00 %	100.00 %	66.67 %	16,67 %
	3	66.67 %	100.00 %	100.00 %	33,33 %		3	66.67 %	66.67 %	83.33 %	0,00 %
	4	100.00 %	100.00 %	100.00 %	83.33 %		4	83.33 %	33.33 %	100.00 %	0,00 %
total		68.67 %	86.67 %	100.00 %	50.00 %	total		66,7 %	85.00 %	95.00 %	25.00 %
3cm	0	83.33 %	100.00 %	83.33 %	50,00 %	3cm	0	83.33 %	100.00 %	55.00 %	83,00 %
	1	100.00 %	100.00 %	83.33 %	66,67 %		1	83.33 %	100.00 %	66.67 %	5.00 %
	2	83.33 %	100.00 %	100.00 %	16,67 %		2	83.33 %	100.00 %	83.33 %	0,00 %
	3	83.33 %	100.00 %	100.00 %	66.67 %		3	66.67 %	83.33 %	33.33 %	0,00 %
	4	100.00 %	100.00 %	100.00 %	66.67 %		4	66.67 %	55.00 %	66.67 %	0,00 %
total		90.00 %	100.00 %	93.33 %	53.34 %	total		76,6 %	86.70 %	65.00 %	16.67 %

Note: % of correctly classified groups of each commercial brand of biscuits after a multivariable analysis of the variables generated by three-point bending of types E, F, G, H of biscuits, at three breaking distances 1cm ,2cm, 3cm; throughout the storage period

It was observed that the percentage of correctly classified cases of the biscuits broken at a distance of 1 cm, presented a great variability as function of storage time, thus the use of this distance for the instrumental analysis was discarded. The effect of improvement of the classification percentage with increasing distance between the stands, from 2 cm onwards, was caused by the decrease of the coefficient of variation of the data of maximum breaking force and rigidity (Table 2).

3.2.1.5. Analysis by neural networks of three-point bending of the biscuits

Throughout the storage period, an statistical analysis by neural networks was applied to the three texturometric variables (Maximum breaking force, Deformation, Rigidity), obtained by TPB at the different holding distances (1cm, 2 cm, 3 cm). Individually, for each commercial brand of biscuits the results of the discriminant function analysis are shown in Table 3.

It was observed that the distance 2 cm that this was the most exact distance for the performance of the analysis, since the variation of the texture throughout the storage period was detected,

separating the groups with a high percentage of success. According to the studies by Mandala (2006), Burt & Fearn, (1983), Ahmad *et al.*, (2001) and Faridi, (1994), it is very difficult to classify commercial biscuits by means of texturometric analysis, even if they have the same formulation, the process of oven-baking and cooling may have been different, since they may present different moisture contents. However, the present study shows that the conditions for and the type of texture analysis can be adjusted and a good classification among several commercial brands can be achieved.

It can also be observed how the percentages of classification for the biscuit groups E, F, and G, at all analysis distances, decreased throughout the storage period, due to the increase in moisture of the biscuits which made the final texture of these biscuit very similar. This effect was also detected in the results of maximum breaking force and compression rigidity as an increase of the coefficient of variation of the data with the time of storage. The holding distance of 2 cm resulted in the best coefficients of separation, throughout the storage period.

3.2.2. Puncture test

The puncture test is based on the measurement of the maximum shear force necessary to completely pass through a section of the product, with a puncture probe. Therefore, there is a relationship between the shear force applied and the resistance of the product.

Similar to the TPB test, the precision of the classification of the groups formed by the different commercial brands of biscuits might vary according to the penetration area and the speed applied during the analysis. Thus, a combination of both parameters has to be established, in order to obtain:

- The average values of each texturometric variable, their coefficient of variation and the similarity of the formed groups, statistical analysis by one-way ANOVA was applied. For each time of analysis, it is stablished as more precise measurements of a texturometric variable those with a lower coefficient of variation and more number of homogenous groups for each time of analysis.

-To obtain the percentages of classification of the groups of biscuit, all their texturometric variables are compared at the same time by two complementary statistical analyses (discriminant function analysis and analysis by neural networks), combining the setting of puncture analysis (position of

the puncture in the biscuit (centre, intermediate and radius) and speed (3.75 mm/s and 3 mm/s) or time lapse (8 s and 10 s) and, as result of it a higher percentage of classification of the groups of biscuits may be related to a the best combination of texturometric variables.

3.2.2.1. Puncture test, centre area

Table 4 show the texturometric variables (Maximum force, Hardness, Rigidity) generated by analysis of the central area of the biscuits at a speed of 3 mm/s and 3,75 mm/s.

It is observed that throughout the storage period and commercial brands, how the average values of each texturometric variables (maximum force of deformation (N), hardness (g.sec), and rigidity (g.sec)) differ depending on the speed of the analysis. Being the slowest speed setting (3mm/s), which obtains the highest average results, for all the texturometric variables. Its explanation may be that employing the slowest setting, the structure of the biscuit can create internal stable and resistant structures which create higher opposition to the puncturing needle.

Table 4: Texturometricalvariables obtained by Puncture Analysis of the center area of the biscuits brands.

Time of analysis: 8s					Time of analysis: 10s				
Storage	Biscuit				Storage	Biscuit			
(months)	E	F	G	H	(months)	E	F	G	H
Maximum force of deformation (g)					Maximum force of deformation (g)				
0	4135.89 ± 12.79 %^{ab1}	4471.66 ± 14.13 %^{b1}	6011.03 ± 19.67 %^{a3}	5503.85 ± 14.26 %^{a}	0	4888.45 ± 12.68 %^{ab1}	4671.17 ± 13.49 %^{a1}	7382.70 ± 18.01 %^{a2}	8735.65 ± 9.00 %^{a3}
1	3762.27 ± 15.34 %^{a1}	4142.64 ± 15.28 %^{ab1}	6938.46 ± 14.04 %^{b3}	6894.80 ± 9.05 %^{b3}	1	4592.80 ± 16.35 %^{a1}	4865.74 ± 16.15 %^{ab1}	8285.65 ± 12.84 %^{b2}	8584.13 ± 9.89 %^{a2}
2	3806.78 ± 11.70 %^{a1}	4626.38 ± 13.85 %^{bc2}	8022.32 ± 12.25 %^{c4}	7243.95 ± 8.45 %^{d3}	2	4511.04 ± 11.51 %^{a1}	5643.64 ± 14.76 %^{c2}	9833.45 ± 12.05 %^{c3}	9058.13 ± 9.20 %^{b3}
3	4103.38 ± 14.35 %^{ab1}	4827.24 ± 13.01 %^{c2}	8321.79 ± 12.27 %^{c4}	7593.09 ± 11.20 %^{e3}	3	4868.59 ± 13.58 %^{ab1}	5487.81 ± 12.93 %^{bc2}	9921.10 ± 13.58 %^{c3}	9433.02 ± 12.89 %^{bc3}
4	4496.74 ± 15.54 %^{bc1}	5003.53 ± 15.57 %^{cd1}	8498.24 ± 16.75 %^{d2}	8226.18 ± 9.60 %^{f2}	4	5338.96 ± 16.04 %^{bc1}	5909.29 ± 11.44 %^{cd1}	10122.90 ± 16.99 %^{d2}	10148.71 ± 11.21 %^{c2}
Hardness (g.sec)					Hardness (g.sec)				
0	391.11 ± 16.72 %^{a1}	567.93 ± 11.68 %^{c2}	508.81 ± 15.45 %^{a2}	598.36 ± 16.52 %^{a3}	0	791.51 ± 15.55 %^{b1}	1038.05 ± 22.39 %^{c2}	1049.23 ± 25.23 %^{a2}	1117.64 ± 15.96 %^{a2}
1	371.53 ± 16.94 %^{a1}	441.35 ± 13.29 %^{a2}	672.79 ± 22.58 %^{b3}	729.13 ± 14.14 %^{b3}	1	648.23 ± 19.16 %^{a1}	766.16 ± 17.29 %^{a2}	1196.88 ± 19.13 %^{a3}	1134.15 ± 11.50 %^{a3}
2	387.78 ± 12.54 %^{a1}	482.66 ± 12.49 %^{b2}	699.06 ± 11.60 %^{c3}	822.76 ± 14.92 %^{c4}	2	808.70 ± 12.75 %^{b1}	883.32 ± 13.48 %^{b2}	1503.26 ± 19.27 %^{b3}	1262.82 ± 11.10 %^{ab3}
3	425.36 ± 16.45 %^{b1}	508.86 ± 14.74 %^{b1}	725.32 ± 11.23 %^{c2}	872.97 ± 14.67 %^{d3}	3	831.37 ± 15.51 %^{b1}	1001.51 ± 13.71 %^{c2}	1706.23 ± 16.94 %^{c3}	1377.51 ± 14.14 %^{bc2}
4	434.15 ± 14.79 %^{b1}	514.66 ± 13.79 %^{b1}	797.37 ± 12.99 %^{d2}	848.8 ± 15.39 %^{c2}	4	955.89 ± 16.37 %^{c1}	1025.49 ± 15.76 %^{c1}	1647.93 ± 14.82 %^{bc2}	1437.54 ± 13.50 %^{c2}
Rigidity (g.sec)					Rigidity (g.sec)				
0	1502.68 ± 16.47 %^{b1}	1845.83 ± 23.76 %^{a2}	1975.41 ± 13.98 %^{ab2}	1891.01 ± 23.45 %^{a2}	0	1572.97 ± 15.64 %^{ab1}	1985.01 ± 23.79 %^{b2}	2094.11 ± 35.08 %^{c3}	1609.53 ± 25.98 %^{ab1}
1	1027.94 ± 21.22 %^{a1}	1413.81 ± 24.57 %^{a2}	1391.11 ± 16.25 %^{a2}	2412.69 ± 25.84 %^{b3}	1	1119.21 ± 18.29 %^{a1}	1515.47 ± 22.39 %^{ab12}	2574.82 ± 24.44 %^{b3}	1961.07 ± 18.99 %^{b2}
2	3177.17 ± 11.09 %^{c2}	2972.48 ± 13.81 %^{b1}	3564.94 ± 6.76 %^{c2}	4342.24 ± 15.55 %^{c3}	2	3652.98 ± 9.75 %^{c1}	3505.40 ± 15.65 %^{c1}	6087.74 ± 14.47 %^{c3}	4521.22 ± 12.34 %^{c2}
3	4267.82 ± 9.69 %^{d1}	4473.56 ± 15.75 %^{c1}	5738.81 ± 8.76 %^{d2}	5481.78 ± 12.59 %^{d2}	3	5135.61 ± 8.72 %^{d1}	5296.66 ± 9.59 %^{d1}	6086.35 ± 13.70 %^{c2}	6196.61 ± 18.60 %^{d2}
4	4746.79 ± 15.37 %^{e1}	4665.11 ± 12.58 %^{c1}	5481.31 ± 15.85 %^{d2}	6078.37 ± 14.31 %^{e2}	4	5581.83 ± 13.27 %^{e1}	5667.50 ± 13.72 %^{de1}	6715.23 ± 11.44 %^{ce2}	6728.48 ± 7.76 %^{de3}

Note: Texturometrical variables obtained by Puncture Analysis from the center of biscuit brands studied (E, F, G, H) during storage (months). Values are mean ± Standard Deviation (SD). (n= 3); significant ($p< 5.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 5.05$) differences between biscuit types (rows) are indicated with different numbers.

Also for each group of biscuits individually, it can be observed how for both speed settings the average values of each texturometric variable increased during the storage. This effect is obviously related to the increase of moisture in the center of the biscuit, as it is observed how the different moisture content of commercial biscuit define the texturometric variables, being the biscuit with more moisture (H) which had higher texturometric values than the other biscuit.

At the beginning of storage, the different commercial brands showed significant differences in texture ($p<0.05$). As a result of the increasing moisture of the biscuits, trapping ambient humidity, these differences kept decreasing until they were not significant, for biscuits E, F, G. Again, biscuit H behaved very differently and these biscuits were the most different from the rest of the biscuits analysed, possibly due to their higher moisture content (Table 1). These results are in agreement with studies carried out by other authors, who have used the same puncture technique with formulation of biscuits in which the wheat flour partially was replaced by other ingredients. Then it was detected too how an increasing moisture increases the hardness of the biscuits, and then so too its maximum force of deformation (Filipčev *et al.*, 2011).
On regard to the coefficient of variation: Comparing the coefficient of variation of each texturometric variables applying two different analysis speeds, it was observed that both speed settings obtained similar coefficient of variation for the variable Maximum force of deformation, however for the variables Hardness and Rigidity the coefficient of variation are higher if they are obtained by the slowest setting (10s)

For each brand of biscuits and setting speed, it is observed how the coefficient of variation are stable for the variables hardness and maximum force of deformation during the storage. On the contrary the coefficient of variation of the variable Rigidity decreased during the storage, for all the commercial biscuits. It may be caused by the increasing moisture which homogenized the result of the variable rigidity.

These similarities between coefficients of variation may be related to the way central area of the biscuit is shaped during rotatory moulding. As the commercial logo of each brand of biscuit is moulded in the central area, this area is the most compressed during the rotatory moulding and

where the height of biscuit is the tightest, not allowing the gas to expanse during oven baking. This homogeneity makes the variables maximum force of deformation and hardness very similar between biscuit of the same brand.

Comparing the result of each brand of biscuits individually for each texturometric variable and speed settings, it was observed that for the variable maximum force the homogenous groups obtained for both speed setting during the storage has a similar number. On the contrary for the variables harness and rigidity, the number of homogenous groups were higher for the slowest setting speed (10s).

Comparing the evolution of the brands of biscuit with each other it is observed that at the end of the storage the number of homogenous groups decreased. As mentioned before, this effect is due to the moisture increase to similar percentages and how it equaled the texturometric variables. These two homogenous groups are composed for the biscuit with less moisture (E, F) and with more moisture (G, H).

3.2.2.2. Puncture test, analysis time 8s, intermediate area

Table 5 show the texturometric variables (Maximum force, Hardness, Rigidity) generated by analysis of the intermediate area of the biscuits at a speed of 3 mm/s and 3,75 mm/s.

In the intermediate area, it is also observed how throughout the storage period and for all the commercial brands, the average values of each texturometric variables differed depending on the speed of the analysis. Being too the slowest speed setting (3mm/s) which obtains the highest average values for the variables maximum force and rigidity for all the times of analysis, on the contrary the variable hardness is not affected at all by the speed setting.

Table 5: Texturometrical variables obtained by Puncture Analysis of the intermediate area of the biscuit brands.

Time of analysis: 8s					Time of analysis: 10s				
Storage	Biscuit brands				Storage	Biscuit brands			
(months)	E	F	G	H	(months)	E	F	G	H
Maximum force of deformation (g)					Maximum force of deformation (g)				
0	3913.11 ± 16.72 %^{ab1}	4421.35 ± 13.29 %^{a2}	5981.36 ± 16.52 %^{a4}	5083.81 ± 15.45 %^{a23}	0	4727.92 ± 15.87 %^{a1}	5061.06 ± 14.08 %^{a2}	7459.07 ± 14.41 %^{a3}	8604.89 ± 15.26 %^{a4}
1	3716.53 ± 16.94 %^{a1}	4822.66 ± 12.49 %^{b2}	7297.13 ± 14.14 %^{b4}	6724.79 ± 12.58 %^{b3}	1	4582.57 ± 16.01 %^{a1}	5842.31 ± 13.90 %^{b2}	8697.82 ± 13.66 %^{b3}	8572.21 ± 14.88 %^{b3}
2	3874.78 ± 12.54 %^{ab1}	5078.86 ± 14.74 %^{b2}	8223.76 ± 14.92 %^{c4}	6992.06 ± 11.60 %^{b3}	2	4724.11 ± 11.47 %^{a1}	5945.64 ± 15.33 %^{b2}	9995.49 ± 14.68 %^{c3}	8931.30 ± 12.06 %^{c3}
3	4201.36 ± 16.45 %^{bc1}	5147.66 ± 13.79 %^{bc2}	8721.97 ± 14.67 %^{d4}	7259.32 ± 11.23 %^{c3}	3	5056.72 ± 16.75 %^{ab1}	6182.17 ± 15.05 %^{bc2}	10435.20 ± 14.02 %^{d4}	9236.80 ± 11.95 %^{d3}
4	4343.15 ± 14.79 %^{c1}	5672.93 ± 11.68 %^{c2}	8483.80 ± 15.39 %^{cd4}	7975.37 ± 12.99 %^{d3}	4	5287.89 ± 14.07 %^{b1}	6711.44 ± 11.91 %^{d2}	10146.50 ± 15.30 %^{d3}	9981.67 ± 12.94 %^{e3}
Hardness (g.sec)					Hardness (g.sec)				
0	722.18 ± 21.56 %^{a1}	809.68 ± 25.21 %^{a12}	1045.08 ± 25.81 %^{a3}	867.54 ± 19.81 %^{a2}	0	722.18 ± 21.56 %^{a1}	796.68 ± 25.13 %^{a1}	1007.20 ± 19.57 %^{a2}	1162.83 ± 22.46 %^{a2}
1	738.88 ± 16.78 %^{a1}	877.67 ± 12.11 %^{b2}	1342.73 ± 16.81 %^{b3}	1131.22 ± 24.49 %^{ab3}	1	738.86 ± 16.78 %^{ab1}	889.49 ± 13.00 %^{b1}	1342.73 ± 16.81 %^{b2}	1138.74 ± 21.29 %^{a2}
2	832.09 ± 14.51 %^{bc1}	1062.04 ± 12.39 %^{c2}	1588.79 ± 15.54 %^{c23}	1203.75 ± 11.37 %^{b2}	2	822.98 ± 14.09 %^{bc1}	1062.14 ± 12.39 %^{c2}	1588.79 ± 15.54 %^{bc3}	1235.89 ± 11.20 %^{a2}
3	906.38 ± 16.21 %^{cd1}	1019.72 ± 16.01 %^{c12}	1755.48 ± 19.67 %^{cd3}	1276.28 ± 9.60 %^{b2}	3	906.87 ± 16.21 %^{cd1}	1019.22 ± 16.11 %^{c2}	1765.88 ± 25.20 %^{d4}	1304.29 ± 9.66 %^{bc23}
4	936.98 ± 19.52 %^{d1}	1037.01 ± 14.44 %^{c1}	1656.92 ± 17.06 %^{c3}	1432.66 ± 14.22 %^{c2}	4	936.83 ± 19.52 %^{de1}	1037.01 ± 14.44 %^{c2}	1661.82 ± 17.16 %^{c4}	1442.49 ± 13.49 %^{cl3}
Rigidity (g.sec)					Rigidity (g.sec)				
0	1305.34 ± 23.88 %^{a1}	1854.43 ± 22.13 %^{b2}	1984.41 ± 25.70 %^{a2}	2021.03 ± 11.11 %^{b2}	0	1411.60 ± 22.48 %^{a1}	2025.20 ± 21.10 %^{b23}	2205.52 ± 21.37 %^{a3}	1765.92 ± 25.82 %^{a1}
1	1265.63 ± 19.91 %^{a1}	1486.42 ± 25.85 %^{a12}	2575.51 ± 23.67 %^{b3}	1505.21 ± 28.21 %^{a12}	1	1344.69 ± 19.36 %^{a1}	1623.38 ± 24.25 %^{ab2}	2766.15 ± 23.48 %^{b3}	1722.52 ± 26.26 %^{a2}
2	3354.32 ± 12.32 %^{b2}	2869.25 ± 15.62 %^{c1}	5649.29 ± 14.44 %^{c4}	3238.09 ± 8.81 %^{c2}	2	3892.27 ± 12.15 %^{b2}	3407.33 ± 15.03 %^{c1}	6371.24 ± 14.05 %^{c3}	3893.37 ± 8.11 %^{b2}
3	4735.72 ± 11.61 %^{c1}	4846.21 ± 15.00 %^{d1}	7688.61 ± 16.59 %^{d3}	4975.97 ± 7.27 %^{d2}	3	5619.68 ± 15.97 %^{c1}	5773.75 ± 13.82 %^{d1}	8942.50 ± 14.60 %^{d3}	5969.12 ± 9.01 %^{c12}
4	4936.73 ± 11.27 %^{c1}	4662.25 ± 13.99 %^{d1}	7507.24 ± 15.89 %^{d3}	5364.76 ± 15.70 %^{e2}	4	5888.96 ± 11.00 %^{c1}	5623.28 ± 13.08 %^{d1}	8751.67 ± 9.93 %^{d3}	6444.35 ± 15.72 %^{d2}

Note: Texturometrical variables obtained by Puncture Analysis from the intermediate area of biscuit brands studied (E, F, G, H) during storage (months). Values are mean ± Standard Deviation (SD). (n= 3); significant ($p< 5.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 5.05$) differences between biscuit types (rows) are indicated with different numbers.

Comparing the average values obtained for the central and intermediate areas it is concluded that both areas have similar average values for each texturometric variable and time of analysis. Also as it was previously observed in the centre area, for each brand of biscuits individually. It can be observed how the average value of each texturometric variable increased during the storage, due to the increasing of the moisture during the storage modifies the texturometric variables of the biscuit, and how the biscuits with more moisture (G, H) had higher texturometric values than the other biscuits, explaining why having the same moisture the biscuits E and F behaved the same way. These results are in agreement with studies carried out by other authors, who have used the same puncture technique with formulation of biscuits in which the wheat flour partially was replaced by other ingredients. Then it was detected too how an increasing moisture increases the hardness of the biscuits, and then so too its maximum force of deformation (Filipčev *et al.*, 2011).

On regard to the coefficient of variation, it is observed that for each group of commercial biscuits, the coefficients of variation of all the texturometric variables obtained by both speed settings are similar for each time of analysis. Nevertheless if compared with their initial values, the coefficient of variation decreased throughout the storage, being the global texture of each brand of biscuits of the storage more similar at the end on the storage.

Similar to the results obtained by the analysis of the centre of the biscuits the coefficients of variation of the texturometric variables were very similar among times of analysis and commercial brands of biscuits. No commercial brand highlighted for having different coefficients of variation. However, equally to what happened during the analysis in the centre of the biscuits, the coefficient of variation of the rigidity decreased throughout the storage period for all types of biscuit.

On regard to the individual comparison of each brand of biscuits throughout the homogenous groups created by statistical analysis of each texturometric variable and speed settings. It was observed that for each variable the homogenous groups obtained for both speed settings are in similar number. On the contrary to the central area where for the variables harness and rigidity, the number of homogenous groups were higher for the slowest setting speed (10s).

Comparing the evolution of the brands of biscuit with each other for both speed settings, it is observed that the number of homogenous groups for each texturometric variable remained

similar for both speed setting and time of analysis, being the texturometric evolution of this area very similar for each group of biscuit. At this area three homogenous groups are created, one composed the biscuit with less moisture (E, F) and other two different with each other H and G.

3.2.2.3 Puncture test, analysis time 8s, radial area

Table 6 show the texturometric variables (Maximum force, Hardness, Rigidity) generated by analysis of the radial area of the biscuits at a speed of 3 mm/s and 3,75 mm/s.

In table 6 can be observed that the radial area of all the brands of biscuits, the average values of all the texturometric variables are higher than those obtained in the central and intermediate areas. As occurred in the intermediate area, for all the times of analysis the slowest speed setting (3mm/s) obtained the highest average values for the variables maximum force and rigidity, while harness wasn't affected by the speed of analysis.

Observing individually the evolution of each brand of biscuit during the storage, due to the moisture caption it is observed how the average values of the variable hardness and rigidity increased during the storage for all the brands of biscuit. Peculiarly for the brands E and F, the variable maximum force obtained by both speed did not increased during the storage, and it may be explained in first place by the way in which the biscuits get toaster and harder on their edges, as result of losing rapidly more moisture during oven-baking than the other areas, and secondly by the higher compression forces on this area during the rotatory moulding, which reduce the expansion of gases during baking. Therefore, the radial area is hardest area and less hydroscopic.

Analogous to what was obtained during the analyses of the center and intermediate areas of the biscuits, it is observed that for each group of commercial biscuits, the coefficients of variation of all the texturometric variables obtained by both speed settings are similar for each time of analysis, thus the speed setting do not modified the coefficient of variation. However while the coefficients of variation of the variables maximum force of deformation and hardness, obtained at both speeds settings, remain constant throughout the storage time, the coefficients of variation of the variable rigidity decreased with the storage time.

Table 6: Texturometrical variables obtained by Puncture Analysis of the radial area of the biscuits.									
Time analysis:8s					Time analysis:10s				
Storage	Biscuit				Storage	Biscuit			
(months)	E	F	G	H.	(months)	E.	F.	G.	H.
Maximum force of deformation (g)					Maximum force of deformation (g)				
0	4599.42 ± 14.61 %^{c1}	4752.79 ± 15.04 %^{c1}	6461.54 ± 16.24 %^{a3}	5344.39 ± 15.22 %^{a2}	0	5413.92 ± 13.95 %^{bc1}	5626.08 ± 15.21 %^{d1}	7503.83 ± 16.03 %^{a2}	9407.78 ± 15.27 %^{a3}
1	3975.34 ± 19.50 %^{a1}	3972.46 ± 13.54 %^{ab1}	7286.54 ± 11.32 %^{b2}	7552.06 ± 14.74 %^{b2}	1	4785.29 ± 19.92 %^{ab1}	4697.77 ± 13.73 %^{a1}	8275.96 ± 11.59 %^{b2}	9541.75 ± 15.19 %^{a3}
2	3817.78 ± 15.96 %^{a1}	4244.02 ± 17.36 %^{bc2}	7786.67 ± 13.53 %^{c3}	7564.28 ± 9.17 %^{b3}	2	4541.76 ± 13.71 %^{a1}	5107.04 ± 16.92 %^{bc1}	9028.39 ± 14.51 %^{c2}	9425.05 ± 9.67 %^{a2}
3	4531.33 ± 13.47 %^{c1}	4653.59 ± 15.85 %^{c1}	8406.86 ± 12.28 %^{d3}	7576.51 ± 8.91 %^{b2}	3	5311.14 ± 11.10 %^{b1}	5435.49 ± 16.72 %^{cd1}	9625.58 ± 13.64 %^{d2}	9432.32 ± 15.59 %^{a2}
4	4188.17 ± 15.50 %^{b1}	4575.92 ± 21.94 %^{c1}	8175.73 ± 13.52 %^{cd2}	8293.21 ± 11.54 %^{c2}	4	5106.74 ± 14.52 %^{b1}	5488.19 ± 21.28 %^{cd1}	9396.30 ± 14.90 %^{cd2}	10366.60 ± 12.47 %^{b3}
Hardness (g.sec)					Hardness (g.sec)				
0	884.33 ± 19.56 %^{cd1}	864.96 ± 17.19 %^{b1}	1025.01 ± 18.57 %^{a2}	923.98 ± 14.39 %^{a2}	0	884.33 ± 19.56 %c^{1}	864.95 ± 17.19 %^{b1}	1253.43 ± 18.35 %^{a2}	1285.19 ± 15.68 %^{a2}
1	755.83 ± 14.31 %^{a1}	714.67 ± 16.71 %^{a1}	1403.45 ± 16.44 %^{b2}	1285.17 ± 15.68 %^{b2}	1	765.74 ± 13.23 %^{a1}	715.32 ± 17.87 %^{a1}	1403.4 ± 16.44 %^{ab2}	1299.81 ± 15.69 %^{a2}
2	826.17 ± 15.91 %^{bc1}	877.33 ± 17.27 %^{bc1}	1611.55 ± 15.15 %^{c2}	1333.94 ± 15.34 %^{b2}	2	826.17 ± 15.92 %^{bc1}	876.33 ± 17.27 %^{bc1}	1611.49 ± 15.15 %^{b2}	1339.63 ± 15.24 %^{a2}
3	983.37 ± 13.96 %^{e1}	973.53 ± 17.98 %^{d1}	1784.55 ± 16.13 %^{c3}	1387.71 ± 11.16 %^{b2}	3	987.91 ± 12.90 %^{e1}	975.94 ± 17.93 %^{d1}	1795.46 ± 15.94 %^{c3}	1399.08 ± 11.13 %^{a2}
4	915.62 ± 17.39 %^{d1}	955.81 ± 22.36 %^{d1}	1764.68 ± 17.38 %^{c3}	1552.15 ± 9.44 %^{c2}	4	913.18 ± 17.37 %^{d1}	956.81 ± 22.36 %^{d1}	1764.68 ± 17.38 %^{c2}	1552.46 ± 11.28 %^{c2}
Rigidity (g.sec)					Rigidity (g.sec)				
0	1793.99 ± 23.94 %^{b1}	1721.57 ± 23.58 %^{b1}	2893.06 ± 23.82 %^{a2}	2508.99 ± 22.48 %^{b2}	0	1901.95 ± 22.14 %^{b1}	1843.93 ± 22.51 %^{b1}	3238.27 ± 25.19 %^{a2}	2053.27 ± 24.27 %^{a2}
1	1415.05 ± 26.10 %^{a1}	1363.31 ± 25.92 %^{a1}	3182.50 ± 26.84 %^{b2}	1894.34 ± 22.36 %^{a1}	1	1533.05 ± 24.68 %^{a1}	1463.62 ± 25.59 %^{a1}	3294.57 ± 25.83 %^{b3}	2061.08 ± 24.43 %^{a2}
2	3565.74 ± 15.88 %^{c1}	3188.98 ± 18.16 %^{c1}	5905.55 ± 14.30 %^{c3}	3831.17 ± 15.09 %^{c12}	2	4143.62 ± 9.10 %^{c2}	3656.78 ± 17.54 %^{c1}	6549.98 ± 13.16 %^{c3}	4463.60 ± 9.78 %^{b2}
3	5194.74 ± 12.10 %^{d1}	5161.38 ± 11.65 %^{e1}	8389.17 ± 13.28 %^{d3}	5768.01 ± 9.27 %^{d2}	3	6075.65 ± 15.39 %^{d1}	6039.46 ± 15.81 %^{e1}	9611.47 ± 12.21 %^{d2}	6873.93 ± 8.41 %^{c1}
4	5172.41 ± 14.27 %^{d1}	4914.58 ± 13.68 %^{d1}	8361.86 ± 15.14 %^{d3}	6146.08 ± 12.57 %^{e2}	4	6154.02 ± 12.52 %^{d1}	5877.34 ± 12.97 %^{d1}	9519.71 ± 14.24 %^{d3}	7181.59 ± 12.50 %^{d2}

Note: Texturometrical variables obtained by Puncture Analysis from the radial area of biscuit brands studied (E, F, G, H) during storage (months). Values are mean ± Standard Deviation (SD). (n= 3); significant ($p< 5.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 5.05$) differences between biscuit types (rows) are indicated with different numbers.

On regard to the comparison of each brand of biscuits individually throughout the homogenous groups created by statistical analysis of each texturometric variable and speed settings. As it was observed in the intermediate area, employing both speed settings the number of homogenous groups obtained was similar for each texturometric variable. On the contrary to the central area where for the variables harness and rigidity, the number of homogenous groups were higher for the slowest setting speed (10s). Comparing the evolution of the brands of biscuit with each other for both speed settings, it is observed that the number of homogenous groups for each texturometric variable remained similar for both speed setting and time of analysis, being the texturometric evolution of this area very similar for each group of biscuit. At this area three homogenous groups are created, one composed the biscuit with less moisture content (E, F) and other two different groups with each H and G.

The results obtained by puncture in three areas of the biscuits at two speeds of analysis (3.75 mm/s and 3 mm/s), indicate that employing a speed setting of 3.75mm/s (8s) at the intermediate area of the biscuits, which corresponds to the softest area in all commercial brands of biscuits, produced a good differentiation between the different brands throughout the storage period with similar and low coefficient of variation during the storage time.

These results are in agreement with the studies by Ahmad *et al.*, (2001) who observed that the spongiest and softest structure was produced in the centre of the biscuit. However, they considered that it is difficult to determine the sensitivity of the method when changes in the moisture content occur (for example 3 – 5 % b.s) when the texture analysis used is (TPB). Therefore it is concluded that the puncture technique would be more precise for identifying changes in moisture of the biscuits.

The different moisture and texture in the centre of the biscuits was explained by Burt & Fearn (1983) as a result of the different gelatinisation of the starch during oven-baking, due to a higher moisture content in the geometric centre of the biscuits. Also, the moulding of a logo, characteristic of each commercial brand, would accentuate these differences, by creating compression and evaporation lines unique for each trademark of biscuit and hence emphasising the differences between commercial brands. On the contrary, the radial area is the area that is dehydrated most

during oven-baking and the area where the commercial logo is not moulded, and therefore the texture would be more similar between the different commercial brands, as was already observed by other authors like Mamat *et al*., (2010) and Lara *et al*., (2011).

Due to the complexity of comparing three texturometric variables for each puncture area at the same time, a statistical analysis by means of discriminant function analysis and analysis of neural networks was carried out. The results obtained are presented simultaneously in table 7 in order to facilitate the comparison of both analyses.

3.2.2.4. Discriminant function analysis of puncture of the biscuits

The goal of applying discriminant function analysis to each commercial brand of biscuit was to assess what the effect on the correct classification percentage of the groups was cause by the penetration area, the analysis speed and the time of storage.

The results of the separate discriminant function analysis of each commercial brand of biscuit, in three puncture areas (centre, intermediate, radius), applying two analysis speeds (3 mm/s (total analysis time 10 s), and 3.75 mm/s (total analysis time 8 s), throughout the storage period, are shown in Table 7.

From the results of the discriminant function analysis of each type of biscuit it can be observed that the correct classification of each brand of biscuit diminished throughout the storage period. In general, the analyses in the central area of the biscuits best classified each commercial brand of biscuits throughout the storage period and best maintained the classification percentage for both analysis speeds. As was mentioned before, this is explained by chemical changes produced by the gelatinization of the starch during oven-baking (Burt & Fearn, 1983). Different moisture content is produced between the centre and the radius of the biscuits and the moulding of the commercial logo, characteristic of each commercial brand, would accentuate these differences.

When the two types of analysis speed are compared, it is observed that the majority of the commercial brands of biscuit (E, F, G) did not show a better percentage of classification as a function of analysis speed, thus the classification percentage was independent of test speed. However, commercial brand H did present differences between the different test speeds. The speed of 3.75 mm/s best classified this group.

Table 7: Analysis multivariable of the texturometric variables generated by puncture									
Analysis time Area	**Storage**	Discriminant function analysis				Neural networks analysis			
		Biscuit brand				Biscuit Brand			
	(months)	E.	F	G	H	E.	F	G	H
8s Centre	0	77.78 %	77.78 %	61.11 %	88.89 %	76.47 %	88.24 %	52.94 %	100.00 %
	1	83.33 %	83.33 %	66.67 %	100.00 %	83.33 %	72.22 %	55.56 %	94.44 %
	2	94.44 %	88.89 %	94.44 %	100.00 %	94.44 %	77.78 %	77.78 %	100.00 %
	3	77.78 %	61.11 %	72.22 %	77.78 %	61.11 %	55.56 %	27.78 %	83.33 %
	4	66.67 %	55.56 %	61.11 %	77.78 %	66.67 %	61.11 %	55.00 %	72.22 %
total		**82.22 %**	**74.44 %**	**71.11 %**	**88.89 %**	**76.40 %**	**75.79 %**	**52.81 %**	**89.89 %**
8s Intermediate	0	61.11 %	22.22 %	75.00 %	97.22 %	69.44 %	85.56 %	72.22 %	94.44 %
	1	52.78 %	61.11 %	85.56 %	94.44 %	63.89 %	86.11 %	86.11 %	91.67 %
	2	94.44 %	55.00 %	83.33 %	100.00 %	86.11 %	88.89 %	75.00 %	94.44 %
	3	38.89 %	55.56 %	36.11 %	75.00 %	38.89 %	58.33 %	47.22 %	69.44 %
	4	58.33 %	100.00 %	55.00 %	61.11 %	61.11 %	35.56 %	58.33 %	58.33 %
total		**63.33 %**	**57.78 %**	**65.00 %**	**85.56 %**	**63.89 %**	**68.89 %**	**67.78 %**	**81.67 %**
8s Radius	0	66.67 %	77.78 %	74.07 %	96.30 %	69.44 %	72.22 %	75.37 %	94.44 %
	1	72.22 %	75.93 %	64.81 %	98.15 %	63.89 %	79.63 %	61.11 %	91.67 %
	2	98.15 %	95.74 %	94.44 %	100.00 %	86.11 %	88.89 %	79.63 %	94.44 %
	3	55.00 %	57.41 %	48.15 %	79.63 %	38.89 %	75.37 %	57.41 %	69.44 %
	4	57.41 %	57.41 %	48.15 %	66.67 %	61.11 %	64.81 %	45.74 %	58.33 %
total		**68.89 %**	**73.33 %**	**71.85 %**	**88.15 %**	**66.30 %**	**75.19 %**	**61.85 %**	**84.81 %**
10s Centre	0	83.33 %	77.78 %	61.11 %	72.22 %	100.00 %	100.00 %	100.00 %	55.56 %
	1	83.33 %	77.78 %	66.67 %	55.00 %	100.00 %	88.89 %	61.11 %	44.44 %
	2	94.44 %	94.44 %	94.44 %	100.00 %	100.00 %	100.00 %	77.78 %	88.89 %
	3	16.67 %	66.67 %	55.56 %	77.78 %	100.00 %	94.44 %	38.89 %	55.00 %
	4	72.22 %	55.56 %	55.56 %	61.11 %	94.44 %	72.22 %	83.33 %	55.00 %
total		**85.00 %**	**73.33 %**	**66.67 %**	**72.22 %**	**98.88 %**	**91.01 %**	**71.91 %**	**57.95 %**
10s Intermediate	0	63.89 %	33.33 %	75.00 %	47.22 %	61.11 %	85.56 %	75.00 %	11.11 %
	1	52.78 %	55.56 %	77.78 %	58.33 %	55.56 %	86.11 %	77.78 %	8.33 %
	2	94.44 %	55.56 %	91.67 %	100.00 %	97.22 %	88.89 %	77.78 %	91.43 %
	3	52.78 %	72.22 %	38.89 %	69.44 %	33.33 %	41.67 %	47.22 %	63.89 %
	4	58.33 %	94.44 %	52.78 %	61.11 %	47.22 %	47.22 %	52.78 %	58.33 %
total		**64.44 %**	**62.22 %**	**67.22 %**	**67.22 %**	**58.89 %**	**68.89 %**	**66.11 %**	**46.37 %**
10s Radius	0	75.37 %	77.78 %	72.22 %	75.93 %	62.11 %	75.93 %	64.81 %	11.11 %
	1	74.07 %	72.22 %	64.81 %	14.81 %	56.66 %	75.37 %	62.96 %	8.33 %
	2	100.00 %	94.44 %	96.30 %	72.22 %	87.32 %	92.59 %	87.04 %	91.43 %
	3	48.15 %	61.11 %	55.56 %	75.37 %	37.93 %	66.67 %	51.85 %	63.89 %
	4	68.52 %	64.81 %	51.85 %	31.48 %	56.62 %	64.81 %	37.04 %	58.33 %
total		**72.22 %**	**74.07 %**	**68.15 %**	**52.96 %**	**67.41 %**	**75.19 %**	**65.74 %**	**46.30 %**

Correctly classified groups of each commercial brand of biscuits after a multivariable analysis of the variables generated by puncture at 3.75mm/s (8s) and 3mm/s (10s), in three areas (center, intermediate, radius), throughout the storage period (months)

The analysis of neural networks aims at studying the effects of the area of puncture and the speed of the analysis on the correct classification of the biscuits throughout the storage period. These results indicate that, when each commercial brand of biscuit is analysed individually, the combination of “central position” of the biscuit and “speed of 3.75 mm/s” shows the best classification percentages for both statistical analyses.

4. CONCLUSIONS

It was to optimize and determine the best method for measuring the texture of biscuits, in order to establish it as a parameter for quality control of digestive type biscuits. It was observed that an increasing moisture content of the biscuit, caused by humidity absorption, entails texture changes during the storage time.

Thus the optimum test settings of the two most commonly used texturometric techniques would be as follows:

For the three-point bending technique, the use of a stand distance of 2 cm best describes the evolution of the texture of each brand of biscuits individually and it shows differences between commercial brands, with a relative low coefficient of variation.

Regarding the puncture technique, it was observed that for a cylindrical P/2 probe applied in the central and intermediate area of the biscuits with a test speed of 3.75 mm/s (8 seconds of analysis), was the most efficient method to determine the evolution of the texture of each brand of biscuits individually and to compare different commercial brands, and it was the most accurate method to determine and compare the evolution of the texture of each brand and between different commercial brands of biscuit, respectively.

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CAPÍTULO 3.-CARACTERIZACIÓN DE LAS GALLETAS CON FIBRA DE ALCACHOFA

CAPÍTULO 3.- CARACTERIZACIÓN DE LAS GALLETAS CON FIBRA DE ALCACHOFA

En este capítulo se presentan los estudios realizados para la obtención del tercero, cuarto y quinto objetivos de esta Tesis Doctoral:

- Evaluación de los parámetros de calidad (textura, color, etc.) de galletas enriquecidas con un 4 % de fibra (fibra comercial de referencia: fibra de guisante (P), fibre rich powders de alcachofa (FRPA) obtenidos por dos métodos de extracción acuosos (en agua destilada (W) y en disolución de 1 %(w/w) $CaCl_2.5H_2O$ (pH: 6,5) (CA)).
- Determinar la concentración de compuestos fenólicos y la actividad antioxidante de las galletas elaboradas con fibras de alcachofas extraídas de los subproductos del procesado de conservas de alcachofas y estudiar su evolución a lo largo del almacenamiento.
- Determinación de la bio-actividad de los compuestos antioxidantes, a lo largo del tiempo del almacenamiento y el proceso digestivo posterior a su consumo, se sometieron a un modelo de digestión humana y a un proceso extractivo químico.

Los resultados obtenidos han puesto de manifiesto que los parámetros de la textura y del color en las galletas sin fibra y con fibra comercial de referencia se modifican a lo largo del almacenamiento, presentando al final del mismo (7 meses) diferencias significativas ($p<0.05$), mientras que para las galletas formuladas con fibre rich powders extraídos de subproductos de alcachofa (W, CA) se comportan de manera más constante durante el almacenamiento.

Complementariamente, tanto el contenido en polifenoles como la actividad antioxidante en los extractos fisiológicos fueron significativamente superiores en comparación con la que presentaban los extractos químicos. Tanto inicialmente, como durante el almacenamiento, las galletas formuladas con FRPA (W, CA) presentaron mayor contenido en polifenoles biodisponibles y mayor actividad antioxidante tanto frente a las galletas control sin fibra (B) como a las formuladas con fibra comercial de referencia (P, fibra de guisante). En conclusión, los FRPA son una muy buena fuente de fibra con actividad antioxidante y bio-asimilable, en especial los FRPA (CA) y presentan una buena alternativa al empleo de fibra comercial de guisante.

Modelo de Digestión humana

Para la digestión de las muestras de galletas seguimos el modelo utilizado por Glahn *et al.*, 2000[290]. Este modelo intenta imitar las etapas de la digestión humana tales como la digestión ácida en el estómago, la neutralización con pepsina y pancreatina y, por último, la digestión intestinal. Una vez terminada en modelo de digestión el extracto de digestión (**Ext.1**), se almacena congelado a -4ºC, hasta su análisis.

Extracción compuestos polifenólicos en una matriz alimentaria (Galletas)

La extracción de compuestos fenólicos en alimentos está influenciada por su naturaleza química, método de extracción y disolvente empleado, el tamaño y la superficie de partícula del soluto, tiempo y condiciones de almacenamiento de producto alimenticio, presencia de sustancias interferentes (Naczk & Shahidi, 2006)[291], etc. Por lo tanto, no existe ningún procedimiento ideal y estandarizado para la extracción fenólica de matrices alimentarias.

En nuestro caso galletas enriquecidas con FRPAs se emplean en la formulación de con alta concentración polifenólica, aplicamos el método de extracción, propuesto por Vitali *et al.*, (2009)[31], para muestras de alimentos baja humedad como cereales o galletas. La extracción de los fenoles solubles de las galletas, se realiza mediante un proceso de extracción con una mezcla de HCl_{conc}/metanol/agua (1:80:10, v/v) durante un periodo de 24 h, a 20ºC, con constante agitación. Tras centrifugación (3500 rpm, 10 min) el sobrenadante de extracción (**Ext.2**) se almacena congelado a -4ºC, hasta su análisis. Debido a que las condiciones de extracción utilizadas no consiguen extraer la totalidad la composición polifenólica de los alimentos, porque éstos se hayan unidos a estructuras de la fibra que habría que hidrolizar. La extracción de fenoles insolubles se realiza según el procedimiento de Hartzfeld (Hartzfeld *et al.*, 2002)[292]. Abreviadamente, el soluto de la primera extracción se somete a un segundo proceso de extracción con una disolución de metanol y ácido sulfúrico cc. (10:1, v/v) durante un periodo de 20 h, a 85 ºC. (**Ext.3**) Tras centrifugación (3500 rpm, 10 min) el sobrenadante de extracción se almacena congelado a -4ºC, hasta su análisis.

3.1.-Variation during storage of the functional properties of biscuit prepared with fibre of artichoke (*Cynara Scolymus* L.)

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ABSTRACT

The objective of this study was to determine the phenolic compounds and the antioxidant activity in biscuits made with fibre-rich powders extracted from by-products of artichokes (FRPA), and to evaluate their evolution throughout storage. Biscuits were prepared with FRPA extracted by two different extraction solvents: Water (W) and a solution of 1 %$CaCl_2.5H_2O$ (CA) and compared with biscuits made with Pea fibre (P) and control biscuits (B) without fibre added.

Initially and during storage, the biscuits enriched with FRPA (W, CA) showed a higher content of bioavailable polyphenols and antioxidant activity compared to the control biscuits (B) and the reference fibre (P, Pea fibre). In conclusion, FRPA are an excellent source of bioavailable fibre with antioxidant activity, especially the FRPA extracted with 1 %$CaCl_2{\cdot}5H_2O$ (CA), and they present a good alternative to the use of Pea fibre.

Keywords: polyphenols, antioxidant activity, Pea fibre.

1. INTRODUCTION

In recent years biscuits with high fibre content have been established as one of the most stable sectors in the functional food industry. Since the population of the Western world attempts to consume products that supply fibre, which their lifestyle and current diet do not provide (Bes-Rastrollo *et al.,* 2006, Slavin, 2005). Therefore, the enrichment of food products like biscuits, with new sources of dietary fibres obtained from vegetal by-products, could be a good alternative to complement the needs of the fibre of the population through the consumption of a daily product (Guillon *et al*., 2011). Moreover, it could yield the environmental management of these by-products profitable and reduce illnesses, suffered by populations in industrialized countries, like: gastric cancer, diabetes, constipation, obesity, irritable bowel syndrome, etc. (Zhang *et al.,*2013, McEvoy & Woodside, 2015, Brownlee, 2011, Jaakkola *et al.,* 2013, Reicks *et al.,* 2014, Pezdirc *et al.,*2015, Aller *et al.,* 2004).

Since vegetal by-products have a rich content in antioxidant compounds, the fibre-rich powders obtained from vegetal by-products could be a good alternative to enrich food products with antioxidant compounds. In their turn, these could both reduce processes of rancidification or fat oxidation during storage of the biscuits (Talbot, 2010) and complement the diet with these nutrients. In the literature, a number of studies can be found in the production of fibre-rich powders from by-products of several products of vegetal origin (O'Shea *et al.,* 2012, Elleuch *et al.,* 2011). Chantaro *et al.,*(2008) extracted fibre-rich powder with a high antioxidant activity from carrot peels, Nilnakara *et al.,*(2009) from the outer leaves of cabbage, Lecumberri *et al*.,(2007) extracted fibre from by-products of the production of cacao, Pérez Jiménez *et al.,*(2008) and Zhu, *et al.,* 2015) from grape seeds, Nandi & Gosh (2015) from sesame seeds, Fuentes-Aleventosa *et al.,*(2009) from asparagus by-products, López-Vargas *et al.,*(2013) from by-products of the production of fruit juices: yellow passion fruit, Amaya-Cruz *et al.,*(2015), Navarro-González *et al.,* (2011) from by-products of tomato and Garau *et al.,* (2007) from orange by-products. Regarding the application of compounds rich in fibres with a high antioxidant power, in oven-baked products like biscuits (Manley, 2011), in recent years products have been used from extracts of fruit and wild plants (Reddy *et al.,*2005) and of tea leaves (Sharma & Zhou, 2012).With respect to the present study on the production of biscuits with antioxidant fibre-rich powders we can highlight the studies by Vitali

et al., (2009) who utilised fibre from apple by-products and Ajila *et al.*,(2007) who employed mango by-products.

Currently, several vegetal fibres are already marketed for the enrichment of biscuits with high fibre content, as is the case of beetroot (Fibrex®), chicory (Raftilosa®) and pea (Raymundo *et al.*,2014). However, we are not aware of studies regarding the formulation of biscuits with fibre-rich powders obtained from the revaluation of artichoke by-products, generated by the canning industries. On the other hand, there are some studies on the functional properties and content of fibre-rich powders of artichoke (FRPA) extracted by different methods (Ruiz-Cano *et al.,* 2014), which support the possible use of these fibre-rich powders in the formulation of novel products.

The objective of this study was to determine the phenolic compounds and the antioxidant activity of biscuits produced with fibre-rich powder of artichoke, extracted from the by-products of artichoke, and to investigate their evolution during storage. Biscuits were made with artichoke fibres, extracted by two distinct methods: water (W) and 1 %$CaCl_2 \cdot 5H_2O$ (CA), and compared with biscuits prepared with Pea fibre. For the control biscuits, no fibre was added. Two types of storage were carried out: one at room temperature (25 ºC, 7 months) and another accelerated (45 ºC, 6 months).

To test whether these antioxidant compounds could maintain their effect and bioactivity throughout the storage period and the digestive process after their consumption, they were subjected to a model of human digestion and an extractive chemical process.

2. MATERIAL AND METHODS

2.1 Sample preparation

*A standard form*ulation similar to the fibre rich biscuits sold in the Spanish market was used (Whitley, 1970), with slight modifications in the percentages of the ingredients.

Three different batches were prepared for each type of biscuits on various days. The biscuit types were: control biscuits without fibre (B), biscuits with Pea fibre (P), and biscuits enriched with FRPA obtained in our laboratory by liquid extraction with distilled water (W) and with a solution of 1 %

(w/w) $CaCl_2 \cdot 5H_2O$ (pH 6,5) (CA) following the method applied by Fuentes-Alventosa, 2009[b]. Each batch of biscuits was analysed separately and in triplicate.

Table 1 shows the formulation used for the preparation of the biscuits. The biscuits were formulated with 4 % of fibre (fibre weight /wet weight). For the control biscuits (B) the fibre were replaced by the same weight of wholemeal flour.

Table 1. Formula of the biscuits	
Ingredient	%
Water	7.45
Glucose-fructose syrup	1.45
Sunflower oil	12.02
Whey	3.52
Sodium and ammonium bicarbonate	1
salt	0.23
Lecithin	0.1
Wholemeal wheat flour	61.2
Fiber: Pea fibre(P) or FRPA (W,CA)	4
	100

The dough is kneaded with a pilot mixer for 10 min at minimum speed. Subsequently, the dough was laminated to a thickness of 5 mm, using a manual sheet pasta machine. The round form of the biscuits was obtained manually, using a cutting cylinder (die) of 60 mm in diameter.

After making 8 evaporation holes in the dough with a fork, the biscuits were baked in an electrical convection oven at a temperature of 180 ± 10 °C during 17 minutes. Afterwards, the biscuits were cooled at room temperature (18 – 20 °C) and wrapped in heat-sealed bags of high-density polyethylene. Until analysis the biscuits were divided into two groups as a function of the storage conditions.

As a control for each group of biscuits, the values were taken at time 0, which was the day after the preparation.

2.2 Storage

The wrapped and coded biscuits were stored in two incubation chambers (Incudigit Selecta Spain, where temperature and humidity were kept constant. One of the chambers simulated storage at room temperature (25 °C, RH 55 %), while the other simulated conditions of accelerated storage (45 °C, RH 55 %) (Yang *et al.*, 2013), forcing the appearance of defects in the biscuits by the increase in

temperature. The biscuits stored under ambient conditions were analysed monthly, during the 7 months of the duration of this study, while the biscuits stored under accelerated conditions were analysed every 3 months until completion of the six months of storage.

By comparing the results of both storage procedures, we intend to investigate at which time under ambient conditions the defects are detected, generated under accelerated storage.

2.3. Determination of Moisture

Moisture (M) in the biscuits was determined by the method described by Nollet (1996) with slight modifications. Biscuit samples (250 mg) were weighed in nonuplicate on an analytical balance (Mettler Toledo, USA) and they were dried for 24 hours at 90ºC. Subsequently, moisture was calculated as the difference in weight (M= (Wet weight-Dry weight)/ weight sample).

2.4. Determination of the functional properties of the biscuits

To investigate the effect of the functional properties of the biscuits enriched with FRPA, the working method proposed by Vitali *et al.*, (2009) was followed, with some modifications (fig. 1). A human digestive model was performed, with which an extract (**Ext.1**) was obtained, and the content of bioavailable polyphenols was determined. A chemical extraction of the polyphenolic compounds was performed with which two extracts were obtained for the determination of the polyphenolic content: extractable (**Ext.2**) and hydrolysable (**Ext.3**). All the extractions were carried out in triplicate.

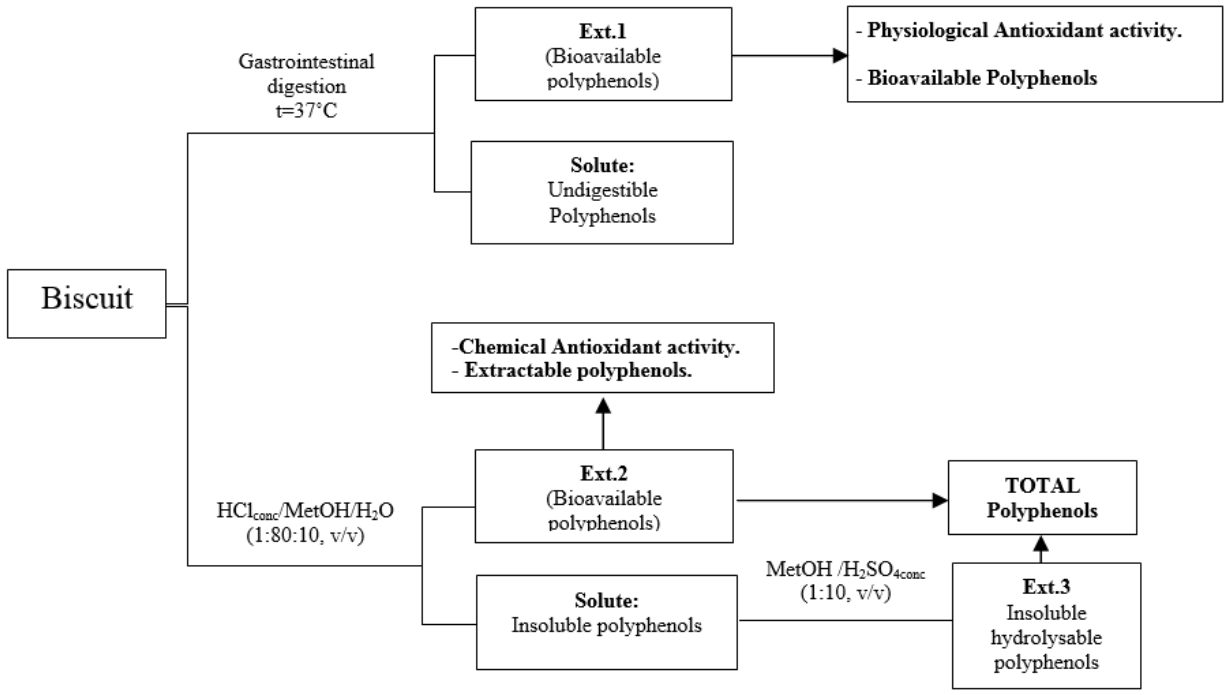

Fig. 1: the Schematic procedure of analyses. Vitali*et al.,* (2009)

2.4.1. Model digestion *in vitro*

For the digestion of the biscuit samples the method of Glahn *et al.,* (2000) was utilized, proposed for the in vitro study of the availability of iron in food products. This model attempts to imitate the phases of the human digestion such as acid digestion in the stomach, neutralization with pepsin and pancreatin and, finally, intestinal absorption. Once the digestion model was terminated, the digestion extract (**Ext.1**) was stored frozen at -4ºC until analysis (polyphenols and antioxidant activity).

2.4.2. Extraction of polyphenolic compounds from a food matrix

The extraction method described by Vitali et al., (2009) was utilized, proposed for samples of food products with little moisture like cereals or biscuits. The extraction of soluble phenols from the biscuits was performed by an extraction with a mixture of HCl_{conc}/methanol/water (1:80:10, v/v) during 24 h, at 20ºC, with constant shaking. After centrifugation (3500 rpm, 10 min) the supernatant (**Ext.2**) was frozen at -4ºC until analysis. The extraction conditions applied are not able to extract

the total phenolic content of the food products, since the phenols are bound to structures of the fibre, which has to hydrolyse. The extraction of the insoluble phenols was carried out by the procedure of Hartzfeld *et al.*, (2002). Briefly, the pellet of the first extraction was subjected to a second extraction process with a mixture of methanol and sulphuric acid (10:1, v/v) during 20 h, at 85 ºC. (**Ext.3**). After centrifugation (3500 rpm, 10 min) the supernatant was stored frozen at -4ºC, until analysis.

2.4.3. Polyphenols

The content of total polyphenols was calculated as the sum of the fractions of extractable (**Ext.2**) and hydrolysable (**Ext.3**) polyphenols, while for the content of polyphenols with physiological antioxidant activity after digestion **Ext.1** was analysed. Polyphenols were estimated using the method proposed by Singelton & Rossi (1965), measuring absorbance at a wavelength of 765 nm. The results were compared with a calibration curve, previously carried out with gallic acid, using concentrations of between 0 and 0.1 mg of gallic acid/mL and were expressed as mg of gallic acid per mL of extract.

2.4.4. Antioxidant activity

For the determination of the total antioxidant activity of the biscuits, **Ext.2** and **Ext.3** were used, while for the antioxidant activity after digestion, Ext.1 was used. Several methods were performed for the analysis of antioxidant activity, $ABTS^{\cdot+}$, FRAP and $DPPH^{\cdot}$.

2.4.4.1. $ABTS^{\cdot+}$

This method was determined according to the method of Re et al., (1999).The variation in the absorbance of the samples was measured at a wavelength of 732 nm, using a spectrophotometer, after 7 and 30 minutes of incubation of the extracts in the dark.

The calibration curve was prepared using Trolox as a standard. Antioxidant activity of the extracts was expressed as TEAC, which is the antioxidant activity in Trolox equivalents (mEq Trolox /g dry weight of biscuit).

2.4.4.2. DPPH·

This method was determined according to the method of Brand-Williams *et al.*, (1995), with some modifications (SanJosé*et al.*, 2018). Absorbance in the extracts was measured at a wavelength of 517 nm using a spectrophotometer. The calibration curve was performed with concentrations of between 25 and 800 mM of Trolox, obtained by different dilutions of a Trolox stock solution of 4 mM (1 mg/mL). The results were expressed as TEAC.

2.4.4.3. FRAP

The FRAP method was carried out according to Benzie & Strain (1996) with some modifications (SanJosé*et al.*, 2018). Absorbance in the extracts was measured at a wavelength of 593 nm using a spectrophotometer. The results were expressed as TEAC.

2.5. Statistical analysis

Statistical analysis was performed using the software Statgraphics Centurion XVI 16.1.03 version (Statpoint Technologies Inc.). Results were expressed as mean values ± standard deviations. The differences between the variables and samples were analysedusing one-way ANOVA and in the event of significant differences ($p < 0.05$) the post-hoc LSD test.

3. RESULTS AND DISCUSSION

3.1 Moisture

Table 2 shows the results obtained of moisture, for the different types of biscuits studied, throughout the storage period at ambient and accelerated temperature. The biscuits formulated with artichoke fibre (W, CA) had higher initial moisture content than the control biscuits (B) and the biscuits with Pea fibre (P). During storage at room temperature, the moisture content of the biscuits decreased, with some fluctuations. During storage at accelerated temperature, all groups of biscuits with fibre showed a significant reduction in moisture content.

Table 2. Moisture (w % w) of the biscuits B, P, W, CA, throughout storage at ambient and accelerated temperature

Stored (25 ºC) (Month)	B	P	W	CA
0	2.34 ± 0.35^{cd1}	2.58 ± 0.43^{e2}	2.63 ± 0.28^{g3}	2.61 ± 0.22^{f3}
1	1.88 ± 0.30^{a1}	1.79 ± 0.35^{a1}	2.07 ± 0.16^{bc2}	1.94 ± 0.30^{ab1}
2	2.44 ± 0.22^{d2}	1.98 ± 0.20^{ab1}	1.87 ± 0.30^{a1}	2.25 ± 0.36^{de2}
3	2.41 ± 0.22^{d2}	2.44 ± 0.24^{de2}	2.41 ± 0.17^{f2}	2.28 ± 0.23^{de1}
4	2.25 ± 0.21^{bcd1}	2.34 ± 0.29^{cde1}	2.27 ± 0.07^{def1}	2.17 ± 0.15^{bcd1}
5	2.04 ± 0.27^{ab1}	2.18 ± 0.20^{bcd2}	2.09 ± 0.14^{bcd1}	2.20 ± 0.12^{cde2}
6	2.10 ± 0.25^{abc2}	2.01 ± 0.20^{ab1}	2.20 ± 0.12^{cde2}	2.01 ± 0.26^{bc1}
7	1.85 ± 0.13^{a12}	2.02 ± 0.21^{ab3}	1.93 ± 0.14^{ab23}	1.76 ± 0.31^{a1}
Stored (45 ºC) (Month)	B	P	W	CA
3MA	2.09 ± 0.25^{abc1}	2.48 ± 0.43^{de2}	2.34 ± 0.26^{ef2}	2.35 ± 0.34^{de2}
6MA	2.05 ± 0.41^{ab1}	2.08 ± 0.40^{abc1}	2.09 ± 0.14^{bcd1}	2.01 ± 0.26^{bc1}

Values are mean ± Standard Deviation (SD). (n= 3); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. Storage codification, letter (fibre source), number (months stored at room temperature), AM accelerated Month). Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2.5H_20$).

3.2. Phenolic content and bioavailability and antioxidant activity of the biscuits studied

The total phenolic content was calculated as the sum of the extractable (Ext.2) and hydrolysable (Ext.3) polyphenolic fractions, according to Pérez -Jimenez & Saura-Calixto (2005). the consistency of our results was estimated and confirmed by comparing with data obtained for polyphenol contents of samples similar to ours (Vitali *et al.*, 2009, Beta *et al.*, 2005, Sudha *et al.*, 2007, Saura-Calixto *et al.*, 2007).

To evaluate whether the fibre-enriched biscuits may be a good source of bioavailable polyphenolic compounds. The polyphenolic content was evaluated of the extracts obtained after having subjected the biscuits to a human digestion model (**Ext.1**). In this way we also wanted to investigate whether the usual approach of estimating soluble phenols in foodstuffs accurately reflects the content of bioavailable polyphenols.

Table 3: Polyphenol content (mg of gallic acid/mL of extract) of the biscuits studied (B, P, W, CA) during storage.				
Ext.1				
Stored (t:25 ºC) (T:Month)	B	P	W	CA
0	3.20 ± 0.35^{ef1}	3.69 ± 0.45^{c2}	4.30 ± 0.33^{c3}	4.84 ± 0.53^{d4}
1	3.61 ± 0.54^{g2}	3.41 ± 0.30^{c1}	4.08 ± 0.25^{c3}	4.93 ± 0.79^{d4}
2	$3.27 \pm 0.23f^{g2}$	2.72 ± 0.24^{b1}	2.81 ± 0.41^{b1}	3.20 ± 0.44^{c2}
3	3.19 ± 0.31^{ef1}	3.02 ± 0.34^{b1}	4.06 ± 0.33^{c3}	3.06 ± 0.24^{c2}
4	2.72 ± 0.43^{d1}	2.87 ± 0.15^{b12}	3.10 ± 0.29^{b2}	2.56 ± 0.30^{b1}
5	2.72 ± 0.43^{cd1}	2.87 ± 0.35^{b12}	3.07 ± 0.30^{b2}	2.46 ± 0.24^{b1}
6	2.87 ± 0.28^{de1}	2.80 ± 0.29^{b1}	3.15 ± 0.21^{b2}	3.01 ± 0.33^{c2}
7	1.95 ± 0.13^{a1}	2.38 ± 0.18^{a2}	3.08 ± 0.22^{b3}	3.03 ± 0.33^{c3}
Stored (t:45 ºC) (T:Month)	B	P	W	CA
3MA	2.46 ± 0.34^{bc1}	2.75 ± 0.22^{b2}	2.87 ± 0.36^{b2}	2.48 ± 0.32^{b1}
6MA	2.29 ± 0.29^{ab1}	2.31 ± 0.20^{a12}	2.21 ± 0.28^{a1}	2.47 ± 0.20^{a2}
Ext.2+Ext.3				
Stored (t:25 ºC) (T:Month)	B	P	W	CA
0	7.86 ± 1.36^{e1}	8.41 ± 0.59^{d2}	8.45 ± 0.54^{f2}	8.65 ± 0.76^{f23}
1	7.69 ± 0.70^{de1}	8.07 ± 1.04^{d2}	8.26 ± 1.4^{ef3}	7.91 ± 1.04^{e2}
2	7.93 ± 0.63^{e2}	8.14 ± 0.89^{23}	7.77 ± 0.67^{cd1}	8.04 ± 0.49^{e2}
3	6.83 ± 0.47^{c1}	7.75 ± 0.77^{b23}	7.52 ± 0.36^{cd2}	8.01 ± 0.89^{e3}
4	7.21 ± 0.51^{cd1}	7.73 ± 0.74^{d23}	7.59 ± 0.85^{def2}	7.70 ± 0.80^{de23}
5	7.12 ± 0.50^{cd2}	6.94 ± 0.48^{c1}	7.26 ± 0.30^{de23}	7.44 ± 0.48^{de3}
6	6.82 ± 0.46^{c12}	6.62 ± 0.78^{c1}	7.17 ± 0.91^{c2}	6.66 ± 0.88^{c1}
7	5.41 ± 0.29^{b1}	5.34 ± 0.44^{b1}	5.96 ± 0.50^{b2}	5.94 ± 0.59^{b2}
Stored (t:45 ºC) (T:Month)	B	P	W	CA
3MA	5.22 ± 0.27^{b1}	5.08 ± 0.91^{d1}	6.80 ± 0.81^{d2}	7.15 ± 0.27^{cd3}
6MA	3.49 ± 0.12^{a1}	3.55 ± 0.71^{a12}	3.56 ± 0.39^{a12}	3.66 ± 0.48^{a2}

Values are mean ± Standard Dev. (n= 9); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. **Ext.1** (model of human digestion) and **Ext 2 + Ext. 3** (chemical extraction) Storage codification, letter (fibre source), number (months stored at room temperature), AM accelerated Month). Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2 \cdot 5H_20$).

The results obtained from the content of polyphenols in the control biscuits without fibre (B) and in the biscuits prepared with the fibre of artichoke (W, CA) and Pea fibre (P), throughout the storage period, are shown in Table 3.The initial polyphenolic concentration of biscuits subjected to a human digestion model was higher in all biscuits prepared with fibre than in the control biscuits without fibre (B), and the groups with fibre showed significant differences amongst each other. Therefore, the increase in polyphenol content is attributed to the enrichment of the formulation of the biscuits with vegetal fibre. The polyphenol content of the biscuits without fibre is mainly the result of the polyphenol content of the wheat bran (Doğan & Gökmen, 2015, Arranz & Saura, 2010). It is noteworthy that at the start of storage, the biscuits formulated with artichoke fibre (CA and W) had a significantly higher polyphenol content than the biscuits formulated with the reference fibre (P).

At room temperature, the polyphenol content of **Ext.1** of all groups of biscuits showed a significant decrease throughout the storage period. The greatest decrease was observed in biscuits B (39 %) and the smallest decrease was found in biscuits W (28 %). The biscuits enriched with FRPA maintained a significantly higher content of polyphenols than the biscuits enriched with Pea fibre. However, at the end of accelerated storage, no significant differences in polyphenol content were observed between the control biscuits and the rest of the biscuits, except for the biscuits enriched with artichoke fibre CA, which had the highest content of total polyphenols.

The total polyphenol contents (**Ext.2+Ext.3**) of the biscuits studied are shown in Table3. It can be observed that the content of total polyphenols of all biscuits showed a great difference with the one obtained after digestion of the biscuits (**Ext.1**). This is due to the liberation of the insoluble polyphenols, bound to the plant structures, in the process of acid extraction. Similar to what was found for Ext.1, the initial polyphenol content of the biscuits prepared with fibre was significantly higher than of the control biscuits without fibre. Consequently, the increase in polyphenol content is the results of the polyphenolic content provided by the vegetal fibre added to the formulation. The initial contents of the biscuits formulated with FRPA (W, CA) and Pea fibre (P) was significantly higher than the initial content of biscuits P. Throughout storage at room temperature, the concentration of extractable polyphenols (**Ext.2+Ext.3**) significantly decreased in all groups of biscuits (≈30 %) until the end of the storage period (7 months). The biscuits enriched with FRPA (W, CA) had a higher content of total polyphenols than the other biscuits (B and P), showing a significant difference at the end of the storage period. Similar to what was observed during storage at room temperature, at accelerated temperature a reduction of the polyphenolic content was observed for all groups of biscuits. When comparing the results of storage at ambient and accelerated temperature, it is evident that the reduction of the total polyphenol content that occurred during storage at accelerated temperature for three months. It corresponded with the reduction observed after seven months of storage at room temperature, for the groups of biscuits that were not formulated with FRPA, i.e. B and P.

The results obtained suggest that the products enriched with FRPA could be considered as food products rich in polyphenols and could contribute to complement the requirements of daily intake (DRI) of dietary polyphenols. Curiously, although polyphenols are essential dietary components, at present their DRI's have not been established yet (Williamson & Holst, 2008), Sies (2010).

3.3. Antioxidant activity

The main ingredient of the biscuits is wholemeal flour, which contains phytochemicals deriving primarily from the layer of bran of the whole cereal grain. These compounds present antioxidant properties, and therefore biscuits could be considered as a foodstuff with potential antioxidant properties. To determine the antioxidant activity of the biscuits, different methods were used. The radical-sequestering activity was elevated when the methods $ABTS^{\cdot+}$ and DPPH· were used, based on the discoloration of the cation radical. The FRAP method was used to determine the ferric reducing power of the analysed samples.

To compare the concentration of antioxidants liberated from the food matrix during the human digestion conditions. The antioxidant activity was determined in two different extracts. (**Ext.1**) with the antioxidants that are solubilized by the solvent (MeOH: water: HCl) of the chemical extraction (**Ext.2**).

3.3.1. $ABTS^{\cdot+}$

Table 4 shows the results obtained for the antioxidant capacity in **Ext.1** by the $ABTS^{\cdot+}$ method, throughout the storage period at room temperature. As can be observed, the biscuits enriched with FRPA (W and CA) initially showed a higher antioxidant activity than the other two types of biscuits, while the control biscuits (B) had the lowest antioxidant activity. The control biscuits (B) presented antioxidant activity as a result of the presence of antioxidant compounds in the sunflower oil (vitamin E) and the antioxidant content of the wheat bran fibre (Manley, 2011). Therefore, in the rest of the biscuits that were enriched with Pea fibre (P) and FRPA (W, CA), the increment of the antioxidant activity would be the result of the contribution of each fibre.

These primary differences levelled off throughout the storage period for all groups of biscuits. However, the antioxidant activity of the biscuits formulated with (P, CA, W) was more stable throughout storage.

The antioxidant activity of the extracts by chemical extraction (**Ext.2**) was higher in the biscuits prepared with fibre (P, W, CA) than in the biscuits without fibre (B), due to the contribution of the vegetal fibres to the content of the biscuits. Initially, a significantly higher antioxidant activity was observed of the FRPA than the reference fibre (P). At the end of storage at room temperature, the antioxidant activity values of the biscuits formulated with vegetal fibres (P, W, CA) levelled off, but the trend of significantly lower values in the control biscuits without fibre (B) remained. During

of the accelerated storage is observed an increase of the activity antioxidant in physiological (**Ext.1**) and chemical (**Ext.2**) extracts. This effect may be due to the temperature of the accelerated storage, which would generate the same antioxidant compounds produced during baking. Reports in the literature suggest that the increase in antioxidant activity could be attributed to the formation of certain products from the Maillard reaction or to oxidation products of lipids under the influence of high temperature, which can occur during oven-baking (Jing & Kitts, 2004, Sun *et al.*, 2006, Wagner, *et al.*, 2007, Yilmaz & Toledo, 2005).

During accelerated storage (45 ºC), after three months an increase was observed in the antioxidant activity of the extracts by chemical extraction (**Ext.2**) of the biscuits, which could be due to the formation of Maillard compounds, as mentioned before. However, after six months of accelerated storage antioxidant activity of all the biscuits decreases, which is probably the result of oxidation of the fats. Nevertheless, the antioxidant activity of biscuits with fibre (P, W, CA) was still significantly higher than of the biscuits without fibre (B).

When comparing the results obtained for the extracts of the digestion model (**Ext.1**, Table 4) with those of the chemical extraction (**Ext.2**, Table 4). It is evident that the free radical antioxidant activity of the digestive extracts of the biscuits (**Ext.1**) was significantly higher than that of the chemical extracts (**Ext.2**). This difference could be caused by the contribution of hydrolysable antioxidant compounds liberated by the acid digestion step that imitates the action of gastric HCl in combination with the digestive enzymes (pepsin and trypsin).

Although there was a general correlation between TEAC values obtained in chemical and physiological extracts, some discrepancies were observed due to the increasing antioxidant activity of the biscuits throughout the storage period at room temperature.

Table 4: Evolution of antioxidant activity by ABTS.+ ((mEq Trolox /g of biscuit) of the biscuits studied (B, P, W, CA) during storage.				
Ext.1				
Store (t:25 ºC) (T:Month)	B	P	W	CA
0	400.12 ± 30.76^{a1}	500.82 ± 30.04^{a2}	559.13 ± 95.05^{cd3}	613.17 ± 73.58^{bc4}
1	494.23 ± 43.49^{a1}	516.44 ± 45.60^{c2}	476.64 ± 104.56^{ab1}	529.60 ± 47.66^{ab3}
2	438.51 ± 70.16^{ab1}	513.44 ± 61.61^{a3}	448.52 ± 103.16^{a1}	484.44 ± 72.66^{a2}
3	596.23 ± 95.39^{d1}	624.54 ± 53.96^{b2}	618.02 ± 111.24^{de12}	628.10 ± 62.81^{cd2}
4	501.05 ± 70.14^{bc1}	605.36 ± 60.83^{b3}	673.50 ± 60.61^{e4}	573.85 ± 51.64^{bc2}
5	713.62 ± 64.22^{e3}	627.57 ± 51.96^{b1}	675.59 ± 74.31^{e2}	696.84 ± 62.71^{e23}
6	695.94 ± 69.59^{e2}	634.51 ± 39.46^{b1}	682.14 ± 34.10^{e2}	$687,84 \pm 75.66^{de2}$
7	513.99 ± 30.83^{c1}	568.78 ± 39.81^{a2}	605.01 ± 48.40^{de3}	613.17 ± 73.58^{cd3}
Store (t:45 ºC) (T:Month)	B	P	W	CA
3MA	508.85 ± 71.23^{a1}	557.69 ± 89.23^{cd3}	549.61 ± 76.94^{bcd3}	539.40 ± 70.12^{ab2}
6MA	500.82 ± 30.04^{a2}	400.12 ± 30.76^{a1}	559.13 ± 95.05^{cd3}	613.17 ± 73.58^{bc4}
Ext.2				
Stored (t:25 ºC) (T:Month)	B	P	W	CA
0	119.61 ± 9.09^{a1}	123.62 ± 7.12^{a2}	136.65 ± 20.29^{a3}	145.37 ± 13.66^{a4}
1	132.32 ± 18.52^{a1}	157.73 ± 22.41^{b2}	156.16 ± 20.19^{ab2}	171.30 ± 29.12^{b3}
2	130.00 ± 7.33^{a1}	153.35 ± 7.08^{b2}	168.90 ± 6.70^{b3}	178.46 ± 17.84^{bc4}
3	308.55 ± 12.99^{f1}	334.24 ± 36.06^{f2}	326.27 ± 18.07^{e2}	358.75 ± 26.54^{g3}
4	272.57 ± 17.49^{e2}	264.55 ± 16.56^{e1}	271.37 ± 38.83^{d2}	296.46 ± 35.57^{f3}
5	273.86 ± 22.53^{e1}	274.60 ± 13.73^{e1}	308.00 ± 24.23^{e2}	311.88 ± 31.18^{f2}
6	214.16 ± 27.83^{d1}	240.23 ± 19.21^{d2}	253.66 ± 42.48^{d3}	268.00 ± 33.41^{e4}
7	170.00 ± 10.40^{b1}	195.56 ± 12.73^{c2}	199.65 ± 16.41^{c2}	199.11 ± 16.84^{de2}
Stored (t:45 ºC) (T:Month)	B	P	W	CA
3MA	220.35 ± 15.60^{d1}	268.86 ± 28.92^{e3}	264.30 ± 34.43^{d3}	237.53 ± 22.94^{d2}
6MA	194.47 ± 19.52^{c1}	208.41 ± 7.27^{c2}	222.65 ± 8.92^{c3}	248.25 ± 23.58^{c4}

Values are mean ± Standard Dev. (n= 9); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. **Ext.1** (extracts obtained from the model of human digestion) and **Ext.2** (chemical extraction). Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2.5H_20$). Storage codification, letter (fibre source), number (months stored at room temperature), AM accelerated Month).

3.3.2. DPPH·

Table 5 shows the results of antioxidant activity measured by DPPH· analysis of the control biscuits without fibre (B), with FRPA (W, CA) and with Pea fibre (P), subjected to a model of human digestion (**Ext.1**). It can be observed that the control biscuits (B) and the biscuits formulated with the reference fibre (P) were very similar to each other and significantly lower than the biscuits enriched with FRPA (W and CA). Thus, the chemical content of the FRPA contributed in a significant way to the antioxidant effect of the formulation of the biscuits, compared to the reference fibre (P).Throughout storage at room temperature, although with some fluctuation of the values, the antioxidant activity of all the biscuits tended to decrease. However, the antioxidant activity of the

biscuits formulated with FRPA (CA, W) was more stable. On the contrary, in the biscuits subjected to accelerated storage an increase in antioxidant activity was observed.

Table 5: Evolution of antioxidant activity by $DPPH^{\cdot}$ (mEq Trolox /g of biscuit) of the biscuits studied (B, P, W, CA) throughout storage.

Ext.1				
Stored (t:25 ºC) (T:Month)	B	P	W	CA
0	163.82 ± 16.38^{c1}	163.48 ± 22.56^{cd1}	194.76 ± 28.33^{f2}	196.13 ± 28.53^{c2}
1	$247,22 \pm 27.19^{d1}$	269.02 ± 38.46^{e2}	269.24 ± 38.14^{g2}	279.67 ± 39.65^{e3}
2	$161,74 \pm 21.02^{c1}$	178.58 ± 17.38^{d2}	187.85 ± 17.53^{ef3}	238.09 ± 38.09^{d4}
3	89.62 ± 0.89^{a1}	103.56 ± 17.72^{a2}	137.75 ± 21.72^{a3}	159.34 ± 27.49^{ab4}
4	$112,76 \pm 17.78^{b1}$	166.68 ± 22.93^{d3}	163.90 ± 18.02^{bcd3}	157.97 ± 17.37^{ab2}
5	102.82 ± 11.31^{ab1}	136.97 ± 17.38^{b2}	142.55 ± 19.95^{ab3}	157.14 ± 17.71^{ab4}
6	$108,64 \pm 17.38^{b1}$	131.16 ± 20.67^{b2}	166.51 ± 24.47^{cde3}	180.52 ± 24.78^{bc4}
7	119.76 ± 11.97^{b1}	129.54 ± 8.66^{b2}	146.77 ± 14.67^{ab3}	144.59 ± 11.56^{a4}
Stored (t:45 ºC) (T:Month)	B	P	W	CA
3MA	119.45 ± 21.49^{b1}	144.43 ± 18.94^{bc2}	152.91 ± 18.34^{abc3}	158.70 ± 19.04^{ab3}
6MA	172.45 ± 22.41^{c1}	171.52 ± 9.74^{d1}	180.63 ± 23.48^{def12}	193.95 ± 170.28^{c2}
Ext. 2				
Stored (t:25 ºC) (T:Month)	B	P	W	CA
0	67.58 ± 4.59^{cd1}	68.31 ± 3.41^{cd1}	84.67 ± 8.32^{bcd2}	91.22 ± 7.93^{def3}
1	64.55 ± 2.67^{c1}	72.72 ± 8.72^{ef2}	81.96 ± 7.31^{bc3}	86.57 ± 10.84^{cd3}
2	58.61 ± 2.76^{b1}	68.62 ± 4.66^{d2}	87.32 ± 1.96^{bcd3}	90.17 ± 7.21^{de3}
3	57.76 ± 3.52^{b1}	61.75 ± 2.23^{b1}	72.31 ± 7.11^{a2}	80.21 ± 9.62^{bc3}
4	69.89 ± 7.68^{de1}	64.41 ± 4.75^{bc1}	73.61 ± 7.86^{a2}	92.39 ± 9.88^{def3}
5	71.09 ± 3.59^{e1}	70.83 ± 3.27^{de1}	89.13 ± 7.80^{d2}	97.88 ± 7.14^{f3}
6	68.87 ± 8.26^{cde1}	76.40 ± 4.53^{f2}	88.92 ± 6.51^{d3}	96.10 ± 4.43^{ef4}
7	52.47 ± 2.74^{a1}	57.51 ± 1.82^{a1}	68.15 ± 3.61^{a2}	72.42 ± 4.26^{f3}
Stored (t:45 ºC) (T:Month)	B	P	W	CA
3MA	60.16 ± 1.70^{b1}	70.88 ± 3.08^{de2}	80.08 ± 7.88^{b3}	78.74 ± 7.10^{ab3}
6MA	67.82 ± 6.51^{cde1}	69.38 ± 1.89^{de1}	86.13 ± 1.64^{cd2}	97.89 ± 6.99^{a3}

Values are mean ± Standard Dev. (n= 9); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. **Ext.1** (extracts obtained from the model of human digestion) and **Ext. 2**(chemical extraction). Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2.5H_20$). Storage codification, letter (fibre source), number (months stored at room temperature), AM accelerated Month).

Regarding the results of the chemical extraction (**Ext.2**), the primary antioxidant activity of the biscuits prepared with FRPA (W, CA) was significantly higher than that of the control biscuits (B) and the biscuits formulated with reference fibre (P), due to the enrichment with vegetal fibre. Equal to what was observed with the $ABTS^{+\cdot}$ method, during storage at room temperature the values of antioxidant activity of all the biscuits analysed by DPPH decreased with time of storage. The

biscuits formulated with FRPA (W, CA) maintained higher antioxidant activities throughout the storage period. When comparing the results of antioxidant activity of the **Ext.1** of the biscuits with the **Ext 2**, it is also confirmed that the process of digestion liberated hydrolysable antioxidants, reactive with $DPPH^{\cdot}$, from the plant structures and therefore **Ext.1** showed higher values of antioxidant activity compared to **Ext.2**, for all biscuits. The trend throughout storage time was maintained at both storage temperatures.

3.3.3. FRAP

The results obtained from antioxidant activity, using the FRAP method, of the biscuits, subjected to a model of human digestion (**Ext.1**) are shown in Table 6. It can be observed that the control biscuits, without fibre (B), and the biscuits formulated with reference fibre (P) had similar initial values, which were significantly lower to those of the biscuits enriched with FRPA (W and CA). These differences between the different types of biscuits were maintained throughout storage at room temperature. These results show the same trends as those obtained with the other analysis techniques of antioxidant activity and once more indicate that this higher antioxidant activity was due to the antioxidant content of the FRPA (W and CA) added to the biscuits.

Regarding the results of antioxidant activity from the chemical extraction (**Ext 2**) initially, the different types of biscuits showed significant differences amongst each other. The biscuits prepared with FRPA (W, CA) had a higher antioxidant activity than the control biscuits (B) and those formulated with reference fibre (P). During storage at room temperature, the values of antioxidant activity of all groups of biscuits decreased significantly. At the end of storage, the antioxidant activity values of the biscuits formulated with FRPA (W, CA) were still substantially higher than those of the control biscuits without fibre (B) and those formulated with Pea fibre (P).

When comparing the results obtained of **Ext.1** (digestion) and **Ext.2** (chemical extraction), it is confirmed as well that the digestion process liberates hydrolysable antioxidants, reactive with FRAP, from the plant structures, and therefore **Ext.1** showed higher values.

Table6. Evolution of antioxidant activity by FRAP (mEq Trolox /g of biscuit) of the biscuits studied (B, P, W, CA) during storage.				
Ext.1				
Stored (t:25 ºC) (T:Month)	B	P	W	CA
0	47.62 ± 6.19^{a1}	56.26 ± 7.31^{a2}	70.74 ± 9.19^{a3}	109.62 ± 14.25^{abc4}
1	96.67 ± 11.60^{e2}	93.22 ± 11.18^{b2}	80.9 ± 12.13^{a1}	110.14 ± 16.52^{abc3}
2	62.64 ± 7.51^{ab1}	60.43 ± 9.66^{a1}	70.94 ± 7.09^{a2}	90.83 ± 13.62^{ab3}
3	47.78 ± 6.21^{a1}	56.81 ± 7.38^{a2}	$74,38 \pm 9.66^{a3}$	84.07 ± 10.92^{a4}
4	64.83 ± 4.54^{bc2}	57.54 ± 9.20^{a1}	$72,15 \pm 11.54^{a3}$	161.85 ± 17.80^{de4}
5	92.64 ± 12.96^{de1}	118.93 ± 16.65^{d2}	132.67 ± 13.26^{c3}	158.36 ± 25.33^{de4}
6	116.40 ± 18.62^{f1}	116.72 ± 18.67^{d1}	132.21 ± 21.15^{c2}	133.04 ± 21.28^{cd2}
7	79.88 ± 10.38^{cd1}	95.03 ± 8.55^{bc2}	106.49 ± 11.71^{b3}	119.26 ± 13.11^{e4}
Stored (t:45 ºC) (T:Month)	B	P	W	CA
3MA	89.66 ± 13.44^{de1}	114.17 ± 17.12^{cd3}	105.36 ± 9.48^{b23}	113.44 ± 13.61^{abc3}
6MA	105.2 ± 15.73^{de1}	117.61 ± 11.40^{d2}	136.58 ± 12.29^{c3}	177.54 ± 26.63^{bc4}
Ext. 2				
Stored (25 ºC) (Month)	B	P	W	CA
0	46.78 ± 6.54^{de1}	54.19 ± 3.25^{cd2}	68.29 ± 9.29^{c3}	77.78 ± 7.93^{de4}
1	46.61 ± 41.94^{de1}	50.83 ± 7.96^{c2}	68.53 ± 7.17^{c3}	70.75 ± 10.51^{d3}
2	27.16 ± 2.65^{a1}	38.03 ± 8.97^{b2}	51.61 ± 8.72^{b3}	54.29 ± 8.32^{bc3}
3	29.92 ± 3.29^{ab1}	31.89 ± 2.51^{a2}	44.87 ± 8.03^{a3}	48.75 ± 9.10^{ab3}
4	40.90 ± 4.09^{c1}	39.76 ± 3.63^{bc1}	51.70 ± 8.06^{ab2}	59.88 ± 4.28^{abc3}
5	$42,34 \pm 7.19^{cd}$	43.62 ± 3.69^{c}	54.64 ± 6.51^{b}	53.27 ± 11.77^{abc}
6	54.53 ± 7.08^{f1}	58.94 ± 5.46^{e1}	73.03 ± 10.65^{c2}	81.70 ± 9.06^{e3}
7	32.97 ± 3.29^{b1}	39.85 ± 3.39^{bc1}	48.36 ± 4.57^{ab2}	51.99 ± 6.35^{e2}
Stored (25 ºC) (Month)	B	P	W	CA
3AM	30.92 ± 1.85^{ab1}	40.97 ± 7.61^{bc2}	51.63 ± 5.35^{ab4}	45.98 ± 4.90^{a3}
6AM	49.51 ± 7.92^{ef1}	56.22 ± 4.21^{e2}	66.32 ± 9.86^{c3}	80.84 ± 12.02^{abc4}

Values are mean ± Standard Dev. (n= 9); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. **Ext.1** (extracts obtained from the model of human digestion) and **Ext. 2**(chemical extraction). Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2.5H_20$). Storage codification, letter (fibre source), number (months stored at room temperature), AM accelerated Month).

The trends detected with the previously described methods were similar. The biscuits in accelerated storage had an increased antioxidant activity, and the biscuits formulated with fibre (P, W, CA) had a higher antioxidant activity than the control biscuits (B), due to the content of the fibre utilized. As was observed by Vitali *et al.*, (2009) the increase in antioxidant activity (in chemical and physiological extracts) is explained by the increase in extractable (or bioavailable) phenolic content during accelerated storage, due to the formation of certain products of the Maillard reaction.

The biscuit CA presented the highest antioxidant activity. The differences found between the values of antioxidant activity, obtained by the different antioxidant analysis methods were in agreement with the studies performed by Rivero *et al.*, (2007) and could be correlated to the higher retention

capacity of the FRPA of CA. B retaining the water of the formulation for a longer period during oven-baking, a pronounced heating of the biscuits and a loss of the polyphenol content and antioxidant activity of the fibre are avoided.

4. CONCLUSIONS

In order to evaluate the bioavailability of the functional compounds of biscuits enriched with FRPA, a model of human digestion was carried out and an extract was obtained (**Ext.1**). In parallel a chemical extraction was carried out of the same biscuits, to determine the bioavailable polyphenolic content, and two extracts were obtained for the determination of total polyphenol content: extractable (**Ext.2**) + hydrolysable (**Ext.3**).

In all studied groups of biscuits correlated well with the content of total polyphenols, expressed as the sum of extractable soluble polyphenols (**Ext.2**) and the hydrolysable (**Ext.3**).However, the effect of acid hydrolysis, used for **Ext.3**, on the plant structures produced a greater liberation of polyphenolic compounds, leading to significant differences between the bioavailable and the total polyphenols. Both initially and during storage, the biscuits formulated with FRPA (W, CA) showed a higher content of bioavailable polyphenols compared to both the control biscuits without fibre (B) and the ones formulated with reference fibre (P, Pea fibre). Consequently, the FRPA present an excellent alternative to the use of reference fibre.

From the results obtained from antioxidant activity by the different methods used: $ABTS^{\cdot+}$, $DPPH^{\cdot}$ and FRAP, we conclude that despite the fact that different values were obtained depending on the method utilized, the results showed the same trends in all the types of biscuits studied and they corresponded with the results achieved from the polyphenol content. Both initially and during storage, the biscuits formulated with FRPA (W, CA) showed a higher antioxidant activity compared to both the control biscuits without fibre (B) and the biscuits formulated with reference fibre (P, Pea fibre). The antioxidant activity of the physiological extracts of digestion, obtained by the model of gastrointestinal digestion (**Ext.1**), was significantly higher than the activity found in the extracts obtained by chemical extraction (**Ext.2 + Ext.3**). Therefore, the use of chemical extraction methods only, for the estimation of antioxidant activity of cereal-based foods, underestimates the antioxidant activity of the substances liberated by the effect of the gastrointestinal digestion. In conclusion,

FRPA are an excellent source of bioavailable fibre with antioxidant activity, especially the FRPA (CA), and present a good alternative to the use of reference fibre from peas.

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3.2. Shelf life of functional biscuits enriched with vegetal fibre extracted from by-products of artichoke (*Cynara scolymus* L.)

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ABSTRACT

To boost industrial application and revaluation of fibre rich powder extracted from artichoke by-products, in novel fibre-enriched products. In this research, it was studied how the evolution of the quality characteristics was affected by the addition of fibre-rich powders of artichoke, obtained by a simple liquid extraction technique employing two extraction liquids: distilled water (W) and an aqueous solution of $CaCl_2 \cdot 5H_2O$ (CA).

The results indicate that texture and colour of the biscuits without fibre (B) and with commercial reference (P) changed throughout the storage period, showing significant differences ($p<0.05$) at the end of the study, while for the biscuits enriched with fibre-rich powders extracted from artichoke by-products (W, CA) were more constant during storage. This confirms the possibility of the revaluation of artichoke by-products in fibre-rich powders, and its potential use in oven-baked products with high fibre content, like wholemeal biscuits.

Keywords: Texture analysis, digital image analysis, novel food.

1. INTRODUCTION

Biscuits with a high fibre content, wholemeal biscuits constitute one of the growing sectors of the food industry in recent years, due to its varied tastes, long shelf life and relatively low costs. As a consequence, it has been tried to improve the functionality and the nutritional value of the biscuits by modifying their composition, for example by increasing the content of dietary fibre (Smith, 2011). In this way, the population of the developed countries could complement the consumption of fibre, which occidental diet does not supply (Milte *et al.,* 2015). The low consumption of fibre has been associated with an aggravation of diseases like diabetes, constipation, obesity, irritable bowel syndrome, etc. (McEvoy & Woodside, 2015).

The utilization of fibre from different plant by-products could be a good alternative to enrich foods, not only with fibre but also with antioxidant compounds (Bilgic, Ibanoglu, & Nur Herken, 2005), present in vegetable by-products. We may highlight the studies of extraction of fibre-rich powders with high antioxidant powers of: carrot peel (Chantaro *et al.,* 2008), outer leaves of cabbage (Nilnakara *et al.,* 2009), and asparagus (Fuentes-Aleventosa *et al.,* 2009).

Regarding the application of compounds with a high antioxidant power, in oven-baked products like biscuits (Manley, 2011), extracts from wild plants (Reddy *et al.,* 2005) and tea leaves (Sharma, & Zhou, 2012) have been used. However, due to the fact that the plant extracts have to be immobilized to increase their functionality and dispersion in the dough, other authors used vegetal fibres obtained from by-products with their own antioxidant effect,), by-products of mango and orange juice industries (Ajila *et al.,* 2007), and fibre extracted from pea pods (Raymundo *et al.,* 2014). The present study focuses on this last way of incorporating antioxidant fibre-rich powders (artichoke).

At the moment, several vegetal fibres are marketed for the enrichment of biscuits with high fibre content: of beetroot (Fibrex®), chicory (Raftilosa®), pea (PEAFIBFG®), etc. However, there are not bibliographic references to studies on biscuits formulated with fibre-rich powders extracted from artichoke by-products.

The aim of this study was to determine the shelf life of biscuits made with fibre-rich powders extracted from artichoke by-products and as a commercial reference was used Pea fibre (P). Two

types of storage were carried out: room temperature (25 °C, RH 55 %) and accelerated storage (45 °C, RH 55 %).

2. Material and methods

2.1 Ingredients and preparation of the biscuits

A standard formulation similar to the fibre rich biscuits sold in the Spanish market was used (Whitley, 1970), with slight modifications in the percentages of the ingredients (Table 1). Fibre was added at a concentration of 4 % (w/w): commercial Pea fibre (P) and two types of artichoke fibre of our own production which were obtained by liquid extraction from artichoke by-products with two different extraction liquids: distilled water (W) and a solution of 1 % (w/w) $CaCl_2 \cdot 5H_2O$ (pH 6.5) (CA) The control biscuits did not contain fibre (B) and the 4 % (w/w) of fibre was replaced by wheat flour.

Table 1. Formula of the biscuits (Whitley, 1970)

Ingredient	%
water	7.45
glucose-fructose syrup	1.45
sunflower oil	12.02
whey	3.52
Sodium and ammonium bicarbonate	1
salt	0.23
lecithin	0.1
wholemeal wheat flour	61.2
fiber: Pea fibre(P) or FRPA (W,CA)	4
	100

All ingredients were mixed and kneaded with a pilot plant mixer for 10 min at minimum speed. Subsequently, the dough was laminated to a thickness of 5 mm, using a manual sheet pasta machine. The round form of the biscuits was obtained manually, using a cutting cylinder (die) of 60 mm in diameter.

After making 8 evaporation holes in the dough with a fork, the biscuits were baked in an electrical convection oven at a temperature of 180 ± 10 °C during 17 minutes. Afterwards, the biscuits were cooled at room temperature (18–20 °C) and wrapped in heat-sealed bags of high-density

polyethylene. They were stored until analysis. For each formulation of biscuit three production batches were carried out.

2.2 Storage

The sealed and coded biscuits were stored in two incubation chambers (Incudigit Selecta Spain) where temperature and humidity were kept constant. One of the chambers simulated room temperature conditions (25 °C, RH 55 %), while the other simulated accelerated storage conditions (45 °C, RH 55 %) (Yang *et al.*, 2013), which forced the appearance of defects in the biscuits by the increased temperature.

The biscuits stored under ambient conditions (7 months) were analysed monthly, while the biscuits stored under accelerated conditions (6 months) were analysed every three months. As a control for each group of biscuits, the values were taken at time 0, which was the day after the preparation. By comparing the results of both storage procedures, we intend to investigate at which time under ambient conditions the defects are detected, generated under accelerated storage.

2.3 Moisture determination

The determination of moisture (M) in the biscuits was performed by the method of oven-drying (Nollet, 1996) with slight modifications. Biscuit samples (250 mg) were weighed in triplicate on an analytical balance (Mettler Toledo, USA) and dried for 24 hours at 90ºC. Subsequently, moisture was calculated as the difference in weight (%M= (Initial weight-Final weight)*100 / Initial weight sample).

2.4 Texture analysis

Texture analysis was carried out with a Ta-XT-plus Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a load cell of 5 kg. The puncture test is based on the measurement of the maximum shearing force necessary to completely pass through a section of the product with a cylindrical probe. The higher force obtained, the higher is the resistance of the product. The biscuits were placed on a Heavy Duty Platform (HDP/90), and the puncture analysis was applied to several points within the mediate zone of the biscuits, using a cylindrical probe P/2 (2 mm diameter). According to the optimization study carried out before the analysis (San José *et al.*, 2018), the analysis settings which best describes the texturometric qualities of the biscuits is as follows: Pre-Test Speed 0.8 mm/s, Test Speed 0.8 mm/s, Post-Test Speed 10.0 mm/s, Distance 5 mm. Values were obtained for Maximum Force (g), Area (g·sec) and Linear Distance (g·sec). For

each formulation of biscuit 5 replicate measurements were made of each of the three production batches. The analyses were performed at a temperature of 20 ± 1ºC

2.5. Colour

Colour was measured using digital image analysis. The digital images were obtained with a table-top scanner with cold cathode light, Epson Perfection V10 Photo (Epson America, USA), with a minimum resolution of 400 dpi (See figure 1). Of each sample, a square of 256 pixels was extracted with the help of the image treatment program Photoshop cs2 9.0 (Adobe, USA). The colour values were calculated from the RGB coordinates, and the system was calibrated with 300 standard images corresponding to the Spanish norm of colour UNE 48-103-94 (AENOR, 1994). In Figure 1 examples are shown in the picture of the scanned biscuits and the samples taken for subsequent digital treatment. For each type of biscuit and production batch three replicate measurements were performed in three different biscuits.

2.6. Statistical analysis

Statistical analysis of the results obtained was carried out with the statistical package STATGRAPHICS Centurion XVI, version 16.1.03 (Statpoint Technologies Inc.) for Windows. Data were expressed as mean ± coefficient of variation (CV). Analysis of variance (ANOVA) and LSD post-hoc tests were performed, as well as discriminant function analysis for the correlation between parameters. Significant differences ($p < 0.05$) were identified with an alphabetical scale and an increasing numeric scale.

3. RESULTS AND DISCUSSION

3.1 Moisture analysis

The results obtained for moisture of the biscuits (Table 2) indicate that the biscuits formulated with fibre from artichoke (CA, W) or pea (P) had a higher initial moisture content (w/w) than the control biscuits (B). This is explained by the water holding capacity of the vegetal fibres, which causes the water in the formulation to be more firmly retained in the biscuit during oven-baking. The resulting difference in moisture may affect the physicochemical phenomena that occur during storage of the biscuits (Belcourt & Labuza, 2007).

Table 2. Evolution of moisture (w % w) of biscuits B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2 \cdot 5H_20$), throughout the storage period at room temperature and accelerated storage -

Stored (25 ºC) (Month)	B	P	W	CA
0	2.34 ± 0.35^{cd1}	2.58 ± 0.43^{e2}	2.63 ± 0.28^{g3}	2.61 ± 0.22^{f3}
1	1.88 ± 0.30^{a1}	1.79 ± 0.35^{a1}	2.07 ± 0.16^{bc2}	1.94 ± 0.30^{ab1}
2	2.44 ± 0.22^{d2}	1.98 ± 0.20^{ab1}	1.87 ± 0.30^{a1}	2.25 ± 0.36^{de2}
3	2.41 ± 0.22^{d2}	2.44 ± 0.24^{de2}	2.41 ± 0.17^{f2}	2.28 ± 0.23^{de1}
4	2.25 ± 0.21^{bcd1}	2.34 ± 0.29^{cde1}	2.27 ± 0.07^{def1}	2.17 ± 0.15^{bcd1}
5	2.04 ± 0.27^{ab1}	2.18 ± 0.20^{bcd2}	2.09 ± 0.14^{bcd1}	2.20 ± 0.12^{cde2}
6	2.10 ± 0.25^{abc2}	2.01 ± 0.20^{ab1}	2.20 ± 0.12^{cde2}	2.01 ± 0.26^{bc1}
7	1.85 ± 0.13^{a12}	2.02 ± 0.21^{ab3}	1.93 ± 0.14^{ab23}	1.76 ± 0.31^{a1}
Stored (45 ºC) (Month)	B	P	W	CA
3MA	2.09 ± 0.25^{abc1}	2.48 ± 0.43^{de2}	2.34 ± 0.26^{ef2}	2.35 ± 0.34^{de2}
6MA	2.05 ± 0.41^{ab1}	2.08 ± 0.40^{abc1}	2.09 ± 0.14^{bcd1}	2.01 ± 0.26^{bc1}

Values are means ± Standard Dev. (n= 3); significant differences ($p< 0.05$) between storage time within biscuit types (columns) are indicated with different letters, differences between biscuit types (rows) are indicated with different numbers.

During the first month at room temperature, the moisture of the biscuits decreased, while during the second and third month it increased due to the hygroscopy of the biscuits. These results coincide with those of studies carried out by other authors who attribute the increase in moisture to the use of water-permeable films or the absence of packaging (Piga *et al.,* 2005). The presence of amorphous sugars also produces an initial absorption of moisture through their hydroxyl groups (Labuza *et al*., 2004). The subsequent loss of moisture is caused by desorption of moisture due to a recrystallization of sugars (Piga, *et al.,* 2005). Although the film has a low permeability to water vapor, after so many months in a forced-air environment the packaging loses part of its moisture.

3.2. Texture analysis

The texture analysis is particularly challenging in biscuits because of their form and complex, fragile and brittle structure, which impedes the measuring probe to easily flow upon forces of pressure (Gaines, 1994). Therefore, to determine the texture of wholemeal biscuits instrumentally, texture techniques have to be optimized customizing the settings of analysis for this type of biscuit. The results of the variables obtained by puncture analysis will be shown separately.

3.2.1. Maximum force of deformation (F.Max)

The maximum force of deformation is the strength required to penetrate the surface of the biscuits during the first bite with the incisive teeth. Therefore, it is the first sensation and the dominant perception (Milford, 2011). Regarding the graphic created by the texturometer, Maximum force is the maximum peak of the graph (breaking point) resulting from the compression of the cylindrical probe on the biscuit (Bourne, 2002).

It can be observed in Table 3 that initially, the biscuits formulated with fibre-rich powders from artichoke by-products (W, CA) had a greater hardness than the rest of the biscuits (P, B). Authors, like Gupta & Tiwari (2014), obtained similar results of a significant increment of the maximum force, increasing with a percentage similar to the percentage of fibre in the formulation of biscuits. On the other hand, other authors (Baltsavias *et al.,* 1999) found that during the first months of packaging all groups of biscuits undergo an increase in the maximum force of deformation. This increase occurred in the second month of storage for the biscuits with artichoke fibre, while for biscuits P and B it occurred in the first month. This change in maximum force of the biscuits is explained by the change in mechanic properties during storage, due to the crystal transition of the ingredients and the loss of moisture of the product. The increase in force presented by the biscuits is in agreement with the studies carried out by Raymundo *et al.,* (2014). They demonstrated that biscuits with a content of between 3-6 % w/w of *psyllium* fibre increased their firmness significantly ($p < 0.05$) due to the positive effect on the strengthening of the network of gluten during storage. In the succeeding months, the maximum force of all the groups remained constant with small fluctuations.

Table 3. Maximum force (g) of the biscuits studied, during storage at room temperature (25 ºC) and accelerated (45 ºC)				
Stored (25 ºC) (Month)	B	P	W	CA
0	1731.76 ± 170.62^{a1}	1997.67 ± 194.20^{b2}	2390.66 ± 249.69^{e3}	2269.19 ± 272.53^{a3}
1	2103.06 ± 225.09^{e1}	2512.46 ± 195.88^{e2}	2444.33 ± 328.00^{ef2}	2826.54 ± 395.95^{e3}
2	1865.48 ± 204.72^{b1}	2167.72 ± 206.09^{c2}	2513.77 ± 261.61^{f3}	2506.56 ± 346.60^{c3}
3	1938.98 ± 161.19^{cd2}	2047.74 ± 211.00^{b3}	1818.70 ± 272.54^{b1}	2347.65 ± 305.48^{ab4}
4	1935.92 ± 233.51^{cd1}	2005.13 ± 208.43^{b1}	2187.19 ± 270.51^{d2}	2478.52 ± 255.16^{c3}
5	1945.51 ± 240.64^{d12}	2013.75 ± 221.16^{b2}	1927.12 ± 173.61^{c1}	2314.29 ± 289.38^{a3}
6	2271.28 ± 286.90^{f2}	2168.36 ± 169.53^{c1}	2177.87 ± 216.90^{d1}	2417.11 ± 271.91^{bc3}
7	1843.38 ± 207.79^{b1}	2168.50 ± 222.50^{c2}	2264.20 ± 248.59^{d3}	$2411.76 \pm 271.399^{bc4}$
Stored (45 ºC) (Month)	B	P	W	CA
3MA	1874.10 ± 167.02^{bc1}	1874.88 ± 163.45^{a2}	1706.68 ± 143.40^{a1}	2283.69 ± 298.27^{a3}
6MA	2263.06 ± 199.28^{f1}	2385.65 ± 244.75^{d1}	2456.10 ± 273.45^{ef1}	2634.81 ± 277.13^{d1}

Values are means ± Standard Dev. (n= 3 significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. Biscuit codification: B (without fibre), P (Pea fibre), W (water), CA (Dis 1 % $CaCl_2.5H_20$)

For the biscuits stored under accelerated storage conditions (AM), it is observed that the values of maximum force of all groups after three months of storage were lower than the values obtained for biscuits without acceleration. This might be caused by the obstruction of the glass transition of starch by the increase in temperature. After 6 months of accelerated storage, the maximum force in all groups of biscuits increased, due to the substantial impact of water loss on texture, until the values were equal between biscuit groups.

3.2.2. Moment of force or rigidity (L.Dist)

The variable rigidity, expressed in g·sec, indicates in what distance of penetration the maximum peak of the force of a biscuit is produced. In those biscuits in which the maximum force is produced, the sensorial perception of the moment of the force will be related to the hardness of the biscuits. Consequently, biscuits with low values of maximum force and rigidity will be crispier. On the contrary, the combination of high maximum forces and high rigidity will create biscuits with little brittleness, or put in another way, hard biscuits have big graphic areas. Therefore, hardness and rigidity are correlated.

Table 4: Rigidity (g.sec) of the biscuits studied during storage at room temperature (25 ºC) and accelerated (45 ºC).				
Stored (25 ºC) (Month)	B	P	W	CA
0	5585.32 ± 801.24^{bc1}	5949.90 ± 696.55^{c2}	6587.44 ± 759.61^{d3}	6181.76 ± 886.72^{ab2}
1	6330.96 ± 985.12^{d1}	7243.02 ± 822.34^{e3}	6689.70 ± 1133.63^{d2}	7402.39 ± 1061.96^{d3}
2	5046.62 ± 767.12^{a1}	6472.37 ± 858.48^{d2}	6739.08 ± 899.371^{d2}	6448.49 ± 997.15^{bc2}
3	5417.86 ± 753.67^{b1}	5642.03 ± 977.53^{ab12}	5705.25 ± 814.75^{b2}	6016.75 ± 970.62^{a3}
4	5777.26 ± 962.77^{c1}	5932.40 ± 880.48^{c1}	6541.02 ± 1026.84^{d2}	6535.66 ± 957.30^{c2}
5	5847.7 ± 897.10^{c2}	5879.38 ± 918.75^{bc2}	5444.94 ± 712.42^{ab1}	5958.93 ± 863.95^{a2}
6	6860.96 ± 1155.76^{e2}	5976.63 ± 823.57^{c1}	6158.45 ± 845.82^{c1}	6063.90 ± 830.14^{a1}
7	5366.68 ± 922.04^{b1}	5937.15 ± 825.72^{c2}	6245.70 ± 843.18^{c3}	6024.61 ± 843.08^{a23}
Stored (45 ºC) (Month)	B	P	W	CA
3MA	5309.51 ± 711.51^{ab1}	5605.19 ± 714.57^{a2}	5203.46 ± 643.28^{a1}	6637.15 ± 1084.16^{c3}
6MA	6725.20 ± 890.50^{e1}	6531.78 ± 908.29^{d1}	6782.25 ± 927.50^{d1}	6627.07 ± 927.39^{c1}

Values are means ± Standard Dev. (n= 3); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2.5H_20$).

In Table 4, rigidity (g.sec) is shown for the biscuits studied (B, P, W, CA) during storage at room temperature (25 ºC) and accelerated storage (45 ºC). Initially, the biscuits enriched with fibre showed a higher rigidity than the control biscuits (B). Biscuits CA and P were the most similar. During the first months of storage at room temperature, the same increase was observed of the initial rigidity values, as was observed for the values of maximum force. The increase of the values occurred in the second month in the biscuits with artichoke fibre, while it occurred in the first months for biscuits P and B. During the following months the value of rigidity diminished in all groups of biscuits, corresponding with the moment when the biscuits started losing moisture.

After three months of accelerated storage (3MA), rigidity had decreased in all types of biscuit, except in type CA, and was different from each other. However, after six months of accelerated storage, rigidity was significantly higher in all types of biscuits and there were no significant differences between them.

3.2.3. Hardness (Area: g.sec)

The variable hardness indicates the mechanical resistance to the penetration of the probe and may be the area below the penetration graphic from the point of contact until the point of maximum puncture force (maximum peak of the graph). Therefore, hardness is correlated with the maximum force and the moment of force. Thus the combination of maximum force and high rigidity explain the hardness of the biscuits.

In Table 5 the results are shown of hardness (g.sec) of the biscuits studied (B, P, W, CA) during storage at room temperature (25 ºC) and accelerated storage (45 ºC). When the evolution of hardness is compared for each type of biscuits, throughout the storage period, it is observed that at each moment of analysis hardness of the biscuits formulated with fibre-rich powders (W, CA), was always higher than of the rest of the groups. Moreover, the value of hardness of the biscuits formulated with artichoke fibre significantly decreased with time of storage, while for the biscuits P and B the value of hardness remained constant in time. This is explained by a reduction of F max and L.Dist throughout the storage period. At the end of the storage, the biscuits with fibre-rich powders of artichoke had a greater hardness than the rest of the groups of biscuits.

Table 5. Hardness (g.sec) of the biscuits studied during storage at room temperature (25 ºC) and accelerated (45 ºC).

Stored (25 ºC) (Month)	B	P	W	CA
0	294.41 ± 45.48^{ab1}	355.55 ± 58.00^{cd2}	511.92 ± 69.34^{ef4}	428.29 ± 75.73^{c3}
1	341.75 ± 64.00^{f1}	420.64 ± 60.48^{g2}	484.14 ± 82.84^{d3}	480.32 ± 65.89^{e3}
2	281.56 ± 48.12^{a1}	401.59 ± 63.14^{f2}	530.43 ± 83.80^{f3}	462.00 ± 81.16^{de4}
3	320.92 ± 47.31^{de1}	343.52 ± 52.71^{bc2}	419.46 ± 65.93^{b3}	401.26 ± 69.02^{ab3}
4	315.63 ± 64.78^{cde1}	341.16 ± 58.82^{bc1}	488.16 ± 78.47^{d3}	409.59 ± 73.35^{abc2}
5	330.62 ± 65.49^{ef1}	330.58 ± 52.075^{ab1}	395.97 ± 64.64^{ab2}	406.46 ± 63.31^{ab2}
6	464.47 ± 69.32^{g3}	373.91 ± 54.64^{e1}	451.88 ± 64.92^{c2}	396.76 ± 55.80^{a1}
7	297.09 ± 65.03^{abc1}	368.16 ± 61.15^{de2}	474.45 ± 78.10^{cd4}	394.28 ± 58.72^{a3}
Stored (45 ºC) (Month)	B	P	W	CA
3MA	309.52 ± 55.48^{bcd1}	319.88 ± 46.13^{a1}	372.96 ± 57.51^{a2}	456.20 ± 81.86^{d3}
6MA	547.39 ± 101.23^{h4}	393.07 ± 55.74^{f1}	493.71 ± 59.10^{de3}	420.37 ± 59.98^{bc2}

Values are means ± Standard Dev. (n= 3); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2.5H_20$).

When the results obtained during accelerated storage are compared with the initial rigidity and at the same storage time without acceleration, it is observed that after 3 months of accelerated storage

the hardness of biscuits P and B remained constant, while biscuits W became softer and biscuits CA became harder. After 6 months of accelerated storage all types of biscuits showed a greater hardness.

3.2.4. Discriminant function Analysis

Due to the complexity of simultaneously comparing the texturometric variables (F.Max, L.Dist, Area), discriminant function analysis was applied. By this analysis, it can be studied whether the changes in the three variables throughout the storage period correctly describe each group of biscuits correctly.

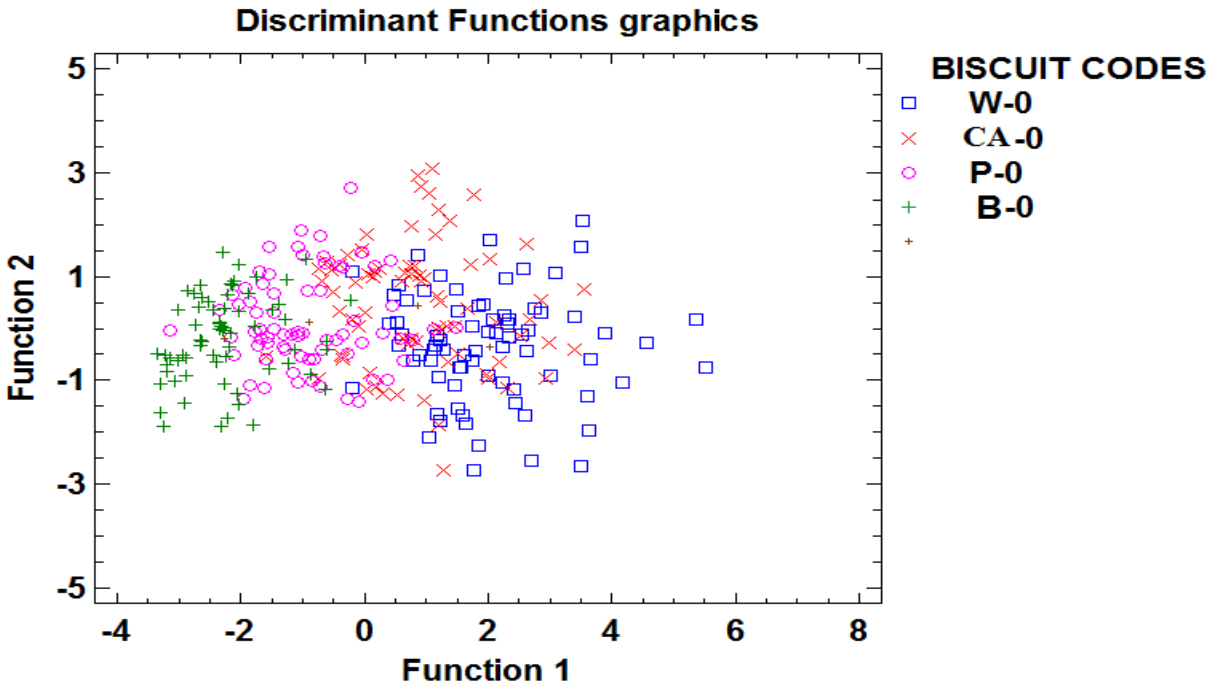

Figure 1. Dispersion function graphic for the biscuits studied (B, P, W, CA); initial time of storage.

When looking at the dispersion diagrams and the discriminant function graphics, it is observed that initially biscuits B and W were the biscuits with the texturometric variables that most deviated from the total, due to the variable rigidity (Data not shown). Therefore, these biscuits had the highest classification percentages (Table 6). Biscuits P and CA were less well classified, because of their similarities with biscuits B and W, respectively. As it can be seen on the discriminant function graphic (Figure 1), where the groups of biscuits P and B, and the groups W and CA are plotted

close together, being the texturometric qualities of biscuits with fibre CA were similar to those of the biscuits formulated with the commercial reference fibre (P).

At the end of storage, the correct classification of biscuits B and W increased (Table 6), because their qualities got less similar to the rest of biscuits. As it could be observed on the dispersion function graphic at the end of the storage (Data not shown) where the plotted values of both groups (B, W) get closer to the center of the function 2 while the values of the groups P and CA got closer to the centre of the function 1. However at the end of the storage, the texturometric variables of the biscuits with fibre (W, CA, P) were very similar amongst each other. Therefore, it can be concluded that the evolution of the texturometric properties of the biscuits with artichoke fibre (W, CA) during storage were similar to the biscuits formulated with the reference fibre (P) and that the texturometric technique applied had a high differentiating capacity between groups with similar formulation.

Table 6: Correctly classified biscuits (B, P, W and CA) during storage at room temperature (25 ºC) and accelerated (45 ºC).

Correctly classified %										
Biscuit	*Stored (25 ºC) (Month)*								*Stored (45 ºC) (Month)*	
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*3MA*	*6MA*
B	79.41 %	77.63 %	80.52 %	64.94 %	61.04 %	51.14 %	66.67 %	80.00 %	57.47 %	57.33 %
P	54.79 %	67.44 %	63.16 %	36.49 %	30.77 %	47.44 %	70.51 %	65.38 %	50.00 %	67.95 %
W	73.68 %	64.38 %	61.64 %	73.61 %	68.75 %	61.19 %	42.11 %	75.44 %	75.00 %	52.63 %
CA	52.63 %	65.48 %	39.71 %	70.51 %	69.01 %	69.23 %	67.57 %	61.33 %	68.92 %	62.67 %

Biscuit codification: (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2 \cdot 5H_20$).

During accelerated storage, the percentages of correct classification of biscuits P and B decreased during the first three months of storage (Table 6). The texturometric evolution of biscuits B increased their similarity with biscuits P (Data not shown). For the biscuits with artichoke fibre-rich powders (CA, W), the decrease of the texturometric values of biscuits W resulted in the differentiation from biscuits CA, which had more constant texturometric values during accelerated storage. After six months of accelerated storage, the texturometric values of biscuits B, P, and W had increased, while biscuits CA remained more constant throughout storage time (Data not shown). These texturometric changes prevented the differentiation between the biscuits. It is concluded that the accelerated evolution of texture by the higher storage temperature provoked

similar texturometric changes in biscuits W and P, which make them difficult to differentiate from biscuits CA, which were more constant group during storage.

3.3. Colour

The colour of biscuits is one of the most important sensorial variables for determining the preference of consumers (Laguna *et al.,* 2011) and provides information regarding freshness and shelf life (Fiszman*et al.,* 2013). One of the factors that contribute to the colour of biscuits is the one caused by the Maillard reaction between the sugar and the amino acids (Mundt & Wedzicha, 2007). Thus, the colour of biscuits increases as a function of temperature and time of cooking and the amounts of sugars and proteins present in the ingredients used for the cooking. Figure 2shows the photos of the different biscuit studiedafter oven-baking. The monitoring of the values of the CIELab coordinates corresponds to a classic analysis of product quality. Table 7 shows the results of the chromatic parameters obtained for the biscuits during storage at ambient and accelerated temperature. Initially, the combination of the coordinates +a* (red) and +b* (yellow) corresponded with an orange colour, more closely to yellow, which would be the proper colour of the biscuits upon the generation of melanoidins by the Maillard reaction during oven-baking. The major differences in colour are attributed to the coordinate +a*. Remarkably, despite the green colour of the artichoke fibre-rich powder, the biscuits W and CA, after oven-baking, had a colour very similar to the biscuits formulated without fibre (B) (figure 2).

Regarding the coordinates L and Chroma, initially the biscuits formulated with fibre-rich powders W and CA have a darker colour than biscuits B, caused by the incorporation of fibre, as has been described by various authors like Raymundo et *al.,* (2014) and Sudha *et al.,* (2007). These authors explained that this non-enzymatic browning is more pronounced when the wheat flour is replaced by a fibre with a different sugar composition and by the colour of the utilized fibre itself. However, in our case, the difference in colour between the biscuits with Pea fibre and the ones formulated with artichoke fibre could be due to the greater capacity to retain water, shown by the artichoke fibres. This capacity to retain moisture would prolong the evaporation of water from the formulation during oven-baking, reducing the internal temperature of the biscuits and consequently reduces the Maillard reaction that is produced during oven-baking (Köksel & Gökmen, 2008). The lower chromaticity of the biscuits with artichoke fibre-rich powders, compared to biscuits P and B, could be caused by a rougher texture of the biscuits with fibre-rich powders, due to their higher content

of insoluble fibre. This could produce a reduction of the chroma and colour of the biscuits with artichoke fibre-rich powders.

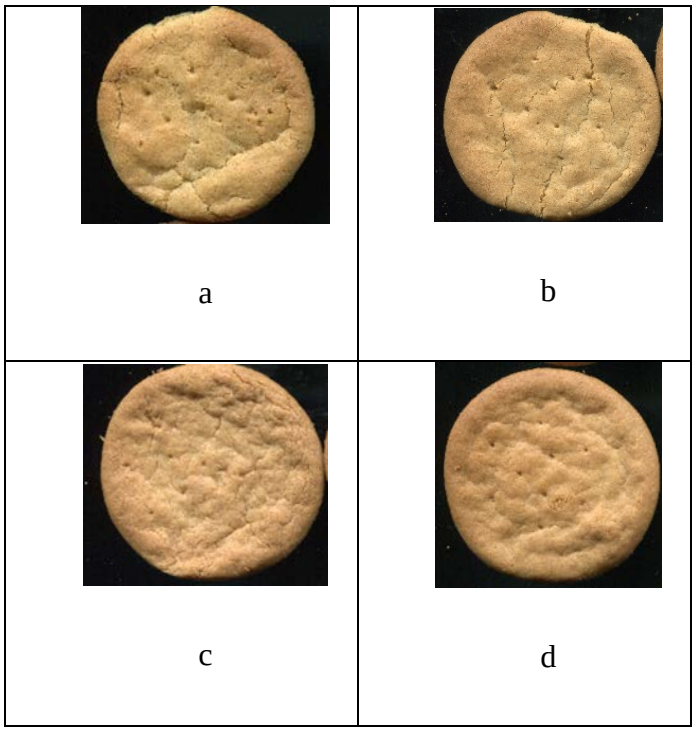

Figure 2.Scanned picture to determine the colour of the biscuits after oven-baking. a) Biscuit W, b) Biscuit CA, c) Biscuit B, d) Biscuit P. Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2 \cdot 5H_20$).

At the end of storage at room temperature, the parameter a* of the biscuits with fibre had experienced only minor variation, while parameter b*, L and chroma decreased significantly. These results are in agreement with the studies carried out by Krystyjan *et al.*, (2015). Since these changes were not correlated with changes in moisture, they could be caused by oxidation processes of the oil in the biscuits, which produced the darkening of the biscuits during storage. It is observed that at the end of storage the values of the colorimetric variables of the biscuits with artichoke fibre were similar to the values of the biscuits formulated with the commercial reference fibre.

During accelerated storage the darkening caused by oxidation is forced. It is observed (Table 7) that the results obtained after three months of accelerated storage were similar to the ones obtained after six months of room temperature and therefore the data at 6 months of accelerated temperature would be outside the range of storage performed at room temperature. In all groups of biscuits, it was

observed a decrease of the colorimetric constants, which rendered a darkening of the colour of the biscuits. This oxidative darkening was more pronounced in biscuits formulated without fibre (B) than in biscuits formulated with fibre. Specifically, the biscuits formulated with artichoke fibre (W, CA) presented a lighter colour after six months of accelerated storage than the biscuits formulated with Pea fibre (P).

Table 7. Colour of the biscuits (B, P, W y CA) throughout storage at room temperature (25 ºC) and accelerated (45 ºC)

Stored (25 ºC) (Month)	**a***				**b ***			
	B	P	W	CA	B	P	W	CA
0	11.8 ± 1.7^{a3}	13.4 ± 1.3^{de4}	11.0 ± 1.3^{c2}	10.2 ± 1.6^{b1}	33.0 ± 1.8^{bc2}	33.1 ± 1.1^{c2}	31.9 ± 1.5^{bc1}	31.8 ± 1.3^{a1}
1	12.7 ± 1.4^{c3}	11.8 ± 1.2^{a2}	10.6 ± 1.8^{bc1}	10.3 ± 1.5^{b1}	33.8 ± 1.6^{cd2}	34.8 ± 1.6^{d2}	32.6 ± 2.1^{c1}	34.2 ± 1.3^{b2}
2	13.0 ± 1.6^{c2}	13.8 ± 1.4^{ef3}	10.1 ± 1.9^{b1}	13.8 ± 1.4^{e23}	35.4 ± 1.9^{e2}	34.8 ± 2.1^{d12}	34.2 ± 1.4^{de1}	34.5 ± 1.8^{bc1}
3	14.3 ± 1.6^{d4}	12.7 ± 1.7^{c3}	11.1 ± 2.0^{c2}	10.3 ± 1.0^{bc1}	35.9 ± 1.8^{ef3}	34.7 ± 1.4^{d2}	33.7 ± 1.9^{d1}	35.4 ± 1.3^{d23}
4	14.2 ± 1.2^{d3}	13.5 ± 1.4^{de2}	13.6 ± 1.4^{e23}	12.0 ± 2.1^{d1}	34.4 ± 2.2^{d1}	35.4 ± 1.5^{de12}	35.7 ± 2.2^{f2}	37.1 ± 1.6^{e3}
5	14.0 ± 1.6^{d12}	14.2 ± 1.4^{e2}	11.9 ± 1.4^{d1}	12.7 ± 2.2^{c12}	36.6 ± 1.9^{f3}	35.4 ± 1.5^{de2}	34.4 ± 1.6^{e1}	35.8 ± 3.3^{d23}
6	12.1 ± 1.6^{ab2}	12.7 ± 1.0^{c2}	10.6 ± 1.6^{bc1}	10.3 ± 1.0^{b1}	33.0 ± 1.7^{bc2}	30.7 ± 2.3^{ab2}	31.1 ± 1.5^{b1}	32.2 ± 1.3^{b1}
7	13.0 ± 2.0^{b2}	12.9 ± 2.0^{cd2}	10.4 ± 2.0^{b1}	10.4 ± 2.0^{b1}	32.4 ± 2.8^{ab2}	30.3 ± 2.5^{ab1}	30.8 ± 2.6^{a1}	31.9 ± 2.3^{b2}
Stored (45 ºC) (Month)	B	P	W	CA	B	P	W	CA
3MA	12.0 ± 1.6^{b2}	12.4 ± 1.2^{bc23}	12.9 ± 1.4^{e3}	$10.7 \pm 2.1b^{c1}$	32.84 ± 2.6^{ab2}	30.5 ± 1.7^{ab1}	33.5 ± 2.6^{de3}	35.7 ± 1.8^{d4}
6MA	11.8 ± 1.8^{a2}	11.9 ± 1.5^{ab2}	8.7 ± 1.4^{a1}	9.6 ± 1.4^{a1}	31.40 ± 1.9^{a3}	29.8 ± 1.8^{a1}	30.4 ± 1.5^{a2}	30.2 ± 1.3^{a2}
	L				**Chroma**			
Stored (25 ºC) (Month)	B	P	W	CA	B	P	W	CA
0	67.0 ± 7.4^{c3}	62.5 ± 4.0^{ab1}	64.1 ± 6.1^{b2}	63.9 ± 4.0^{ab2}	35.0 ± 2.1^{b3}	35.7 ± 1.3^{b3}	31.0 ± 1.6^{a1}	33.4 ± 1.5^{a2}
1	66.5 ± 5.9^{bc1}	70.4 ± 5.7^{f2}	64.9 ± 6.0^{c1}	66.6 ± 6.0^{cd1}	36.13 ± 1.8^{c2}	36.1 ± 1.7^{b2}	34.3 ± 2.1^{c1}	35.7 ± 1.4^{c2}
2	70.3 ± 5.1^{d3}	65.4 ± 6.0^{bc2}	67.0 ± 6.7^{e3}	61.1 ± 5.4^{a1}	37.79 ± 2.2^{d2}	37.7 ± 2.1^{cd2}	35.7 ± 1.6^{d1}	37.2 ± 1.8^{d2}
3	66.5 ± 6.2^{bc1}	70.8 ± 5.9^{f2}	66.7 ± 5.5^{de1}	$68.0 \pm 4.6d^{e1}$	38.72 ± 1.9^{e3}	37.0 ± 1.5^{c2}	35.5 ± 1.8^{d1}	37.0 ± 1.3^{d2}
4	64.0 ± 5.9^{a1}	70.4 ± 5.3^{ef2}	70.7 ± 5.2^{f2}	69.1 ± 6.5^{ef2}	37.26 ± 2.2^{d1}	37.9 ± 1.7^{cd12}	38.2 ± 2.2^{f34}	39.0 ± 1.5^{f4}
5	70.0 ± 4.8^{d2}	69.4 ± 4.3^{ef12}	68.1 ± 5.0^{e1}	70.2 ± 3.9^{f2}	39.23 ± 1.9^{e2}	38.2 ± 1.7^{e12}	36.4 ± 1.5^{e1}	38.6 ± 1.4^{e2}
6	68.8 ± 4.8^{cd2}	63.8 ± 3.8^{ab1}	63.0 ± 5.1^{a1}	63.7 ± 4.4^{ab1}	34.27 ± 1.9^{a3}	33.3 ± 2.2^{a12}	32.9 ± 1.3^{b1}	33.9 ± 1.4^{a23}
7	68.0 ± 6.0^{cd2}	61.5 ± 6.5^{a1}	$63.1 \pm \mathbf{6}.8^{a1}$	63.1 ± 5.8^{a1}	34.47 ± 2.7^{ab3}	33.1 ± 2.6^{a2}	32.9 ± 2.7^{b1}	33.4 ± 2.3^{a2}
Stored (45 ºC) (Month)	B	P	W	CA	B	P	W	CA
3MA	68.0 ± 5.8^{cd3}	62.1 ± 5.6^{ab1}	64.6 ± 5.9^{bc2}	63.4 ± 5.3^{ab2}	34.99 ± 3.1^{b2}	33.0 ± 1.5^{a1}	35.9 ± 2.4^{de3}	34.4 ± 1.8^{b4}
6MA	64.4 ± 4.8^{ab3}	61.4 ± 4.4^{a1}	63.1 ± 5.0^{a12}	63.2 ± 4.2^{a12}	34.00 ± 2.0^{a3}	32.7 ± 1.9^{a3}	33.0 ± 1.5^{b1}	33.3 ± 1.3^{a2}

Values are means ± Standard Dev. (n= 3); significant ($p< 0.05$) differences between storage time within biscuit types (columns) are indicated with different letters, significant ($p< 0.05$) differences between biscuit types (rows) are indicated with different numbers. Biscuit codification (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2 \cdot 5H_20$).

4. CONCLUSIONS

This study of the shelf life of the different types of biscuits (B (without fibre), P (Pea fibre), W (water), CA (1 % $CaCl_2 \cdot 5H_20$)) indicates that, during storage at room temperature (25 ºC, 7 months), the utilization of fibre-rich powders from artichoke by-products increases the texture

variables (hardness, maximum force of deformation and rigidity) in a similar way as commercial reference vegetal fibres (P). Concerning colour, the substitution of 4 % of wholemeal flour by artichoke fibre-rich powders (with green colour) changes the colour of the biscuits in a similar way as the commercial reference fibre (P). The advantage of the artichoke fibre-rich powders (W, CA), compared to the reference fibre (P), is that the dough of biscuits formulated with artichoke fibre has a higher capacity to retain moisture. This retention of moisture helps to slow down the evaporation of water during baking in the oven, which reduces the non-enzymatic reactions during baking and therefore yields a less dark-toasted colour.

In conclusion the utilization of fibre-rich powders of artichoke by-products is a possible alternative for the fibre enrichment of oven-baked products like biscuits.

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CAPÍTULO 4.- EVALUACIÓN SENSORIAL DE LAS GALLETAS CON FIBRA DE ALCACHOFA

CAPÍTULO 4.- EVALUACIÓN SENSORIAL DE LAS GALLETAS CON FIBRA DE ALCACHOFA

En este capítulo se presentan los estudios realizados para la obtención del sexto objetivo de esta Tesis Doctoral:

De la descripción hedónica y sensorial las galletas enriquecidas con FRPAs, comparadas con galletas formuladas con la fibra comercial de referencia (guisante) y el control galletas sin fibra, se concluye que durante el almacenamiento acelerado, la mayor pérdida de calidad sensorial se debe a un aumento en el color y aparición de sabores y olores no característicos, sobre todo en las galletas Sin Fibra (B). La aparición de estos defectos en las cualidades sensoriales de las galletas B es más perceptible por los catadores debido a su color más claro y sabor más neutro, que el de las galletas con fibra. Se puede concluir que el buen comportamiento sensorial de las galletas con fibra de alcachofa durante el almacenamiento a temperaturas aceleradas, las hacen apetecibles a los consumidores. Con ello, se puede afirmar que es muy plausible la revalorización de los subproductos de alcachofa, utilizándolos en productos horneados, como galletas integrales con alto contenido en fibra.

Aplicación del análisis sensorial en la tesis

El modo en el que los subproductos de alcachofa son transformados en FRPAs, modifica las propiedades funcionales de la fibra alimentaria en la formulación de galletas, y por tanto puede modificar su percepción sensorial. En la actualidad, en la industria galletera se utilizan fibras vegetales de guisante, para el enriquecimiento de galletas con alto contenido de fibra,

El panel sensorial consistió en 9 jueces entrenados el uso del método de perfiles sensoriales (Lawless & Heymann, 2010)[293]. Los panelistas fueron entrenados con galletas comerciales y prototipos elaborados en planta piloto, ya que las galletas formuladas con FRPA serían también se elaboran en planta piloto. Mediante este entrenamiento se consensuó para cada atributo, una terminología propia para las características y rangos sensoriales. Todos los jueces entrenados del panel sensorial interno han pasado la prueba de gusto básico, la prueba de olor y la prueba de visión de color, y su capacidad de evaluación se revisa rutinariamente mediante tarjetas de control individua. Los vocabularios de los atributos sensoriales fueron consensuados para describir las

diferencias entre las muestras. La intensidad de los atributos fueron clasificados en escalas de intensidad no estructurado, gráfico continuo. Las escalas fueron de 10 cm de longitud y verbalmente se consensuo cada extremo, el lado izquierdo de la escala correspondiente a la intensidad más baja (valor 0) y el lado derecho para la intensidad más alta del atributo (valor 10), siendo el punto medio el valor de las galletas estándar comercial (valor 5).

Se evaluó la frescura de la muestra por la presencia o ausencia de olor/sabor característico, color, textura, apariencia, aceptabilidad global. Para las muestras de galletas sin acelerar una muestra fresca debe tener un sabor y un olor característico similar al estándar, por lo que la ausencia de olor y sabor característico se valora por debajo del valor estándar, mientras que el olor y sabor se valora por encima del valor estándar, hasta muy tostado o quemado como extremo superior de la escala.

El color definirá en el grado de tostado y los extremos de color irían de la corteza de pan de molde y al color del pan tostado integral. La dureza se expresa como la resistencia de la galleta a la rotura al presionar los incisivos durante la mordedura con los incisivos; por lo que para una galleta dura se incrementa la fuerza necesaria para el corte (los extremos estándar de textura irían de la dureza de biscotes de pan tostado y los biscotes integrales tipo “grisinni” de 0,5 cm de diámetro.

La apariencia se relaciona con la geometría y la estructura de las galletas, los extremos de apariencia irían desde una galleta irregular, con superficie irregular producida por burbujas de evaporación, a una galleta muy compacta y de superficie lisa.

La aceptación global sería una mezcla de todos los atributos sensoriales, y por lo tanto los extremos de la escala serían: de muy poco conforme al estándar comercial de galleta, color muy claro, poco olor y gusto a galleta, galleta muy desmenuzable y muy blanda. A su extremo superior, donde las galletas son muy oscura, con fuerte sabor tostado, muy dura y compacta.

Para poder describir los posibles defectos sensoriales, como la aparición de sabores y olores extraños provocados por el almacenamiento acelerado, se matizó el consenso de las variables sensoriales sabor y olor. Para los atributos olor y sabor característico se valorara la frescura de la muestra, como el punto medio de la escala no estructurada. Por lo que la ausencia de olor y sabor característico se valora por debajo del valor estándar, como pérdida del olor o sabor característico si se detectan sabores u olores relacionados con procesos oxidativo, siendo extremo inferior de la

escala como fuerte olor y sabor a rancio. Este matiz en la descripción de los extremos sensoriales de olor y gusto provocan la redefinición de la aceptabilidad global de las galletas, en su extremo inferior de la escala de aceptabilidad global, donde las galletas son muy claras, con fuerte sabor y olor a rancio, muy blandas y desmenuzables.

La presentación de las muestras de galletas se realiza en ciego, codificando cada muestra con un código numérico aleatorio de tres dígitos. El orden de presentación de las galletas se balancea empleando en la hoja de cata, todas las combinaciones de presentación posibles. La caracterización de productos se llevó a cabo bajo iluminación de luz diurna y en cabinas portables dentro de un laboratorio sensorial. La ración de consumo es una galleta entera de cada referencia, termostatada a temperatura de 25 ºC. Se sirvió agua a los evaluadores para la limpieza de la boca entre las diferentes muestras. Las muestras fueron juzgadas sin realizar replica. Con el objetivo de comprobar la fiabilidad de los resultados, la galleta de control fue introducida en las evaluaciones dos veces, al azar entre otras muestras.

4.-Sensory evaluation of biscuits enriched with artichoke fibre-rich powders *(Cynara scolymus* L.)

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ABSTRACT

The artichoke by-products from the canning industry are mainly used for silage, being minimally revaluated. The ways of extraction of by-products of artichoke into fibre-rich powders modify their industrial applications in biscuits, as the sensory evaluation may change compared with the reference fibre (Pea fibre, P) used with commercial biscuit. In this sensory study biscuits enriched with fibre-rich powders of artichoke (W, Ca) are compared with biscuits with the same percentage of the reference fibre (P) and control biscuits without fibre (B). For most of the sensory attributes of the biscuits enriched with artichoke fibre-rich powders were perceived similar to the biscuits with the commercial reference fibre (P). The good sensory behavior of the biscuits with artichoke fibre-rich powders during two storage conditions applied may confirm that the artichoke by-products would be a suitable substitute for Pea fibre in oven-baked products, like wholemeal biscuits with high fibre content.

Practical Applications: A sensory evaluation technique is applied in this article to determine the sensory perception of a novel biscuit, in which the reference fibre (Pea) predominantly used in the bakery industry, is substituted by two experimental fibre-rich powders of artichoke. The potential use in bakery products of fibre-rich powders extracted from artichoke could add functional value to current products while an industrial by-product is revaluated profitably.

Keywords. *Revaluation; by-product; trained panel, functional ingredient.*

1. INTRODUCTION

Whole meal biscuits with high fibre content constitute one of the rising sectors of the food industry in recent years, because of their possibilities of formulation and relatively low production costs. Consumer trends demand more natural products with better nutritional characteristics which can complement nutritional deficiencies, like the low of fibre ingestion (Milte *et al.,* 2015, Mongeau, Brassard, & Verdier 1989, Arrillaga & Martínez, 2006).

In this respect, the simple incorporation of dietary fibre in the formulation of biscuits is an easy method to increase the functionality of the biscuits, with minimal costs (Gallagher *et al.,* 2003, Smith, 2011). Numerous studies confirm that vegetable fibre prevents diseases such as gastrointestinal disorders, duodenal ulcer, constipation, hemorrhoids, Type II diabetes, obesity , cardiovascular diseases (Afaghi *et al.,*2015), (Vitaglione, Napolitano & Fogliano, 2008) and kidney stones (Sorensen *et al.,*2014).

The dietary fibre composition of the artichoke is high; singling out its high composition of inulin, which can be considered its most important industrial and functional composite and a prebiotic compound itself (Muzzarelli *et al.,* 2012).

From an environmental perspective, the revaluation of vegetable by-products is also critical, since it increases the economic viability of waste minimization processes. Usually, those by-products (70 % by weight are by-products of artichoke) are only used as animal feed, being minimum their economic revaluation. Assuming that the by-products have a composition similar to the edible part of the artichokes, these by-products can be a promising source of new value-added compounds such as phytochemicals and fibre (Ruiz-Cano *et al.,* 2014). Note that recent publications of revaluation

indicate that vegetable by-products of the canning industry (asparagus) (Nindo *et al.*, 2003, Fuentes-Alventosa *et al.*,2009), chard, cardoon, green beans, etc. (Randhawa *et al.*,2015) have good functional qualities, such as Water or Oil holding capacity, and high content of antioxidant compounds (polyphenols, etc.) and fibre (soluble and insoluble). All of them qualities really demanded by manufactures of enriched products (such as biscuits, meat, dairy products) who eventually could select vegetal fibre-rich-powder over other fibres extracted from grains (Rodríguez *et al.*,2006), rice (Gul *et al.*,2015), tubers or roots as (Fibrex® (sugar beet), Raftilosa® (chicory) with less functional qualities.

However, there are not any commercial products of food fibre-rich powders of artichoke yet, although it would be very interesting to study their possible industrial appliance. Thus the aim of this study was to describe the biscuits enriched with artichoke fibre-rich powders, compared to biscuits formulated with commercial reference fibre (Pea Fibre) and control biscuits without fibre. The shelf-life study of the biscuits was carried out by investigating the evolution of the sensorial qualities during storage at ambient (25 ºC) and accelerated (45 ºC) temperature.

2. MATERIAL AND METHODS

2.1 INGREDIENTS AND PREPARATION OF THE BISCUITS

The biscuits were formulated in triplicate following the recipe of Whitley (1970) with slight modifications in the percentages of the ingredients since 4 % (w/w) of plant fibre was added. The choice of this percentage of fibre was determined by an optimization study from which it was concluded that with 4 % of plant fibre of both varieties (commercial reference fibre P and fibre-rich powders from artichoke Ca and W) obtained the maximum sensorial acceptability (data not shown). The commercial Pea fibre (P) was obtained on the Spanish market of food additives and the two fibre-rich powders were produced in our laboratory by liquid extraction of artichoke by-products. Two different extraction liquids were used: distilled water (W) and a solution of 1 % (w/w) of $CaCl_2 \cdot 5H_2O$ (pH 6.5) (Ca). In the control biscuits, the 4 % (w/w) of fibre was replaced by wheat flour.

The dough was kneaded with a pilot mixer during 10 min at minimum speed. Subsequently, the dough was laminated to a thickness of 5 mm, using a manual sheet pasta machine. The round form

of the biscuits was obtained manually, using a cutting cylinder (die) of 60 mm in diameter. After making 8 evaporation holes in the dough with a fork; the biscuits were baked in an electrical convection oven at a temperature of 180 ± 10 °C during 17 minutes (Figure 1).

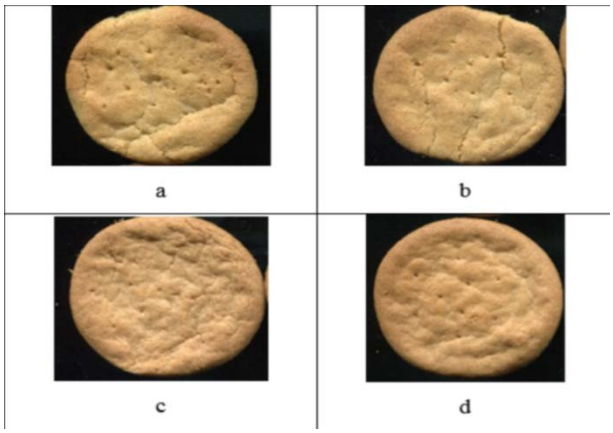


Fig. 1 Biscuits after oven-baking. a) Biscuit W, b) Biscuit Ca, c) Biscuit B, d) Biscuit P. Biscuit codification (B (without fibre), P (Pea fibre), W (water), Ca (1 % $CaCl_2.5H_20$).

Afterwards, the biscuits were cooled at ambient temperature (18 – 20 °C) and wrapped in heat-sealed bags of high-density polyethylene. The biscuits were divided into two groups as a function of the storage conditions. As the control for each group of biscuits, the values were taken at time 0, which was the day after preparation.

2.2 STORAGE

The wrapped biscuits were stored in two incubation chambers (Incudigit Selecta Spain), where temperature and humidity were kept constant.

One of the chambers simulated storage at room temperature (25 °C, RH 55 %), while the other simulated conditions of accelerated storage (45 °C, RH 55 %) (Yang *et al., 2013*), forcing the appearance of defects in the biscuits by the increase in temperature. The biscuits stored under ambient conditions were analysed bimonthly, during the 4 months, while the biscuits stored under accelerated conditions were analysed every 3 months until completion of the six months of storage. By comparing the results of both storage procedures, it intended to conclude at which time under ambient conditions the defects were detected, generated under accelerated storage.

2.3 SENSORY ANALYSIS

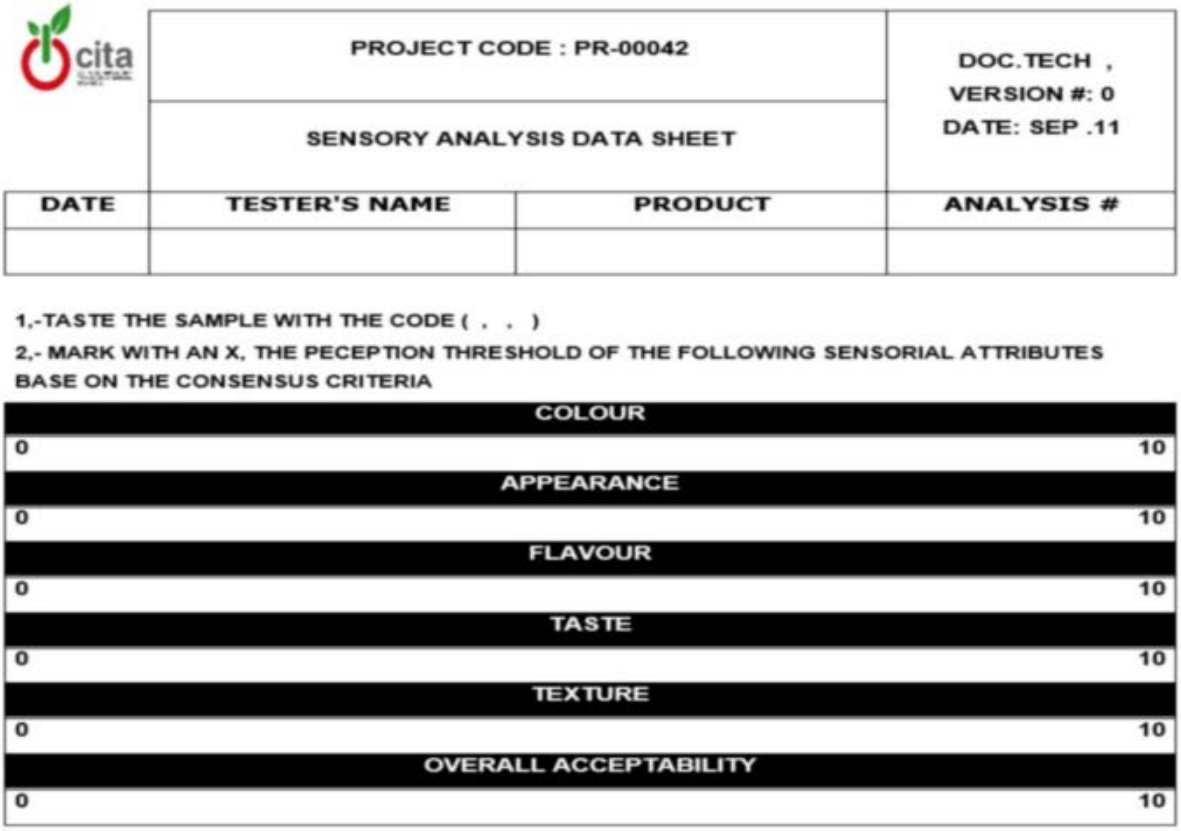
The sensory evaluation was carried out in the tasting room of CITA-CTIC La Rioja. The sensory panel consisted of 9 judges trained in the use of the sensory profiles method (Lawless & Heymann, 2010). The panelists were trained with commercial biscuits and prototypes prepared in pilot plants, since the biscuits formulated with fibre-rich powder were also prepared in a pilot plant. By means of this training, a specific terminology for the sensory characteristics and ranges for each attribute was agreed upon. All the trained judges of the internal sensory panel passed the basic taste test, the odour test and the colour vision test, and their evaluation capacity was routinely verified by way of individual control cards. The vocabularies of the sensory attributes were agreed upon in order to describe the differences between samples. The intensity of the attributes was classified on non-structured, continuous graphic scales. The scales were 10 cm in length and it was verbally agreed that the end on the left-hand side of the scale corresponded to the lowest intensity (value 0) and the end on the right-hand side to the highest intensity of the attribute (value 10); while the intermediate point was the value of the commercial standard biscuits (value 5).

The freshness of the samples was evaluated by the presence or absence of characteristic flavour/taste, colour, texture, appearance, overall acceptance. For the biscuit samples without accelerated storage, a fresh sample should have a taste and flavour similar to the standard. Therefore, the absence of characteristic flavor and taste was rated below the standard value, while flavor and taste were rated above the standard value, up to very roasted or burnt as the upper end of the scale. Colour defined the degree of toasting and the outer ends of colour went from bread crust of white tin loaf to toasted whole meal bread. Hardness was expressed as the resistance of the biscuit to breaking upon pressure of the front teeth during biting. For a hard biscuit, the strength needed for breaking would increase (the standard outer ends of texture went from the hardness of toasted bread rusks to that of whole meal “grissini”-type rusks of 0.5 cm in diameter).

Appearance is related to the geometry and the structure of the biscuits, and the outer ends of appearance went from an irregular biscuit with an irregular surface produced by evaporation bubbles, to a very compact biscuit with a smooth surface.

Overall acceptability is a mixture of all of the sensory attributes and therefore the outer ends of the scale go from: very little like the commercial standard biscuit, with a very light colour, little flavor and taste of biscuit, a very crumbly and soft biscuit, to a very dark biscuit, with a strong taste of toasted, a very hard and compact biscuit, at the upper end. In order to be able to describe the possible sensory defects, like the appearance of strange flavors and odors caused by the accelerated storage,

the consensus on the sensory variables taste and flavor was differentiated. For the attributes characteristic flavor and taste, the freshness of the sample was rated as the point in the middle of the non-structured scale. Thus, the absence of characteristic flavor and taste was rated below the standard value, like loss of characteristic flavor or taste when tastes or smells related to oxidative processes were detected, being a strong flavor and taste of rancidity the lower end of the scale. This nuance in the description of the sensory outer ends of flavor and taste caused the redefinition of the overall acceptability of the biscuits, in the lower end of the scale of overall acceptability, where the biscuits have a very light colour, a strong taste and smell of rancidity, and are very soft and crumbly (Figure 2).



cita	PROJECT CODE : PR-00042		DOC.TECH , VERSION #: 0 DATE: SEP .11
	SENSORY ANALYSIS DATA SHEET		
DATE	TESTER'S NAME	PRODUCT	ANALYSIS #

1,-TASTE THE SAMPLE WITH THE CODE (. .)
2,- MARK WITH AN X, THE PECEPTION THRESHOLD OF THE FOLLOWING SENSORIAL ATTRIBUTES BASE ON THE CONSENSUS CRITERIA

COLOUR	
0	10
APPEARANCE	
0	10
FLAVOUR	
0	10
TASTE	
0	10
TEXTURE	
0	10
OVERALL ACCEPTABILITY	
0	10

Fig. 2. Tasting ballot.

The presentation of the biscuit samples was performed in a blind fashion, coding each sample with a random numeric code of three digits. The order of presentation of the biscuits was balanced by using all of the presentation combinations possible on the tasting sheet. The characterization of the products was carried out under daylight lighting and in portable cabins within the sensory

laboratory. The ration of consumption was one whole biscuit of each reference, maintained at a temperature of 25 ºC. Water was served to the evaluators for cleaning of the mouth between the different samples. The samples were judged without replicate. With the aim of testing the reliability of the results, the control biscuit was introduced two times in the evaluations, randomly between other samples.

2.4 SENSORY SAMPLES

The samples tested during the sensory analysis were samples randomly selected from each of the three pilot plant batches. The three session of each replica of samples were performed with a time lapse of two days. Before the sensory analysis sessions, the samples were tempered for 30 minutes, at a temperature of the sensory analysis room (25 ºC). The presentation of the sample to the panelist was as follows: using white light, the four hold biscuits were presented together on a piece of white paper divided into four equal parts, in which the codes of the biscuit were printed randomly. The panelists were instructed to taste the samples clockwise starting from the upright quarter.

2.5 STATISTICAL ANALYSIS

Statistical analysis of the sensorial measurements was carried out with the sensory analysis software Panel Check. The data were expressed as Mean ± Coefficient of variation (CV). 2-way ANOVA (1 rep) sample means & LSD, and principal component analysis (PCA-statis) were applied. The PCA decomposes the viability of a group of data in principal components and tracks the panelist in the principal component space according to their valuations of the samples together with the average classifications of the samples or attributes.

3. RESULTS AND DISCUSSION

3.1 SHELF-LIFE TEST AT ROOM TEMPERATURE

As can be observed in Figures 3 and 4, colour and taste were the two sensory qualities for which all the groups of biscuits were significantly different ($p<0.001$). With respect to colour, as was

expected, the biscuits without fibre (B) had a lighter colour than the rest, followed by the pea biscuits (P), while the biscuits formulated with artichoke fibre-rich powders (W, Ca) were significantly darker, mainly due to the darker colour of the artichoke fibre-rich powders (Figure 1).

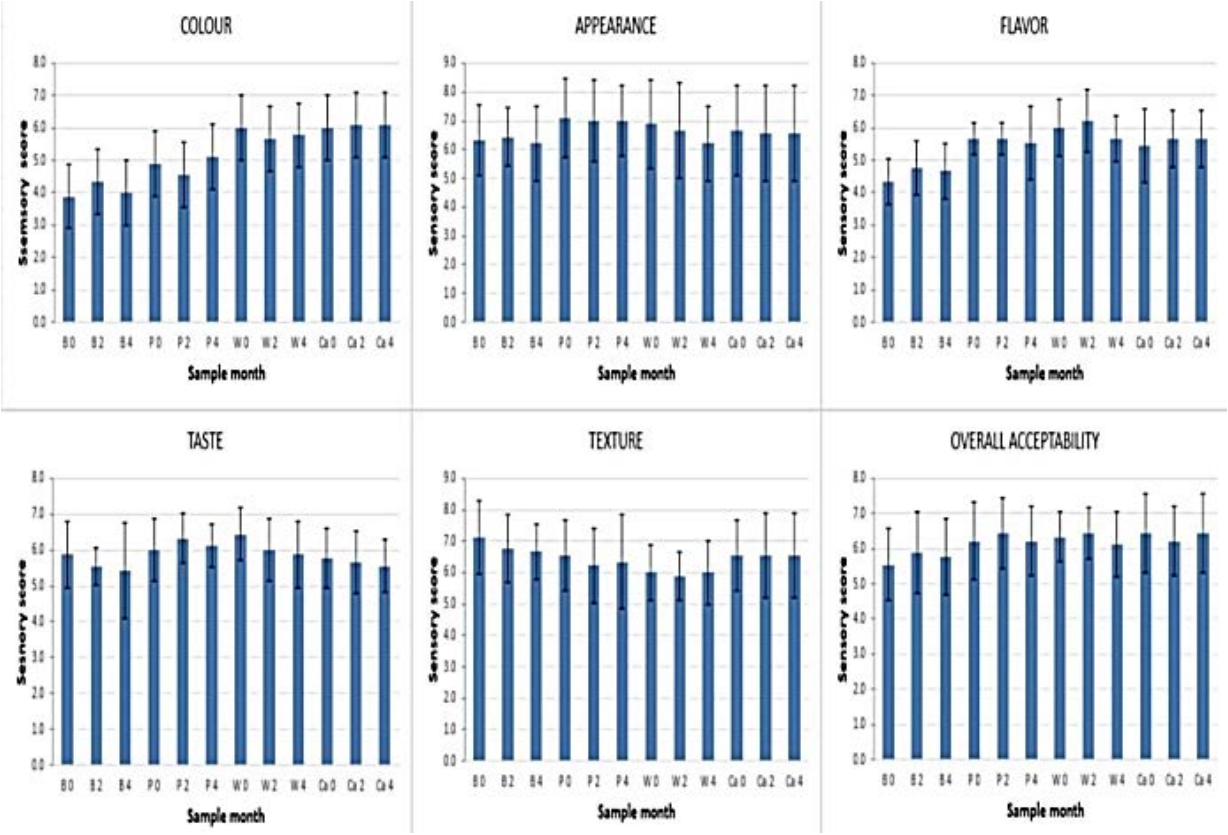


Fig. 3. Organoleptic properties (Colour; Appearance, Taste, Flavor, Texture, Overall acceptability) of biscuits formulated without fibre (B), and with fibre of Pea (P) and fibre-rich powder of Artichoke (W, Ca), storage at room temperature (0, 2 and 4 months). Values are means ± Standard Dev. (n= 9)

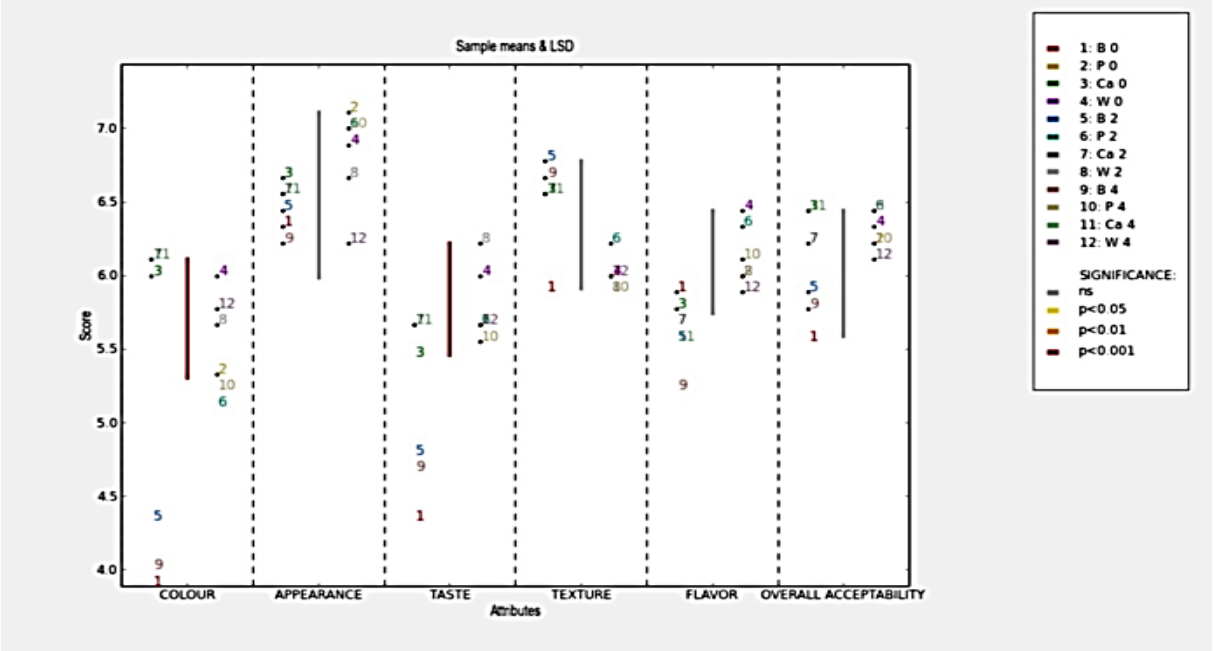


Fig. 4.2-way ANOVA (1rep) sample means & LSD. Storage of biscuits formulated without fibre (B), and with fibre of Pea (P) and fibre-rich powder of Artichoke (W, Ca), at room temperature and with accelerated storage for (0, 2, 4) months Values are means ± Standard Dev. (n= 9)

Regarding the variable taste, it was observed that all the biscuits enriched with fibre had a more intense taste than the biscuits without fibre (B). When comparing the biscuits with artichoke fibre-rich powders (W, Ca) with the biscuits formulated with Pea fibre, it was observed that their taste was similar.

With respect to the overall acceptability, biscuits B were more similar to the standard biscuit, because of their more neutral taste and colour. Similar results were found by other authors for whole meal wheat biscuits in which part of the flour was replaced by another functional ingredient (Akubor & Badifu, 2004, Kohajdová *et al.*, 2013, Cheng & Bhat, 2016). The panelists preferred the lighter colour of the whole meal wheat biscuits when a high level of replacement of wheat flour caused a low acceptance by the panelists with respect to the attributes of colour. In our case, the acceptability of the biscuits formulated with artichoke fibre-rich powders was similar to that of the biscuits with Pea fibre. It can also be observed, from the texture, that the biscuits with fibre were significantly harder than the biscuits without fibre. For the rest of the sensory qualities (appearance and flavor), all groups of biscuits were similar and their values remained constant during storage at ambient temperature.

PCA-statistical analysis explained 89.8 % of the variation in the sensory attributes of the biscuits; principal component 1 (PC1) explained 75.5 % and the second component (PC2) 14.3 % (Figure. 5).

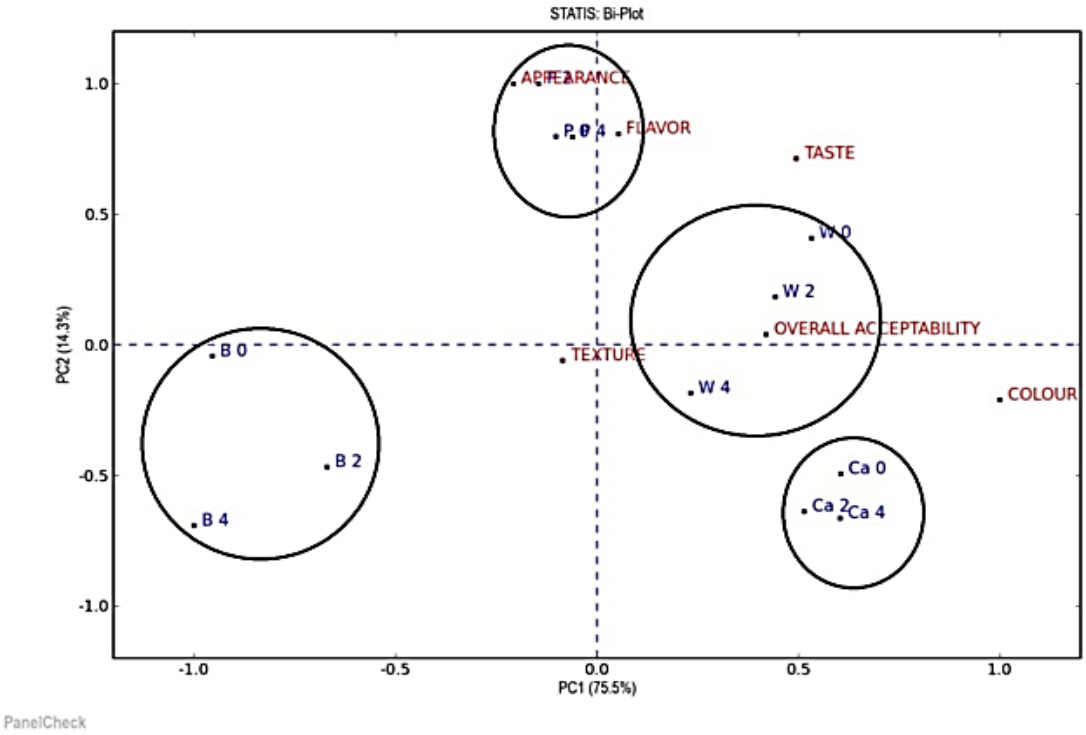


Fig. 5. PCA-statis plot of the sensory profile of the biscuits by descriptive method. Storage of biscuits formulated without fibre (B), and with fibre of Pea (P) and fibre-rich powder of Artichoke (W, Ca), at room temperature and with accelerated storage for (0, 2, 4) months. Values are means ± Standard Dev. (n= 9).

In the first principal component, the variable colour and overall acceptability were correlated with the biscuits formulated with artichoke fibre-rich powders (Ca, W) separating them from biscuits B and P. Thus, if the colour of the biscuits is darker, this is related to an increase in the values of overall acceptability.

Regarding the second principal component, taste is the sensory variable that produced the major differentiation of the groups, separating them in biscuits with Pea fibre, biscuits with artichoke fibre-rich powders and biscuits without fibre. When looking at the groups that were formed in the PCA-statis plot individually, it is observed that throughout the four months of storage at ambient temperature the groups of biscuits formulated with vegetal fibres remained grouped more closely. This is explained by a lack of significant variation of the sensory qualities and therefore, due to an

effect of protection of the sensory qualities by the enrichment of the biscuits with vegetal fibre, or at least an effect of masking the changes in colour and taste.

3.2 ACCELERATED SHELF-LIFE TEST

As can be observed in Figures 6 and 7, during accelerated storage the score of the biscuits without fibre (B) significantly decreases for the sensory variables taste and flavor, diminishing their overall acceptability. This change in sensory perception is caused by the formation of aromatic compounds, of unpleasant taste and flavor, by-products of lipid oxidation, (Talbot, 2010) like 2,4-decadienal y 2,4- heptadienal (Cheng & Bhat 2016) and hexanal (Sakač *et al.*,2016).

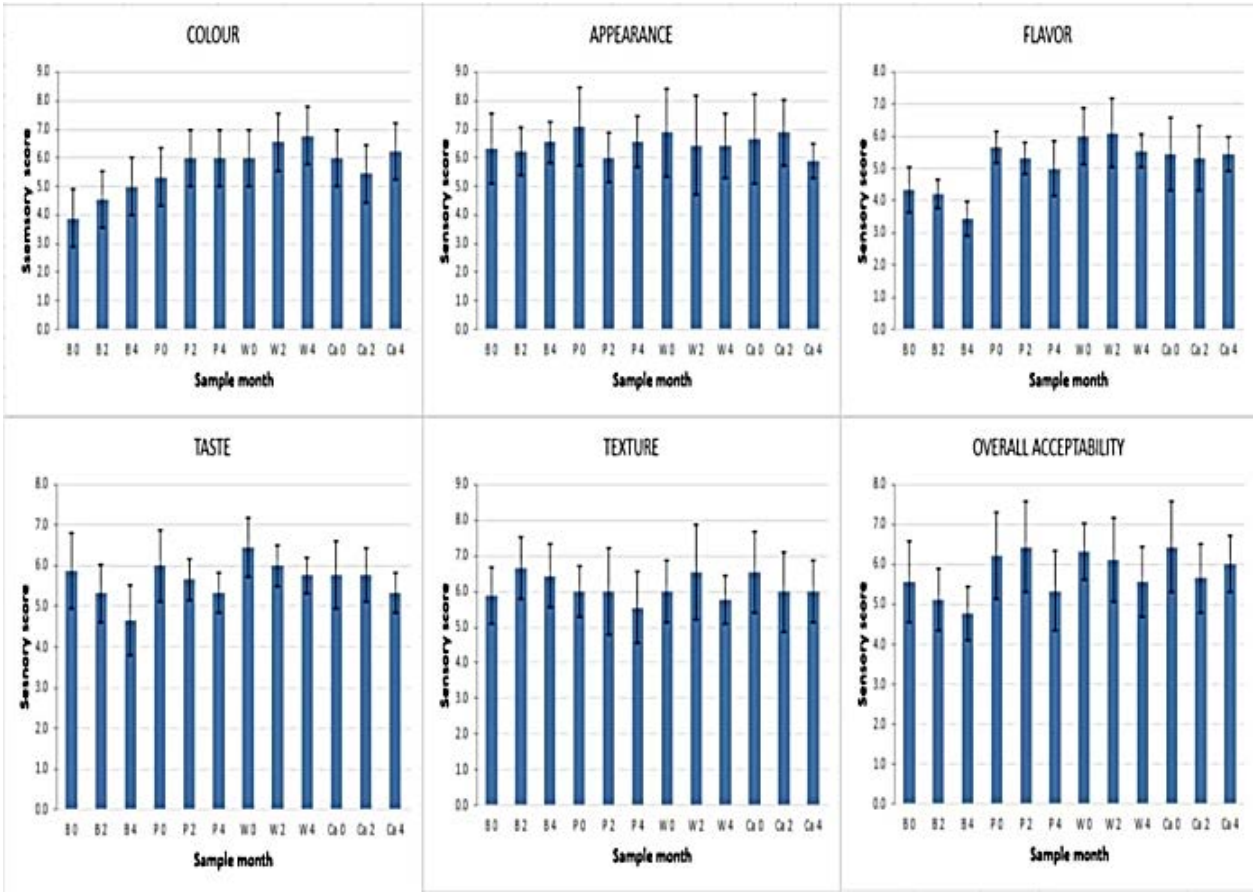


Fig. 6. Organoleptic properties (Colour; Appearance, Flavor, Taste, Texture, Overall acceptability.) Storage at accelerated temperature of biscuits formulated without fibre (B), and with fibre of Pea (P) and fibre-rich powder of Artichoke (W, Ca), for 0, 2, 4 months Values are means ± Standard Dev. (n= 9)

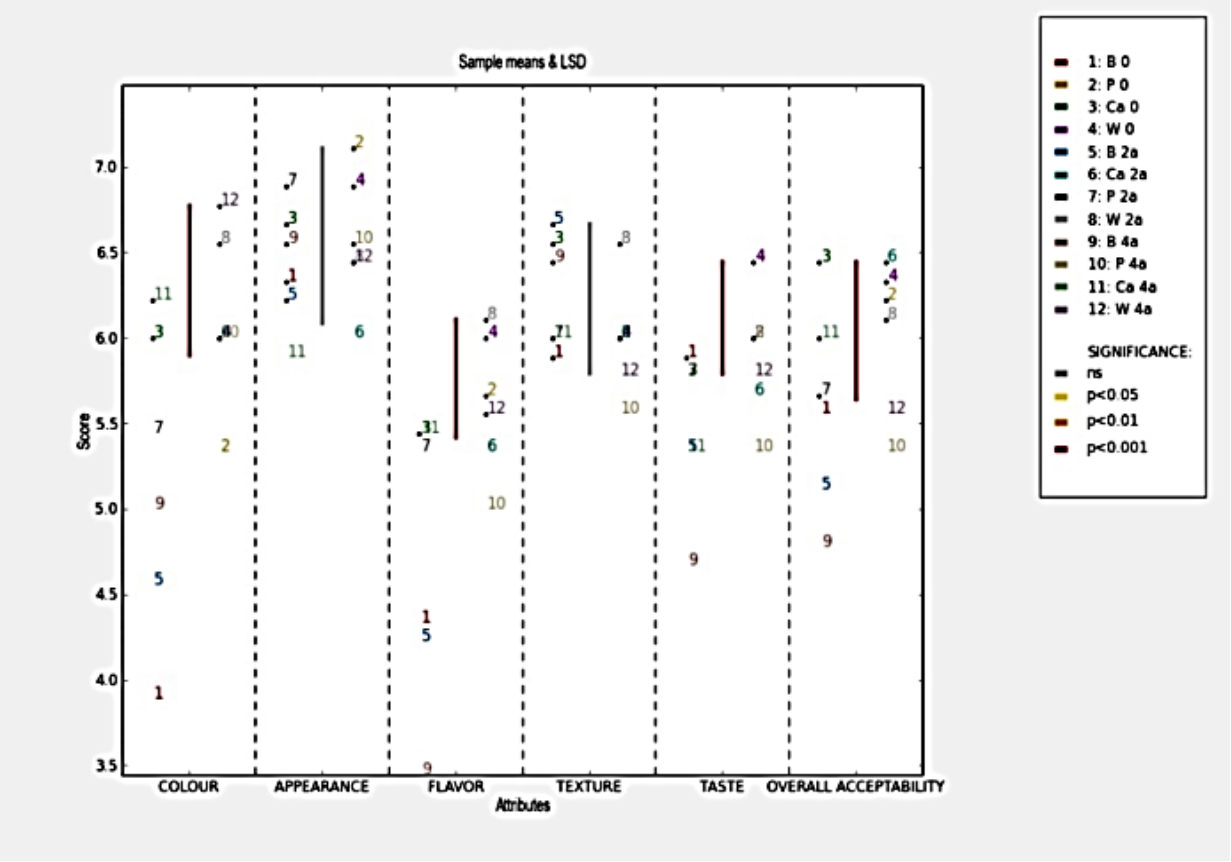


Fig. 7. 2-way ANOVA (1rep) sample means & LSD. Storage at accelerated temperature of biscuits formulated without fibre (B), and with fibre of Pea (P) and fibre-rich powder of Artichoke (W, Ca), for 0, 2, 4 months Values are means ± Standard Dev. (n= 9

With respect to colour, only the colour of biscuits B significantly increased during accelerated storage. This was partly because of the fact that the initial colour of the biscuits B was lighter than that of the rest and therefore small variations in colour were more perceptible by the panelist than in the biscuits with plant fibre, which were darker due to the colour of the fibre.

When the sensory valuation of texture and appearance is observed during accelerated storage, no significant differences are found between the groups of biscuits. The biscuits maintained similar values throughout the storage period and both sensory variables had a constant behavior during accelerated storage. With respect to the overall acceptability, only the biscuits without fibre had a significantly lower appreciation at the end of accelerated storage.

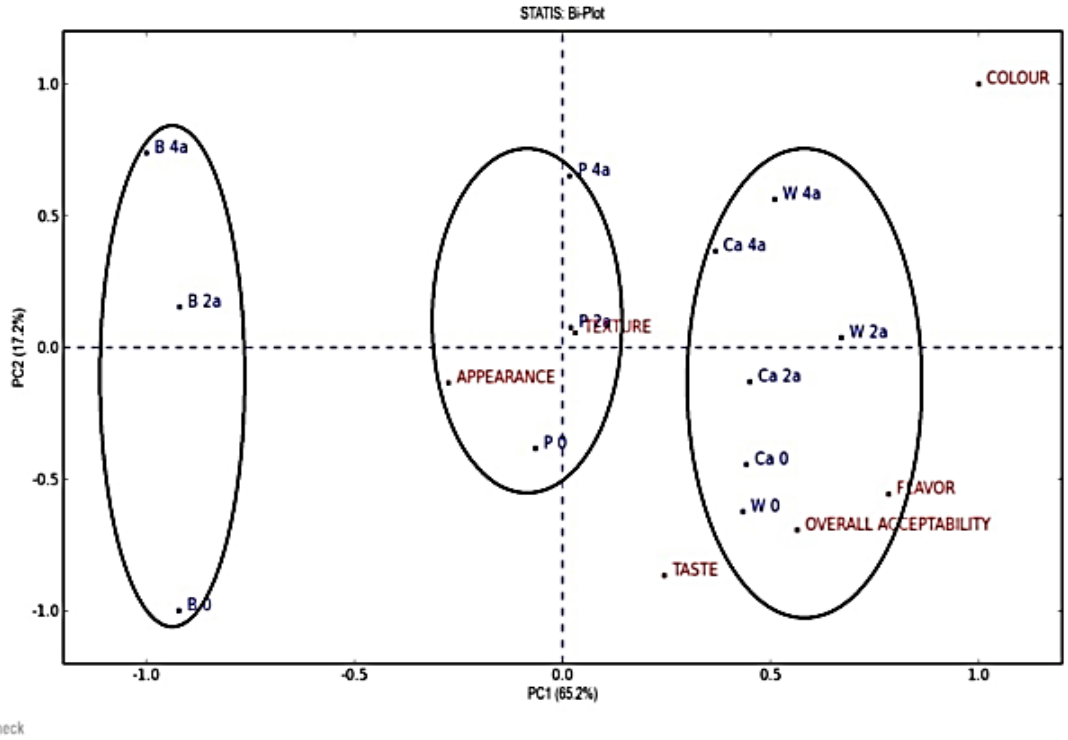


Fig. 8. PCA-statis biplot of the sensory profile of the biscuits by descriptive method. Storage at accelerated temperature of biscuits formulated without fibre (B), and with fibre of Pea (P) and fibre-rich powder of Artichoke (W, Ca), for 0, 2, 4 months Values are means ± Standard Dev. (n= 9)

For the biscuits subjected to accelerated storage, the PCA-statis analysis explained a total of 82.4 % of the variation in the sensory attributes of the biscuits; the first principal component 1 (PC1) explained 65.2 % and the second (PC2) 17.2 % (Fig. 8). This effect on the decrease of the percentage of explanation of the first principal component is caused by the conditions of accelerated storage and the increase of the explanation of the model of more sensory variables, like taste and flavor.

When comparing figure 8 with figure 5, it is observed that in both figures the explanation of the different biscuit groups along the first principal component was due to the colour of the fibre used. Since these clear differences between biscuits without fibre and the rest were maintained throughout accelerated storage, and because the biscuits formulated with FRPAs (W, Ca) could not be distinguished, the percentage of explanation of the first principal component decreased.

On the contrary, the second principal component increases the explanation of each group of biscuits, as a result of the darkening of the biscuits during storage, and due to that accelerated at two and four months (2a, 4a) cookies are separated vertically cookies control (0). Feel this differentiation

greater in them cookies of a colour clearer, as the biscuits B and P, where small changes in colour are more perceptible to them panelist.

The sensory variables overall acceptability, taste and flavor were correlated with the biscuits enriched with artichoke fibre-rich powder at the beginning of the accelerated test (Ca0, W 0). On the contrary, the variables appearance and texture are not explained well by the model, since they are close to the centre of both principal components.

In conclusion, the evolution of the sensory qualities of the biscuits formulated with artichoke fibre-rich powders (W, Ca) was similar to that of the biscuits formulated with pea commercial reference fibre (P), and thus that its better sensory appreciation would be maintained throughout the accelerated storage.

4. CONCLUSIONS

The sensory description of the biscuits with fibre showed that the variables taste and colour were the sensory qualities that significantly differentiate them from the biscuits without fibre (B).

The taste of all biscuits enriched with fibre was appreciated as similar by the panelist. With respect to colour, the use of both artichoke fibre-rich powders (Ca, W) modifies the colour of the biscuits, making them darker than the biscuits formulated with Pea fibre, due to its more neutral colour. However, initially, the overall acceptability of the biscuits formulated with both artichoke fibre-rich powders was similar to that of the biscuits formulated with Pea fibre (P).

When the biscuits were subjected to accelerate storage, significant changes in taste and colour were produced in the biscuits without fibre (B), since colour and taste in these biscuits are more neutral, and therefore small variations are more easily detected by the panelist. It was also demonstrated that the biscuits formulated with artichoke fibre-rich powders (Ca, W) had a similar behavior during storage to that of the biscuits formulated with the commercial reference fibre (P).

These results conclude that the utilization of fibre-rich powders from artichoke by-products (Ca, W) can be a viable alternative for the enrichment of biscuits as well as other oven-baked products and that it could be a suitable substitute for commercial Pea fibre.

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CONCLUSIONES

Las conclusiones más importantes que se deducen delos resultados obtenidos en el estudio llevado a cabo en esta Tesis Doctoral son las siguientes:

En cuanto a cualidades funcionales de los FRPAs:

- Las propiedades funcionales de los FRPA (extractos ricos en fibra obtenidos de subproductos de Alcachofa) están influenciadas por el método y el solvente de extracción utilizado.
- El método de extracción se debe modificar en función de requisitos funcionales de los FRPAs. Aquellos productos que requieran mayor "WHC" y "SWC" es más convenientes la extracción húmeda utilizando alcohol como solvente de extracción; para productos que requieren que una mayor "OHC" es preferible una extracción húmeda con agua, como solvente de extracción.
- En relación con el contenido de fibra de FRPA, los valores FDT son intermedios en comparación con la fibra de referencia comercial (Fibra de guisante), la cual estáubicada en el rango máximo. Esta fibra comercial presenta un desequilibrio entre fibra insoluble y soluble que afecta a sus cualidades funcionales y algunas aplicaciones industriales.
- Los FRPAs obtenidos de subproductos de alcachofa tienen una mayor concentración de polifenoles totales y una mayor capacidad antioxidante que la fibra de guisante comercial.
- El secado directo es el mejor método de extracción para obtener FRPAs con capacidad antioxidantes como; alternativa para sustituir a la fibra comercial de referencia (fibra de guisante) y reducir el impacto medioambiental de los subproductos de la alcachofa.

Se puede concluir que un método sencillo de extracción como es el método de secado directo permite revalorizar los subproductos de alcachofa producidos en las industrias de conservas obteniendo los FRPAs, y proporcionando una alternativa viable a la fibra de referencia comercial utilizada actualmente en la industria alimentaria (fibra de guisante) como fibra alternativa al salvado de cereales.

En relación con el uso FRPAs en la formulación de galleta:

- Las variables sensoriales sabor y color son las cualidades sensoriales que diferencian significativamente las galletas con fibra de las galletas sin fibra.

- La aceptación sensorial de las galletas formulada con ambos FRPAs (CA, extractos ricos fibra obtenidos por extracción humeda con una diolucion de 1 % (w/w) $CaCl_2$ * $5H_2O$ (pH: 6.5); W, extractos ricos en fibra obtenidos por extracción humeda W) fue similar a la de las galletas formuladas con fibra de guisante. El sabor de todas las galletas enriquecidas con fibra fue apreciado como similares por el panel sensorial.
- Durante el almacenamiento a temperatura ambiente (25 ºC, 7 meses), la utilización de FRPAs (CA, W) aumenta las variables de textura (dureza, fuerza máxima de deformación y rigidez) de manera similar a la fibra de referencia comercial (P). Con respecto al color, la sustitución de 4% de harina integral por FRPAs cambia el color de las galletas de una manera similar a la fibra de referencia comercial (P).

Se puede concluir que la utilización de FRPAs (CA, W) puede ser una alternativa viable para el enriquecimiento de galletas, y otros productos horneados, y que podría ser un sustituto adecuado para comercial fibra de guisante.

Por lo tanto se han logrado los objetivos de esta Tesis Doctoral y se puede concluir que es factible la revalorización de subproductos de la alcachofa, obtenidos en la producción de alcachofas en conserva, en forma de FRPAs; y que éstos se podrían utilizar en la elaboración de galletas, sustituyendo a la fibra comercial de referencia y aportando valor añadido para productos enriquecidos o funcionales.

CONCLUSSIONS

The most important conclusions that are subtracted from the results obtained in this PhD Thesis are the following:

Regarding functional qualities of the FRPA:

- Functional properties of the fibre-rich powders obtained from by-products of artichoke (FRPAs) are influenced by the method and the solvent of extraction used.
- The method of extraction could be customized base on functional fibre-rich requirements. For example, for those products requiring greater “WHC” and “SWC” wet extraction using alcohol as extraction solvent would be more suitable ; on the other hand for products requiring a greater “OHC” water would be preferaed as extraction solvent.
- In regard to the fibre content of FRPAs, FDT values are intermediate compared with the commercial reference fibre (Pea fibre), which is located in the maximum range. It has been proven that this commercial fibre presents an imbalance between insoluble and soluble fibre that may affect its functional qualities and some industrial applications
- All the FRPAs have a higher total polyphenol concentration and a higher antioxidant capacity than the commercial Pea fibre, independently of the methodology used.
- The direct drying method results in FRPAs with higher concentrations of polyphenolic and antioxidant compounds. Which would be an excellent alternative to commercial reference fibre (Pea fibre) and reducing environmental impact of by-products of artichoke.

It can be concluded that a simple method of extraction allows revaluing artichoke by-products produced in canning industries as fibre-rich powders, providing viable alternative to commercial reference fibre (Pea fibre) used as an alternative to cereal bran, in the food industry.

Regarding the use FRPA in the formulation of biscuit:

- The variables taste and colour were the sensory qualities that significantly differentiate them from the biscuits without fibre.

The sensorial preference of the biscuits made with both FRPAs (CA, fibre rich powders of artichoke obtained by wet extraction using a dissolution of 1 % (w/w) $CaCl_2$ * $5H_2O$ (pH: 6.5) as extraction solvent ; W, fibre rich powders of artichoke obtained by wet extraction using distilled water as extraction solvent) similiar to the biscuits made with pea fiber. The flavor of all the biscuits enriched with fibre was perceived as similar by sensorial panel.

- During the storage a room temperature (25 ºC, 7 months), the utilization of FRPAs (CA, W) increases the texture variables (hardness, maximum force of deformation and rigidity) in a similar way to the commercial reference fibre (P). Concerning colour, the substitution of 4 % of whole meal flour by FRPAs changes the colour of the biscuits in a similar way to the commercial reference fibre (P).

In summary these results conclude that the utilizationof FRPAs (CA, W) can be a viable alternative for the enrichment of biscuits, as well as other oven-baked products, and that it could be a suitable substitute for commercial Pea fibre.

Based on the results, it would be proved that the objectives of this thesis has been achievedand it can be concluded that it is feasible to reevaluate by-products of the artichoke, obtained in the canning production of preserved artichokes, in the form of FRPAs; and that these could be used in the manufacture of biscuits, while replacing the commercial reference fiber and providing added value to enriched or functional products.

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