



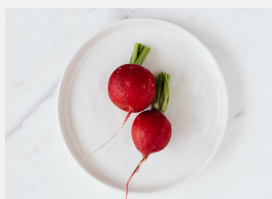
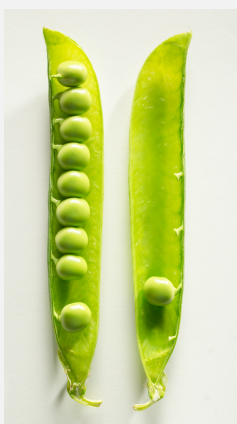
UNIVERSITAT  
POLITÈCNICA  
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# DESARROLLO DE PREPARADOS TEXTURIZANTES *CLEAN LABEL*

Programa de Doctorado en Ciencia, Tecnología y Gestión Alimentaria

Autora: Ana Teresa Noguerol Meseguer

Directora: Prof. Dr. M<sup>a</sup> Jesús Pagán Moreno



Valencia, enero 2022





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Hace constar que:

La memoria titulada “Desarrollo de productos texturizantes *Clean Label*” que presenta **D<sup>a</sup> Ana Teresa Noguero Meseguer** para optar al grado de Doctor por la Universitat Politècnica de València, ha sido realizada en el Departamento de Tecnología de Alimentos de la Universitat Politècnica de València bajo su dirección y que reúne las condiciones para ser defendida por su autora.

Valencia, enero 2022

Fdo. M<sup>a</sup> Jesús Pagán Moreno



*A mis padres*





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**Resumen,  
Abstract  
y Resum**



## Resumen

Los consumidores preocupados por la salud, sostenibilidad y bienestar animal han aumentado en los últimos años. Por ello, las nuevas tendencias *clean label* y *plant-based* están en alza. Cada vez son más las empresas que quieren lanzar al mercado productos sin aditivos y conservantes, más naturales y menos procesados. Además, el interés por los productos puramente vegetales está creciendo, debido al aumento de población que quiere eliminar por completo el consumo de carne de su dieta o reducirlo.

Para la elaboración de productos alimentarios es necesario el uso de aditivos, ya que estos no solo aseguran su inocuidad y alargan su vida útil, sino que también tienen funciones de texturizantes, espesantes y gelificantes. La elaboración de análogos de productos de origen animal (derivados cárnicos y otros como el queso) requiere del uso de este tipo de ingredientes para conseguir que el producto tenga una textura similar a los productos originales. Por ello, al igual que los aditivos y conservantes, en ocasiones estos productos no están bien vistos por los consumidores, que prefieren que sean lo más naturales posible. Sin embargo, existen ingredientes procedentes de las plantas con poder texturizante, gelificante y/o espesante, que podría ser utilizados con tal finalidad.

Por todo ello, el objetivo de esta tesis doctoral fue la ampliación y modificación de la gama de preparados ofertados por la empresa Productos Pilarica S.A. en base a los criterios *clean label* para la elaboración de productos *plant-based*.

Inicialmente se planteo un estudio sobre la visión general de los productos *plant-based* con etiqueta limpia por parte de diferentes grupos de consumidores (omnívoros, veganos, vegetarianos y flexitarianos). Los resultados obtenidos indicaron que la percepción de este tipo de productos

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era diferente en función de la dieta de los consumidores. Los omnívoros no diferenciaron entre los productos veganos y vegetarianos *clean label* y con etiqueta original; sin embargo, los consumidores que reducen o eliminan el consumo de carne y derivados percibieron los productos *clean label* de manera diferente a los productos con etiqueta original, prefiriendo los productos “más naturales”. Además, se vio que esta población parece estar más concienciada con el medio ambiente.

Seguidamente, se procedió a realizar una búsqueda de ingredientes vegetales naturales con poder texturizante, siendo los ingredientes seleccionados fibras alimentarias de diferente procedencia (fibra de guisante, fibra de patata, fibra de bambú, fibra de cítricos, tres tipos de fibras de *Plantago ovata*, y dos mezclas de fibras, una con bambú, psyllium y cítricos, y otra con guisante, caña de azúcar y bambú). Para conocer su capacidad texturizante se procedió a estudiar sus características tecno-funcionales y, especialmente, su capacidad de gelificación. Como resultados más relevantes se observó que las fibras procedentes de *P. ovata* o psyllium, formaban geles estables a concentraciones más bajas que las demás fibras, con o sin tratamiento por calor. Además, el tratamiento con calor aumentaba la dureza de estos geles. También se vio que el contenido en minerales como el hierro y la capacidad antioxidante de estas fue destacable.

Una vez seleccionadas las fibras de *P. ovata* como principales texturizantes se procedió a la evaluación de estas en diferentes matrices alimentarias (untables y salchichas tipo Frankfurt). En ambas matrices se observó que la adición de psyllium, a diferentes concentraciones, aumentaba la firmeza y la consistencia de los productos. Se debe resaltar el uso de la fibra de psyllium PW (*Plantago White*), ya que, además de funcionar como texturizante, fue la que minimizó las diferencias de color con respecto a las muestras control.

Palabras clave: clean label; plant-based; fibra; texturizante; gelificante; veganos; vegetarianos; flexitarianos; omnívoros; *Plantago ovata*; análogos de productos de origen animal.

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## **Abstract**

Consumers concerned about health, sustainability and welfare have increased in recent years. For this reason, the new clean label and plant-based trends are on the rise. More and more companies want to launch products without additives and preservatives, more natural and less processed. In addition, the interest in purely vegetable products is growing, due to the increase population that wants to completely eliminate or reduce meat consumption from their diet.

The use of additives is necessary for the formulation of food products since these not only ensure their safety and extend their useful life, but also have texturizing, thickening and gelling functions. The analogue elaboration of animal origin products (meat derivatives and others such as cheese) requires the use of this type of ingredients to ensure that the product has a texture similar to the original product. Therefore, as well as additives and preservatives, animal origin products sometimes are not well seen by consumers, who prefer these kinds of products to be as natural as possible. However, there are ingredients from plants with texturizing, gelling and/or thickening capacity, which could be used for this purpose.

Therefore, the objective of this doctoral thesis was the expansion and modification of the range of preparations offered by the collaborated company based on the clean label criteria for the production of plant-based products.

Initially, a study was proposed on the general vision of plant-based products with a clean label by different groups of consumers (omnivores, vegans, vegetarians and flexitarians). The results obtained indicated that the perception of this type of products was different depending on the diet of the consumers. Omnivores did not differentiate between clean label and original label vegan and vegetarian products. However, consumers who reduce or



eliminate their consumption of meat and meat products perceived clean label products differently than products with the original label, preferring “more natural” products. In addition, it was seen that this population seems to be more aware of the environment.

Next, a search was carried out for natural vegetable ingredients with texturizing power. The selected ingredients being food fibres from different sources (pea fibre, potato fibre, bamboo fibre, citrus fibre, three types of *Plantago ovata* fibres, and two fibre mixtures, one with bamboo, psyllium, and citrus, and the other with pea, sugarcane, and bamboo). In order to know its texturizing capacity, we proceeded to study their techno-functional characteristics and, especially, their gelling capacity. As more relevant results, it was observed that the fibres from *P. ovata* or psyllium more formed stable gels at lower concentrations than the other fibres, with or without heat treatment. Furthermore, heat treatment increased the hardness of these gels. It was also seen that the content of minerals such as iron and their antioxidant capacity was remarkable.

Once the *P. ovata* fibres were selected as the main texturizers, they were evaluated in different food matrices (spreads and sausages). In both matrices it was observed that the addition of psyllium, at different concentrations, increased the firmness and consistency of the products. The use of psyllium fibre PW (Plantago White) should be highlighted, since, in addition to working as a texturizer, it was the one that minimized the colour differences with respect to the control samples.

**Keywords:** clean label; plant-based; fibre; texturizing; gelling; vegans; vegetarians; flexitarians; omnivores; *Plantago ovata*; analogues of products of animal origin.

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## Resum

Els consumidors que es preocupen per la salut, sostenibilitat i benestar animal han augmentat en els últims anys. Per això, les noves tendències *clean label* i *plant-based* estan a l'alça. Cada vegada són més les empreses que volen llançar al mercat productes sense additius ni conservants, més naturals i menys processats. A més, l'interés pels productes purament vegetals està creixent, a causa de l'augment de població que vol eliminar per complet el consum de carn de la seua dieta, o reduir-lo.

Per a l'elaboració de productes alimentaris és necessari l'ús d'additius, ja que aquests no tan sols asseguren la seua innocuïtat i allarguen la seua vida útil, sinó que també tenen funcions texturitzants, espessidores i gelificants. L'elaboració d'anàlegs de productes d'origen animal (derivats carnis o altres com el formatge) requereix l'ús d'aquests tipus d'ingredients per aconseguir que el producte tinga una textura semblant als productes originals; per això, com ocorre amb els additius i conservants, de vegades no estan ben mirats pels consumidors, els quals volen que els productes siguen el més natural possible. No obstant això, n'hi ha ingredients procedents de les plantes amb poder texturitzant, gelificant i/o espessidor, que podrien ser utilitzats amb aquesta finalitat.

Per tot això, l'objectiu d'aquesta tesi doctoral va ser l'ampliació i modificació de la gamma de preparats oferits per l'empresa col·laboradora basant-se en els criteris *clean label* per a l'elaboració de productes *plant-based*.

Inicialment es va plantejar un estudi sobre la visió general dels productes *plant-based* amb etiqueta neta per part de diferents grups de consumidors (omnívors, vegans, vegetarians i flexitaris). Els resultats obtinguts van indicar que la percepció d'aquest tipus de productes era diferent en funció de

la dieta dels consumidors. Els omnívors no van diferenciar entre els productes vegans i vegetarians *clean label* i amb etiqueta original; no obstant això, els consumidors que reduïxen o eliminen el consum de carn i derivats van percebre els productes *clean label* de manera diferent als productes amb etiqueta original, preferint els productes “més naturals”. A més, es va veure que aquesta població pareix estar més conscienciada amb el medi ambient.

A continuació, es va procedir a realitzar una recerca d'ingredients vegetals naturals amb poder texturitzant, sent els ingredients seleccionats fibres alimentàries de diferent procedència (fibra de pèsol, fibra de creïlla, fibra de bambú, fibra de cítrics, tres tipus de fibres de *Plantago ovata*, i dues mescles de fibres, una amb bambú, psyllium i cítrics, i una altra amb pèsol, canya de sucre i bambú). Per a conèixer la seua capacitat texturitzant es va procedir a estudiar les seues característiques tècnic-funcionals i, especialment, la seua capacitat de gelificació. Com a resultats més rellevants es va observar que les fibres procedents de *P. ovata* o psyllium formaven gels estables a concentracions més baixes que les altres fibres, amb tractament amb calor o sense. A més, el tractament amb calor augmentava la duresa d'aquests gels. També es va veure que el contingut en minerals, com el ferro, i la capacitat antioxidant d'aquests va ser destacable.

Una vegada seleccionades les fibres de *P. ovata* com a principals agents texturitzants es va procedir a l'avaluació d'aquestes en diferents matrius alimentàries (aliments d'untar i salsitxes tipus Frankfurt). A ambdues matrius es va observar que l'addició de psyllium a diferents concentracions augmentava la fermesa i la consistència dels productes. S'ha de ressaltar l'ús de la fibra de psyllium PW (*Plantago White*), ja que, a més de funcionar com texturitzant, va minimitzar les diferències de color respecte a les mostres control.

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Paraules clau: *clean label*; *plant-based*; fibra; texturitzant; gelificant; vegans; vegetarians; flexitarians; omnívors; *Plantago ovata*; anàlegs de productes d'origen animal.

# **Capítulo 1**

## **Introducción**



## **1. Productos *clean label***

En la actualidad, existe una, cada vez más creciente preocupación de los consumidores por su salud (Li & Nie, 2016), cuidando en particular su dieta, y también preocupándose por la sostenibilidad de la forma de vida que llevan (Aschemann-Witzel, Varela & Peschel, 2019). Por ello, está aumentando cada vez más la demanda de productos más saludables (Li & Nie, 2016), más naturales y ecológicos, y menos procesados, es decir, libres de ingredientes o aditivos artificiales que son percibidos de manera negativa por parte de los consumidores (Aschemann-Witzel et al., 2019), lo que provoca en muchas ocasiones el rechazo de estos. Esto puede ser debido a la industrialización y la globalización, que han aumentado la percepción de los ciudadanos sobre el riesgo en el consumo de algunos alimentos (Asioli et al., 2017). Por ejemplo, existe una gran preocupación de los consumidores por el uso de pesticidas en las prácticas agrícolas convencionales e intensivas (Aktar, Sengupta & Chowdhury, 2009), y el uso de hormonas en las prácticas de ganadería intensiva (Devcich, Pedersen & Petrie, 2007), lo que hace que el consumidor prefiera los productos ecológicos. También, el uso de ingredientes artificiales, aditivos, colorantes y la adopción de tecnologías alimentarias como OMG (Organismos Modificados Genéticamente) hace que los consumidores estén preocupados por los efectos adversos que puedan ocasionar en su salud (Asioli et al., 2017). Además, hay una tendencia creciente en la preocupación sobre el efecto de la producción de alimentos en el cambio climático y sus efectos negativos para la sostenibilidad del planeta (Godfray et al., 2010) al igual que por razones éticas, es decir, el impulso de los consumidores por la elección de una dieta vegetariana o vegana (Aschemann-Witzel & Peschel 2019). Este hecho se ve reflejado, ya no solo en las personas que deciden no comer carne ni productos cárnicos, sino también en el aumento de la población que intenta reducir el consumo de carne, pues son conscientes de la

contaminación que implica su producción. Al igual que también se ve reflejado en el incremento de la búsqueda de alimentos de proximidad o locales (Asioli et al., 2017), que ayudan en la reducción de la huella de carbono.

Grunert (2013) indica que el consumo de alimentos por parte de las sociedades industrializadas se ve particularmente afectado por tres tendencias principales: la preocupación por la salud, la sostenibilidad y la conveniencia. Aunque, sin embargo, otros autores como Frewer & van Trijp (2007) y MacFie (2007) indican que los alimentos son elegidos por los consumidores por su poder saciante y sus nutrientes, y por otros factores como el sabor y el precio. Además, es una realidad que cada vez se conocen más casos de enfermedades relacionadas con los alimentos como la diabetes o la obesidad, y que también pueden ser debidas al estilo de vida (Kearney, 2010) así como alergias e intolerancias a algunos alimentos o componentes como el gluten (Asioli et al., 2017) o la lactosa. La tendencia a la elección de los alimentos por conveniencia está relacionada por la cantidad de comidas que actualmente se realizan fuera de casa, ya que cada vez más tienden a superar a las realizadas en casa (Lachat et al., 2012).

Todos estos factores han incrementado el interés de los consumidores por productos más saludables, que sean fáciles de preparar, es decir, que ahorren tiempo en su preparación y que a la vez reduzcan el riesgo de enfermedades y no perjudiquen el medio ambiente. Además, recientemente, la discusión sobre el impacto climático y la contaminación producida para generar productos de origen animal ha cambiado el foco de interés a los productos de origen vegetal y a base de plantas, evitando el uso de ingredientes de origen animal (Aschemann-Witzel & Peschel, 2019; Aschemann-Witzel et al., 2019).



### 1.2. ¿Qué se entiende por productos “etiqueta limpia” o “clean label”?

Algunos autores como Vandevijvere et al. (2019) indican que las dietas de la población actual están basadas en alimentos altamente procesados, incluyendo como ingredientes de estos alimentos: aceites y grasas, harinas y almidones, azúcar y proteínas, pero sobre todo aceites y grasas hidrogenados, almidones modificados, proteínas hidrolizadas y "mezclas" trituradas o extruidas de despojos de carne o restos. Sin embargo, autores como Tarnavölgyi (2003) destacan que el uso de aditivos causa aversión en los consumidores, ya que piensan que estos ingredientes son innecesarios y, en algunos casos, perjudiciales para la salud, lo que como indica Varela & Fiszman (2013), al final se convierte en un tema emocional que preocupa a los consumidores. Así, las nuevas tendencias, salud y sostenibilidad, han llevado a la industria alimentaria a la producción de alimentos con una llamada “etiqueta limpia”, o llamada más comúnmente en inglés “*clean label*” (Asioli et al., 2017; Varela & Fiszman, 2013), eliminando o sustituyendo ingredientes por otros considerados como “más limpios”, es decir, los que no provocan un rechazo por parte del consumidor. Diferentes autores subrayan que los consumidores leen las etiquetas en busca de ingredientes simples y conocidos (Aschemann-Witzel & Peschel 2019), que les resulten familiares (Husein et al., 2019; Aschemann-Witzel et al., 2019; Aschemann-Witzel & Peschel 2019) y que sean cortos (Aschemann-Witzel et al., 2019). Además, buscan la ausencia de ingredientes artificiales, de sonido químico (Aschemann-Witzel et al., 2019), y difíciles de pronunciar (Varela & Fiszman, 2013).

El término “*clean label*” o “etiqueta limpia” apareció por primera vez durante los años 80 cuando los consumidores comenzaron a evitar los ingredientes con números E-, porque se creían que provocaban efectos negativos en su salud (Asioli et al., 2017). Pero en los últimos años este fenómeno se ha incrementado drásticamente y la industria no para de lanzar

productos con declaraciones como “libre de” o “100% natural”. Datos recogidos por Ingredion (2014) mostraron que en el año 2013 casi el 27% de los nuevos productos alimentarios lanzados en Europa tenían algún tipo de declaración de etiqueta limpia. A pesar de esta nueva tendencia de las empresas alimentarias por elaborar productos “clean label”, aún no existe una definición acordada ni regulaciones/legislaciones específicas sobre qué se considera este tipo de producto, lo que lo deja en base a la interpretación de consumidores y empresas alimentarias (Busken et al., 2013; Asioli et al., 2017; Aschemann-Witzel et al., 2019). Autores como Aschemann-Witzel et al. (2019) indican que en general se entiende como “clean label” aquellos productos libres de ingredientes percibidos negativamente por los consumidores como los alérgenos, aditivos, ingredientes procesados industrialmente, o aquellos desconocidos para los consumidores. De esta manera, se entiende por productos “*clean label*” los que en su composición contienen ingredientes percibidos como naturales, inofensivos, simples y que los consumidores conocen y usan habitualmente, identificándolos conjuntamente en algunos casos con productos ecológicos, o con declaraciones como “natural” y “libre de” (Ingredion, 2014). Pero debido a la falta de regulación en este aspecto, diferentes autores como Katz & Williams (2011) y Asioli et al. (2017) resaltan que se necesita una definición clara y una regulación específica que pueda mejorar la comprensión y comportamiento del consumidor, guiar a los fabricantes en el desarrollo y la comunicación de los alimentos. Además, Asioli et al. (2017) explica que un producto alimentario nunca va a poder llevar una declaración “*clean label*” como reclamo, sino que en la actualidad es un término, que está generalizando su uso tanto por parte de fabricantes e investigadores, como por los consumidores, todos ellos buscando una etiqueta lo más limpia posible. A continuación, se va a tratar de diferenciar entre la preferencia por los productos “naturales” y por los que incluyen como reclamo “libre de”.

### 1.2.1. Preferencia por lo “natural”.

La tendencia de los consumidores por evitar ciertos alimentos ha hecho que se eleve el interés por los productos etiquetados como “100% natural” o “naturales” (Dominik, Fullerton, Widmar & Wang, 2017; Aschemann-Witzel et al., 2019).

Diferentes estudios recogen lo que en general se considera la etiqueta “todo natural”, cada uno de ellos recoge un aspecto diferente, como alimentos orgánicos y productos no modificados genéticamente (no OMG) (Asioli et al., 2017), asociación con términos como frescura (Rozin, Fischler & Argelès, 2012), y también productos que no contienen ingredientes artificiales ni colorantes, que estén mínimamente procesados (Dominik et al., 2017), y que además estén libres de conservantes sintéticos, edulcorantes artificiales, saborizantes, no contener hormonas de crecimiento, antibióticos, aceites hidrogenados, así como estabilizadores y emulsionantes (Amos, Pentina, Hawkins & Davis, 2014). Algunos de estos estudios a su vez, respaldan la idea de que la etiqueta “todo natural” también hace referencia a la preocupación de los consumidores por el bienestar animal (Dominik et al., 2017), que estos productos preservan mejor la biodiversidad (Rozin et al., 2012), incluso que los productos así etiquetados son más saludables, atractivos, sabrosos, y respetuosos con el medio ambiente (Asioli et al., 2017; Liu, Hooker, Parasidis & Simons, 2017). Un ejemplo de esto es el estudio de Dominik et al. (2017) en el que concluyeron que la elección de la etiqueta “todo natural” estuvo asociada con un mejor sabor, una mejor calidad nutricional y de seguridad, y además una mejora en el ámbito del bienestar animal.

Como indica Rozin et al. (2012) tanto en Estados Unidos como en Europa occidental, en la actualidad se presta mucha atención a lo “natural”, sobre todo en el ámbito de la alimentación y la salud. El problema del término “natural”, es que al igual que el término “*clean label*”, no está debidamente regulado, y

que no necesariamente estos productos son más saludables ni tienen por qué ser ecológicos, ya que puede que los productos “naturales” y “no naturales” no presenten diferencias (Amos et al., 2014; Asioli et al., 2017). Esto se traduce en que la percepción y elección de los consumidores por este tipo de productos “naturales” es debida a los sentimientos culturales y sus propios criterios (Amos et al., 2014). Aunque por un lado Tarnavöglyi (2003) indica que la lista de números E- debería estar disponible, Rozin et al. (2012) expone que hay una falta de conocimiento general sobre el tema de los aditivos y que, aunque en la actualidad haya gran cantidad de información fácilmente disponible, los consumidores no prestan interés en descubrirla.

En cuanto a este interés por los productos “todo natural” se ha demostrado en diferentes estudios que las mujeres son más receptivas a este tipo de etiquetas que los hombres (Dominik et al., 2017), es decir, las mujeres muestran una mayor disposición a pagar por este tipo de productos, y esto podría deberse a que las mujeres son más sensibles que los hombres (Asioli et al., 2017).

### *1.2.2. Preferencia por lo “libre de”.*

Los aditivos alimentarios son sustancias que se añaden a los productos alimentarios para mejorar sus atributos intrínsecos debido a sus funciones tecnológicas y sensoriales, aumentando su vida útil, mejorando el sabor y restaurando los colores (Asioli et al., 2017). Los aditivos alimentarios se pueden clasificar en función de dos características diferentes: su origen y función. En función del origen existen aditivos artificiales y naturales y en términos de función se pueden identificar seis categorías: conservantes, aditivos, colorantes, saborizantes, texturizantes y agentes misceláneos (Carocho et al., 2014). A pesar de estas ventajas, la relación entre los consumidores y los aditivos alimentarios siempre ha sido problemática (Carocho et al., 2015), a este problema se le añade la terminología europea

utilizada para identificar los aditivos en las etiquetas de los alimentos, es decir, los números E, los cuales son percibidos de manera negativa por los consumidores (Asioli et al., 2017). Esto impulsa a muchos consumidores a elegir productos “sin aditivos”, ya sean naturales o artificiales, incluso si no hay diferencia entre ellos (Tarnavöglyi, 2003; Asioli et al., 2017).

Como recoge Asioli et al. (2017), la elección de los consumidores por estos productos es debida a que existen muchas fuentes de información negativas, como Internet y otros medios de comunicación que influyen directamente en ellos. Otro factor que contribuye es la preocupación por la salud y sobre los posibles efectos negativos que los aditivos e ingredientes artificiales pueden causar, y al igual que en el caso de “natural” la familiaridad de los consumidores con los ingredientes desempeña un papel clave en su aceptación o rechazo, ya que los consumidores tienen miedo de los raros y desconocidos (Varela & Fiszman, 2013).

Otro factor que se repite, como en el caso del término “natural” es que las mujeres perciben más riesgo que los hombres, por lo que tenderán a comprar más alimentos libres de aditivos (Asioli et al., 2017). Además, la edad también es un factor que influye en la elección de estos productos, siendo los jóvenes los menos preocupados por la seguridad alimentaria (Asioli et al., 2017).

### *1.3. Hidrocoloides.*

Los hidrocoloides son un grupo heterogéneo de polímeros de cadena larga con una solubilidad total o parcial y propensos a hincharse en el agua. Además, cambian las propiedades físicas de la solución formando geles, espesando, emulsionando, recubriéndola o estabilizándola (Phillips & Williams, 2000; Varela & Fiszman, 2013; Li & Nie, 2016). Estos hidrocoloides son muy utilizados en la industria alimentaria, formando parte importante de nuestra dieta diaria, ya que la modificación de las propiedades reológicas

permite mejorar las propiedades sensoriales de los productos a los que se añaden (Li & Nie, 2016). Cada hidrocoloide es totalmente diferente, confiriendo propiedades fisicoquímicas distintas y, por lo tanto, comportandose de manera diferente en presencia de agua y confiriendo texturas y propiedades reológicas diferentes (Martínez, Vicente, De Vega & Salmerón, 2019). Otros beneficios que aportan estos ingredientes y que deben ser destacados son, por ejemplo, la ayuda que proporcionan en los procesos de fabricación, el aumento de la vida útil de los productos en los que se añaden, la ayuda al mantenimiento de las propiedades de los productos durante los procesos de calentamiento (Varela & Fiszman, 2013), los cambios de viscosidad para mejorar ciertas características sensoriales y la estabilización de los productos (Wangler & Kohlus, 2018).

La viscosidad que proporcionan los hidrocoloides a los alimentos hace que estos tengan alta capacidad saciante. Como indica Morell, Fiszman, Varela & Hernando, (2014), esta saciedad proporcionada por los hidrocoloides está relacionada con la disminución de la eficacia de la acción enzimática, y a su vez con el retraso del vaciado gástrico. Además, varios estudios han mostrado que el consumo de hidrocoloides podría regular la composición de la flora bacteriana, promoviendo bacterias beneficiosas como bifidos y lactobacilo. También, el consumo de estos podría aumentar la actividad fermentativa y la producción de ácidos grasos de cadena corta, que tienen efectos beneficiosos sobre la modulación de la proliferación celular, apoptosis y angiogénesis (Viebke, Al-Assaf & Phillips, 2014; Li & Nie, 2016).

La elección de los hidrocoloides para la formulación de productos alimentarios depende de la característica técnica que se busque y de su precio. Por ello, los hidrocoloides más utilizados son los almidones dado su bajo coste. Otro aspecto muy importante para la selección de hidrocoloides es la seguridad. Los hidrocoloides menos costosos son los exudados de goma

vegetal y las harinas de semillas, pero a muchos de estos no se les ha dotado del estatus “GRAS” (Generally Recognized As Safe) (Li & Nie, 2016), es decir, reconocidos como seguros para su uso en los productos alimentarios.

### *1.3.1. Clasificación de los hidrocoloides.*

La clasificación tradicional de los hidrocoloides agrupa a estos polisacáridos según su fuente, quedando por un lado el grupo de los exudados de árboles (goma ghatti, goma arábica y otras gomas); el grupo de las algas (agar agar, alginato, y carragenato entre otras); grupo de las plantas (pectina y psyllium); y grupo animal (gelatina y quitina) (Li & Nie, 2016). Los hidrocoloides derivados de las plantas se usan principalmente para estabilizar las emulsiones de aceite en agua, en cambio, los hidrocoloides derivados de animales generalmente forman emulsiones de agua en aceite (Razavi, 2019).

Sin embargo, dado que la clasificación tradicional no incluía las gomas sintéticas Glicksman (1969) propuso otra clasificación compuesta por tres categorías: gomas naturales, gomas modificadas (semisintéticas) y gomas sintéticas (Nussinovitch & Hirashima, 2014; Li & Nie, 2016). Los hidrocoloides sintéticos son los sintetizados completamente por las industrias mediante materiales base derivados del petróleo, es decir, mediante combinaciones químicas para obtener productos con estructura similar a los polisacáridos naturales. Los hidrocoloides sintéticos y semisintéticos son buenos emulsionantes, siendo mejores los primeros, pero su coste tan elevado hace que la industria prefiera utilizar con más frecuencia los hidrocoloides semisintéticos (Razavi, 2019).

Por otro lado, la creciente demanda de comidas preparadas y, a la vez, la creciente demanda por comidas más naturales y saludables, como se ha mencionado anteriormente, han aumentado el uso de los hidrocoloides naturales, como la fibra dietética, que está reemplazando gradualmente a los

hidrocoloides sintéticos y semisintéticos. Sin embargo, estos hidrocoloides vegetales de origen natural tienen las desventajas de ser requeridos en grandes cantidades para ser efectivos como emulsionantes, en comparación con los semisintéticos o sintéticos (Li & Nie, 2016; Razavi, 2019).

### *1.3.2. Propiedades funcionales de los hidrocoloides en productos alimentarios.*

La capacidad de los hidrocoloides de modificar la textura y las propiedades reológicas de los productos a los que se añaden hacen que sean ampliamente utilizados por la industria alimentaria. Como se ha mencionado anteriormente, los hidrocoloides tienen propiedades gelificantes, espesantes, emulsionantes, sustitutos de la grasa, agentes de recubrimiento, agentes aglutinantes e inhibidores de la sinéresis. Su importancia es debida a que el perfil de textura en un alimento, es decir, dureza, masticabilidad, gomosidad, adhesividad, cohesividad, elasticidad y fracturabilidad, es uno de los atributos que determinan la palatabilidad de los alimentos (Li & Nie, 2016). La aceptación de los productos alimentarios está relacionada directamente con la textura, ya que los consumidores sienten gran placer al percibir los cambios de textura al comer los alimentos (Li & Nie, 2016). Además, el uso de hidrocoloides para la modificación de este atributo es de vital importancia para los ancianos y los pacientes con dificultades para masticar y/o tragar, población que está creciendo continuamente a nivel mundial, por lo que hay que tener en cuenta de manera urgente estos requerimientos nutricionales (Aguilera & Park, 2016).

La Tabla 1 muestra un resumen de la función de los hidrocoloides y ejemplos de productos alimentarios donde se utilizan. Generalmente, la propiedad espesante de los hidrocoloides viene referida a su capacidad de mejorar la viscosidad de los productos alimentarios (Li & Nie, 2016). Algunos hidrocoloides, además de espesar, tienen una característica muy importante,



la de formar geles. Un gel es una dispersión coloidal donde una matriz sólida es la fase continua y un líquido es la fase discontinua (Saha & Bhattacharya, 2010). Es importante destacar que algunos hidrocoloides cambian de estado en presencia de temperatura (Li & Nie, 2016). La capacidad emulsionante está directamente relacionada con la capacidad estabilizante, ya que algunos hidrocoloides promueven la formación de emulsiones y, a su vez, tienen la capacidad de mantener esta mezcla uniforme, reduciendo la tensión interfacial entre las dos fases (Dickinson, 2003) o, en otros casos los hidrocoloides son adsorbidos en las interfaces de aceite-agua estabilizando las emulsiones (Li & Nie, 2016).

Por otro lado, las grasas presentes en los productos alimentarios proporcionan un sabor, una textura y una apariencia deseables (Li & Nie, 2016). La necesidad de disminuir la grasa en la dieta actual es debida a que muchas investigaciones correlacionan el consumo de grasa con enfermedades como las cardiovasculares (principalmente grasas saturadas y colesterol), cáncer, diabetes tipo 2 y otras relacionadas con la obesidad (Schmiele, Mascarenhas, Barreto & Pollonio, 2015). La funcionalidad de los hidrocoloides como sustitutos de la grasa se deben a su capacidad para la formación de geles y al aumento de la viscosidad, además, proporcionan sabor y textura, y aumentan la capacidad de retención de agua (de Moraes Crizel et al., 2013). Sin embargo, la reducción del contenido de grasa en los alimentos generalmente disminuye sus cualidades sensoriales. Por ello, es necesario diseñar productos con bajo contenido de grasa que posean cualidades sensoriales similares a los que la contienen (Li & Nie, 2016).

**Tabla 1.** Función de los hidrocoloides y ejemplos donde se utilizan (Adaptación de Razavi, 2019).

| Función                                      | Ejemplo  |   |
|--|--|---|
|  | Hidrocoloide   | Producto  |
| Espesante/sensación de piel/tampón del pH    | Pectina  | Lociones, cremas para después del afeitado, geles   |
| Espesante/agente de suspensión/estabilizante | Xantana  | Lociones, protectores solares, rimel, jabones, pastas dentales  |
| Adhesivo                                     | Almidón y almidones modificados, alginato                          | Esmaltes, glaseados, pasta adhesiva, adhesivo para madera   |
| Agente de unión                              | $\lambda$ -carragenano, gelatina                                   | Alimentos para mascotas, compuestos aromáticos  |
| Agente de batido                             | Almidón, proteína de soja  | Toppings, malvaviscos, panes sin gluten   |
| Inhibidor de cristalización                  | $\kappa$ -carragenano, quitosano, gelatina                         | Helados, siropes, productos congelados  |
| Agente clarificante                          | Xantana, pectina   | Cerveza, vino, frutas, zumos de verduras  |
| Agente de turbidez                           | Aloe Vera, quitosano, pectina, gelatina, goma arábica              | Bebidas de frutas, bebidas  |
| Agente de recubrimiento                      | Inulina, beta glucano, hidroxipropilmetilcelulosa, goma arábica    | Confitería, aros de cebolla, zanahorias, manzanas Fuji, fruta fresca  |
| Fibra dietética                              | Gelatina, pectina, quitosano, agar, $\kappa$ -carragenano, almidón | Cereales, panes, yogures, pasta de arroz, pasteles  |
| Formador de films                            | Almidón  | Tripas para embutidos, recubrimientos protectores, películas de embalaje  |
| Moldeador                                    | $\kappa$ -carragenano, gelatina                                    | Dispersión de gomas, gelatinas  |
| Agente de suspensión                         | Almidón, almidones modificados, alginato                           | Chocolate con leche   |
| Hinchamiento                                 | Carragenina  | Productos lácteos procesados  |
| Inhibidor de sinéresis                       | Carragenano, gelatina, metilcelulosa                               | Queso, yogur, productos congelados, crema de cacahuete, tofu, salchichas de pavo, masas, mariscos fritos                      |
| Encapsulante                                 | Alginato, agar, $\kappa$ -carragenano, pectina, almidón, gelatina  | Microencapsulación de sabores, aceite de pescado, aceite esencial, enzimas y minerales, medicamentos, asfalto, gel de relleno |
| Forma poros                                  | $\kappa$ -carragenano  | Membranas   |
| Potenciador de resistencia a la compresión   | Carragenina  | Construcciones de adobe   |
| Estabilidad del color                        | Carragenina  | Filetes de ternera  |
| Sustitutos de grasa                          | Carragenina, pectina, almidón                                      | Salchichas, mayonesa, aderezo para ensaladas Nuggets de pescado rebozado  |
| Agentes pegante                              | Hidroxipropilmetilcelulosa (HPMC)                                  | Nuggets de pescado rebozados  |

**Tabla 1 (continuación).** Función de los hidrocoloides y ejemplos donde se utilizan (Razavi, 2019).

| Función   | Ejemplo   |   |
|---|---|---|
|   | Hidrocoloide  | Producto  |
| Agente gelificante  | Agar, alginato, κ-carragenano, gelatina, bajo y alto metoxi pectina, hidroxipropil metil celulosa (HPMC)                                    | Puddings, postres, confitería, jaleas, postres lácteos, productos de panadería, tofu, batidos, coberturas, glaseados, queso, leche acidificada, tratamiento de aguas residuales, inmovilización de enzimas, bebidas lácteas, aerogel, geles de rellenos |
| Emulsificante   | Goma arábica, nanocristales de almidón  | Aderezo para ensaladas, salsa de tomate, mayonesa, refrescos emulsionados   |
| Espesante   | Carboximetilcelulosa (CMC), xantana, goma guar, goma karaya, goma ghatti, goma garrofin, goma de tragacanto, goma tara, konjac, carragenina | Helados, yogurt, salsas, mermeladas, rellenos de tarta, sopas, aderezos para ensaladas, masas de pastelería, fideos, bebidas lácteas  |
| Espesante/emulsificante   | Hidroxipropilcelulosa (HPC), goma de fenogreco, carragenano λ, pectina de remolacha azucarera   | Quesos, leche en polvo, yogurt, ketchup, nanoemulsión   |
| Espesante/emulsificante/gelificante                                     | Metilcelulosa (MC)  | Pasteles  |
| Espesante/gelificante   | Celulosa microcristalina, almidón, almidones modificados  | Empanadas de carne fritas, postres lácteos, salsas para postres, snacks   |
| Emulsionante/estabilizador de espuma                                    | Xantano, goma guar, alginato de propilenglicol, agar  | Ingredientes batidos, crema batida, helado, cerveza, bebidas de naranja emulsionadas  |
| Espesante/capacidad de formar films                                     | Goma de celulosa  | Lociones, geles de baño, pasta de dientes   |
| Espesante/estabilizante/forma geles/capacidad de formar films           | Carragenina   | Lociones, champús, geles de afeitador, pasta de dientes   |
| Agente de suspensión/estabilizante, formador de geles/formador de films | Gelatina  | Protector solar, jabones corporales, pasta de dientes   |

Otra de las funcionalidades de los hidrocoloides es su capacidad de encapsular, proceso mediante el cual los ingredientes activos u otros materiales quedan atrapados dentro de un material para su protección y/o

posterior liberación (Li & Nie, 2016). Los hidrocoloides se emplean para crear encapsulados debido a que son comestibles, biodegradables y capaces de formar una barrera entre el núcleo y sus alrededores (Nedovic, Kalusevic, Manojlovic, Levic & Bugarski, 2011).

También son utilizados como adhesivos, manteniendo unidos los materiales mediante fuerzas atractivas, interacciones físicas y enclavamientos mecánicos, este último producido por la penetración de materiales adhesivos en poros microscópicos y otras irregularidades de la superficie (Nussinovitch, 2010).

### *1.3.3. Fibra alimentaria.*

Además de los diferentes aspectos funcionales, los hidrocoloides han recibido una gran acogida debido, en gran medida, a su papel como fibras dietéticas, por ello en algunas ocasiones son utilizados para aumentar el contenido de fibra en los productos (Li & Nie, 2016). La fibra fue uno de los primeros ingredientes asociados con la salud en la década de 1980 y ha sido desde entonces utilizada por la industria alimentaria (de Moraes Crizel et al., 2013). Para los adultos se recomienda un consumo de fibra de entre 25-36 g/día, siendo el 70-80% de esta fibra insoluble (Li & Nie, 2016; Han & Bertram, 2017). Según la definición de la Comisión del Codex Alimentarius (2009), las fibras dietéticas se definen como polímeros de carbohidratos con 10 o más unidades monoméricas que no son hidrolizadas por las enzimas endógenas en el intestino delgado de los humanos. Otras definiciones de fibra dietética están más relacionadas con criterios analíticos que son buenos para fines de etiquetado (particularmente solubilidad en agua), pero, además, hay otras funciones no tan informativas como las propiedades fisicoquímicas, viscosidad y fermentabilidad, que pueden afectar a la función gastrointestinal (Kristensen & Jensen, 2011).

Las fibras dietéticas desempeñan funciones tecnológicas como la absorción y retención de agua, compensando los cambios de textura no deseados sin afectar a las propiedades sensoriales del producto final (Han & Bertram, 2017). Una "fibra dietética ideal" debe tener características importantes como un sabor, color y olor agradables, una composición equilibrada, una cantidad adecuada de compuestos bioactivos, una larga vida útil, compatibilidad con el procesamiento de los alimentos, efectos fisiológicos favorables en el cuerpo humano y un precio razonable (de Moraes Crizel et al., 2013). Por esto, hay diferentes estudios en los que se evalúan varias fibras dietéticas, solas o en combinación, para sustituir la grasa tanto en productos cárnicos, para cambiar los atributos de salud (Henning et al., 2016; Kehlet et al., 2017; Oz et al., 2016; Han & Bertram, 2017), como en productos horneados (Colla, Costanzo & Gamlath, 2018; Andrade et al., 2018; Azmoon et al., 2021) para mantener las propiedades texturales deseables como resultado de sus diferentes propiedades funcionales de retención de agua, estabilidad de la emulsión, lubricación, modificación de textura y sabor neutro. En los últimos años, los extractos de fibra de frutas (por ejemplo, cítricos, manzana, kiwi) se han considerado nuevas fuentes de fibra dietética que pueden conferir beneficios adicionales para la salud debido a su contenido en compuestos bioactivos, como flavonoides y carotenoides, su menor contenido calórico y su mejor capacidad nutricional (Tarrega, Quiles, Morell, Fiszman & Hernando, 2017). Otra razón importante para el uso de fibras dietéticas es que generalmente provienen de subproductos agrícolas que son relativamente baratos, y la incorporación en diferentes productos pueden reducir los costos generales de producción (Han & Bertram, 2017), y la reutilización de estos subproductos, evitando así generar desperdicios.

Según el Instituto de Tecnología Alimentaria (IFT), el sesenta y tres por ciento de los productores de alimentos intentan agregar más fibra, el doce por

ciento de los consumidores sigue una dieta alta en fibra, y en la actualidad se están lanzando diferentes productos con un alto contenido en fibra, como suplementos elaborados con cáscara de *psyllium* y azúcar, siendo estos suplementos los más utilizados seguidos de enzimas digestivas, colágeno y queratina.

#### *1.3.4. Beneficios nutricionales de los hidrocoloideos.*

Muchos estudios mencionan que un aumento en la ingesta de fibra dietética esta relacionado con ciertos beneficios para la salud, como un menor riesgo de padecer enfermedades cardiovasculares, enfermedades coronarias, una menor incidencia de diabetes tipo II y de ciertos tipos de cáncer, como el cáncer de colon (Kristensen & Jensen, 2011; Moraes Crizel et al., 2013; Li & Nie, 2016; Han & Bertram, 2017; Tarrega et al., 2017), una reducción de los niveles de colesterol y de padecer estreñimiento (Sosulski & Cadden, 1982), una menor incidencia de trastornos intestinales (de Moraes Crizel et al., 2013), e interviene también en la regulación del apetito, en la función intestinal y en la reducción del riesgo de padecer osteoporosis (Li & Nie, 2016). Además, en los últimos años, las fibras dietéticas han recibido una mayor atención por su potencial en la regulación del peso, ya que la ingesta elevada de fibra se ha asociado con un menor aumento de peso en los estudios prospectivos de observación (Kristensen & Jensen, 2011), es decir, reduciendo el riesgo de padecer obesidad (Han & Bertram, 2017; Tarrega et al., 2017).

##### *1.3.4.1. Anti-estreñimiento.*

Existen hidrocoloideos que son ampliamente utilizados como medicamentos por las empresas farmacéuticas, como la semilla de *Psyllium* (*Plantago ovata*), se trata de un arabinoxilano conocido también como “*psyllium husk*” (cáscara de *psyllium*), o mucílago de *psyllium*, el cual es fibroso, hidrófilo, y es capaz de formar un gel mucilaginoso incoloro al absorber

agua (Li & Nie, 2016). Eldesoky et al. (2018) indicaron que otra variedad, *Plantago major*, se usa en muchas partes del mundo para el tratamiento de enfermedades, como enfermedades de la piel, digestivas, respiratorias y reproductivas, además de tumores, fiebre y dolor. Por otro lado, el *psyllium* se ha propuesto como medicamento laxante para aliviar el estreñimiento debido a que después de la ingestión se expande y forma una masa gelatinosa en el colon (Mishra, Sinha, Dey & Sen, 2014). El estreñimiento crónico causa tensión abdominal, provoca presiones altas prolongadas dentro de la luz del colon y en la parte inferior del abdomen, lo que aumenta el riesgo de degeneración muscular, incluidas las varices, hemorroides, hernia de hiato y divertículos del colon (Allen & Prentice, 2005; Li & Nie, 2016). Además, cualquier hidrocoloide que no pueda fermentarse bien puede tener una capacidad de retención de agua residual adecuada, aumentando así la producción de heces (Li & Nie, 2016).

### *1.3.4.2. Efecto prebiótico.*

Por otro lado, también se ha prestado atención a diferentes hidrocoloides debido a los efectos beneficiosos para la salud acortando la duración en las diarreas por rotavirus, reduciendo el eccema atópico en niños lactantes, y protegiendo contra enfermedades intestinales como la colitis (Caballero et al., 2003). El interés de estos hidrocoloides viene dado por su posible uso como prebióticos, es decir, un ingrediente alimentario que no es hidrolizado por enzimas digestivas humanas en el tracto gastrointestinal superior y afecta beneficiosamente al huésped al estimular selectivamente el crecimiento y/o actividad de un número limitado de bacterias (*Bifidobacterias* o *Lactobacilos*) (Milani & Golkar, 2019). Los criterios para otorgar la categoría de prebiótico a los ingredientes alimentarios son: I) no deben hidrolizarse ni absorberse en la parte superior del tracto intestinal; II) deben ser fermentados parcialmente por ciertas bacterias potencialmente beneficiosas para el colon; III) deben mejorar

la microflora del colon; y IV) deben promover preferentemente efectos beneficiosos para la salud de los consumidores (Slavin, 2013; Gibson, 1999).

Se ha estudiado y demostrado la capacidad de alterar favorablemente la microflora intestinal utilizando varias fuentes de fibra y alimentos vegetales (Milani & Golkar, 2019). La inulina es el prebiótico más estudiado, además está legalmente clasificado como alimento o ingrediente alimentario en todos los países donde se usa, y se acepta para uso alimentario sin ninguna limitación (Li & Nie, 2016). Aunque hay estudios que hacen referencia a otros hidrocoloides con función prebiótica. Champ et al. (2003) informaron sobre el papel específico del almidón en la estimulación de bacterias capaces de producir ácido butírico. Elli et al. (2008) observaron que el *psyllium* tiene un efecto prebiótico en mujeres sanas. Además, existen algunos informes sobre el papel de los prebióticos en la prevención del cáncer, beneficios en la enfermedad atópica y, la reducción del colesterol y los triglicéridos en la sangre (Milani & Golkar, 2019).

Aunque el prebiótico tiene muchas ventajas referidas a las funciones fisiológicas, como se ha comentado anteriormente, la fermentación que producen puede conllevar a la aparición de efectos secundarios, como los gases producidos en la fermentación, que, según la dosis, pueden crear efectos no deseados como flatulencias y calambres (Li & Nie, 2016).

#### *1.3.4.3. Efecto hipoglucémicos y actuación contra la diabetes mellitus.*

La diabetes mellitus es un trastorno metabólico crónico caracterizado por el alto nivel de glucosa en sangre (hiperglucemia) debido a la deficiencia de insulina y/o resistencia a la insulina (Cunha et al., 2017). Se clasifica en dos grupos: la diabetes tipo 1 que se caracteriza por la destrucción de las células  $\beta$  pancreáticas, y tipo 2 que es la principal forma de diabetes mellitus y, es causada por la resistencia a la insulina y la producción, secreción y función de



esta (Milani & Golkar, 2019). Se trata de una enfermedad propagada en todo el mundo, afectando a aproximadamente el 4% de la población y se espera que continúe aumentando (Milani & Golkar, 2019).

En la actualidad, hay evidencias de que una dieta baja en índice glucémico o en carga glucémica tiene un papel muy importante en la prevención de diabetes tipo 2, enfermedades cardiovasculares, obesidad, cáncer de colon y cáncer de mama (Li & Nie, 2016). Es decir, dietas más altas en carbohidratos no disponibles mejoran la resistencia a la insulina (Livesey et al., 2008), ya que una ingestión de carbohidratos disponibles hace que aumente la glucosa en sangre (Caballero et al., 2003).

Para la terapia de diabetes mellitus se han utilizado diferentes medicamentos durante décadas, pero estos pueden ocasionar efectos adversos, hasta llegar a producir problemas graves. Por ello, la nueva tendencia es que los pacientes usen alimentos funcionales como medicina complementaria o alternativa, es decir, alimentos que naturalmente contienen compuestos activos, como los polisacáridos y las fibras dietéticas, que tienen un alto potencial en el tratamiento de la diabetes mellitus (Wang et al., 2016; Milani & Golkar, 2019).

Por ejemplo, la goma guar parece inhibir los procesos asociados con la digestión y la absorción de los carbohidratos disponibles en el intestino y, por lo tanto, disminuye la absorción de glucosa que se produce en una comida rica en almidón (Li & Nie, 2016). Los fragmentos de almidón quedan atrapados en la red viscosa formada por el gel de guar, haciendo que estos estén menos accesibles a las enzimas digestivas (como la amilasa), ralentizando así su difusión hacia el intestino delgado (Li & Nie 2016), lo que limita el aumento de la glucosa en sangre que se observa después de una comida. De esta manera, se ha demostrado que las formas viscosas de fibra dietética mejoran el control de la glucosa en sangre al atrapar los carbohidratos ingeridos dentro del gel

viscoso formado después de la digestión (Milani & Golkar, 2019). Lattimer & Haub (2010) añaden que la fibra insoluble ayuda a reducir el apetito y la ingesta de alimentos y, además que los ácidos grasos de cadena corta, obtenidos por la fermentación de esta, reducen la respuesta de glucosa posprandial.

Existen una serie de investigaciones científicas donde se enfatiza la relación entre la diabetes y otros hidrocoloides, la mayoría de estas se han realizado con el  $\beta$ -glucano de la avena para observar la atenuación de las respuestas de glucosa en sangre e insulina en individuos sanos y diabéticos (Li & Nie, 2016). También se ha observado este efecto en otros hidrocoloides como la inulina, levan, *psyllium* y goma arábica, entre otros (Li & Nie, 2016).

#### *1.3.4.4. Prevención contra el cáncer.*

Hay un creciente interés en la investigación de antioxidantes naturales, ya que pueden proteger al cuerpo humano de los radicales libres y, retrasar el progreso de muchas enfermedades crónicas y de enfermedades como el cáncer (Xing et al., 2005). Recientemente, se ha demostrado que algunos polisacáridos naturales y sus derivados poseen actividad antioxidante y tienen aplicación como antioxidantes (Abdel-Fattah, Gamal-Eldeen, Helmy & Esawy, 2012; Milani & Golkar, 2019). Déléris, Nazih & Bard (2016) resumen las estrategias del tratamiento de cáncer en: (1) prevención, basada en promover estilos de vida asociados con bajos riesgos de tumorigénesis; (2) cirugía, que a menudo consiste en la eliminación del tumor, idealmente antes de la transición epitelial-mesenquimatosa que conduce a metástasis; y (3) al inducir la muerte de las células tumorales mediante radio o quimioterapia dirigida.

Si existen disfunciones en el colon pueden aparecer enfermedades sustanciales, que abarcan desde infecciones agudas (diarrea o estreñimiento), hasta enfermedades crónicas como enfermedades inflamatorias del intestino

(EII), síndrome del intestino irritable (SII) o cáncer de colon (Li & Nie, 2016). La neoplasia colorrectal está relacionada con una defecación poco frecuente, ya que el estreñimiento provoca la exposición prolongada de las células epiteliales del colon a productos químicos mutagénicos, que podrían iniciar el cáncer (Li & Nie, 2016), tratándose de la segunda causa más común de muerte por cáncer (Ricci-Vitiani et al., 2007).

Diferentes estudios epidemiológicos han sugerido que existe una asociación inversa entre la ingesta de fibra dietética y el riesgo de cáncer de colon, ya que la primera aumenta el volumen fecal, reduce el tiempo de tránsito del colon y el contenido diluido de toxinas fecales, lo que reduce la exposición de la mucosa del colon a los carcinógenos lumbinales (Milani & Golkar, 2019). A su vez, se ha observado que la fibra dietética también previene el cáncer debido a la reducción del pH del colon, a que adsorbe o diluye los carcinógenos fecales, modifica el metabolismo de los ácidos biliares y aumentar la producción de ácidos grasos de cadena corta (Li & Nie, 2016). Por todo esto, cualquier hidrocoloide con la función de promover la defecación podría tener un efecto positivo sobre el cáncer de colon (Femia et al., 2002).

### *1.3.4.5. Reductor del riesgo de padecer obesidad.*

La obesidad se ha convertido en una epidemia mundial que afecta a personas en todos los continentes, pero el principal inconveniente es que la obesidad está asociada con una alta incidencia de riesgos para salud, como síndrome metabólico, la diabetes tipo 2 y problemas cardiovasculares (Milani & Golkar, 2019). Por ello, hay un incremento en el interés de la industria alimentaria por el uso de fibras dietéticas (Redgwell & Fischer, 2005), para prevenir el aumento de peso, mediante la elaboración de productos con altas capacidades saciantes y bajos de calorías. Según Lattimer & Haub (2010) un aumento en el consumo de fibra puede disminuir la absorción de energía, ya

que se diluyen los componentes más energéticos mientras se mantienen intactos otros nutrientes importantes.

En general, la capacidad de la fibra dietética para disminuir el peso corporal podría estar relacionada con la fermentación de fibra soluble que produce una serie de péptidos que juegan un papel importante en la saciedad; puede disminuir significativamente la ingesta de energía; y, además, puede disminuir la energía metabolizable de la dieta, que es la energía bruta menos la pérdida en las heces, orina y gases combustibles (Lattimer & Haub, 2010). Por otro lado, como se ha mencionado anteriormente, los hidrocoloides pueden desempeñar un papel clave en la modulación del comportamiento del intestino delgado (Milani & Golkar, 2019).

#### *1.3.4.6. Reductor del colesterol y de enfermedades cardiovasculares.*

A pesar del aumento en la prevención, las enfermedades cardiovasculares siguen siendo la principal causa de muerte en la mayoría de los países occidentales, debido a la ingesta de colesterol y de grasas saturadas (Theuwissen & Mensink, 2008; Milani & Golkar, 2019).

Hidrocoloides como la goma de psyllium y el  $\beta$ -glucano de avena son las fuentes más utilizadas de fibra soluble y han sido aprobados por la FDA por sus beneficios en salud, relacionados con la protección contra enfermedades cardiovasculares (Milani & Golkar, 2019). Muchos estudios afirman que la fibra puede reducir los niveles de colesterol en sangre al alterar la absorción de colesterol y ácidos biliares y, por sus efectos sobre la producción de lipoproteínas hepáticas y la síntesis de colesterol (Redgwell & Fischer, 2005; Buttriss & Stokes, 2008; Milani & Golkar, 2019).

Theuwissen & Mensink (2008) describen el proceso por el que la fibra dietética disminuye el colesterol. En este proceso las fibras viscosas solubles en agua forman una gruesa capa en la luz intestinal, disminuyendo así la

(re)absorción de colesterol y ácidos biliares, lo que conduce a un aumento de la producción fecal de estos dos componentes. Como resultado, aumenta la conversión hepática del colesterol en ácidos biliares, disminuyen las reservas hepáticas de colesterol libre y, aumenta la síntesis de colesterol endógeno. Además, los receptores hepáticos de colesterol LDL se regulan para restablecer las reservas de colesterol libre hepático. Todos estos procesos conducirán a una disminución de las concentraciones séricas de colesterol LDL.

### **2. Dietas alternativas**

En junio de 2019 la Organización de las Naciones Unidas estimó que la población mundial aumentará en 2.000 millones de personas en los próximos 30 años, de 7.700 millones actualmente a 9.700 millones en 2050. Además, indicó que la población mundial está envejeciendo, siendo el grupo de edad de 65 años o más el que más rápido crece. Asociados a esta problemática, se están desarrollando e investigando diferentes alternativas al consumo carne, buscando que tengan un valor biológico similar. Existen distintas alternativas ya en marcha de las que se han realizado estudios significativos, como el consumo de carne de cultivo o de laboratorio, algas, insectos o alternativas basadas en plantas (van der Weele et al., 2019).

#### *2.2. Consumo de carne*

La carne es una parte integral de la dieta humana y constituye una fuente importante de proteínas y grasas para las poblaciones de todo el mundo (Boada, Henríquez-Hernández & Luzardo, 2016). Además, es una fuente importante de nutrientes esenciales, incluidos hierro, zinc y vitamina B12, si se consume en cantidades no excesivas (Kim, Coelho & Blachier, 2013). En España, el consumo de carne sigue siendo más alto que el consumo en hortalizas y verduras, en 2018 se destacó un descenso del consumo de carne

fresca, pescado fresco y congelado del 2,6% con respecto al año 2017. Sin embargo, creció el consumo de carne transformada aproximadamente un 1,9%. Estos transformados o también llamados ultraprocesados se han caracterizado por ser hiper-apetecibles, cuasi-adictivos y social y ambientalmente destructivos (Vandevijvere et al., 2019).

Por otro lado, muchos estudios plantean la preocupación de que la ingesta elevada de carne roja y/o procesada esté asociada con enfermedades crónicas como la obesidad, la diabetes tipo 2, las enfermedades cardiovasculares y una variedad de cánceres (Klurfeld, 2015). Además, esta ingesta también se ha asociado con un mayor riesgo de mortalidad total, cardiovascular y por cáncer (Wang et al., 2015). El cáncer más asociado con el consumo de carne roja y/o procesada es el cáncer de colon (CCR). Entre los factores ambientales que intervienen en la modulación del riesgo de CCR, los parámetros dietéticos desempeñan un papel importante (WCRF/AICR, 2007). Este tipo de cáncer se ha incrementado con rapidez especialmente en países económicamente desarrollados (Kim, Coelho & Blachier, 2013). La variación de la incidencia de cáncer entre países parece estar asociada en gran medida a diferencias en los hábitos alimentarios (Armstrong & Doll, 1975).

### *2.3. Dietas alternativas*

Debido al crecimiento de la población, la seguridad alimentaria y nutricional se convertirán en los mayores problemas que la humanidad deberá abordar a corto y largo plazo (Dillard, 2019). Los científicos reconocen la urgencia de los desafíos alimentarios actuales y futuros y están buscando soluciones técnicas, así como identificando políticas y acciones claras y precisas que se pueden implementar para abordar los problemas mundiales de los alimentos (IFPRI, 2016). Esta urgencia se ve intensificada por los efectos del cambio climático los cuales ya están alterando los agroecosistemas y desafiando la capacidad para alimentar al mundo (Dillard,

2019). Los sistemas de producción de alimentos han tenido un gran impacto ambiental global en términos de contaminación y agotamiento de los recursos naturales (Marini et al., 2021). Por todos estos motivos, en los últimos años se han investigado y promovido patrones dietéticos más saludables y con un menor impacto ambiental (Ulaszewska et al., 2017). Al mismo tiempo, uno de los factores más importantes para adoptar dietas vegetarianas o basadas en plantas han sido la conciencia sobre las dietas sostenibles, las motivaciones éticas contra la producción de proteínas animales y las preocupaciones por la salud (Marini et al., 2021; Danish Vegetarian Association, 2019). Aunque la dieta omnívora sigue siendo la dieta más común, la adopción de una dieta vegetariana es cada vez más frecuente en las sociedades occidentales (Rosenfeld & Burrow, 2017a; Rosenfeld & Burrow, 2017b; Lavalée et al., 2019; Dorard & Mathieu, 2021).

En el 2019, un 7,9% de los españoles adultos se declaraba flexitariano, un 1,5% vegetariano y un 0,5% vegano, sumando un total de 9,9% de españoles *veggies*, entendiéndose por *veggies* personas que colocan el reino vegetal en el centro de su alimentación. Esto supuso un crecimiento total del 27% entre 2017 y 2019 (Lantern, 2019). Las 3 principales dietas *veggies* son: la vegana, donde se eliminan totalmente los productos de origen animal tanto en la alimentación, consumiendo una dieta 100% vegetal, como en la vida diaria; la vegetariana, se consumen mayoritariamente productos de origen vegetal, junto con algunos productos derivados de los animales, como serían los lácteos, huevos o miel; y la flexitariana, se da preferencia a los productos vegetales como frutas, hortalizas o legumbres, consumiendo solo ocasionalmente carne o pescado (Derbyshire, 2017; Rosenfeld, Rothgerber, & Tomiyama, 2020a).

#### *2.4. Motivaciones*

Numerosos estudios han investigado las principales motivaciones de los consumidores para eliminar de la dieta el consumo de carne. Estos estudios han demostrado que las motivaciones son el bienestar animal, la protección del medio ambiente y la salud (Ruby, 2012; Rosenfeld, 2018; Nogueroles et al., 2021), siendo el bienestar animal el motivo principal de gran parte de los veganos y vegetarianos. Sin embargo, no se han encontrado diferencias entre las motivaciones para seguir una dieta vegana o vegetariana. Algunos autores han indicado que las tres motivaciones principales para seguir una dieta vegana son las preocupaciones éticas, los motivos de salud y, por último, la preocupación por el medio ambiente y el cambio climático (Timko et al., 2012; Dyett et al., 2013; Janssen et al., 2016). Además, se ha observado una relación positiva entre el consumo de alimentos orgánicos y el vegetarianismo impulsada por la ética y la preservación del medio ambiente (Lacour et al., 2018; Rabès et al., 2020).

Otros autores identificaron otros motivos por los que eliminar totalmente el consumo de carne y derivados, como las creencias y prácticas religiosas (Fiestas-Flores & Pyhälä, 2018), los relacionados con el gusto (Kessler et al., 2016) y la influencia del entorno familiar y social (Fresán et al., 2020).

La decisión de las personas de seguir una dieta vegetariana no es necesariamente una decisión permanente (Kilian & Hamm, 2021). Algunas personas vuelven a consumir carne después de un tiempo, debido principalmente a motivos de salud, como preocupaciones por la desnutrición (Haverstock & Forgays, 2012). Al igual que la decisión de seguir una dieta vegana o vegetariana está influida por el entorno social, volver al consumo de carne también (Hodson & Earle, 2018).



Por otro lado, las razones para reducir el consumo de carne, es decir, para seguir una dieta "flexitariana", suelen ser similares a las razones para eliminar por completo la carne de la dieta. Sin embargo, el bienestar animal es menos importante para los flexitarianos que para los vegetarianos y veganos (De Backer & Hudders, 2014; De Backer & Hudders, 2015; Noguerol et al., 2021). Izmirli & Phillips (2011) identificaron que el motivo más importante para adoptar una dieta flexitariana fue la preocupación por el medio ambiente. Aleksandrowicz et al. (2016) demostró que las dietas que reducen la cantidad de alimentos de origen animal tienen los mayores beneficios ambientales, no solo en términos de emisiones de gases de efecto invernadero, sino también en términos de uso de la tierra y demanda de energía. Aunque otros estudios han encontrado que esta preocupación medioambiental es generalmente menos importante para los flexitarianos que para los vegetarianos (De Backer & Hudders, 2014; Mullee et al., 2017; Hagmann et al., 2019). Otro de los motivos por los que los consumidores deciden reducir el consumo de carne es la salud (Forestell et al., 2012; Mylan, 2018; Noguerol et al., 2021), ya que se ha demostrado que una ingesta elevada de frutas, verduras y cereales integrales protege contra el cáncer de colon (Kim, Coelho & Blachier, 2013). Sin embargo, existe poca información sobre las características de este subgrupo de consumidores y de por qué los reductores de carne están cambiando su comportamiento sobre el consumo de la misma (Rosenfeld, Rothgerber & Tomiyama, 2020b; Malek & Umberger, 2021).

### 2.5. ¿Son saludables?

Según la Asociación Americana de Dietética *"las dietas vegetarianas adecuadamente planificadas, incluyendo las dietas vegetarianas totales o veganas, son saludables, nutricionalmente adecuadas, y pueden proporcionar beneficios para la salud en la prevención y el tratamiento de ciertas enfermedades"*. Conociendo de manera correcta las combinaciones de grupos

de alimentos e incluyendo suplementos cuando se requieran, es posible llevar una dieta vegana de forma saludable (Díaz, 2019). La Comisión EAT-Lancet sobre Dietas Saludables de Sistemas Alimentarios Sostenibles (Willett et al., 2019) describe de forma cuantitativa que una dieta saludable universal de referencia debe basarse en el aumento del consumo de alimentos de origen vegetal (verduras, frutas, cereales integrales, legumbres y frutos secos), y la disminución del consumo de alimentos menos saludables (carnes rojas, azúcar y cereales refinados). Sin embargo, el principal inconveniente de una dieta vegana es la biodisponibilidad de diferentes nutrientes. Cubrir las necesidades en vitamina B12, vitamina D, calcio, zinc y hierro, en los casos de una dieta vegana puede resultar difícil, así como asegurar el aporte de ácidos grasos esenciales (Farran et al., 2015).

En cuanto a la vitamina B12, las dietas veganas pueden causar una deficiencia debido a la falta de ingesta dietética (Donaldson, 2000). La cobalamina es sintetizada por microorganismos (Pawlak et al., 2013) por lo que se encuentra en cantidades sustanciales únicamente en alimentos de origen animal (Herrmann & Geisel, 2002). Un déficit de B12 puede provocar anemia, trastornos neuropsiquiátricos y alteración de la función cognitiva (Buil Arasanz et al., 2009), por ello es recomendable el uso de suplementos. La ingesta de suplementos de vitamina B12 también se recomiendan para aquellas personas que lleven una dieta vegetariana, ya que la ingesta de huevos/lácteos no suele ser lo suficientemente elevada como para cubrir las necesidades de esta vitamina (Pawlak et al., 2013).

La disponibilidad de vitamina D depende de la exposición solar y de la ingesta de alimentos (Vega & Ferreira, 2016). Esta vitamina se encuentra principalmente en alimentos de origen animal, por lo que las personas que reducen o eliminan el consumo de carne tienen más probabilidades de déficit cuando además la síntesis cutánea de vitamina D se ve menguada como por

ejemplo en invierno. Con el fin de asegurar unos niveles adecuados, se aconseja la exposición solar y la ingesta de alimentos fortificados con vitamina D (bebidas vegetales, zumos y cereales de desayuno). Si existe riesgo de déficit de esta vitamina, sería necesaria la suplementación diaria (García-Maldonado et al., 2019), ya que unos bajos niveles de vitamina D causan raquitismo en niños y, al disminuir la densidad ósea, puede provocar osteoporosis y fracturas en adultos. Además, se han asociado con un mayor riesgo de cáncer, enfermedades autoinmunes, hipertensión y enfermedades infecciosas (Holick & Chen, 2008).

El calcio es necesario para los huesos, la función nerviosa y muscular y la coagulación de la sangre (Herrmann & Geisel, 2002). Su biodisponibilidad depende de variables fisiológicas como la edad, el embarazo y la lactancia, y de variables dietéticas, que incluyen aquellos compuestos inhibidores o promotores de la absorción. Los principales responsables de la inhibición de la absorción del calcio en personas cuya dieta esta basada en alimentos vegetales son los fitatos y los oxalatos. En este caso, se ha de tener en cuenta el procesado, la cocción y la digestión de estos alimentos, así como la elección de alimentos vegetales con calcio de elevada biodisponibilidad o fortificados con este mineral (García-Maldonado et al., 2019). Algunas opciones vegetales ricas en calcio biodisponible son las crucíferas (kale, coliflor, brócoli, etc.), las almendras y el tofu entre otros (García & Martínez, 2015).

El zinc es importante para el crecimiento óptimo de las células, la rápida cicatrización de las heridas y el buen funcionamiento del sistema inmunitario (Herrmann & Geisel, 2002). Este oligoelemento se encuentra tanto en alimentos de origen animal como de origen vegetal, siendo los productos lácteos la principal fuente de zinc en vegetarianos y los cereales, en veganos (Vega & Ferreira, 2016). Sin embargo, su biodisponibilidad en los alimentos vegetales se ve reducida por el fitato de las legumbres y los cereales

integrales. Es necesaria la fermentación de estos últimos y de la soja para aumentar su absorción. Este es el caso de productos como el miso y el tempeh, consumidos de forma habitual en dietas veganas (Herrmann & Geisel, 2002). También mejoran la biodisponibilidad del zinc el tratamiento térmico y la hidrólisis enzimática de los alimentos (García-Maldonado et al., 2019).

El hierro no hemínico (Fe-No Hem) es el que se encuentra en los alimentos de origen vegetal y algunos alimentos de origen animal como la leche y los huevos (Gaitán et al., 2006). Su biodisponibilidad está condicionada principalmente por el ácido fítico y, aunque el procesado de los alimentos elimina gran parte de los fitatos, también disminuye el contenido de otros nutrientes beneficiosos tales como el hierro y el zinc. También son inhibidores de la absorción del hierro los polifenoles, los cuales están presentes en productos como el café o el té y los oxalatos, presentes en verduras como espinacas y acelgas. La vitamina C, sin embargo, es el mayor potenciador de la absorción del hierro, pudiendo incrementarla hasta 6 veces (Rojas Allende et al., 2017). Por lo tanto, para evitar déficits de hierro en personas que reducen o eliminan la carne de su dieta, se recomienda separar la ingesta de comidas principales, de productos ricos en polifenoles, así como consumir los alimentos vegetales ricos en hierro de forma conjunta con los ricos en vitamina C (García-Maldonado et al., 2019).

Los omega-3 son un tipo de ácido graso que existe tanto en alimentos de origen vegetal como animal. Sin embargo, los omega-3 EPA y DHA solo existen en productos de origen animal, principalmente en pescado azul, carne de animales alimentados con pasto y en la leche materna. En los alimentos de origen vegetal como las nueces, algunas algas y las semillas de lino y chía, se encuentra una variante, el alfa-linolénico (ALA). A pesar de que el organismo de una persona es capaz de convertir el ALA en DHA y EPA, este lo hace de manera ineficaz e ineficiente. Sin embargo, no se suele recomendar la

suplementación de estos ácidos grasos (Saunders et al., 2013; García-Maldonado et al., 2019).

Sin embargo, son muchos los estudios que establecen los beneficios que conlleva la reducción o eliminación de la carne de la dieta. Las dietas que limitan o excluyen el consumo de carne y derivados ofrecen beneficios para la salud incluyendo un menor riesgo de sobrepeso y obesidad, diabetes tipo 2, enfermedad coronaria y ciertos cánceres (World Health Organization, 2015; Derbyshire, 2017; Malek & Umberger, 2021). Pérez-Martínez et al. (2017) indicaron que existen poblaciones asiáticas con un perfil de riesgo cardiometabólico más favorable y de un menor riesgo de síndrome premenstrual entre los individuos que siguen dietas basadas en plantas (veganos y vegetarianos) en comparación con los omnívoros. A su vez, Viguiouk et al. (2019) informó que el consumo de un patrón dietético vegetariano se asocia con un menor riesgo de diabetes de tipo 2, enfermedad coronaria, obesidad, hipertensión, y mortalidad cardiovascular. Los datos sugieren que las dietas veganas pueden reducir en un 40% el riesgo de enfermedades coronarias y en un 29% el riesgo de enfermedades vasculares (Kahleova, 2017). Además, este tipo de dieta mejora el control glucémico, los lípidos en sangre, el peso corporal y la presión arterial en individuos con diferentes fenotipos metabólicos (Viguiouk et al., 2019).

No obstante, los beneficios que supone la reducción de carne de la dieta no se limitan únicamente a la salud de la población, sino que también alcanzan a la salud del planeta y el medioambiente. La FAO en 2010 indicó que, además del valor nutricional de la dieta, también debía considerarse su sostenibilidad ambiental. En este sentido, una “dieta sostenible” se define como aquella dieta con un bajo impacto medioambiental que contribuye a la seguridad nutricional y alimentaria y optimiza el uso de recursos humanos y naturales de forma que las generaciones presentes y futuras puedan llevar una vida saludable.

Además, protege y respeta la biodiversidad y los ecosistemas, es culturalmente aceptable, accesible y justa (Pérez-Cueto, 2015).

En referencia a los problemas medioambientales, Greenpeace afirmó que una reducción de la producción y el consumo de productos animales del 50%, esto supondría una reducción del 64% en la emisión de gases de efecto invernadero en 2050. Además, el cambio de dieta tendría efectos sobre la biodiversidad, puesto que el sector ganadero, a través de la emisión de gases de efecto invernadero, provoca una degradación de las tierras, deforestación y disminución de la flora y fauna autóctona (Martínez, 2020). Por todo ello, la tendencia de eliminar o reducir el consumo de carne de la dieta como movimiento y forma de alimentación se puede relacionar directamente con el cumplimiento de varios de los Objetivos de Desarrollo Sostenible (ODS) impulsados por la ONU (Gil, 2018), los cuales pretenden erradicar la pobreza, proteger el planeta y asegurar la prosperidad común.

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## **Capítulo 2**

### **Justificación y objetivos**



### **1. Justificación**

Esta tesis se enmarca dentro de un proyecto con empresa, financiado por la Generalitat Valenciana (Subvenciones para la formación de doctores y doctoras en empresas valencianas, FEDEGENT/2018/014).

El creciente interés de la población por la salud y lo natural, junto con la demanda de productos de origen vegetal, dirigidos a aquellas personas que quieren reducir o eliminar por completo la carne y sus derivados de la dieta, lleva a la empresa colaboradora a querer desarrollar preparados que cumplan estas dos marcadas tendencias. Generalmente, las etiquetas de los productos vegetales dirigidos a veganos y vegetarianos tienen etiquetas largas y difíciles de leer. Por ello, se crea la necesidad de cambiar las formulaciones reduciendo el número de ingredientes, y a su vez de los texturizantes utilizados. La utilización de fibras vegetales como texturizantes es la opción elegida por la empresa colaboradora, pero para ello se necesita conocer las características tecno-funcionales de cada una de ellas, ya que la composición y tipo de extracción cambia las propiedades de estas, por lo que hay que estudiarlas individualmente.

### **2. Objetivos**

Por todo esto, el objetivo general de estas tesis fue la ampliación y/o modificación, en su caso, de la gama de preparados ofertados por la empresa colaboradora en base a los criterios de etiqueta limpia o *clean label*, es decir, sin aditivos ni alérgenos, y enfocados a productos vegetarianos/veganos.

Para la consecución de dicho objetivo, se plantearon los siguientes objetivos específicos:

- Percepción por parte de todo tipo de consumidores (omnívoros, veganos, vegetarianos y flexitarianos) de productos veganos/vegetarianos *clean label*.
- Búsqueda y caracterización de diferentes fibras alimentarias vegetales para su posible utilización como ingredientes texturizantes naturales en la producción de preparados para veganos/vegetarianos.
- Desarrollo, formulación, caracterización y aplicación de preparados veganos/vegetarianos utilizando los ingredientes texturizantes naturales seleccionados en las fases anteriores, mediante análisis fisicoquímico, mecánico y sensorial.



## Capítulo 3

# Percepción de productos *clean label* a base de plantas

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Green or Clean? Perception of clean label plant-based products by omnivorous, vegan, vegetarian and flexitarian consumers.

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### **Abstract**

Consumers are increasingly interested in health and sustainability aspects of their diets. Meat reduction diets have gained popularity with some consumers, leading to an increase in plant-based products in the markets. Additionally, the demand for more natural and healthier products is associated with the clean label trend. But how these two trends relate to each other, has not yet been widely investigated. The aim of the present research was to explore the perception of different consumers (vegans, vegetarians, flexitarians and omnivores), towards clean label, plant-based products in order to better understand their motivations and attitudes. Consumers in Spain, followers of these four diets, participated in a projective mapping task - categorisation of twenty plant-based products (ten clean label and ten original products) - and answered a health and sustainability attitudes survey. The results showed that according to the diet followed by the consumers, they categorised and perceived the products differently, in line with their attitudes. Meat-reducer and avoider consumers paid more attention to quality and health and presented a greater concern for animal welfare and sustainability. Also, they focused on the clean label status for product categorization, while omnivores did not separate between original and clean label products. The present study shows a first exploration of how consumers with different relation to meat (frequent consumption-reduction-avoidance) perceive clean label plant-based products, in relation to their attitudes to health and sustainability, a building block on the way to support consumers in the transition to healthier, more sustainable diets.

## 1. Introduction

In recent years, meat reduction diets have gained popularity with some consumers striving to shift to more sustainable and healthier diets. Depending on the levels of strictness and adherence to dietary meat restriction there are different diets (Eveleigh, Coneyworth, Avery & Welham, 2020). Typically, vegans do not consume any animal-derived products, vegetarians exclude meat and fish but may consume milk and eggs and flexitarians who are semi-vegetarian because do not exclude meat products (red meat or other meats) but limit their consumption (Derbyshire, 2017; Rosenfeld, Rothgerber & Tomiyama, 2020). Lantern Study (2019) indicated an increase in Spain of 27% in this type of diets, with 0.5% vegans, 1.5% vegetarians and 7.9% flexitarians, being these percentages lower than in other places in Europe such as Germany and England. Despite these data, 87.8% of the population declares itself omnivores (Lantern Study, 2019). The health benefits of limiting or excluding meat and meat derived products include a lower risk of overweight and obesity, type 2 diabetes, coronary heart disease and certain cancers such as colorectal cancer (World Health Organization, 2015; Malek & Umberger, 2021). Furthermore, following these diets also has environmental benefits such as the reduction of greenhouse gas emissions (GHG) and land use demand (Tilman & Clark, 2014; Rabès et al., 2020).

This reduction in meat consumption entails having to increase the intake of plant-based proteins and fresh fruits and vegetables (Kumar, 2016), thus, this consumption is replaced by plant-based meat analogues. Aschemann-Witzel (2020) indicated that the term “plant-based” is used to describe a recent consumer trend of avoiding animal-based products and choosing plant-based alternatives instead, reducing the amount of animal-based foods in diets; so it can be understood in two ways, excluding or reducing animal-based products. Fardet (2017) classified “plant-based foods” into fruits, vegetables, legumes,

grains, nuts, and seeds; their derived processed counterparts (breads, pasta, breakfast cereals, cooked and fermented vegetables and legumes, and fruit purées, juices, and jams); and their derived ingredients (oleaginous seed-derived oils, sugars, and some herbs and spices). However, these products are usually produced using a significant amount of water, flavourings, oil or fat, binding agents, and colouring agents, apart from protein (textured and non-textured form) (Kyriakopoulou, Keppler & van der Goot, 2021), what can be perceived as negative.

From a technological point of view, food additives play an important role in the development of food products, but these additive names, sometimes difficult to pronounce, give rise to the impression of unfamiliarity, which in turn results in perceptions of higher health risk (Varela & Fiszman, 2013). Thus, the trend of clean label products has led consumers to consider what components are used in the food products that they eat in everyday life (Asioli et al., 2017). This trend has emerged due to the concern of consumers about healthiness and sustainability of food products (Euromonitor International, 2016). Despite this trend, there is still no definition or specific regulations/legislation on what is considered as clean label (Aschemann-Witzel, Varela & Peschel., 2019). However, it is generally known that these products contain ingredients perceived as natural, harmless, simple, and those that consumers know and use regularly (Ingredion, 2014). In particular, many consumers trying to reduce meat consumption to shift to healthier and more sustainable diets, find it that highly processed, plant-based meat analogues can be a contradiction, perceiving them as not healthy, and full of additives (Varela et al., 2021). How these two trends (plant-based and clean label) relate to each other, has not yet been widely investigated. Consumer research, thus, needs to support consumers to enhance trust and acceptance towards more sustainable alternatives (Aschemann-Witzel et al., 2019).

Hereby, the aim of this research was to explore the perception of consumers following different diets (vegan, vegetarian, flexitarian and omnivorous), towards clean label, plant-based products as compared with their additive-added counterparts, in order to better understand their motives and attitudes, with the view of a transition to healthier and more sustainable diets.

## **2. Materials and Methods**

### *2.1. Consumers*

The recruitment goal was to include both consumers interested in plant-based food products and consumers who are generally not interested in this type of food, so consumers were invited that followed different diets with regards to meat consumption (vegan, vegetarian, flexitarian and omnivorous). Participants (n=101) were recruited from vegan and vegetarian consumer groups, university areas and coffee places, who voluntarily filled out the complete survey. As the aim of this research was to find an and spontaneous product differentiations, all participants were untrained or naïve assessors (Dehlholm, 2014). Table 1 shows the socio-demographics information of the 101 respondents, the sample included 32 men, 68 women and 1 who preferred not to indicate their gender. Consumers interviewed were mostly students (46.53%) and people employed full-time (28.71%). Many publications pointed out young people as a motor of change in the dietary green-shift (Lu, Bock & Joseph, 2013; Cerri, Testa & Rizzi, 2018; Lago et al., 2020). Additionally, 50 respondents identified themselves as followers of an omnivorous diet, 24 of a flexitarian diet (they try to reduce the meat and animal by-products consumption), 14 of a vegan diet (purely plant-based), and 13 of a vegetarian diet (they can include egg and dairy products). This implies a good representation of the followers of each diet based on the distribution by Spanish diet in 2019 (Lantern study, 2019).

**Table 1.** Socio-demographic information of 101 respondents.

| <b>Socio-demographic information</b> | <b>Number of respondents</b> | <b>Percentage</b> |
|--------------------------------------|------------------------------|-------------------|
| <b>Gender</b>                        |                              |                   |
| Male                                 | 32                           | 31.68%            |
| Female                               | 68                           | 67.33%            |
| I prefer not to say                  | 1                            | 0.99%             |
| <b>Age</b>                           |                              |                   |
| 18-24                                | 27                           | 26.73%            |
| 25-34                                | 58                           | 57.43%            |
| 35-44                                | 10                           | 9.90%             |
| 45-54                                | 3                            | 2.97%             |
| 55-64                                | 3                            | 2.97%             |
| <b>Education level</b>               |                              |                   |
| Secondary school                     | 9                            | 8.91%             |
| Professional training                | 9                            | 8.91%             |
| Degree                               | 42                           | 41.58%            |
| Master                               | 26                           | 25.74%            |
| Doctorate                            | 14                           | 13.86%            |
| I prefer not to say                  | 1                            | 0.99%             |
| <b>Country</b>                       |                              |                   |
| Spain                                | 82                           | 81.19%            |
| Europe                               | 10                           | 9.90%             |
| Latin America                        | 9                            | 8.91%             |
| <b>Diet</b>                          |                              |                   |
| Vegetarian                           | 13                           | 12.87%            |
| Vegan                                | 14                           | 13.86%            |
| Omnivorous                           | 50                           | 49.50%            |
| Flexitarian                          | 24                           | 23.76%            |
| <b>Employment status</b>             |                              |                   |
| Student                              | 47                           | 46.53%            |
| Unemployed                           | 3                            | 2.97%             |
| Employed Part-time                   | 9                            | 8.91%             |
| Employed Full-time                   | 29                           | 28.71%            |
| Public worker                        | 12                           | 11.88%            |
| Retired                              | 1                            | 0.99%             |
| <b>Salary</b>                        |                              |                   |
| < 500                                | 26                           | 25.74%            |
| 500-1000                             | 23                           | 22.77%            |
| 1000-1500                            | 22                           | 21.78%            |
| 1500-2000                            | 21                           | 20.79%            |
| 2000-2500                            | 5                            | 4.95%             |
| I prefer not to say                  | 4                            | 3.96%             |

**Table 1.** (Continued)

| <b>Socio-demographic information</b> | <b>Number of respondents</b> | <b>Percentage</b> |
|--------------------------------------|------------------------------|-------------------|
| <b>Live status</b>                   |                              |                   |
| Alone                                | 14                           | 13.86%            |
| In couple                            | 29                           | 28.71%            |
| With family                          | 36                           | 35.64%            |
| Sharing floor                        | 22                           | 21.78%            |

## 2.2. Stimuli

As the global demand for more plant-based food alternatives and specially for meat substitutes and ready-to-eat food have been increasing rapidly in Europe in recent years (Lantern study, 2019; EUVEPRO 2019; Aschemann-Witzel et al., 2020). After visiting different supermarkets in Spain (specialised and non-specialised on vegan and vegetarian products), eight vegan and two vegetarian commercial products were selected, to represent a variation of widely available plant-based available products within these categories (sausages, burger, vegetable steak, salami, croquettes, meatballs, quinoa spread, *sobrassada*, pizza and quinoa with vegetables) (Table S1). The ingredient label of each product was presented on two different cards with the same product picture, but different ingredient lists, one presenting a clean label and the other the original label (see an example card in Figure 1). In general, the intention in the clean label version was to take out additives, preservatives, allergens, and all ingredients coming from animals to represent the plant-based and the clean label trends.

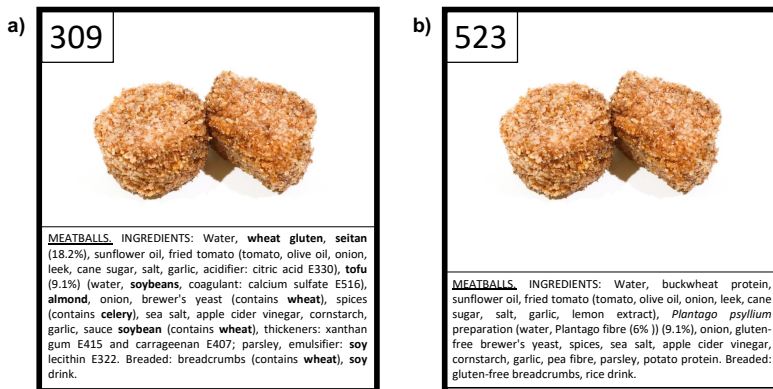
## 2.3. Projective mapping

Projective mapping (Risvik et al., 1994) was used to study the perception of clean label plant-based products and their mainstream counterparts. This methodology was applied due to its success to study undirected, top-of-mind consumer perception, and has been applied before in relation to claims, nutritional info and other product and ingredient features in different food



categories (Varela & Ares, 2012; Carrillo et al, 2012; Varela et al, 2017; Aschemann-Witzel et al., 2019).

Respondents were asked to place the cards on a DIN A2 white sheet according to their perceived similarities and differences. They were asked to complete the task using their own criteria and they were told that there were no right or wrong answers. After completing the projective mapping task, consumers were asked to provide a description to the groups they made on the sheet paper, or the reason why they mapped them in that way. As a help to explain and understand the task an example of projective mapping showing the categorisation of different objects on a sheet was provided.



**Figure 1.** Example of experimental card, (a) original label and (b) clean label (CL).

#### 2.4. Attitude questionnaire

The survey was conducted through a selection of questions from different questionnaires on nutrition knowledge, food choice, and environmental concern, as described below. The participants carried out this questionnaire, using a tablet device, after finishing the projective mapping task.

##### 2.4.1. General nutrition knowledge

The questions about nutrition were selected from the 'General Nutrition

Knowledge Questionnaire' (GNKQ) described by Parmenter and Wardle (1999). Selected questions were relevant to plant-based products consumption. The selected questions from the dietary recommendations section was *"Do you think health experts recommend that people should be eating more, the same amount, or less of these foods?"*; from knowledge of food sources, that is, which foods contain which nutrients were *"Do you think these are high or low in protein?"*, *"Do you think these are high or low in fibre/roughage?"*, *"Do you think experts call these a healthy alternative to red meat?"* and *"Which one of the following has the most calories for the same weight?"*; from the dietary choices were *"Which would be the best choice for a low fat, high fibre snack?"* and *"Which would be the best choice for a low fat, high fibre light meal?"*; and from the diet- disease associations were *"Are you aware of any major health problems or diseases that are related to a low intake of fibre?"*, *"Do you think these help to reduce the chances of getting certain kinds of cancer?"* and *"Do you think these help prevent heart disease?"*.

#### *2.4.2. Motives underlying food choice*

The questions about motivational factors were selected from the 'Food Choice Questionnaire' (FCQ) developed by Steptoe, Pollard and Wardle (1995), which involved nine motivational dimensions (or factors). However, in this study just seven factors were chosen (health, mood, convenience, sensory appeal, natural content, price and ethical concern) and their respective items to evaluate were selected. The survey was evaluated by answering the following question: *'It is important to me that the food I eat on a typical day...'*. In order to know more about the ethical food choice motives, the ethical concern factor was expanded, and the animal welfare factor included according to Lindeman and Väänänen (2000). As described Carrillo, Varela, Salvador and Fiszman (2011) all the questionnaire items were answered on a seven-box scale, labelled from 'not at all important' to 'very important', to increase the

ability to discriminate among food choice motives.

### *2.4.3. Health and Ecological concern*

In order to understand the health concerns related to the naturalness of foods, the Factor 3 labelled *Natural product interest* from the 'Health and Taste Scales' (Roininen, Lähteenmäki & Tuorila, 1999) was selected. This factor was composed of six items involving an interest in eating foods that do not contain additives and are unprocessed. Additionally, to explore general environmental attitudes, 6-items from the 'New Ecological Paradigm Scale' (NEP) described by Dunlap, Van Liere, Mertig and Jones (2000) were chosen. These items were selected to tap into each of the three facets of an ecological worldview: the reality of limits to growth ('the earth has plenty of natural resources if we just learn how to develop them' and 'the earth is like a spaceship with very limited rooms and resources'), anti-anthropocentrism ('plants and animals have as much right as human to exist'), and the possibility of an eco-crisis ('humans are severely abusing the environment', 'the so-called "ecological crisis" facing humankind has been greatly exaggerated' and 'if thing continue on their present course, we will soon experience a major ecological catastrophe'). All the statements were scored on a 7-point scale with the categories ranging from 'strongly disagree' to 'strongly agree'.

### *2.5. Data analysis*

All analyses were performed using the software XLStat 2021.2.1 (Addinsoft, USA).

#### *2.5.1. Projective Mapping*

The x and y coordinates for each product were recorded in the projective maps from all respondents, measured in centimetres as the distance from the lower left corner. Data were collected and recorded for each consumer group (omnivores and other diets). Terms generated in the descriptive step of the PM

were categorized by consensus of two researchers, considering word synonyms and the interpretation of each map as described Aviles et al. (2020), the frequencies of mention of the categorized attributes were counted across all consumers and for each experimental group of the consumer panel, for being able to analyse the results for all consumers and each group separately. To reduce the number of descriptors, only those mentioned by at least 10% of the respondents (in each dietary group) were used (Aschemann-Witzel et al., 2019). A Multifactor Analysis (MFA) was used to analyse the projective mapping task for each consumer group.

#### *2.5.2. General knowledge questions*

In order to analyse the GNKQ, the number of correct answers for each section were counted for each consumer group and an analysis of variance was conducted to observe the differences between the groups.

#### *2.5.3. Attitude questions*

An analysis of variance was performed for each item and factor of the FCQ, 'Health and Taste Scales' and NEP. A factor analysis (FA) with varimax rotation was conducted to study the factor structure.

#### *2.5.4. Consumer segmentation analyses*

Data were analysed based on the *a priori* consumer segmentation, focused on understanding consumers following different diets. Some analyses compare two groups: omnivores vs "other diets", to explore the differences in perception between omnivore consumers and those who completely avoid animal products, avoid or reduce their meat consumption (vegan, vegetarian and flexitarian). This was also to have stable sample configurations in the projective mapping outcomes, having 50 consumers in each group (Vidal et al., 2014). For having more details on the perception of the products and the different consumer motivations by the "other diets" group, it was divided for

further analysis into vegans/vegetarians and flexitarians (the vegan group was too small to draw conclusions on its own).

### **3. Results**

It is important to point out that the purpose of this research was not to draw conclusions on the acceptability of specific products or market implications, but on how the information on the label influences the product descriptions and product choice information by consumers with different diets.

#### *3.1. Perception and categorisation of plant-based products via Projective Mapping: omnivores vs other diets*

##### *3.1.1. Omnivores*

Consumer categorisation showed a clear distinction into three groups of products, which were set apart from each other in the first two dimensions of the MFA (Figure 2a). The first group located in the upper left quadrant contains the four labels of the two plant-based products which are breaded (croquettes and meatballs), without differentiating if they were original or clean label (CL). In the lower left quadrant, the second group was located, comprising both options of pizza and quinoa with vegetables, again without separating the original from the clean label. Finally, the third group located in the upper and lower right quadrant contained the meat analogues like burgers, steaks and cold cuts, and the quinoa spreads, without separation of original and clean labels. The Figure 2b shows the representation of the terms obtained in the descriptive by omnivore group. Sixteen terms were obtained in three categories, their distribution was as follows: use and type of products (7 different terms: “ready-to-eat”, “necessary to heat”, “cold cut”, “spreadable”, “breaded”, “fried”, and “meat analogue”), nutrition and health characteristics (5 different terms: “simple/additive free”, “with additives”, “natural”, “artificial/processed food”, and “healthy”), and composition/ingredients (4

different terms: “allergen”, “gluten”, “vegetable (plant-based)”, and “carbohydrates”). Croquettes and meatballs products were described as breaded, fried and with gluten; the pizza and quinoa with vegetables were described as healthy, ready-to-eat, vegetable and rich in carbohydrates. On the other hand, the third, larger and more heterogeneous group, consisting of burger, sausages, salami, vegetable steak, *sobrassada* and quinoa spread, was described as meat analogue, natural, simple/additive free, necessary to heat, cold cut, spreadable and allergen. Thus, the third group of products was more positively perceived by omnivores although the products of the second group were classified as healthy. The attributes “artificial/processed food” and “with additives”, and “allergens” were located in the middle of the map, not well correlated to the perceptual space, and describing all the items in all the groups (regardless of CL or not). Omnivore consumers did not use these attributes as main drivers for product categorization, but rather classified products by their type and utilization, as highlighted also by the fact that clean label and original ones were mapped together within each category.

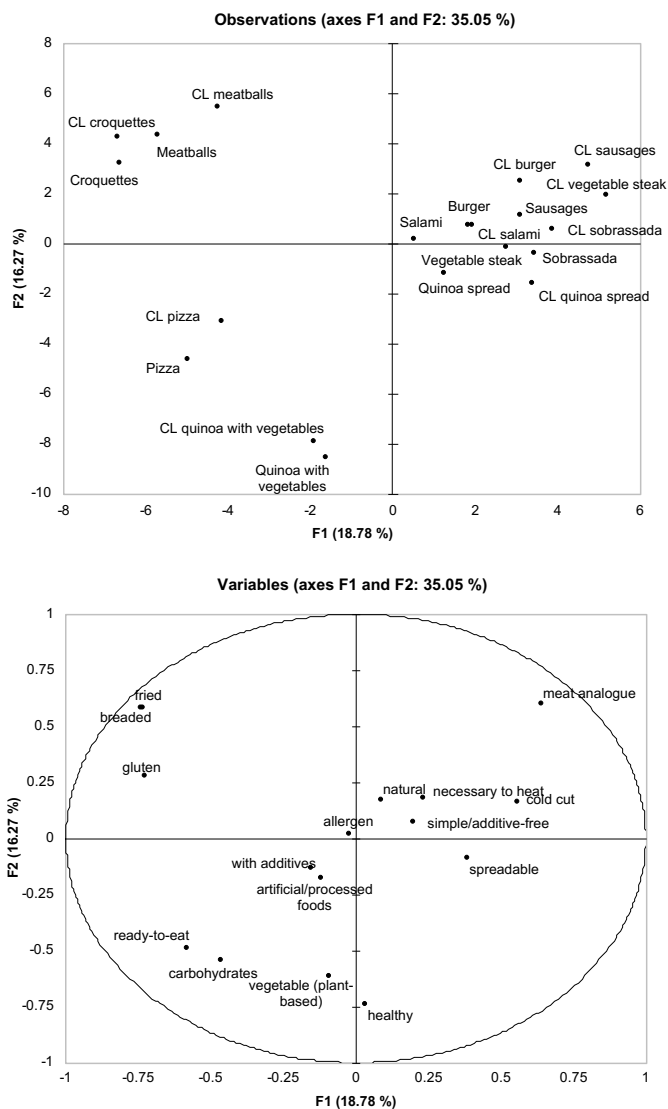
### *3.1.2. Other diets (vegans, vegetarians and flexitarians)*

The categorisation of the “other diets” group (vegan, vegetarian and flexitarian consumers) resulted in four groups (Figure 3a). The map was divided into two large groups. In the upper left part of the map was located the first group which contained the clean-label products and the second group formed by originals products was located in the bottom right part of the map. This shows that for consumers reducing or avoiding meat and other animal products (“other diets”), the status of clean label is an important characteristic for their perception of plant-based foods, quite different to the map obtained for omnivorous consumers.

The descriptors obtained by “other diets” group are shown in Figure 3b. In this case, seventeen terms were collected, and separated in a similar way as

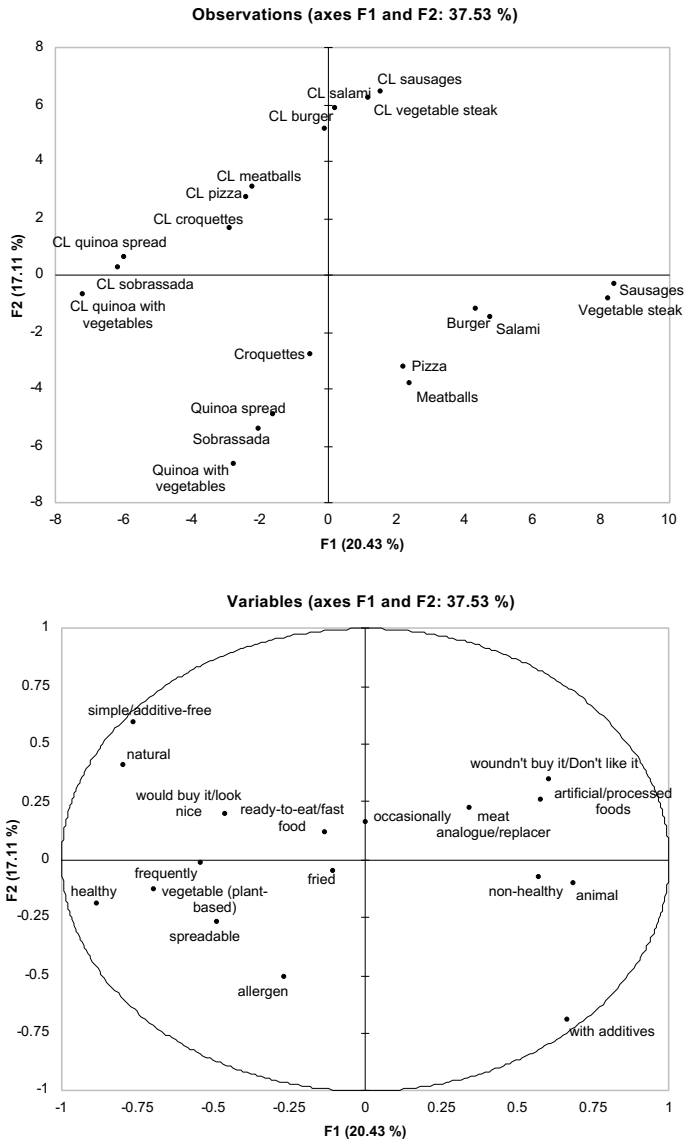
for the omnivore group. Four categories were obtained and their distribution was as follows: use and type of products (4 different terms: “ready-to-eat/fast food”, “spreadable”, “fried”, and “meat analogue/replacer”), nutrition and health characteristics (6 different terms: “simple/additive free”, “with additives”, “natural”, “artificial/processed food”, “non-healthy” and “healthy”), composition/ingredients (3 different terms: “allergen”, “animal”, and “vegetable (plant-based)”) and, preference and consumption (4 different terms: “frequently”, “occasionally”, “would buy it/look nice” and “wouldn’t buy it/don’t like it”).

Clean label products were characterised as additive-free and natural (top left) while the original products were described as with additives (bottom right quadrant). In both groups of products (CL and original), the products on the left (quinoa spread, *sobrassada* and quinoa with vegetables) were perceived as more natural, since descriptors such as simple/additive-free, natural, healthy, would buy it/look nice, and frequently consumption were used. However, products which simulate meat (burger, salami, vegetable steak and sausages) were located on the right and they were more negatively described with terms as with additives, wouldn’t buy it/don’t like it, artificial/processed foods, non-healthy and with animal ingredients, what supposes a rejection towards these types of products by vegan consumers. Products located in the middle of the map were classified by their type and consumption. “Other diet” consumers evaluated the products for their nutritional and health aspects, also highlighted by the fact that the clean label and the originals were mapped separately.



**Figure 2.** Perceptual space determined by the first two factors of the MFA in the projective mapping task by **omnivores**. (a) Representation of the samples and (b) representation of the terms obtained in the descriptive step. Note: CL before the food product name means “clean-label”.





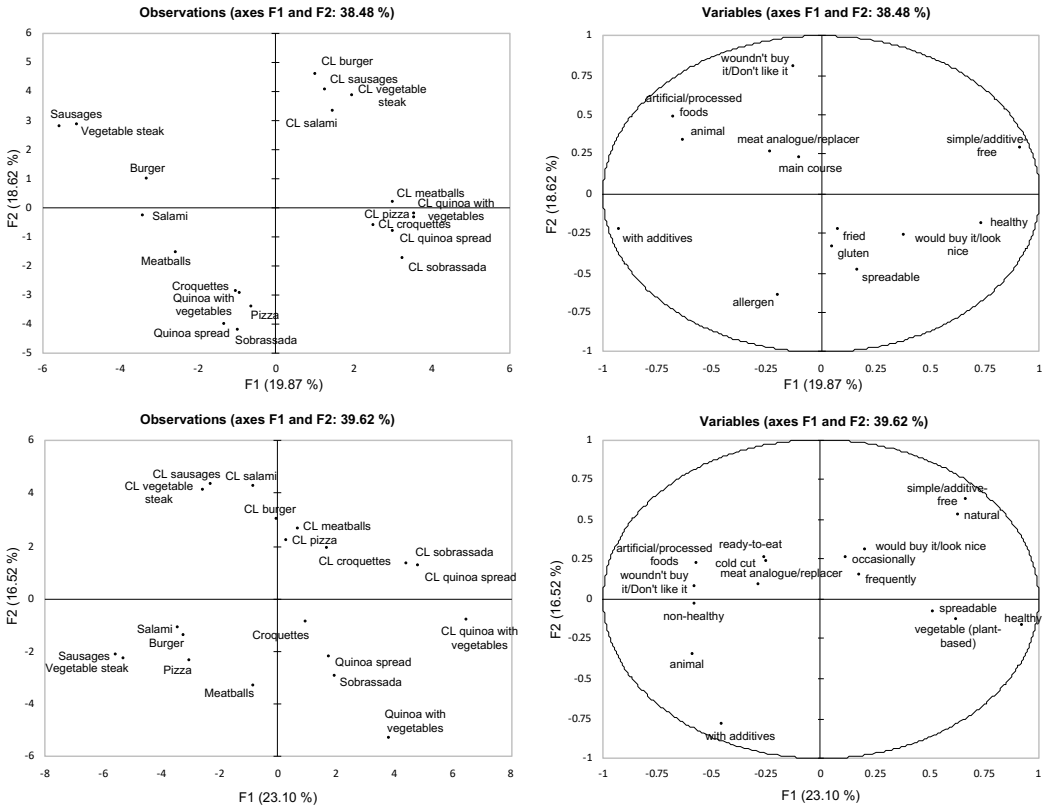
**Figure 3.** Perceptual space determined by the first two factors of the MFA in the projective mapping task by consumers of “other diets” (vegetarian, vegan and flexitarian). (a) Representation of the samples and (b) representation of the terms obtained in the descriptive step. Note: CL before the food product name means “clean-label”.

### *3.2. Perception and categorisation of plant-based products via Projective Mapping: flexitarians vs vegans/vegetarians*

Figure 4a shows the categorisation of the flexitarian group and Figure 4b the descriptors obtained by this group. Flexitarians categorized the products in two large groups, divided according to F1, on the right the clean label products, and on the left the original label products (Figure 4a). The clean label products were associated to positive descriptors as healthy, would buy it/look nice and simple/additive-free; however, the products with original labels were linked to negative descriptors as artificial/processed food, with additives and wouldn't buy it/don't like it (Figure 4b).

The map of vegans and vegetarians also presented two different groups (Figure 4c), the first one was located in the top of the map and consisted of clean label products. On the left were the clean-label products considered more artificial, processed and meat analogues/replacers, and the products perceived as natural, simple/additive-free and healthy were on the right of the map (Figure 4d). The second group was formed by original products and was in the bottom of the map. Products on the left were perceived as non-healthy, with additives and with animal ingredients. However, products on the right were perceived as healthy (Figure 4d).

Even if the two groups of consumers represented here were not extremely different in perception, it is interesting to highlight that flexitarian consumers seemed to have given more importance to the category/product usage, further dividing the CL group in two subgroups of meat-analogues (burger, sausages, steak, salami) from the rest.



**Figure 4.** Perceptual space determined by the first two factors of the MFA in the projective mapping task by flexitarians and vegans/vegetarians. (a) Samples' representation by flexitarians; (b) representation of terms obtained by flexitarians; (c) samples' representation by vegans/vegetarians; (d) representation of terms obtained by vegans/vegetarians.

### 3.3. Attitude questionnaires

#### 3.3.1. Nutritional knowledge

In order to assess the general nutrition knowledge of the two consumer groups, the correct answers for each section of this questionnaire were collected for each; the results from GNKQ for each section are depicted in Fig. 5. It can be observed that both groups presented, in general, similar knowledge about nutrition, since there were no significant differences between them in any

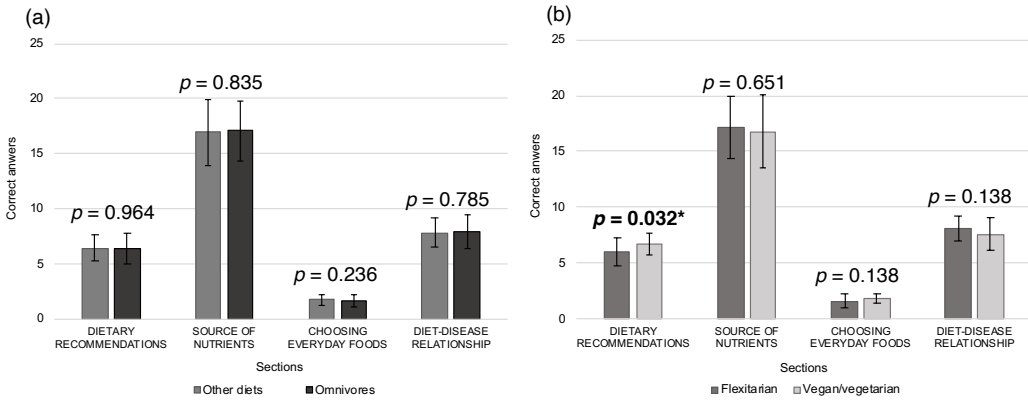
section of the questionnaire ( $p > 0.05$ ) (Figure 5a). However, when the meat reducers group was further divided into vegans/vegetarians and flexitarians, it can be observed that vegans and vegetarians had a greater significant knowledge about dietary recommendations than flexitarians ( $p < 0.05$ ) (Figure 5b).

### *3.3.2. Food choice and environmental attitudes*

Table 2 shows the mean scores for each item of the FCQ and the factor analysis results performed to detect the load of each item and the relationship with the factor. When assessors were divided into omnivores and other diets group, the item “keeps me healthy” was the most important for both groups. On the other hand, the item “has been produced in a way that animals have not experienced pain” was the least important for omnivores, while and for consumers following other diets was the least important item was “takes me no time to prepare”. Moreover, the statistical analysis highlighted significant differences between the food choice motives ( $p < 0.05$ ). People who follow an omnivorous diet indicated a pleasant texture as significantly more important when choosing food than for vegan, vegetarian or flexitarian consumers. In the case of naturalness, vegans, vegetarians and flexitarians thought it was significantly more important than omnivores (“Contains no artificial ingredients”). Moreover, it should be noted that all the items of “Ethical concern/Environmental protection” and “Animal welfare” factors were significantly more important for meat reducer/meat avoiding followers than for omnivores.

Further comparing the subgroups within “other diets”, depending if they are a meat reducer (flexitarians) or meat/animal products avoiders, in the second part of the Table 3 it can be observed that for vegan/vegetarian consumers the “Animal welfare” factor and the items “has been prepared in an environmentally friendly way”, and “has been produced in a way which has not

shaken the balance of nature” were significantly more important than for flexitarians.



**Figure 5.** GNQK correct answers for each section: (a) differences between omnivores and “other diets” group and (b) differences between flexitarians and vegans/vegetarians.

The survey results about health concern and environmentalism are depicted in Table 4. These results showed that other diets consumer group have a higher concern in the natural product interest, since the “I try to eat foods that do not contain additives”, “I do not eat processed foods, because I do not what they contain” and “I would like to eat only organically grown vegetables” items were significantly more important to them than to omnivores group ( $p < 0.05$ ). Additionally, they also presented a higher ecological interest in the abuse of the environment, the plants and animals rights, and the possible ecological catastrophe ( $p < 0.05$ ). However, when this group is divided into vegans/vegetarians and flexitarians it was only observed that all of them think similarly about natural products, but vegan and vegetarian consumers have a higher concern about plants and animals’ rights and the earth’s limited resources ( $p < 0.05$ ).

**Table 2.** FCQ-items means, standard deviations (SD), factor loading and *p*-value for answer to: “ It is important to me that the food I eat on a typical day” for each experimental group.

| Factors and items  | Omnivores      |             | Other diets    |             | Vegans/Vegetarians |             | Flexitarians   |             | <i>p</i> -value    |
|--|----------------|-------------|----------------|-------------|--------------------|-------------|----------------|-------------|--------------------|
|  | Factor loading | Mean (SD)   | Factor loading | Mean (SD)   | Factor loading     | Mean (SD)   | Factor loading | Mean (SD)   |                    |
| Factor 1. Health   |                |             |                |             |                    |             |                |             |                    |
| Keeps me healthy   | 0.442          | 6.38 (0.78) | 0.616          | 6.47 (0.73) | 0.589              | 6.59 (0.69) | 0.578          | 6.33 (0.76) | 0.209              |
| Is high in protein   | 0.415          | 4.80 (1.41) | 0.268          | 4.84 (1.27) | 0.270              | 5.11 (0.89) | 0.312          | 4.54 (1.56) | 0.124              |
| Is high in fibre and roughage  | 0.505          | 4.66 (1.29) | 0.435          | 5.02 (1.27) | 0.623              | 5.04 (1.02) | 0.474          | 5.00 (1.53) | 0.921              |
| Factor 2. Mood   |                |             |                |             |                    |             |                |             |                    |
| Helps me to cope with life   | 0.330          | 5.52 (1.39) | 0.388          | 5.90 (1.30) | 0.189              | 5.93 (1.44) | 0.573          | 5.88 (1.15) | 0.891              |
| Makes me feel good   | 0.391          | 6.22 (1.27) | 0.496          | 6.33 (0.93) | 0.364              | 6.33 (1.00) | 0.574          | 6.33 (0.87) | 1.000              |
| Factor 3. Convenience  |                |             |                |             |                    |             |                |             |                    |
| Is easy to prepare   | 0.707          | 4.88 (1.76) | 0.661          | 4.76 (1.56) | 0.566              | 5.19 (1.39) | 0.733          | 4.29 (1.63) | <b>0.039*</b>      |
| Is easily available in shops and supermarkets                                      | 0.605          | 5.70 (1.27) | 0.426          | 5.55 (1.32) | 0.686              | 5.82 (1.15) | 0.416          | 5.25 (1.45) | 0.127              |
| Takes me no time to prepare  | 0.633          | 4.72 (1.62) | 0.693          | 4.65 (1.65) | 0.546              | 4.89 (1.53) | 0.764          | 4.38 (1.77) | 0.270              |
| Factor 4. Sensory appeal   |                |             |                |             |                    |             |                |             |                    |
| Has a pleasant texture   | -0.038         | 6.06 (1.08) | 0.654          | 5.35 (1.43) | 0.621              | 5.22 (1.70) | 0.577          | 5.50 (1.06) | 0.482              |
| Looks nice   | 0.222          | 5.44 (1.25) | 0.740          | 5.00 (1.39) | 0.600              | 4.96 (1.45) | 0.675          | 5.04 (1.33) | 0.842              |
| Factor 5. Natural content  |                |             |                |             |                    |             |                |             |                    |
| Contains no additives  | 0.511          | 4.32 (1.76) | 0.377          | 4.90 (1.63) | 0.441              | 4.89 (1.78) | 0.625          | 4.92 (1.47) | 0.952              |
| Contains natural ingredients   | 0.560          | 5.60 (1.21) | 0.399          | 5.98 (1.10) | 0.597              | 6.00 (1.24) | 0.637          | 5.96 (0.96) | 0.895              |
| Contains no artificial ingredients   | 0.573          | 4.64 (1.71) | 0.416          | 5.51 (1.29) | 0.761              | 5.52 (1.28) | 0.692          | 5.50 (1.32) | 0.960              |
| Factor 6. Price  |                |             |                |             |                    |             |                |             |                    |
| Is not expensive   | 0.649          | 4.76 (1.60) | 0.617          | 5.25 (1.34) | 0.541              | 5.41 (1.42) | 0.598          | 5.08 (1.25) | 0.394              |
| Is good value for money  | 0.722          | 5.84 (1.22) | 0.279          | 5.92 (0.98) | 0.513              | 5.85 (1.03) | 0.268          | 6.00 (0.93) | 0.594              |
| Factor 7. Ethical concern/Environmental protection                                 |                |             |                |             |                    |             |                |             |                    |
| Is packaged in an environmentally friendly way                                     | 0.710          | 5.14 (1.47) | 0.752          | 6.00 (1.15) | 0.744              | 6.26 (1.06) | 0.665          | 5.71 (1.20) | 0.087              |
| Has been prepared in an environmentally friendly way <sup>a</sup>                  | 0.815          | 5.16 (1.49) | 0.840          | 6.10 (1.22) | 0.786              | 6.56 (0.85) | 0.856          | 5.58 (1.38) | <b>0.005*</b>      |
| Has been produced in a way which has not shaken the balance of nature <sup>a</sup> | 0.905          | 4.98 (1.45) | 0.874          | 5.86 (1.22) | 0.796              | 6.33 (0.92) | 0.864          | 5.33 (1.31) | <b>0.003*</b>      |
| Factor 8. Animal welfare   |                |             |                |             |                    |             |                |             |                    |
| Has been produced in a way that animals have not experienced pain <sup>a</sup>     | 0.636          | 4.51 (1.92) | 0.569          | 6.02 (1.53) | 0.483              | 6.85 (0.46) | 0.478          | 5.08 (1.77) | <b>&lt;0.0001*</b> |
| Has been produced in a way that animals' rights have been respected <sup>a</sup>   | 0.780          | 4.84 (1.49) | 0.751          | 6.24 (1.09) | 0.576              | 6.74 (0.66) | 0.793          | 5.67 (1.20) | <b>0.000*</b>      |

Mean values in bold type correspond to the highest mean for each factor.

\* indicates significant differences at *p*-value < 0.05

<sup>a</sup> items from Lindeman and Väänänen (2000).

**Table 3.** Natural products interest and NEP-items means, standard deviations (SD), factor loading and *p*-value for each experimental group.

| Factors and items  | Omnivores   |                | Other diets        |                | Vegans/Vegetarians |                | Flexitarians |                | <i>p</i> -value |
|--|-------------|----------------|--------------------|----------------|--------------------|----------------|--------------|----------------|-----------------|
|  | Mean (SD)   | Factor loading | Mean (SD)          | Factor loading | Mean (SD)          | Factor loading | Mean (SD)    | Factor loading |                 |
| Natural product interest <sup>a</sup>  |             |                |                    |                |                    |                |              |                |                 |
| I try to eat foods that do not contain additives   | 4.14 (1.90) | 0.851          | <b>5.12</b> (1.76) | 0.848          | 5.33 (1.86)        | 0.853          | 4.88 (1.65)  | -0.856         | 0.359           |
| I do not care about additives in my daily diet   | 3.28 (1.59) | -0.583         | 2.92 (1.86)        | -0.824         | 3.15 (1.96)        | -0.687         | 2.67 (1.76)  | 0.885          | 0.362           |
| I do not eat processed foods, because I do not what they contain                                   | 2.80 (1.65) | 0.474          | <b>3.57</b> (1.60) | 0.411          | 3.59 (1.53)        | 0.776          | 3.54 (1.72)  | -0.398         | 0.911           |
| I would like to eat only organically grown vegetables  | 3.66 (1.94) | 0.536          | <b>5.02</b> (1.88) | 0.750          | 5.37 (1.80)        | 0.848          | 4.63 (1.93)  | -0.697         | 0.160           |
| In my opinion, artificially flavoured foods are not harmful for my health                          | 3.30 (1.91) | -0.738         | 3.00 (2.05)        | -0.635         | 3.04 (2.21)        | -0.752         | 2.96 (1.90)  | 0.471          | 0.893           |
| In my opinion, organically grown foods are no better for my health than those grown conventionally | 3.96 (1.91) | -0.629         | 3.26 (2.08)        | -0.728         | 3.56 (2.34)        | -0.791         | 2.92 (1.72)  | 0.751          | 0.277           |
| New Ecological Paradigm scale <sup>b</sup>   |             |                |                    |                |                    |                |              |                |                 |
| Humans are severely abusing the environment  | 6.58 (0.86) | 0.657          | <b>6.88</b> (0.43) | 0.663          | 6.96 (0.19)        | 0.280          | 6.79 (0.59)  | 0.723          | 0.184           |
| The earth has plenty of natural resources if we just learn how to develop them.                    | 5.98 (1.29) | 0.215          | 5.98 (1.46)        | 0.549          | 5.89 (1.58)        | 0.538          | 6.08 (1.35)  | 0.670          | 0.640           |
| Plants and animals have as much right as human to exist  | 5.64 (1.61) | 0.559          | <b>6.35</b> (1.47) | 0.502          | 6.78 (0.64)        | 0.176          | 5.88 (1.94)  | 0.346          | <b>0.038*</b>   |
| The so-called "ecological crisis" facing humankind has been greatly exaggerated                    | 2.88 (1.69) | 0.222          | 2.41 (1.97)        | -0.308         | 2.48 (2.10)        | -0.319         | 2.33 (1.86)  | 0.346          | 0.792           |
| The earth is like a spaceship with very limited rooms and resources                                | 5.76 (1.62) | 0.693          | 5.49 (2.10)        | 0.507          | <b>6.04</b> (1.74) | 0.619          | 4.88 (2.33)  | 0.660          | <b>0.048*</b>   |
| If things continue on their present course, we will soon experience a major ecological catastrophe | 5.74 (1.60) | 0.753          | <b>6.41</b> (0.85) | 0.703          | 6.48 (0.75)        | 0.753          | 6.33 (0.96)  | 0.666          | 0.541           |

Mean values in bold type correspond to the highest mean for each factor.

\* indicates significant differences at *p*-value < 0.05

<sup>a</sup> Factor and items from Roininen, Lähteenmäki and Tuorila (1999).

<sup>b</sup> Items from Dunlap, Van Liere, Mertig and Jones (2000).

#### 4. Discussion

The popularity of plant-based products has been increasing considerably in recent years. However, the results of this study show differences in attitudes, perception and product categorisation depending on the consumer diet (omnivore, flexitarian, vegetarian, vegan). This is in line with recent findings showing that consumers may have different attitudes and motivations depending on the stage of behavioural change towards meat reduction or avoidance (thinking of reducing meat, actually reducing and maintaining that reduction) (Hielkema et al., 2021). More concretely, omnivores in our study separated three groups of plant-based products, based on the category and usage, fried products, products enriched in carbohydrates and the last one as meat analogue products, but clean label and original products were mapped together. However, the meat reducers and avoiders (“other diets”) group, perceived plant-based products in a different way, focusing on the clean label status for their categorization. In this case, they perceived the group which contains CL spread food and quinoa with vegetables as healthy, and they use frequency of consumption descriptors to classify them (i.e., frequently for the CL, would not buy for the original products, and occasionally in the middle). It is a well-known fact that meat reducers or avoiders are more concerned about health and sustainability, as can also be observed in the main motivations displayed by this consumer group when answering to the attitude questions. Omnivores did not use any frequency of consumption descriptors, may be because they thought that these kinds of products, in general, were not directed at them, and perhaps they may not like the texture or flavour and they may prefer to consume meat products instead (Lea, Crawford & Worsley, 2006; Fiestas-Flores & Pyhälä, 2018). Additionally, meat-eaters are more resistant to going vegetarian because they perceive vegetarian diets as less tasty, more expensive, less familiar, less convenient, and less healthful (Fiestas-Flores &



Pyhälä, 2018; Rosenfeld & Tomiyama, 2020). The meat reducers and avoiders in this study differentiated clean label from original products, perceiving clean label products as more natural and simpler. It can be pointed out that most of “other diets” consumers described products which simulate meat products as “replacer” not as “analogue”, this is an important nuance since they perceived these products like an alternative and, with their consumption, they can supply meat products. However, omnivores perceived these products as products that simulate meat or as a “copy-cat” of meat products. Fiestas-Flores & Pyhälä (2018) pointed out that the taste of animal products is the main challenge for Spanish omnivores to choose to reduce or avoid meat from their diet and in that sense, it can be highlighted that Spain has become the country with the largest meat consumption in Europe (Faber et al., 2020). This different categorisation might be explained by the larger importance that meat-reducers give to health and sustainability, and the different degrees of familiarity towards meat analogues. This is interesting, as familiarity (or rather the lack thereof) is one of the big barriers towards meat reduction. Hielkema et al. (2021) confirmed that identity-incongruence (eating foods not familiar or part of their habitual behaviour) inhibits consumers to progress towards meat reduction, and they highlighted that for consumers that were already reducing meat, climate concerns were important drivers, but not for those consumers with no intention to reduce meat.

For most consumers, naturalness is crucial to which food industry’s answer has been the “clean label” trend (Roman et al, 2017), defined by Ingredion (2014) as *“a ‘clean label’ positioned on the pack means the product can be positioned as ‘natural’, ‘organic’ and/or ‘free from additives/preservatives’.”* Our study has shown that consumers may react differently and have a different degree of interest/focus in this trend when categorizing plant-based products. We can confirm that meat-reducers and

meat avoiders are more related to this trend, since they pointed out as main reasons for choosing foods that did not contain additives, that were not processed food and that they preferred organically grown vegetables, also, we saw that those consumers categorized clean label products in a different group than the original counterparts. This stronger focus on clean label vs additive added products in the meat reducers and avoider groups, goes in line to what Clicerì et al. (2018) found in their study on attitudes, vegetarian and meat reducers' attitudes towards healthy and natural food products were more positive than omnivores' attitudes. Furthermore, when it comes to veganism, it has been described as "a way of life rather than a simple dietary choice" (North et al., 2021), many times overlaying with other ideology groups (animal activists, environmentalists, focus on social justice), which is also the case for many vegetarians (Rosenfeld & Barrow, 2017) and these groups can be somehow more absolutists in their dietary and lifestyle choices.

In general, consumers presented a similar knowledge about nutrition; however, the reasons for choosing food vary according to the diet that each consumer follows. The perception of clean label plant-based products by vegans/vegetarians and flexitarians were quite similar, although flexitarian consumers gave a further division between the CL group separating meat-analogues from the rest. Furthermore, vegans and vegetarians showed a higher concern about dietary recommendations; it could be because vegan/vegetarian consumers need to obtain key nutrients such as zinc, vitamin B12 and protein from alternative sources (Sneijder & te Molder, 2009), so that, they are likely to follow the nutritional recommendations more closer than meat eaters. Thus, the different perception of clean label plant-based products could be related to the attitudes of each consumer diet; commonly, the vegetable and fruit consumption is associated with a healthy dietary pattern and more concern about sustainability. An association between dietary patterns and

environmental concern was observed in this study, as results indicated by Asvatourian et al. (2018). Furthermore, our study confirms that reduced-meat diets, vegans and vegetarians, are more motivated by the environmental issues and animal rights than omnivores, in line with that reported by Hopwood, Rosenfeld, Chen and Bleidorn (2021) for vegetarians. Spanish vegan and vegetarians showed having the highest levels of affection towards animals and environmental awareness, while omnivores were least likely to draw similarities between human and non-human animal emotions (Díaz, 2016; Fiestas-Flores & Pyhälä, 2018). Moreover, the ecological drive for vegetarianism has been documented as the most often listed reason. This is based on the fact that meat consumption strongly increases the greenhouse gas emissions, which increase the ecological footprint, as well as the water footprint (De Backer & Hudders, 2014). However, considering the existing literature showing that concerns about animal welfare and environmental impact are the most common reasons for avoiding meat (De Backer & Hudders, 2014; Malek, Umberger & Goddard, 2019; Malek & Umberger, 2021), we observed that what distinguished meat reducers from meat avoiders was the importance that they gave to animal welfare factors. Similarly, Fiestas-Flores & Pyhälä (2018) pointed out that human-like animal attributes directly affected Spanish students' intentions to become vegetarian and vegan.

Attitudes towards a vegetarian lifestyle have been shown to be significantly correlated with nutritional knowledge (Pribis, Pencak, & Grajales, 2010; Corrin & Papadopoulos, 2017). However, in this study no differences were found between omnivores and other diets group related to healthy patterns, it could be because vegans and vegetarians are more likely to cite ethical motivations over health ones (Rosenfeld, 2018). Thus, the increase of plant-based products in markets (Lantern Study, 2019) may promote the benefits of these ones on sustainability, environment, and animal rights, in

addition to health. Both omnivores and meat-reducers and avoiders seem to know well that a meat-reduced diet is positive, since they presented similar nutritional knowledge in our study, as results reported by Asher and Peters (2020). So that, Lea, Crawford and Worsley (2006, p. 835) report that “the primary barrier to eating a vegetarian diet related to taste, whereas taste barriers ranked relatively low in the plant-based diet survey,” which is also in line with the results obtained in our study, since omnivores showed that one of the motives for choosing foods is “have a pleasant texture”, unlike meat-replacers.

On the other hand, most consumers of this study were young people (<44 years), some of them students with low incomes (Table 1). Fuller, Brown, Rowley and Elliott-Archer (2021) indicated that people who follow a vegan diet are particularly young, females and those living in urban areas. University-educated and younger people may be more receptive to information on changing to a plant-based diet and they appeared to be more willing to alter their diet than the non-university educated and oldest groups (Lea, Crawford & Worsley ,2006). Furthermore, although they present low incomes while they are studying, it is to be expected that they would have high socio-economic status and would take up this form of eating first (Lea, Crawford & Worsley ,2006).

It has been suggested that there is not “one size fits all” with regards to plant-based foods and consumers (Aschemann-Witzel et al., 2020); our study further contributes to the understanding that these two big consumer trends growing in the last years, of plant-based foods and clean-label may interact in different ways with regards to consumer perception and attitudes, which would ultimately affect their choices towards healthier and more sustainable foods.

### *4.1. Limitations and future research*

This study is a first visualization of the interaction of the clean label with the perception of plant-based products by consumers in different stages of meat reduction, avoidance and omnivores, but future research might be performed by focusing on other factors such as gender, age, income and/or diet familiarity, among others, as well as focusing on how taste might influence the effects seen in this work, by including product tasting.

It should be noted that this study was performed before the COVID-19 crisis, which has impacted consumer's attitudes and habits in many ways. If this survey were carried out today, the results could be different, since Spanish population during the COVID-19 health crisis has increased its concern for a healthy lifestyle, with an increase of physical activity and fruit and vegetable consumption (Academia Española de Nutrición y Dietética, 2020; López-Bueno et al., 2020). It could be related to the families had more time to cook and improve eating habits, even though this did not increase the overall diet quality of Spanish population (Ruiz-Roso et al., 2020).

This study represents a sample of Spanish consumers; so these results may be different in other cultural contexts and environments settings (Ares, 2018).

## **5. Conclusions**

Consumer categorisation and perception of clean label plant-based products was different depending on the type of diet. The present results show that flexitarian, vegetarian and vegan consumers pay more attention to food naturalness quality and health, following the clean label trend, as compared to omnivorous consumers. At the same time, this group also presents a greater concern for animal welfare and sustainability. Clean label plant-based products were perceived as healthy, simple/additive-free, natural, and would buy it/look

nice by meat reducers and avoiders, while omnivores did not focus on the clean label status when categorizing plant-based products.

We hereby present a first exploration of consumers' categorisation and perception of clean label plant-based products, and its relation to consumer attitudes, depending on their diet, and it can help to the understanding of how different consumers perceive them, at the light of supporting consumers in a transition to healthier, more sustainable diets.

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## 7. Supplementary material

**Table S1.** Original and Clean labels of selected products.

| Products               | Original Label   | Clean Label   |
|------------------------|--|---|
| Sausages               | <b>Egg white powder</b> (rehydrated), water, sunflower oil, salt, aromatic plants, spices, flavourings, glucose syrup, dextrose, stabilizers: carrageenan E407, garrofin gum E410, xanthan gum E415, Konjac gum E425; acidity correctors: sodium acetate E262, Lactic acid E270; carrot powder, colours: radish concentrate, carotene E160a.   | Potato protein (rehydrated), water, sunflower oil, salt, aromatic plants, spices, aromas, glucose syrup, dextrose, Plantago fibre ( <i>Plantago psyllium</i> ), lemon juice, alcohol vinegar, carrot powder, radish concentrate.  |
| Burger                 | Water, pea protein (18%), <b>rapeseed oil</b> , refined coconut oil, aroma, smoke aroma, stabilizers: cellulose E460, methylcellulose E461, gum Arabic E414; potato starch, maltodextrin, yeast extract, salt, sunflower oil, dehydrated yeast, antioxidants: ascorbic acid E300, acetic acid E260; colour: beet juice concentrate E162; modified starch, apple extract, lemon juice concentrate.                  | Water, pea protein (18%), sunflower oil, refined coconut oil, aroma, smoke aroma, Plantago fibre ( <i>Plantago psyllium</i> ) and pea fibre, potato starch, maltodextrin, yeast extract, salt, sunflower oil, lemon extract, beetroot juice, modified starch, apple extract, lemon juice concentrate. |
| Vegetable steak        | <b>Rehydrated egg white</b> , water, sunflower vegetable oil, salt, vegetable fibre, aromatic plants (0.6%), spices, spice extracts, flavourings, sugar, dextrose, thickeners: carrageenan E407, garrofin gum E410, xanthan gum E415; acidity correctors: sodium acetate E262, Lactic acid E270, potassium lactate E326, potassium chloride E508; food colour: radish, apple and blackcurrant concentrate; olives. | Rehydrated potato protein, water, sunflower vegetable oil, salt, aromatic plants (0.6%), spices, spice extracts, aromas, sugar, dextrose, Plantago fibre ( <i>Plantago psyllium</i> ), potato fibre, lemon juice, yeast extract, radish concentrate, apple and blackcurrant; olives.                  |
| Salami                 | Water, canola oil, stabilisers: garrofin gum E410, xanthan gum E415; <b>wheat gluten</b> (4.4%), pea proteins (2.7%), spices, maltodextrin, dextrose, sea salt, vinegar, beet concentrate, colour: paprika extract E160c; tomato concentrate, acidity regulator: calcium citrate E333.   | Water, sunflower oil, potato fibre, buckwheat protein (4.4%), pea protein (2.7%), spices, maltodextrin, dextrose, sea salt, vinegar, red beet concentrate, paprika, tomato concentrate, vinegar extract.  |
| Croquettes             | <b>Soy drink</b> , spinach (20.1%), <b>breaded</b> (breadcrumbs [contains wheat], water, <b>wheat flour</b> , corn starch), <b>wheat flour</b> , <b>tofu</b> (7%) (water, <b>soybeans</b> , stabilizer: calcium sulphate E516), margarine, <b>pine nuts</b> , raisins, sea salt.   | Rice drink, spinach (20.1%), <b>breaded</b> (breadcrumbs [contains wheat], water, <b>wheat flour</b> , corn starch), <b>wheat flour</b> , prepared from <i>Plantago psyllium</i> (water, Plantago fibre (6%)), margarine, <b>pine nuts</b> , raisins, sea salt.                                       |
| Quinoa with vegetables | Cooked red bean, real quinoa (14%), water, tomato, corn, extra virgin olive oil, onion, red pepper, green pepper, lemon juice, vinegar, salt, brown sugar, spices and stabilizer: xanthan gum E415.  | Cooked red bean, real quinoa (14%), water, tomato, corn, extra virgin olive oil, onion, red pepper, green pepper, lemon juice, vinegar, salt, brown sugar, spices and citrus fibre.   |

Table S1. (Continued)

| Products      | Original Label   | Clean Label  |
|---------------|--|--|
| Meatballs     | Water, <b>wheat gluten, seitan</b> (18.2%), sunflower oil, fried tomato (tomato, olive oil, onion, leek, cane sugar, salt, garlic, acid: citric acid E330), <b>tofu</b> (9.1%) (water, <b>soybeans</b> , coagulant: calcium sulphate E516), <b>almond</b> , onion, <b>brewer's yeast (contains wheat)</b> , <b>spices (contains celery)</b> , sea salt, apple vinegar, corn starch, garlic, <b>soy sauce (contains wheat)</b> , thickeners: xanthan gum E415 and carrageenan E407; parsley, emulsifier: soy lecithin E322. Breading: <b>breadcrumbs (contains wheat)</b> , <b>soy milk</b> .   | Water, buckwheat protein, sunflower oil, fried tomato (tomato, olive oil, onion, leek, cane sugar, salt, garlic, lemon extract), prepared <i>Plantago psyllium</i> (water, Plantago fibre (6%)) (9.1%), onion, gluten-free brewer's yeast, spices, sea salt, apple vinegar, corn starch, garlic, pea fibre, parsley, potato protein. Breading: gluten-free breadcrumbs, rice milk.   |
| Quinoa spread | Water, <b>tofu</b> (water, <b>soybeans</b> , gelling agent: E511 magnesium chloride), sunflower oil, kale (10.3%), quinoa (4.3%), potato starch, onion, <b>celery</b> , potato powder, sea salt, agave syrup, lemon juice, garlic, herb mixture (0.67%), thyme (0.18%).  | Water, <b>cashews</b> , sunflower oil, kale (10.3%), quinoa (4.3%), potato starch, onion, potato powder, sea salt, agave syrup, lemon juice, garlic, herb mixture (0.67%), thyme (0.18%).  |
| Sobrassada    | <b>Cashews</b> (33.7%), sunflower seeds (11.2%), sunflower oil, extra virgin olive oil (6%), miso (barley and <b>soybean</b> ), paprika, salt and xanthan gum E415.  | <b>Cashews</b> (33.7%), sunflower seeds (11.2%), sunflower oil, extra virgin olive oil (6%), miso (barley and <b>soybean</b> ), paprika, salt and potato fibre.  |
| Pizza         | <b>Common wheat flour</b> , prepared natural strips (11%) [water, <b>soy protein concentrate</b> , sunflower oil, salt, aroma, spices (paprika, pepper, ginger, nutmeg, cardamom)], tomato sauce, tomato pulp, vegetable preparation (10%) (water, modified corn and potato starch, coconut oil, vegetable protein, salt, vegetable fibre, flavouring, stabilizer: tara gum E417; colour: calcium carbonate E170, b-carotene E160a; preservative: sorbic acid E200; vitamin B12), water, semi-dehydrated tomato (7.5%), rucola (2.5%), sunflower seed oil, extra virgin olive oil, salt, seasoned breadcrumbs [breadcrumbs ( <b>wheat flour, malted wheat flour, rapeseed oil</b> , sunflower seed oil, yeast, dextrose, salt), extra virgin olive oil, onion, garlic, rosemary, parsley, thyme, salt, black olives], yeast, sugar, olive oil, thyme, garlic, onion, parsley, oregano, black pepper and basil. | <b>Common wheat flour</b> , prepared natural strips (11%) [water, pea protein concentrate, sunflower oil, salt, aroma, spices (paprika, pepper, ginger, nutmeg, cardamom)], tomato sauce, tomato pulp, vegetable preparation (10%) [(water, modified corn and potato starch, coconut oil, vegetable protein, salt, plantain vegetable fibre ( <i>Plantago psyllium</i> ) and potato, yeast extract, paprika, fermented dextrose, vitamin B12), water, semi-dehydrated tomato (7.5%), rucola (2.5%), sunflower seed oil, extra virgin olive oil, salt, onion, garlic, rosemary, parsley, thyme, salt, black olives], yeast, sugar, olive oil, thyme, garlic, onion, parsley, oregano, black pepper and basil. |

# **Capítulo 4**

## **Caracterización de fibras**





## Primera parte

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Nutritional, Physico-Chemical and Mechanical Characterization of Vegetable Fibers to Develop Fiber-Based Gel Foods

Este artículo ha sido publicado como:

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### **Abstract**

The aim of this research was to evaluate the nutritional and physico-chemical properties of six different vegetable fibers and explore the possibility of using them as a thickener or gelling agent in food. To determine the technological, nutritional and physical parameters, the following analyses were carried out: water-holding capacity, water retention capacity, swelling, fat absorption capacity, solubility, particle size, moisture, hygroscopicity, pH, water activity, bulk density, porosity, antioxidant activity, phenolic compounds and mineral content. Gels were prepared at concentrations from 4% to 7% at 5 °C and analyzed at 25 °C before and after treatment at 65 °C for 20 min. A back extrusion test, texture profile analysis and rheology were performed and the pH value, water content and color were analyzed. As a result, all the samples generally showed significant differences in all the tested parameters. Hydration properties were different in all the tested samples, but the high values found for chia flour and citrus fiber are highlighted in functional terms. Moreover, chia flour was a source of minerals with high Fe, Mn and Cu contents. In gels, significant differences were found in the textural and rheological properties among the samples, and also due to the heat treatment used (65 °C, 20 min). As a result, chia flour, citrus, potato and pea fibers showed more appropriate characteristics for thickening. Moreover, potato fiber at high concentrations and both combinations of fibers (pea, cane sugar and bamboo fiber and bamboo, psyllium and citric fiber) were more suitable for gelling agents to be used in food products.

## 1. Introduction

Consumer interest in health, sustainability aspects of their way of life and their diet is growing. They demand more natural, less processed food, which are made using ingredients that are not perceived as negative (Aschemann-Witzel, Varela & Peschel, 2019). Moreover, E numbers or a long ingredients list with scientific names shown on labels does not appeal to consumers; this negatively affects consumers' perception regarding their naturalness and their impacts on health and wellness (Kyriakopoukou, Keppler & van der Goot, 2021). Therefore, these ingredients should be replaced with others that consumers perceive as being healthier, such as fibers (Varela & Fiszman, 2013).

It is a well-known fact that hydrocolloids change the physical properties of solutions to form gels, or enable thickening, emulsification, coating and stabilization, and they also provide viscosity and play a role in developing food with high satiating capacity (Li & Nie, 2016). Furthermore, hydrocolloids are of great importance in gel network formation since they can act as carriers for bioactive food ingredients, which usually have low mobility and diffusion rates because they are trapped by gel networks (Mao et al., 2020). Besides functional qualities, hydrocolloids have also received enthusiastic support due largely to their dietary fiber (DF) aspect (Li & Nie, 2016). In this sense, it is important for its beneficial effects on health, as well as prebiotic ingredients and their technological attributes, such as water binding, gelling and structure building. Therefore, they can be used as a low-calorie sweetener, fat re-placer or texture modifier (de Moraes Crizel et al., 2013; Li & Nie, 2016). For this reason, the interest shown in plants high in DF and natural antioxidants has increased in recent years (Zhu et al., 2010). Moreover, eating them offers several health benefits, including body weight control, serum lipid and cholesterol reduction, controlled postprandial glucose responses and

colorectal cancer prevention (Ma & Mu, 2016).

For these reasons, designing future food structures by structuring food colloids such as fiber offers high theoretic and application value to use them, for example, in functional food products for elderly people with swallowing problems, special food products for diabetic patients or artificial plant-based meats for vegan and vegetarians (Lu, Nishinari, Matsukawa & Fang, 2020).

The main purpose of this research was to evaluate the nutritional and physico-chemical properties of different vegetable fibers, and the possibility of using them as a thickener or gelling agent in food.

## 2. Materials and Methods

### 2.1. Raw Materials

The samples used in this study were six different vegetable sources of DFs supplied by the company Productos Pilarica S.A., Paterna, Spain. Table 1 depicts the name, ingredients and proximate compositions of the samples, as indicated by their producers.

**Table 1.** Sample names, ingredients and proximate compositions expressed as g/100 g sample.

| Sample | Name                              | Ingredients                                       | Proteins | Lipids | Carbohydrates | TDF   | IDF   | SDF  |
|--------|-----------------------------------|---|----------|--------|---------------|-------|-------|------|
| FCH    | Chia Flour                        | <i>Salvia hispanica</i> Seed Powder               | 30.95    | 12     | 49.04         | 45.5  | 43.1  | 3.5  |
| FPE    | Pea Fiber                         | Yellow pea fiber Synergetic                       | 5.19     | 0.3    | 93.41         | 43.8  | 36.7  | 7.1  |
| FP     | Potato Fiber                      | combination of potato pure fiber                  | 2.17     | 3.1    | 92.37         | >60.0 | >60.0 | <1.0 |
| FC     | Citrus Fiber                      | Citrus Fiber                                      | 5.89     | 1.02   | 81.34         | 71.1  | 37.6  | 33.3 |
| FBPC   | Bamboo, Psyllium and Citric Fiber | Combination of bamboo, psyllium and citric fibers | 0.613    | 0.5    | 98.287        | 45.6  | 42.1  | 3.5  |
| FPESB  | Pea, cane Sugar and Bamboo Fiber  | Vegetable fibers: pea, cane sugar and bamboo      | 4.7      | 0.9    | 92.11         | >60.0 | >60.0 | <1.0 |

## *2.2. Physico-Chemical Analysis*

Moisture ( $x_w$ ) (g water/100 g sample) was determined by vacuum oven drying (Vaciotem, J.P. Selecta, Spain) at 70 °C until constant weight.

The water activity ( $a_w$ ) of samples was analyzed by AquaLab PRE LabFerrer equipment (Pullman, Washington, DC, USA).

Hygroscopicity (Hg) was determined according to Cai and Corke (2000).

The pH of samples was analyzed on dispersions (10% w/v) in distilled water following Bender et al. (2020).

Sample particle size distribution was determined according to Standard ISO13320 (AENOR 2009) with a particle size analyzer (Malvern Instruments Ltd., Mastersizer 2000, Worcestershire, UK) equipped with a dry sample dispersion unit (Malvern Instruments Ltd., Scirocco 2000). Particle size distribution was characterized by the volume mean diameter ( $D [4, 3]$ ) and standard percentiles  $d (0.1)$ ,  $d (0.5)$  and  $d (0.9)$ .

Porosity ( $\epsilon$ ) was determined from true ( $\rho$ ) and bulk ( $\rho_b$ ) densities according to Agudelo, Igual, Camacho and Martínez-Navarrete (2016) with slight modifications. To determine bulk density ( $\rho_b$ ), about 2 g of powder were placed inside a 10 mL-graduated test tube and the occupied volume was noted. Bulk density was calculated by dividing the mass of powder by the occupied volume, and was expressed as g/L. The real density of samples was established by a helium pycnometer (AccPyc 1330, Micromeritics, Norcross, GA, USA).

## *2.3. Hydration Properties*

Water-holding capacity (WHC) and water retention capacity (WRC) were described in line with by Raghavendra, Rastogi, Raghavarao and Tharanathan (2004) and Chantaro, Devahastin and Chiewchan (2008), respectively.

Swelling water capacity (SWC) and fat adsorption capacity (FAC) were

described according to Navarro-González, García-Valverde, García-Alonso and Periago (2011) with minor modifications. For SWC, 1 g of sample was placed inside a graduated test tube and was hydrated with 20 mL of distilled water. Samples were stored for 18 h at 25 °C after recording the bed volume. SWC was expressed as volume mL/g sample. For the FAC determination, 4 g of samples were placed inside a centrifuge tube with 24x g of sunflower oil. Contents were stirred for 30 sec every 5 min for 30 min. Later samples were centrifuged at 1600x g for 25 min. Free oil was decanted and FAC was expressed as g oil/g sample.

The water solubility index (WSI) was analyzed according to the method of Mahdavi, Jafari, Assadpour and Ghorbani (2016) with minor modifications. Approximately 1 g of sample was mixed in centrifuge tubes with 30 mL of distilled water for 5 min until mixtures were homogeneous. Solutions were then incubated at 37 °C in a water bath for 30 min. Then, tubes were centrifuged at 17,640x g for 20 min at 4 °C. Supernatants were collected and dried in an oven at 100 °C until constant weight was achieved. The results are expressed as a percentage.

### *2.4. Antioxidant Capacity and Phenolic Compounds*

Antioxidant capacity (AC) was assessed using DPPH method following the methodology of Igual, García-Martínez, Camacho and Martínez-Navarrete (2015). UV-visible spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) was used for the absorbance at 515 nm. The final results were expressed as milligram trolox equivalents (TE) per 100 g (mg TE/100 g).

Total phenol content (PC) was carried out according to Agudelo et al. (2016). Absorbance was measured at 765 nm in a UV-visible spectrophotometer (Thermo Scientific, Helios Zeta UV-Vis, Loughborough, UK). The total phenolic content was expressed as mg of gallic acid equivalents

(GAE) (Sigma-Aldrich, Steinheim, Germany) per 100 g of sample.

### *2.5. Ash and Mineral Analysis*

The total ash content was determined following the method 930.05 of AOAC procedures (Horwith & Latimer, 2005). In total, 500 mg of sample were incinerated at high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 24 h at 550 °C, and ash was gravimetrically quantified.

The multimineral determination was analyzed in an inductively coupled plasma optical emission spectrometer, model 700 Series ICP-OES of Agilent Technologies (Santa Clara, USA), equipped with axial viewing and a charge coupled device detector (García-Segovia, Igual, Noguerol & Martínez-Monzó, 2020). Mineral compositions (macro- and microelements) were expressed as mg/100 g.

### *2.6. Gel Preparation*

Samples were dissolved in cold water (5 °C) for 30 min at concentrations of 4%, 5%, 6% and 7% and were then divided into two batches. One was directly stored for 24 h at 5 °C until gel stabilization. However, the other batch was heated at 65 °C for 20 min before being stored for 24 h at 5 °C. The samples were tempered until reaching 25 °C for their analyses.

### *2.7. Gel Analysis: Water Content, pH, Color, Texture and Rheology*

Water content ( $x_w$ ) (g water/100 g sample) was determined as in the powder fibers.

The pH of the gel samples was measured by a pH-meter Crison MultiMeter MM 41 (Hach Lange, Barcelona, Spain).

In order to determine the color of gel translucency, CIE\* $L^*a^*b^*$  colors were measured according to García-Segovia et al. (2020). The color differences ( $\Delta E$ ) associated with heating were calculated for each sample.



Textural characteristics were evaluated by a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). The back extrusion test was performed following the method described by Cevoli, Balestra, Ragni and Fabbri, 2013) with minor modifications. The employed extrusion disc had a diameter of 25.4 mm, and was positioned centrally over the sample's plastic container (diameter: 50 mm, height: 75 mm) with 40 g of sample (approximately 30 mm high). The test was performed at a depth of 50% at the 1 mm/s test speed. The attributes calculated from the force-deformation curve were the area under the curve up or consistency (N s), the maximum force or firmness (N), the maximum negative force or cohesiveness (N) and the resistance to flow off the disc or viscosity (N s).

The flow behavior of gels was measured on samples with back extrusion consistency results lower than 100 N s by following the method described by Cevoli et al. (2013) with slight modifications. Flow curves were performed using a Kinexus pro+ rotational rheometer (Malvern Instruments, Worcestershire, UK), equipped with a system of coaxial cylinders (C25/PC25), and the rSpace software. Then, 20 mL of sample was loaded into the geometry and rested for 3 min to achieve temperature equilibrium (25 °C) and stress relaxation in a heat-controlled sample stage (Peltier Cylinder Cartridge, Malvern Instruments, Worcestershire, UK). Samples were exposed to a logarithmically increase shear rate from 0 to 200 s<sup>-1</sup> in 3 min. Finally, consistency (*k*), the flow behavior index (*n*) and the apparent viscosity at a 50 s<sup>-1</sup> shear rate ( $\eta_{ap}$ ) were calculated (Ribes et al., 2021). The analyses of gels were performed in triplicate for each concentration sample.

In total 40 g of each concentration of the gel preparations was weighed on cylindrical plastic glass (diameter: 50 mm, height: 75 mm). Gel structures were removed from the container before analyzing and a texture profile analysis (TPA) was performed with a TA-XT2 Texture Analyzer (Stable Micro Systems

Ltd., Godalming, UK) and the Texture Exponent software (version 6.1.12.0). A double compression cycle test was run up to 50% strain compression of the original portion height in an aluminum cylinder probe (diameter: 75 mm) following the method described by Ađar, Genęcelep, Saricaođlu and Turhan, 2016). This analysis was performed only on samples with back extrusion consistency results over 100 N s.

### *2.8. Statistical Analysis*

An analysis of variance (ANOVA), with a 95% confidence level ( $p < 0.05$ ), was applied to evaluate the differences among samples using the Statgraphics Centurion XVII software, version 17.2.04. Furthermore, a correlation analysis among parameters, with a 95% significance level, was achieved.

## **3. Results and Discussion**

### *3.1. DF Analysis*

Table 2 shows the physico-chemical properties of the DF samples. In general, significant ( $p < 0.05$ ) differences were found among all the samples. FBPC and FPE showed the highest  $x_w$ , and no significant ( $p < 0.05$ ) differences. The sample with the significantly ( $p < 0.05$ ) lowest  $x_w$  was FC. The FC value was lower than those reported by de Moraes Crizel et al. (2013) for orange fibers. All the DF samples presented significant ( $p < 0.05$ ) differences in the  $a_w$  values, with FPE and FP displaying higher  $a_w$ . This would mean that their shelf life would be shorter because microorganisms could increase during storage. On the contrary, except for FPE and FP, the samples'  $a_w$  values fell within the ideal range (0.11 to 0.40) to avoid microorganism growth and degradation reactions (de Moraes Crizel et al., 2013). Significant differences were also observed between the pH values ( $p < 0.05$ ). FCH was the sample with the highest pH and FC had the lowest pH. The sample with the lowest Hg was FCH. This value was significantly lower than those for the other samples ( $p <$

0.05). According to Moghbeli, Jafari, Maghsoudlou and Dehnad (2020), lower hygroscopicity could be more positive because of its importance for the flowability factor during storage.

**Table 2.** Mean values  $\pm$  standard deviation of moisture ( $x_w$ ) (g<sub>w</sub>/100 g sample), water activity ( $a_w$ ), pH (dispersions 10% w/v), hygroscopicity (Hg) (g water/100 g dry solid), bulk density ( $\rho_b$ ) (g/L), porosity ( $\epsilon$ ), volume mean diameter D [4, 3] ( $\mu\text{m}$ ) and the standard percentiles d (0.1), d (0.5) and d (0.9) of the studied samples.

|            | Vegetable Fiber Samples        |                                  |                                  |                                  |                                  |                                  |
|------------|--------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
|            | FCH                            | FPE                              | FP                               | FC                               | FBPC                             | FPESB                            |
| $x_w$      | 3.47 $\pm$ 0.06 <sup>d</sup>   | 6.62 $\pm$ 0.03 <sup>a</sup>     | 6.13 $\pm$ 0.12 <sup>b</sup>     | 2.686 $\pm$ 0.118 <sup>e</sup>   | 6.676 $\pm$ 0.104 <sup>a</sup>   | 5.7 $\pm$ 0.3 <sup>c</sup>       |
| $a_w$      | 0.372 $\pm$ 0.003 <sup>c</sup> | 0.5200 $\pm$ 0.0012 <sup>a</sup> | 0.4857 $\pm$ 0.0012 <sup>b</sup> | 0.1230 $\pm$ 0.0012 <sup>f</sup> | 0.3590 $\pm$ 0.0012 <sup>d</sup> | 0.342 $\pm$ 0.002 <sup>e</sup>   |
| pH         | 6.063 $\pm$ 0.015 <sup>a</sup> | 5.25 $\pm$ 0.06 <sup>d</sup>     | 4.65 $\pm$ 0.04 <sup>e</sup>     | 4.06 $\pm$ 0.02 <sup>f</sup>     | 5.45 $\pm$ 0.02 <sup>c</sup>     | 5.6633 $\pm$ 0.0115 <sup>b</sup> |
| Hg         | 13.9 $\pm$ 0.3 <sup>d</sup>    | 19.1 $\pm$ 0.6 <sup>c</sup>      | 19.2 $\pm$ 0.8 <sup>c</sup>      | 29.3 $\pm$ 0.3 <sup>a</sup>      | 26.7 $\pm$ 0.7 <sup>b</sup>      | 27.3 $\pm$ 0.2 <sup>b</sup>      |
| $\rho_b$   | 424 $\pm$ 30 <sup>b</sup>      | 348 $\pm$ 19 <sup>c</sup>        | 211 $\pm$ 8 <sup>d</sup>         | 410 $\pm$ 27 <sup>b</sup>        | 489 $\pm$ 17 <sup>a</sup>        | 354 $\pm$ 10 <sup>c</sup>        |
| $\epsilon$ | 70.7 $\pm$ 1.3 <sup>d</sup>    | 76.9 $\pm$ 0.5 <sup>b</sup>      | 86.63 $\pm$ 0.16 <sup>a</sup>    | 73.22 $\pm$ 0.15 <sup>c</sup>    | 69.22 $\pm$ 0.95 <sup>d</sup>    | 77.51 $\pm$ 0.12 <sup>b</sup>    |
| D [4, 3]   | 126.9 $\pm$ 1.3 <sup>e</sup>   | 249 $\pm$ 14 <sup>a</sup>        | 184 $\pm$ 4 <sup>b</sup>         | 71.8 $\pm$ 0.6 <sup>f</sup>      | 142.6 $\pm$ 0.3 <sup>d</sup>     | 156 $\pm$ 2 <sup>c</sup>         |
| d (0.1)    | 23.0 $\pm$ 0.3 <sup>d</sup>    | 99 $\pm$ 3 <sup>a</sup>          | 36 $\pm$ 4 <sup>b</sup>          | 11.92 $\pm$ 0.07 <sup>e</sup>    | 36.1 $\pm$ 0.3 <sup>b</sup>      | 33.0 $\pm$ 0.4 <sup>c</sup>      |
| d (0.5)    | 104.3 $\pm$ 0.7 <sup>e</sup>   | 224 $\pm$ 12 <sup>a</sup>        | 155 $\pm$ 3 <sup>b</sup>         | 60.2 $\pm$ 0.4 <sup>f</sup>      | 124.4 $\pm$ 0.3 <sup>d</sup>     | 132.9 $\pm$ 1.3 <sup>c</sup>     |
| d (0.9)    | 263 $\pm$ 3 <sup>d</sup>       | 438 $\pm$ 29 <sup>a</sup>        | 375 $\pm$ 6 <sup>b</sup>         | 150 $\pm$ 2 <sup>e</sup>         | 276.2 $\pm$ 0.9 <sup>d</sup>     | 313 $\pm$ 6 <sup>c</sup>         |

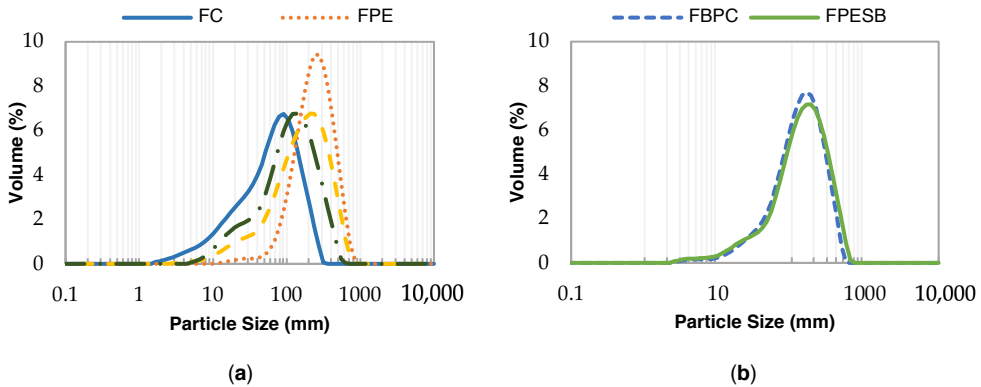
A different letter in the same row denotes a significant difference as determined by the LSD test ( $p < 0.05$ ).

The  $\rho_b$  and  $\epsilon$  values are shown in Table 2. In general, significant differences appeared in these parameters ( $p < 0.05$ ). The sample with the highest  $\rho_b$  was FBPC and, at the same time, it was the sample with the lowest  $\epsilon$ , although no significant differences were observed in  $\epsilon$  between FBPC and FCH ( $p > 0.05$ ). The lowest  $\rho_b$  was for FP. This value was slightly lower than that obtained by Huber et al. (2016) for potato fiber. Both FPESB and FPE had similar  $\rho_b$  values to potato fiber and lower values compared to pea fiber (Huber et al., 2016).

Figure 1 shows the samples' particle size distribution. Figure 1a indicates the vegetable DF samples of a different source. In this figure, the largest and the homogeneous particle size of samples are presented by FPE. The particles range lies between 60 and 830  $\mu\text{m}$ , although there is a narrow particles range

between 12 and 60  $\mu\text{m}$ . This sample also has the highest volume mean diameter value ( $D [4, 3]$ ) and standard percentiles  $d (0.1)$ ,  $d (0.5)$  and  $d (0.9)$  (Table 2). For FP, the particle size is distributed between 2.5 and 724  $\mu\text{m}$ , although it concentrates the most between 72–724  $\mu\text{m}$ . FCH shows a more concentrated particle size range between 40 and 550  $\mu\text{m}$ , but similar to FP, its particles range is narrower, between 5–40  $\mu\text{m}$ . The more marked peak appears for FC, which means that the particle size is more heterogeneous. We can observe that the more concentrated range in FC lies between 40 and 363  $\mu\text{m}$ , but it presents the largest volume of a small particles size, between 1.9 and 40  $\mu\text{m}$ . For both vegetable DF combinations (Figure 1b), the particle size distribution is similar, between 2.9 and 550  $\mu\text{m}$ , but the FPESB sample has a higher  $D [4, 3]$  (Table 2). Finally, in Figure 1a,b, all the samples vastly differ. This is also observed in Table 2, where significant ( $p < 0.05$ ) differences in  $D [4, 3]$ ,  $d (0.1)$ ,  $d (0.5)$  and  $d (0.9)$  are shown for all the samples. According to Rosell, Santos and Collar (2009), it is vital to know the particle size distribution of DFs because this parameter can determine fiber functionality and its role in the digestive tract (transit time, fermentation, fecal excretion).

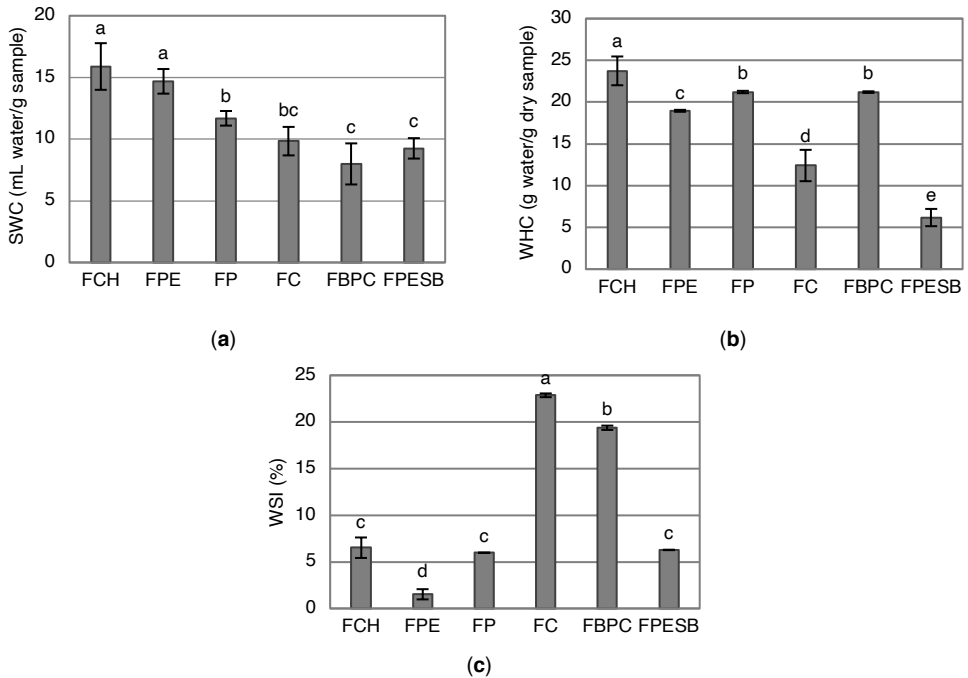
Analyzing the hydration properties is important because they can determine the fate of DF in the digestive tract and explain some of its physiological effects (Guillon & Champ, 2000). Figure 2 shows the hydration properties (a) SWC, (b) WHC and (c) WSI of the tested DF samples. SWC is the volume occupied by a known weight of fiber under the applied condition. The SWC values of FCH and FPE were significantly higher than for the other samples ( $p < 0.05$ ) (Figure 2a). The SWC of FPE was comparable to that shown by Huber et al. (2016) for pea fiber, although the FP results were higher than potato fiber SWC reported by this author, but similar to that indicated by Kaack, Pedersen, Laerke and Meyer (2006) for potato pulp fiber. Wang et al. (2015) obtained higher results than FC SWC for different sources of citrus fiber.



**Figure 1.** Volume of the particle size distributions (representative curves) of the studied fibers, where (a) presents the vegetable DF samples of one different source and (b) denotes the samples made with a combination of vegetable DFs.

Rosell et al. (2009) indicated that WHC was the amount of water retained by samples without being subjected to any stress. All the samples showed significant differences for the water-holding capacity ( $p < 0.05$ ) (Figure 2b), except between FP and FBPC, and the highest WHC was shown by FCH, while FPESB presented the lowest value. Mancebo, Rodríguez, Martínez and Gómez (2018) reported lower WHC for pea and potato fibers than the results herein shown for FPE and FP. However, the results reported by Wang et al. (2015) for different citrus fibers were higher than the FC WHC result.

For WSI (Figure 2c), FC showed higher solubility, with significant ( $p < 0.05$ ) differences among the other samples. The differences in the WSI of samples could be related to the nature of the glycidyl component and the structural characteristics of fiber (de Moraes Crizel et al., 2013). Moreover, high solubility can inhibit the digestion and absorption of nutrients (glucose and cholesterol) from the gut (Guillon & Champ, 2000; Belorio, Marcondes & Gómez (2020). The results of WSI for FC was lower than the results reported by de Moraes Crizel et al. (2013) for DF from orange.



**Figure 2.** Mean values and standard deviations of (a) the swelling capacity (SWC), (b) the water-holding capacity (WHC) and (c) the solubility (WSI) of the DF samples. Letters indicate the homogeneous groups established by the ANOVA ( $p < 0.05$ ).

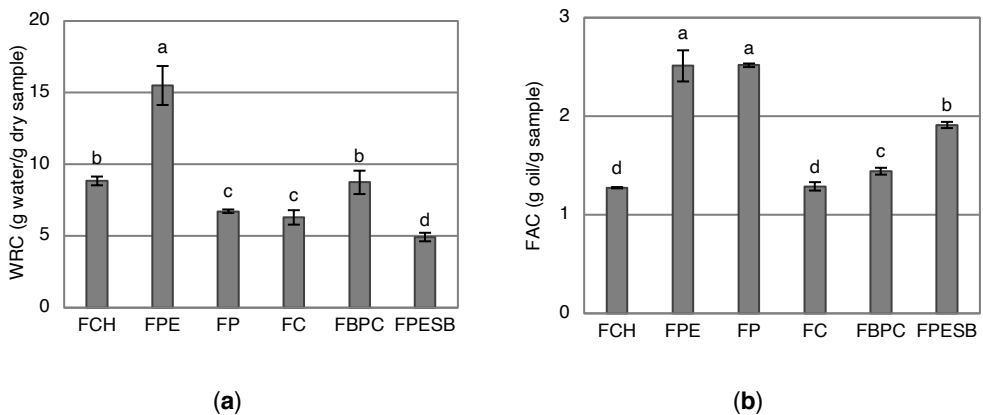
The ability of DF to retain water when subjected to an external force such as centrifugation is presented by WRC (Ma & Mu, 2016). Both SWC and WRC can provide an overview of fiber hydration, and it is vital to know if fiber is suitable to be used in supplemented foods (Guillon & Champ, 2000). Figure 3a depicts the WRC values of studied samples, where we can see that the DF whose WRC was significantly ( $p < 0.05$ ) higher was FPE, and the significant ( $p < 0.05$ ) lowest WRC was for FPESB. These results are notably lower than those reported by Lan, Chen, Chen and Tian, (2012) for the DF isolated from *P. odoratum* by drying in the sun. However, the FPE and FP values were higher than those obtained for potato and pea fibers according to Huber et al. (2016). WRC was lower for FCH than the 15.41 g water/g sample of chia fiber

(Vázquez-Ovando, Rosado-Rubio, Chel-Guerrero & Betancur-Ancona, 2009) Moreover, a DF with high WRC can be used as a functional food ingredient to cut calories, avoid syneresis and modify both the viscosity and texture of processed food (de Moraes Crizel et al., 2013; Grigelmo-Miguel & Martín-Belloso, 1999).

The ability of the fiber to absorb fat or oil lies in FAC. Figure 3b shows the FAC values of the studied samples. The samples with the highest FAC were FPE and FP, and no significant differences were found between them ( $p > 0.05$ ). These results were similar to those shown by Huber et al. (2016) for pea and potato fiber, and also to the pea fiber FAC reported by Mancebo et al. (2018). These values were notably higher than those reported by Navarro-González et al. (2011) in tomato peel fiber. However, FCH and FC had the lowest FAC values, and no significant differences appeared between them ( $p > 0.05$ ); however, the results reported by Wang et al. (2015) for citrus fibers were higher. For FCH, higher results were found for chia fiber (2.02 g oil/g sample) (Vázquez-Ovando et al., 2009). It is vital to know this parameter because it means that high FAC could prevent fat loss during cooking, and also in nutrition by absorbing fat in the intestinal lumen which lowers cholesterol, and can also help to retain food flavor (de Moraes Crizel, 2013; Navarro-González et al., 2011; Belorio et al., 2020). Therefore, the DF samples, which generally presented better hydration properties, were FPE, FP, FCH and FBPC for water binding, and FP and FPE for oil retention.

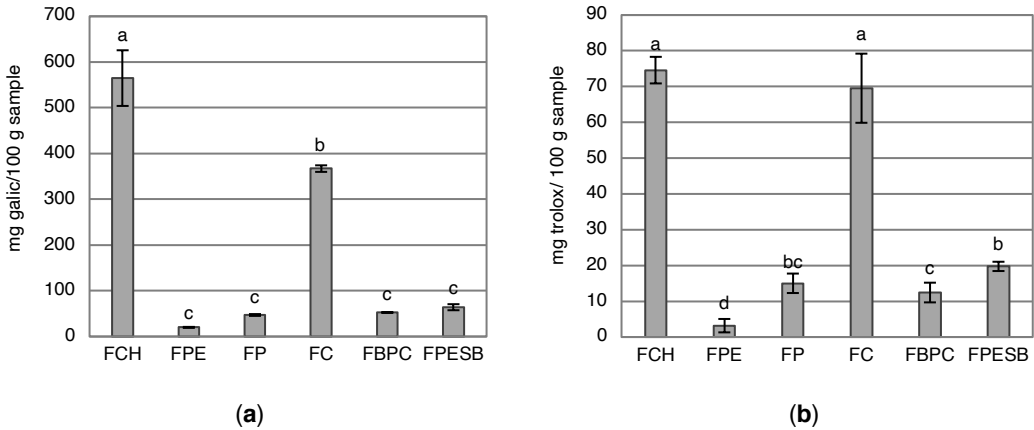
WSI presented a significant negative Pearson's correlation for particle size (D [4, 3], d (0.1), d (0.5) and d (0.9)) ( $p < 0.05$ ), and the higher WSI was, the smaller the particle size became: -0.7902, -0.6083, -0.7576 and -0.8342, respectively. However, FAC and WRC presented a significant ( $p < 0.05$ ) positive correlation with particle size (D [4, 3], d (0.1), d (0.5), d (0.9)): 0.8636, 0.7059, 0.8481 and 0.8873 for FAC and 0.6866, 0.8658, 0.7210 and 0.5847 for

WRC, respectively. This means that when the samples' particle size grew, the absorption capacity of oil and water increased. Authors such as Lan et al. (2012) indicated that an increased particle size is related to a better ability to bind oil and water, although this effect cannot be generalized because the hydration properties of DF were related to the chemical structure of component polysaccharides, and to other factors such as porosity, particle size, ionic forms, pH, temperature, ionic strength, type of ions in solution and stresses on fibers (Elleuch et al., 2011). Furthermore, FAC showed positive correlations with  $\varepsilon$  (0.8219) when  $\varepsilon$  presents high values, and the capacity of DF to absorb oil increased. Finally, Hg showed negative correlations with hydration properties (WHC and SWC),  $-0.7071$  and  $-0.8322$ , respectively, and higher Hg values implied less ability to absorb water. These results showed how variable fibers are and the importance of analyzing the characteristic of each type of fiber, as their composition plays a key role (Chantaro et al., 2008; Rosell et al., 2009; Tejeda-Ortigoza, García-Amezquita, Serna-Saldívar & Welti-Chanes, 2016).



**Figure 3.** Mean values and standard deviations of (a) water retention capacity (WRC) and (b) fat absorption capacity (FAC) of DF samples. Letters indicate homogeneous groups established by the ANOVA ( $p < 0.05$ ).





**Figure 4.** Mean values and standard deviations of (a) the samples' total phenol content and (b) antioxidant capacity. Letters indicate the homogeneous groups established by the ANOVA ( $p < 0.05$ ).

Figure 4 shows the total phenol content (a) and the antioxidant capacity (b) of the tested samples. The higher values of the total phenol content and antioxidant capacity in the FCH and FC samples are quite remarkable. FCH has the highest phenol content with significant differences compared to the other samples (Figure 3a). However, these differences are not significant compared to FC in antioxidant terms (Figure 3b). The FC total phenol content is similar to the total phenol concentrations in grapefruit powder (Agudelo et al., 2016) and for orange freeze-drying (Silva-Espinoza, Camacho & Martínez-Navarrete, 2021). The antioxidant capacity values of FP fall within the same range as other studies performed with soluble dietary potato fiber (Xie et al., 2017). Pearson's correlation between total phenol content and antioxidant capacity was 0.9538. Thus, the total phenol content has a strong effect on the antioxidant capacity, as observed in other studies with Lulo (*Solanum quitoense*) (Igal, Ramires, Mosquera & Martínez-Navarrete, 2014) and grapefruit (Igal et al., 2019). FCH and FC can be an important functional fiber if employed as a thickener or gelling agent because they have a high total

phenol content and a good antioxidant capacity.

Table 3 shows the ash content, total minerals and mineral contents of the DF samples. All the samples generally showed a high mineral content. Both ash content and total mineral content were significantly different in all the studied samples ( $p < 0.05$ ), and the FCH sample presented the highest content for both ash and minerals. It could be related to FCH, which is not an isolated dietary fiber, because it contains chia seed flour, which has a high percentage of total dietary fiber (Table 1). The K value in FP, FBPC and FPESB, whose formulation includes sugar cane, was similar to some results obtained by Chong, Ball, McRae and Packer (2019) for sugar cane fiber, and by Ma and Mu (2016) for DFs, obtained from de-oiled cumin. FPE, FP and FPESB have obtained comparable values for Zn (Chong et al., 2019), as have FPE and FBPC for Ca (Ma & Mu, 2016). Llorent-Martínez, Córdova, Ortega-Barrales, Ruiz-Medina (2013) reported similar chia seed values to those observed for FCH for minerals P, Mg and Zn, but the FCH values for Ca, Cu and Na were higher, and the Na content was much higher in FCH. However, the Fe, Mn and K contents of FCH were lower (Llorent-Martínez et al., 2013). Some values should be highlighted, such as Fe content, and also other trace elements, such as Mn, Zn and Cu for FCH, because Fe and Zn deficiency is a worldwide health problem, with about 30–33% of the world's population at risk, particularly in underdeveloped countries (Rousseau et al., 2019).

SWC presented a significant Pearson's correlation when related to P, Na, Zn and Cu ( $p < 0.05$ ), and the higher SWC, the higher the content of these minerals: 0.6257, 0.8272, 0.6617 and 0.6332, respectively. WRC also correlated positively with Na (0.8153) ( $p < 0.05$ ). However, negative correlations were found between WHC and Se ( $-0.6206$ ), and between WSI and Na ( $-0.6830$ ), and also when FAC was related to K and Ca ( $-0.6885$  and  $-0.6896$ , respectively). Antioxidant capacity also showed positive correlations

when associated with ash, total mineral content, P, K, Ca, Mg, Cu and Mn (0.7928, 0.8770, 0.6995, 0.8211, 0.9449 and 0.6749, respectively) ( $p < 0.05$ ). At the same time, total phenol content also presented positive correlations with ash, total mineral content, P, K, Ca, Mg, Zn, Cu and Mn (0.8895, 0.9561, 0.8464, 0.9217, 0.8621, 0.8101, 0.7792, 0.8196 and 0.7591, respectively) ( $p < 0.05$ ). Although soluble and insoluble DF, and components such as polyphenols, are associated with a lower mineral absorption in the small intestine because of binding and/or physical entrapment, this is believed to be compensated by the fermentation of fibers in the colon by gut microflora, as short-chain fatty acids are produced that can release trapped minerals to increase the absorptive surface area and, hence, improve their absorption. This situation has been observed to be significant if deficient (Baye, Guyot & Mouquet-Rivier, 2016).

**Table 3.** Mean values  $\pm$  standard deviations of ash (%), total minerals (mg/100 g sample) and mineral content (mg/100 g sample).

|                | Vegetable Fiber Samples      |                                |                                  |                                |                                |                              |
|----------------|------------------------------|--------------------------------|----------------------------------|--------------------------------|--------------------------------|------------------------------|
|                | FCH                          | FPE                            | FP                               | FC                             | FBPC                           | FPESB                        |
| Ash (%)        | 8.01 $\pm$ 0.04 <sup>a</sup> | 1.1 $\pm$ 0.2 <sup>d</sup>     | 2.36 $\pm$ 0.03 <sup>c</sup>     | 2.9 $\pm$ 0.5 <sup>b</sup>     | 0.60 $\pm$ 0.03 <sup>e</sup>   | 2.29 $\pm$ 0.04 <sup>c</sup> |
| Total minerals | 2460 $\pm$ 22 <sup>a</sup>   | 453 $\pm$ 79 <sup>d</sup>      | 510 $\pm$ 42 <sup>d</sup>        | 1227 $\pm$ 34 <sup>b</sup>     | 316 $\pm$ 18 <sup>e</sup>      | 746 $\pm$ 29 <sup>c</sup>    |
| P              | 582 $\pm$ 33 <sup>a</sup>    | 29 $\pm$ 10 <sup>c</sup>       | 26 $\pm$ 2 <sup>c</sup>          | 60 $\pm$ 5 <sup>b</sup>        | 7.9 $\pm$ 0.7 <sup>c</sup>     | 32.9 $\pm$ 1.4 <sup>c</sup>  |
| K              | 639 $\pm$ 6 <sup>a</sup>     | 42 $\pm$ 8 <sup>f</sup>        | 101 $\pm$ 10 <sup>e</sup>        | 241 $\pm$ 16 <sup>b</sup>      | 167 $\pm$ 14 <sup>c</sup>      | 141 $\pm$ 7 <sup>d</sup>     |
| Ca             | 672 $\pm$ 25 <sup>b</sup>    | 71 $\pm$ 24 <sup>e</sup>       | 181 $\pm$ 13 <sup>d</sup>        | 784 $\pm$ 26 <sup>a</sup>      | 96 $\pm$ 3 <sup>e</sup>        | 340 $\pm$ 16 <sup>c</sup>    |
| Na             | 170 $\pm$ 12 <sup>b</sup>    | 275 $\pm$ 39 <sup>a</sup>      | 116 $\pm$ 12 <sup>c</sup>        | 73 $\pm$ 2 <sup>d</sup>        | 40 $\pm$ 4 <sup>e</sup>        | 60 $\pm$ 3 <sup>de</sup>     |
| Mg             | 380 $\pm$ 23 <sup>a</sup>    | 33 $\pm$ 12 <sup>d</sup>       | 81 $\pm$ 7 <sup>c</sup>          | 66 $\pm$ 5 <sup>c</sup>        | 3.706 $\pm$ 1.002 <sup>e</sup> | 107 $\pm$ 4 <sup>b</sup>     |
| Zn             | 6.4 $\pm$ 0.9 <sup>a</sup>   | 0.6 $\pm$ 0.3 <sup>b</sup>     | 0.22 $\pm$ 0.05 <sup>b</sup>     | - b                            | - b                            | 0.58 $\pm$ 0.03 <sup>b</sup> |
| Fe             | 6.4 $\pm$ 0.6 <sup>a</sup>   | 1.6 $\pm$ 0.9 <sup>c</sup>     | 4.7 $\pm$ 0.3 <sup>b</sup>       | 2.1 $\pm$ 0.2 <sup>c</sup>     | 1.6 $\pm$ 0.3 <sup>c</sup>     | 4.2 $\pm$ 0.5 <sup>b</sup>   |
| Mn             | 2.74 $\pm$ 0.14 <sup>a</sup> | - c                            | - c                              | - c                            | - c                            | 0.75 $\pm$ 0.06 <sup>b</sup> |
| Cu             | 1.29 $\pm$ 0.07 <sup>a</sup> | - b                            | - b                              | - b                            | - b                            | - b                          |
| Se             | - d                          | 0.046 $\pm$ 0.003 <sup>b</sup> | 0.0278 $\pm$ 0.0009 <sup>c</sup> | 0.058 $\pm$ 0.013 <sup>a</sup> | - d                            | - d                          |

A different letter in the same row is significantly different as determined by the LSD test ( $p < 0.05$ ).

### 3.2. Gel Analysis

Gels were prepared from each fiber sample at concentrations of 4%, 5%, 6% and 7% to know the physico-chemical and mechanical properties and their possible use in food products. Table 4 depicts the physico-chemical properties ( $x_w$  and pH) and the gel samples' color at each concentration. Samples' water content ( $x_w$ ) showed a decrease in both (with and without heat treatment) when fiber concentration rose. Moreover, an interaction took place between the fiber concentration and the DF samples. For fiber sample FPE, the decrease was less intense, and this fiber presented the highest  $x_w$  at all the tested concentrations both with and without heat treatment, with significantly higher values at concentrations of 5%, 6% and 7% than the other fiber samples when gels were heated ( $p < 0.05$ ). This could be because the FPE fiber presented the lowest solubility (Figure 2c), and thus, left more free water. In general terms, no significant effect on the  $x_w$  of the gel concentrations tested per fiber was observed due to heat treatment ( $p > 0.05$ ).

Samples' pH value was significantly different in both cases with and without heat treatment ( $p < 0.05$ ) (Table 4), and the pH range went from 6.56 to 4.14. Only for dispersion, no significant differences were found ( $p > 0.05$ ) for samples 4, 5 and 6% made with FPE and FPESB. For 7% gel, the pH of the FPESB sample was higher pH than the FPE gel ( $p < 0.05$ ). In both cases, the different dispersions made with the FCH sample presented the highest pH, and the lowest pH was observed in the FC fiber dispersions, which agrees with the pH values observed in the 10% w/v dispersion of fibers (Table 2). For pH, an interaction between the concentration and fiber sample also took place, and the pH values were slightly lowered for all the fiber samples ( $p < 0.05$ ) when concentrations rose. As in  $x_w$ , no significant effect on pH was generally observed on the dispersion concentrations for each fiber due to heat treatment, except for FCH, which had a slightly higher pH in the heated sample

## Capítulo 4. Caracterización fibras

dispersions ( $p < 0.05$ ).

**Table 4.** Mean values  $\pm$  standard deviations of the  $x_w$  (g/100 g sample), pH and color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) of the formulated gels.

| Sample | C | $x_w$                                | pH                               | $L^*$                           | $a^*$                              | $b^*$                              | $\Delta E$                      |
|--------|---|--------------------------------------|----------------------------------|---------------------------------|------------------------------------|------------------------------------|---------------------------------|
| FCH    | 4 | 96.35 $\pm$ 0.04 <sup>abA</sup>      | 6.44 $\pm$ 0.02 <sup>aCD</sup>   | 26.0 $\pm$ 0.2 <sup>kC</sup>    | 2.51 $\pm$ 0.09 <sup>dD</sup>      | 9.5 $\pm$ 0.7 <sup>dE</sup>        |                                 |
|        | 5 | 95.48 $\pm$ 0.03 <sup>dB</sup>       | 6.38 $\pm$ 0.13 <sup>bcEF</sup>  | 27.3 $\pm$ 0.3 <sup>hijB</sup>  | 3.07 $\pm$ 0.06 <sup>cC</sup>      | 11.2 $\pm$ 0.4 <sup>bCD</sup>      |                                 |
|        | 6 | 95.37 $\pm$ 0.04 <sup>dB</sup>       | 6.41 $\pm$ 0.05 <sup>bDE</sup>   | 29.4 $\pm$ 0.5 <sup>gA</sup>    | 3.58 $\pm$ 0.14 <sup>aA</sup>      | 12.0 $\pm$ 0.6 <sup>aBC</sup>      |                                 |
|        | 7 | 93.52 $\pm$ 1.06 <sup>IC</sup>       | 6.4 $\pm$ 0.2 <sup>cF</sup>      | 29.0 $\pm$ 0.5 <sup>gA</sup>    | 3.19 $\pm$ 0.13 <sup>bC</sup>      | 11.12 $\pm$ 0.12 <sup>bCD</sup>    |                                 |
| 65FCH  | 4 | 96.21 $\pm$ 0.09 <sup>yxA</sup>      | 6.56 $\pm$ 0.02 <sup>zA</sup>    | 26.6 $\pm$ 0.4 <sup>rBC</sup>   | 2.66 $\pm$ 0.12 <sup>wD</sup>      | 10.7 $\pm$ 0.6 <sup>yD</sup>       | 1.4 $\pm$ 0.7 <sup>efgh</sup>   |
|        | 5 | 95.519 $\pm$ 0.116 <sup>vuB</sup>    | 6.50 $\pm$ 0.19 <sup>yB</sup>    | 28.8 $\pm$ 1.5 <sup>sA</sup>    | 3.7 $\pm$ 0.4 <sup>zA</sup>        | 13.1100 $\pm$ 1.0012 <sup>zA</sup> | 1.7 $\pm$ 0.9 <sup>defg</sup>   |
|        | 6 | 94.94 $\pm$ 0.16 <sup>srqB</sup>     | 6.452 $\pm$ 0.009 <sup>xC</sup>  | 29.2 $\pm$ 0.6 <sup>tsA</sup>   | 3.51 $\pm$ 0.19 <sup>yAB</sup>     | 12.8 $\pm$ 0.8 <sup>zAB</sup>      | 1.3 $\pm$ 0.2 <sup>efghi</sup>  |
|        | 7 | 95.26 $\pm$ 0.06 <sup>utsB</sup>     | 6.4 $\pm$ 0.3 <sup>xCD</sup>     | 29.1 $\pm$ 0.5 <sup>tsA</sup>   | 3.25 $\pm$ 0.19 <sup>xBC</sup>     | 11.2 $\pm$ 0.9 <sup>yCD</sup>      | 1.0 $\pm$ 0.4 <sup>fg hij</sup> |
| FPE    | 4 | 96.7 $\pm$ 0.3 <sup>aA</sup>         | 5.88 $\pm$ 0.03 <sup>gA</sup>    | 33.2 $\pm$ 0.6 <sup>eD</sup>    | 0.10 $\pm$ 0.03 <sup>ID</sup>      | 2.1 $\pm$ 0.3 <sup>mC</sup>        |                                 |
|        | 5 | 96.1 $\pm$ 0.3 <sup>bcB</sup>        | 5.76 $\pm$ 0.13 <sup>hB</sup>    | 39.1 $\pm$ 0.3 <sup>cB</sup>    | 0.14 $\pm$ 0.03 <sup>ID</sup>      | 3.186 $\pm$ 0.113 <sup>jkA</sup>   |                                 |
|        | 6 | 95.57 $\pm$ 0.07 <sup>dC</sup>       | 5.64 $\pm$ 0.06 <sup>iC</sup>    | 35.5 $\pm$ 1.9 <sup>dC</sup>    | 0.11 $\pm$ 0.04 <sup>ID</sup>      | 2.6 $\pm$ 0.5 <sup>B</sup>         |                                 |
|        | 7 | 94.4 $\pm$ 0.5 <sup>eD</sup>         | 5.482 $\pm$ 0.108 <sup>D</sup>   | 41.31 $\pm$ 1.05 <sup>abA</sup> | 0.16 $\pm$ 0.05 <sup>C</sup>       | 3.4 $\pm$ 0.2 <sup>A</sup>         |                                 |
| 65FPE  | 4 | 96.77 $\pm$ 0.13 <sup>zA</sup>       | 5.85 $\pm$ 0.04 <sup>sA</sup>    | 33.1 $\pm$ 1.7 <sup>vD</sup>    | -0.060 $\pm$ 0.012 <sup>poBC</sup> | 0.65 $\pm$ 0.08 <sup>nD</sup>      | 2.1 $\pm$ 0.5 <sup>bcd e</sup>  |
|        | 5 | 96.3845 $\pm$ 0.1103 <sup>zyAB</sup> | 5.61 $\pm$ 0.09 <sup>pC</sup>    | 34.6 $\pm$ 0.7 <sup>wvCD</sup>  | -0.052 $\pm$ 0.009 <sup>poAB</sup> | 0.74 $\pm$ 0.04 <sup>nD</sup>      | 5.5 $\pm$ 0.4 <sup>a</sup>      |
|        | 6 | 96.0 $\pm$ 0.4 <sup>xwBC</sup>       | 5.49 $\pm$ 0.06 <sup>oD</sup>    | 30.7 $\pm$ 1.3 <sup>uE</sup>    | -0.08 $\pm$ 0.03 <sup>oB</sup>     | 0.63 $\pm$ 0.08 <sup>nD</sup>      | 6.5 $\pm$ 0.4 <sup>a</sup>      |
|        | 7 | 94.686 $\pm$ 0.112 <sup>qpoD</sup>   | 5.448 $\pm$ 0.118 <sup>nD</sup>  | 39 $\pm$ 2 <sup>yxB</sup>       | 0.05 $\pm$ 0.03 <sup>qpoA</sup>    | 2.3 $\pm$ 0.4 <sup>pBC</sup>       | 3.7 $\pm$ 0.7 <sup>bc</sup>     |
| FP     | 4 | 96.3 $\pm$ 0.2 <sup>abA</sup>        | 5.04 $\pm$ 0.05 <sup>kA</sup>    | 28.12 $\pm$ 1.18 <sup>hE</sup>  | 0.514 $\pm$ 0.102 <sup>JC</sup>    | 4.4 $\pm$ 0.4 <sup>ID</sup>        |                                 |
|        | 5 | 95.39 $\pm$ 0.14 <sup>dB</sup>       | 4.944 $\pm$ 0.018 <sup>C</sup>   | 33.9 $\pm$ 0.9 <sup>eD</sup>    | 0.912 $\pm$ 0.109 <sup>IAB</sup>   | 7.6 $\pm$ 0.2 <sup>IB</sup>        |                                 |
|        | 6 | 94.9 $\pm$ 0.4 <sup>eBC</sup>        | 4.91 $\pm$ 0.03 <sup>ID</sup>    | 38.35 $\pm$ 1.08 <sup>cC</sup>  | 0.87 $\pm$ 0.12 <sup>fghAB</sup>   | 7.9 $\pm$ 0.6 <sup>IAB</sup>       |                                 |
|        | 7 | 94.6 $\pm$ 0.3 <sup>eC</sup>         | 4.9 $\pm$ 0.9 <sup>mE</sup>      | 41.6 $\pm$ 0.6 <sup>aA</sup>    | 1.08 $\pm$ 0.16 <sup>eA</sup>      | 8.7 $\pm$ 0.7 <sup>eA</sup>        |                                 |
| 65FP   | 4 | 96.5 $\pm$ 0.8 <sup>zyA</sup>        | 5.00 $\pm$ 0.03 <sup>mB</sup>    | 30.25 $\pm$ 1.06 <sup>utE</sup> | 0.6 $\pm$ 0.3 <sup>tsC</sup>       | 6.0 $\pm$ 0.9 <sup>IC</sup>        | 2.9 $\pm$ 0.5 <sup>b</sup>      |
|        | 5 | 95.36 $\pm$ 0.09 <sup>vutB</sup>     | 4.930 $\pm$ 0.014 <sup>ICD</sup> | 33 $\pm$ 2 <sup>vD</sup>        | 0.5 $\pm$ 0.2 <sup>sC</sup>        | 6.1 $\pm$ 0.6 <sup>IC</sup>        | 1.6 $\pm$ 0.6 <sup>cdef</sup>   |
|        | 6 | 94.86 $\pm$ 0.15 <sup>rqpBC</sup>    | 4.882 $\pm$ 0.013 <sup>kE</sup>  | 38 $\pm$ 2 <sup>xC</sup>        | 0.7 $\pm$ 0.2 <sup>utsBC</sup>     | 7.6 $\pm$ 0.9 <sup>vuB</sup>       | 1.3 $\pm$ 0.9 <sup>efghi</sup>  |
|        | 7 | 94.2 $\pm$ 0.6 <sup>nmC</sup>        | 4.9 $\pm$ 0.9 <sup>IE</sup>      | 39.5 $\pm$ 1.6 <sup>yB</sup>    | 0.7 $\pm$ 0.2 <sup>vutBC</sup>     | 8.1 $\pm$ 0.9 <sup>vAB</sup>       | 2.8 $\pm$ 0.9 <sup>b</sup>      |

For the samples with the same conditions, the same lowercase letter in superscript in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ) (a–n for unheated, z–o for heated samples). To compare the same sample with the temperature effect, the same capital superscript letter in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ).  $\Delta E$  indicates color differences associated with the heat treatment. C: Concentration (%).

Table 4. (Continued)

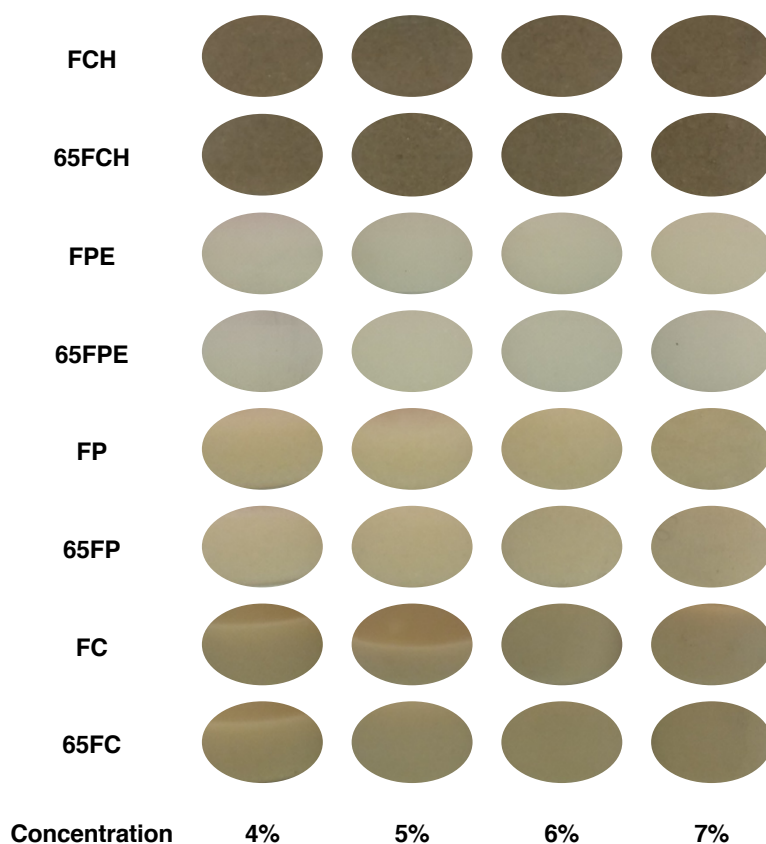
| Sample  | C | $x_w$                         | pH                           | $L^*$                      | $a^*$                          | $b^*$                         | $\Delta E$                 |
|---------|---|-------------------------------|------------------------------|----------------------------|--------------------------------|-------------------------------|----------------------------|
| FC      | 4 | 96.40 ± 0.06 <sup>abA</sup>   | 4.26 ± 0.02 <sup>nA</sup>    | 35.31 ± 0.13 <sup>dG</sup> | -0.12 ± 0.06 <sup>mF</sup>     | 7.49 ± 0.16 <sup>gG</sup>     |                            |
|         | 5 | 95.52 ± 0.04 <sup>dB</sup>    | 4.184 ± 0.009 <sup>oD</sup>  | 38.85 ± 0.12 <sup>ce</sup> | 0.32 ± 0.03 <sup>kD</sup>      | 9.62 ± 0.19 <sup>dE</sup>     |                            |
|         | 6 | 94.50 ± 0.05 <sup>eD</sup>    | 4.180 ± 0.009 <sup>oD</sup>  | 40.5 ± 0.2 <sup>bC</sup>   | 0.41 ± 0.07 <sup>jkC</sup>     | 10.7 ± 0.3 <sup>cC</sup>      |                            |
|         | 7 | 93.80 ± 0.03 <sup>fE</sup>    | 4.14 ± 1.04 <sup>pE</sup>    | 42.1 ± 0.4 <sup>aA</sup>   | 0.79 ± 0.07 <sup>hA</sup>      | 12.09 ± 0.19 <sup>aA</sup>    |                            |
| 65FC    | 4 | 96.32 ± 0.17 <sup>yxA</sup>   | 4.226 ± 0.015 <sup>B</sup>   | 35.2 ± 0.3 <sup>wG</sup>   | -0.06 ± 0.02 <sup>poF</sup>    | 7.20 ± 0.14 <sup>uH</sup>     | 0.43 ± 0.15 <sup>ij</sup>  |
|         | 5 | 95.09 ± 0.06 <sup>tsrC</sup>  | 4.200 ± 0.002 <sup>hC</sup>  | 38.2 ± 0.4 <sup>yxF</sup>  | 0.1940 ± 0.1108 <sup>qE</sup>  | 9.0380 ± 0.1114 <sup>wF</sup> | 0.9 ± 0.4 <sup>ghij</sup>  |
|         | 6 | 94.50 ± 0.03 <sup>ponD</sup>  | 4.180 ± 0.007 <sup>hgD</sup> | 39.4 ± 0.4 <sup>yD</sup>   | 0.54 ± 0.03 <sup>sB</sup>      | 9.9 ± 0.4 <sup>xD</sup>       | 1.4 ± 0.5 <sup>efgh</sup>  |
|         | 7 | 93.72 ± 0.03 <sup>fE</sup>    | 4.15 ± 1.05 <sup>gE</sup>    | 41.06 ± 0.13 <sup>zB</sup> | 0.74 ± 0.06 <sup>vutA</sup>    | 11.28 ± 0.09 <sup>yB</sup>    | 1.3 ± 0.4 <sup>efghi</sup> |
| FBPC    | 4 | 96.50 ± 0.08 <sup>abA</sup>   | 6.09 ± 0.06 <sup>dA</sup>    | 17.0 ± 0.2 <sup>nG</sup>   | -0.21 ± 0.03 <sup>nd</sup>     | 0.43 ± 0.08 <sup>nG</sup>     |                            |
|         | 5 | 95.63 ± 0.03 <sup>cdB</sup>   | 5.98 ± 0.02 <sup>eC</sup>    | 20.3 ± 0.4 <sup>mF</sup>   | -0.118 ± 0.009 <sup>mnC</sup>  | 0.77 ± 0.12 <sup>nF</sup>     |                            |
|         | 6 | 94.68 ± 0.03 <sup>eD</sup>    | 5.99 ± 0.07 <sup>eC</sup>    | 26.7 ± 0.5 <sup>jkB</sup>  | 0.10 ± 0.05 <sup>IB</sup>      | 2.78 ± 0.13 <sup>kiC</sup>    |                            |
|         | 7 | 93.72 ± 0.05 <sup>fF</sup>    | 5.9 ± 0.5 <sup>gD</sup>      | 28.0 ± 0.4 <sup>hiA</sup>  | 0.19 ± 0.03 <sup>IA</sup>      | 3.43 ± 0.15 <sup>IA</sup>     |                            |
| 65FBPC  | 4 | 96.44 ± 0.06 <sup>zyA</sup>   | 6.05 ± 0.05 <sup>wB</sup>    | 21.51 ± 1.18 <sup>oE</sup> | -0.11 ± 0.04 <sup>oC</sup>     | 1.6 ± 0.4 <sup>eE</sup>       | 5.6 ± 0.3 <sup>a</sup>     |
|         | 5 | 95.46 ± 0.09 <sup>vutC</sup>  | 5.97 ± 0.02 <sup>vc</sup>    | 23.0 ± 0.7 <sup>pD</sup>   | -0.08 ± 0.07 <sup>poC</sup>    | 2.2 ± 0.3 <sup>poD</sup>      | 3.1 ± 0.8 <sup>b</sup>     |
|         | 6 | 94.47 ± 0.05 <sup>onE</sup>   | 5.97 ± 0.06 <sup>vc</sup>    | 25.3 ± 0.2 <sup>qC</sup>   | 0.06 ± 0.02 <sup>qpoB</sup>    | 3.14 ± 0.18 <sup>qB</sup>     | 1.5 ± 0.6 <sup>defg</sup>  |
|         | 7 | 93.78 ± 0.16 <sup>fF</sup>    | 5.9 ± 0.5 <sup>uD</sup>      | 28.1 ± 0.3 <sup>sA</sup>   | 0.1060 ± 0.0114 <sup>qpB</sup> | 3.53 ± 0.13 <sup>qA</sup>     | 0.4 ± 0.3 <sup>j</sup>     |
| FPESB   | 4 | 96.15 ± 0.06 <sup>bA</sup>    | 5.93 ± 0.02 <sup>IA</sup>    | 22.1 ± 0.8 <sup>IE</sup>   | 0.45 ± 0.04 <sup>IE</sup>      | 3.23 ± 0.08 <sup>ID</sup>     |                            |
|         | 5 | 95.51 ± 0.09 <sup>dC</sup>    | 5.76 ± 0.02 <sup>hC</sup>    | 27.2 ± 0.4 <sup>ijD</sup>  | 0.67 ± 0.07 <sup>IC</sup>      | 4.6 ± 0.3 <sup>hiC</sup>      |                            |
|         | 6 | 94.77 ± 0.13 <sup>eD</sup>    | 5.67 ± 0.02 <sup>IE</sup>    | 29.2 ± 0.5 <sup>gC</sup>   | 0.81 ± 0.09 <sup>ghB</sup>     | 5.0 ± 0.3 <sup>ghB</sup>      |                            |
|         | 7 | 93.93 ± 0.06 <sup>fE</sup>    | 5.65 ± 0.02 <sup>IE</sup>    | 30.6 ± 0.2 <sup>fB</sup>   | 0.89 ± 0.03 <sup>fgA</sup>     | 5.2 ± 0.3 <sup>gB</sup>       |                            |
| 65FPESB | 4 | 96.23 ± 0.09 <sup>yxA</sup>   | 5.93 ± 0.03 <sup>VA</sup>    | 20.2 ± 0.7 <sup>oF</sup>   | 0.33 ± 0.05 <sup>rF</sup>      | 2.5 ± 0.3 <sup>pE</sup>       | 2.5 ± 0.9 <sup>bcd</sup>   |
|         | 5 | 95.69 ± 0.07 <sup>wwB</sup>   | 5.85 ± 0.04 <sup>utB</sup>   | 26.7 ± 0.4 <sup>rD</sup>   | 0.60 ± 0.03 <sup>tsD</sup>     | 4.28 ± 0.07 <sup>rC</sup>     | 0.4 ± 0.2 <sup>ij</sup>    |
|         | 6 | 94.63 ± 0.09 <sup>qponD</sup> | 5.77 ± 0.02 <sup>rC</sup>    | 29.1 ± 0.4 <sup>tsC</sup>  | 0.81 ± 0.05 <sup>vuB</sup>     | 5.0 ± 0.2 <sup>sB</sup>       | 0.71 ± 0.15 <sup>hij</sup> |
|         | 7 | 93.95 ± 0.16 <sup>mIE</sup>   | 5.72 ± 0.02 <sup>qD</sup>    | 31.6 ± 0.5 <sup>uA</sup>   | 0.88 ± 0.06 <sup>vA</sup>      | 5.8 ± 0.2 <sup>tA</sup>       | 1.3 ± 0.3 <sup>efghi</sup> |

For the samples with the same conditions, the same lowercase letter in superscript in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ) (a–n for unheated, z–o for heated samples). To compare the same sample with the temperature effect, the same capital superscript letter in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ).  $\Delta E$  indicates color differences associated with the heat treatment. C: Concentration (%).

Generally speaking, in the samples with lower fiber content, solid content was precipitated, but the uniformity of suspensions markedly increased when concentration rose. This could be due to the fact that samples are mixtures of whole cells and dispersed cell wall materials, and it has been reported that these materials usually form non homogeneous suspensions, unlike smashed cellular material that forms homogeneous fibrous networks (Wang et al., 2018). However, homogeneity increased while fiber concentration increased due to the mixture's saturation.

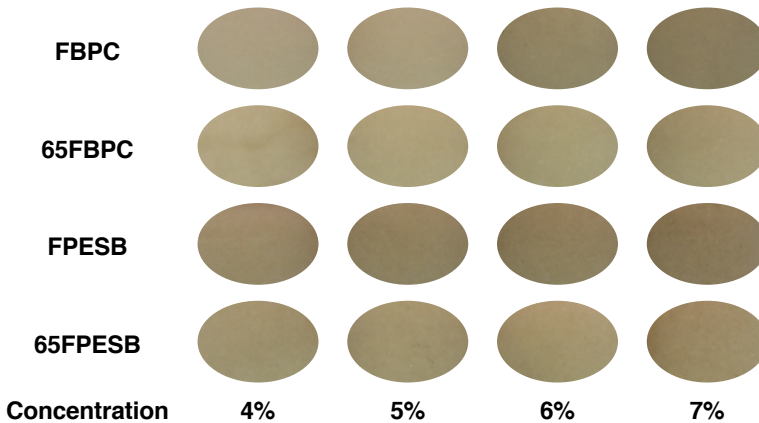
The values of the colorimetric parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) of the formulated gels are presented in Table 4. To illustrate the color of suspensions, the images of each formulated sample are shown in Figure 5, both with and without heat treatment. We can see that samples totally differed, but parameters  $L^*$ ,  $a^*$  and  $b^*$  significantly increased with a higher fiber concentration for all the samples in both cases, with and without heat treatment ( $p < 0.05$ ). It has been reported that color parameters increase in homogenized suspensions and are more saturated in red and yellow (Wang et al., 2018), like the results herein shown. Moreover, an increase in the color parameters was also related to the employed fiber, and the FCH suspensions were those with higher  $a^*$  values in the untreated and heated samples ( $p < 0.05$ ). Yellowness ( $b^*$ ) was the most different value in the samples ( $p < 0.05$ ) in both the heated and unheated samples, except for 6% and 7% of the FPE and FBPC unheated samples and for 7% of the FC and FCH heated samples ( $p > 0.05$ ). Figure 5 generally shows no differences between the heated or unheated samples. However, color differences ( $\Delta E > 3$ ) appeared for samples at 4% and 5% of FBPC, and at 5%, 6% and 7% of FPE (Table 4), which means that these differences are visibly perceptible (Bodart, de Peñaranda, Deneyer & Flamant, 2008). Furthermore, Pearson's correlations were observed between the color parameters of both suspensions (heated and unheated) and mineral content. An increase in  $L^*$  is

related to a higher Se content of (0.7163), while an increase in  $a^*$  (redness) is related to higher P, K, Mg, Zn, Fe, Cu and Mn contents and to total content of minerals (0.9419, 0.9036, 0.9635, 0.9290, 0.8084, 0.9373 and 0.8986, respectively). For  $b^*$ , a positively correlation was also found with P, K, Ca, Fe and Cu (0.6684, 0.7529, 0.8431, 0.6292, 0.6245 and 0.8242, respectively).



**Figure 5.** Color gel images at the tested concentrations.





**Figure 5.** (Continued)

The back extrusion assay was performed as in a previous study to elucidate the mechanical properties of fiber suspensions. This assay showed that not only did the consistency and firmness of gels increase as the concentration of DFs rose, but also the viscosity and cohesiveness in all the samples (Table 5). For all the samples and texture parameters, an interaction occurred between concentration and temperature, which were also related to the used fiber. A significant ( $p < 0.05$ ) increase in all the parameters from the concentration of 4% was observed, except for all the parameters of the heated and unheated suspensions, where FC remained stable at all the concentrations. This increase was significantly greater when suspensions were formulated with the FBPC and FPESB fibers for all the parameters. In this way, the sample which presented the greatest consistency was 65FBPC (7%). The consistency, firmness and cohesiveness values were higher in 65FBPC (4%), but this sample had lower viscosity than that found by Cevoli et al. (2013) for xanthan gum (4%). When comparing it to 65FPESB (4%), both showed similar consistency and cohesiveness, and 65FPESB (4%) obtained a higher strength and lower viscosity values. The sample with the lowest consistency and firmness was 4% FC for both heated and unheated conditions. Angioloni and

Collar (2009) reported a similar result to FC and 65FC (4% and 5%) in a pectin liquid-like gel prepared at 5 °C and 95 °C, and also similar results to 4% FP and FPE in a liquid-like gel prepared with pectin and FOS or GOS. Furthermore, the viscosity index and cohesiveness of these samples were also comparable to those found by these authors (Angioloni & Collar, 2009). Moreover, the importance of determining consistency and viscosity is because they are related to the coverage in mouth of gelled products, as indicated by Igual, García-Martínez, Camacho and Martínez-Navarrete (2011) and Igual, Contreras and Martínez-Navarrete (2014) for jam.

**Table 5.** Mean values  $\pm$  standard deviations of the back extrusion parameters of the formulated gels.

| Sample | C | Consistency (N s)                    | Firmness (N)                     | Viscosity (N s)                  | Cohesiveness (N)                  |
|--------|---|--------------------------------------|----------------------------------|----------------------------------|-----------------------------------|
| FCH    | 4 | 5.4 $\pm$ 0.5 <sup>hijC</sup>        | 0.92 $\pm$ 0.04 <sup>hiE</sup>   | 0.66 $\pm$ 0.05 <sup>fgD</sup>   | 0.63 $\pm$ 0.06 <sup>ghiD</sup>   |
|        | 5 | 6 $\pm$ 2 <sup>hijC</sup>            | 1.0 $\pm$ 0.4 <sup>hiDE</sup>    | 0.74 $\pm$ 0.18 <sup>FD</sup>    | 0.80 $\pm$ 0.15 <sup>fghCD</sup>  |
|        | 6 | 16 $\pm$ 3 <sup>efA</sup>            | 3.1 $\pm$ 0.7 <sup>dAB</sup>     | 1.46 $\pm$ 0.17 <sup>dA</sup>    | 1.9 $\pm$ 0.3 <sup>eA</sup>       |
|        | 7 | 16 $\pm$ 4 <sup>deA</sup>            | 3.3 $\pm$ 0.8 <sup>dA</sup>      | 1.4 $\pm$ 0.2 <sup>dA</sup>      | 1.8 $\pm$ 0.4 <sup>eAB</sup>      |
| 65FCH  | 4 | 6.4 $\pm$ 0.9 <sup>qponC</sup>       | 1.16 $\pm$ 0.17 <sup>onDE</sup>  | 0.81 $\pm$ 0.05 <sup>srCD</sup>  | 0.78 $\pm$ 0.15 <sup>qpCD</sup>   |
|        | 5 | 10.1 $\pm$ 0.6 <sup>rqpoB</sup>      | 1.74 $\pm$ 0.06 <sup>ponCD</sup> | 0.98 $\pm$ 0.03 <sup>tsC</sup>   | 1.09 $\pm$ 0.09 <sup>rqC</sup>    |
|        | 6 | 12.85 $\pm$ 1.19 <sup>srqAB</sup>    | 2.3 $\pm$ 0.3 <sup>qpoBC</sup>   | 1.23 $\pm$ 0.09 <sup>utB</sup>   | 1.47 $\pm$ 0.09 <sup>srB</sup>    |
|        | 7 | 15 $\pm$ 5 <sup>tsrA</sup>           | 3.1 $\pm$ 1.3 <sup>raqAB</sup>   | 1.3 $\pm$ 0.2 <sup>utAB</sup>    | 1.6 $\pm$ 0.5 <sup>srAB</sup>     |
| FPE    | 4 | 1.04 $\pm$ 0.06 <sup>kD</sup>        | 0.20 $\pm$ 0.03 <sup>iC</sup>    | 0.065 $\pm$ 0.009 <sup>ikC</sup> | 0.146 $\pm$ 0.016 <sup>jkC</sup>  |
|        | 5 | 2.0 $\pm$ 0.4 <sup>jkD</sup>         | 0.68 $\pm$ 0.19 <sup>hiC</sup>   | 0.07 $\pm$ 0.02 <sup>kC</sup>    | 0.150 $\pm$ 0.015 <sup>ijkC</sup> |
|        | 6 | 5 $\pm$ 3 <sup>hijBCD</sup>          | 2.0 $\pm$ 1.3 <sup>fgBC</sup>    | 0.15 $\pm$ 0.09 <sup>ijkBC</sup> | 0.3 $\pm$ 0.2 <sup>ijkBC</sup>    |
|        | 7 | 10 $\pm$ 3 <sup>gBC</sup>            | 3.3 $\pm$ 0.7 <sup>dAB</sup>     | 0.31 $\pm$ 0.06 <sup>hijB</sup>  | 0.61 $\pm$ 0.06 <sup>ghiBC</sup>  |
| 65FPE  | 4 | 1.6 $\pm$ 0.3 <sup>onD</sup>         | 0.56 $\pm$ 0.14 <sup>onC</sup>   | 0.062 $\pm$ 0.003 <sup>pC</sup>  | 0.157 $\pm$ 0.009 <sup>oC</sup>   |
|        | 5 | 4.1 $\pm$ 0.9 <sup>ponCD</sup>       | 1.6 $\pm$ 0.4 <sup>ponBC</sup>   | 0.13 $\pm$ 0.04 <sup>pBC</sup>   | 0.25 $\pm$ 0.08 <sup>poC</sup>    |
|        | 6 | 21 $\pm$ 6 <sup>srqAB</sup>          | 5.4 $\pm$ 1.3 <sup>srqAB</sup>   | 0.45 $\pm$ 0.09 <sup>qpB</sup>   | 1.2 $\pm$ 0.3 <sup>qpoAB</sup>    |
|        | 7 | 24 $\pm$ 5 <sup>utsA</sup>           | 6.25 $\pm$ 1.13 <sup>tsA</sup>   | 0.66 $\pm$ 0.18 <sup>rqA</sup>   | 1.5 $\pm$ 0.3 <sup>rQA</sup>      |
| FP     | 4 | 1.07 $\pm$ 0.04 <sup>kD</sup>        | 0.28 $\pm$ 0.06 <sup>iG</sup>    | 0.054 $\pm$ 0.002 <sup>KE</sup>  | 0.145 $\pm$ 0.013 <sup>ikE</sup>  |
|        | 5 | 2.84 $\pm$ 1.07 <sup>jkD</sup>       | 1.4 $\pm$ 0.7 <sup>ghF</sup>     | 0.14 $\pm$ 0.08 <sup>ijkE</sup>  | 0.52 $\pm$ 0.18 <sup>hiDE</sup>   |
|        | 6 | 10.3966 $\pm$ 1.0114 <sup>hijC</sup> | 3.0 $\pm$ 0.4 <sup>deDE</sup>    | 0.66 $\pm$ 0.04 <sup>fgD</sup>   | 1.8 $\pm$ 0.2 <sup>eC</sup>       |
|        | 7 | 19.5 $\pm$ 1.7 <sup>gB</sup>         | 4.6 $\pm$ 0.4 <sup>cC</sup>      | 1.11 $\pm$ 0.13 <sup>eC</sup>    | 3.0 $\pm$ 0.3 <sup>dB</sup>       |
| 65FP   | 4 | 3.90 $\pm$ 1.08 <sup>ponD</sup>      | 2.2 $\pm$ 0.5 <sup>qpoEF</sup>   | 0.29 $\pm$ 0.18 <sup>qpE</sup>   | 0.73 $\pm$ 0.08 <sup>qpoD</sup>   |
|        | 5 | 11.1 $\pm$ 1.4 <sup>srqC</sup>       | 3.9 $\pm$ 0.3 <sup>tsrqCD</sup>  | 0.76 $\pm$ 0.15 <sup>srD</sup>   | 1.9 $\pm$ 0.4 <sup>tsC</sup>      |
|        | 6 | 22 $\pm$ 5 <sup>utB</sup>            | 5.5 $\pm$ 0.9 <sup>utB</sup>     | 1.5 $\pm$ 0.3 <sup>uA</sup>      | 3.2 $\pm$ 0.5 <sup>uB</sup>       |
|        | 7 | 41 $\pm$ 6 <sup>vA</sup>             | 9.7 $\pm$ 1.4 <sup>wA</sup>      | 2.7 $\pm$ 0.6 <sup>wB</sup>      | 5.5 $\pm$ 0.8 <sup>vA</sup>       |

For the samples with the same conditions, the same lowercase letter in superscript in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ) (a–n for unheated, z–k for heated samples). To compare the same sample with the temperature effect, the same capital superscript letter in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ). C: Concentration (%).

**Table 5. (Continued)**

| Sample  | C | Consistency (N s)           | Firmness (N)                    | Viscosity (N s)                | Cohesiveness (N)               |
|---------|---|-----------------------------|---------------------------------|--------------------------------|--------------------------------|
| FC      | 4 | 1.004 ± 0.013 <sup>kC</sup> | 0.177 ± 0.008 <sup>iBC</sup>    | 0.0720 ± 0.0014 <sup>jkE</sup> | 0.153 ± 0.008 <sup>ikD</sup>   |
|         | 5 | 1.058 ± 0.014 <sup>kC</sup> | 0.1860 ± 0.0104 <sup>iC</sup>   | 0.100 ± 0.003 <sup>ikE</sup>   | 0.170 ± 0.008 <sup>ikD</sup>   |
|         | 6 | 1.285 ± 0.009 <sup>kB</sup> | 0.214 ± 0.006 <sup>iB</sup>     | 0.257 ± 0.004 <sup>hijkC</sup> | 0.306 ± 0.013 <sup>ijkC</sup>  |
|         | 7 | 1.68 ± 0.09 <sup>kA</sup>   | 0.26 ± 0.02 <sup>iA</sup>       | 0.37 ± 0.02 <sup>hiB</sup>     | 0.47 ± 0.08 <sup>ijB</sup>     |
| 65FC    | 4 | 0.970 ± 0.009 <sup>nC</sup> | 0.178 ± 0.008 <sup>nBC</sup>    | 0.0756 ± 0.0017 <sup>pE</sup>  | 0.152 ± 0.008 <sup>oD</sup>    |
|         | 5 | 1.05 ± 0.03 <sup>nC</sup>   | 0.182 ± 0.002 <sup>nBC</sup>    | 0.124 ± 0.009 <sup>pD</sup>    | 0.2050 ± 0.0113 <sup>poD</sup> |
|         | 6 | 1.28 ± 0.03 <sup>nB</sup>   | 0.209 ± 0.014 <sup>nB</sup>     | 0.246 ± 0.007 <sup>qpC</sup>   | 0.33 ± 0.04 <sup>poC</sup>     |
|         | 7 | 1.89 ± 0.06 <sup>onA</sup>  | 0.29580 ± 0.01006 <sup>nA</sup> | 0.45 ± 0.03 <sup>qpA</sup>     | 0.662 ± 0.119 <sup>qpoA</sup>  |
| FBPC    | 4 | 6.5 ± 0.5 <sup>hG</sup>     | 1.267 ± 0.106 <sup>ghE</sup>    | 0.71 ± 0.05 <sup>fgF</sup>     | 1.05 ± 0.04 <sup>fF</sup>      |
|         | 5 | 12.4 ± 1.5 <sup>fgFG</sup>  | 2.2 ± 0.3 <sup>efgE</sup>       | 1.33 ± 0.07 <sup>deE</sup>     | 1.91 ± 0.10 <sup>eE</sup>      |
|         | 6 | 27 ± 3 <sup>cDE</sup>       | 4.7 ± 0.4 <sup>cd</sup>         | 2.26 ± 0.15 <sup>cd</sup>      | 3.22 ± 0.13 <sup>dD</sup>      |
|         | 7 | 42 ± 8 <sup>aC</sup>        | 8 ± 2 <sup>aC</sup>             | 3.6 ± 0.5 <sup>bc</sup>        | 5.5 ± 0.7 <sup>bc</sup>        |
| 65FBPC  | 4 | 24 ± 2 <sup>uEF</sup>       | 4.5 ± 0.6 <sup>srD</sup>        | 1.3 ± 0.2 <sup>utE</sup>       | 2.21 ± 0.16 <sup>E</sup>       |
|         | 5 | 39 ± 6 <sup>vCD</sup>       | 7 ± 2 <sup>vC</sup>             | 2.20 ± 0.17 <sup>vD</sup>      | 4.0 ± 0.4 <sup>vD</sup>        |
|         | 6 | 75 ± 13 <sup>xB</sup>       | 14 ± 2 <sup>yB</sup>            | 4.3 ± 0.5 <sup>xB</sup>        | 8.0 ± 0.9 <sup>yB</sup>        |
|         | 7 | 107 ± 21 <sup>zA</sup>      | 20 ± 4 <sup>zA</sup>            | 6.0 ± 0.6 <sup>yA</sup>        | 10.4 ± 1.3 <sup>zA</sup>       |
| FPESB   | 4 | 5.7 ± 0.7 <sup>hiE</sup>    | 1.274 ± 0.103 <sup>ghE</sup>    | 0.49 ± 0.06 <sup>ghE</sup>     | 0.92 ± 0.19 <sup>fgE</sup>     |
|         | 5 | 14 ± 6 <sup>efgD</sup>      | 2.5 ± 0.8 <sup>defDE</sup>      | 1.3 ± 0.2 <sup>deD</sup>       | 2.1 ± 0.4 <sup>eD</sup>        |
|         | 6 | 16.9 ± 0.7 <sup>cd</sup>    | 3.204 ± 0.115 <sup>dD</sup>     | 2.4 ± 0.2 <sup>cC</sup>        | 3.73 ± 0.14 <sup>cC</sup>      |
|         | 7 | 36 ± 6 <sup>aC</sup>        | 6.6 ± 1.5 <sup>bc</sup>         | 4.2 ± 0.6 <sup>aB</sup>        | 6.2 ± 0.6 <sup>aB</sup>        |
| 65FPESB | 4 | 18 ± 2 <sup>utsrD</sup>     | 3.7 ± 0.4 <sup>tsrqD</sup>      | 1.24 ± 0.18 <sup>utD</sup>     | 1.8 ± 0.3 <sup>tsD</sup>       |
|         | 5 | 38 ± 5 <sup>vC</sup>        | 7.01 ± 1.04 <sup>vuC</sup>      | 2.4 ± 0.3 <sup>vvC</sup>       | 3.8 ± 0.4 <sup>vC</sup>        |
|         | 6 | 63 ± 3 <sup>wB</sup>        | 12.08 ± 1.12 <sup>xB</sup>      | 4.5 ± 0.9 <sup>xB</sup>        | 6.4 ± 0.3 <sup>xB</sup>        |
|         | 7 | 94 ± 9 <sup>yA</sup>        | 19 ± 2 <sup>zA</sup>            | 7.3 ± 0.4 <sup>zA</sup>        | 10.1 ± 0.4 <sup>zA</sup>       |

For the samples with the same conditions, the same lowercase letter in superscript in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ) (a–n for unheated, z–k for heated samples). To compare the same sample with the temperature effect, the same capital superscript letter in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ). C: Concentration (%).

In order to continue characterizing samples after obtaining the back extrusion assay results, the samples with a lower consistency than 100 N s were rheologically characterized by the flow curve analysis. The adjustment of shear stress and the shear rate of all the data were fitted to Ostwald’s power law, where  $k$  and  $n$  indicate consistency and flow behavior, respectively. In addition,  $R^2$  (goodness of fit) values over 0.9 were obtained (Table 6). The change in  $k$  showed that increased consistency was related to a higher fiber concentration for all the samples, as Su et al. (2020) indicated for citrus fiber-oil dispersions. At the same time, consistency increased when samples were heated, as shown by the back extrusion results, except for the samples

prepared with FCH (Table 5). The gels formulated with FPBC and FPESB presented higher consistency. Therefore, both these fibers and those with a  $k$  over 20 Pa are identified with semisolid or spoonable foods (Aguayo-Mendoza et al., 2019), and have gelling properties for their possible use in different food products, such as fat replacers and/or to modify the texture of meat analogs, among others. However, the other studied fibers ( $k$  values between 0.21 and 3.6) are associated with liquids or drinkable food, and would be more suitably used as thickeners in soups, sauces and dressings. FC was the fiber with the lowest thickening power and FP had the highest ( $p < 0.05$ ) at the highest tested concentration (7%).  $n$  was lower than 1, which means that samples behaved as pseudoplastic fluids. In general, no changes were observed in  $n$  due to increased fiber content, except for the unheated FC, FPE and FP samples, and the heated FC and FPE samples, for which a decrease occurred with increased fiber. This parameter depended on particle size distribution (Wang et al., 2018). For this reason, negative Pearson's correlations were observed between  $n$  and particle size parameters (D [4, 3], d (0.1) and (0.5);  $-0.6968$ ,  $-0.6934$  and  $-0.7345$ , respectively), which means that diminishing shear thinning behavior (higher  $n$  value) is related to reduced particle size. The result for the consistency of 65FPESB 7% was comparable to that reported by Santo-Domingo, Rojas, Fissore and Gerchenson (2019) for artichoke dietary fiber, but shear thinning behavior (lower  $n$  value) was higher in artichoke fiber. In this study, the sample which displayed high consistency and good shear thinning behavior was 65FP 7%.

Table 6 offers the shear viscosity recorded at the shear rate ( $50 \text{ s}^{-1}$ ) in accordance with the dispersion concentrations. According to Agarwal, Hewson and Foster (2017), shear viscosity depends on the source, processing and microstructure of fibers. This table reveals that shear viscosity is also dependent on treatments, and the heated samples tended to be higher ( $p <$

0.05), except for the FCH sample. At the highest studied concentration (7%), samples FBPC and FPESB displayed higher viscosity, and were higher when gels were heated. These viscosity results obtained with the different gel fibers are required to know in which model systems these fibers can be used as thickeners or gelling agents in order to investigate whether the inherent properties of the materials can impact the sensory characteristics of the texture analysis and taste perception.

**Table 6.** Mean values  $\pm$  standard deviations of the rheology parameters  $k$  (consistency index),  $n$  (flow index) and  $\eta_{ap}$  (shear viscosity at 50 s<sup>-1</sup> shear stress) of the formulated gels.

| Sample | C | Ostwald-De Waele Model            |                                      |  | R <sup>2</sup> |
|--------|---|-----------------------------------|--------------------------------------|--|----------------|
|        |   | $k$ (Pa s <sup>n</sup> )          | $n$                                  | $\eta_{ap}$ (Pa s)                     |                |
| FCH    | 4 | 1.74 $\pm$ 0.05 <sup>ghijDE</sup> | 0.519 $\pm$ 0.008 <sup>dA</sup>      | 0.265 $\pm$ 0.009 <sup>ghD</sup>       | 0.99           |
|        | 5 | 3.1 $\pm$ 0.8 <sup>gC</sup>       | 0.4991 $\pm$ 0.0103 <sup>deB</sup>   | 0.440530 $\pm$ 0.101015 <sup>efC</sup> | 0.99           |
|        | 6 | 3.61 $\pm$ 0.09 <sup>fBC</sup>    | 0.502 $\pm$ 0.006 <sup>defB</sup>    | 0.515 $\pm$ 0.012 <sup>eB</sup>        | 0.99           |
|        | 7 | 5.8 $\pm$ 0.6 <sup>eA</sup>       | 0.485 $\pm$ 0.005 <sup>efghC</sup>   | 0.77 $\pm$ 0.06 <sup>dA</sup>          | 0.99           |
| 65FCH  | 4 | 1.55 $\pm$ 0.02 <sup>onE</sup>    | 0.519 $\pm$ 0.003 <sup>uA</sup>      | 0.2360 $\pm$ 0.0018 <sup>nmd</sup>     | 0.99           |
|        | 5 | 2.199 $\pm$ 0.116 <sup>poD</sup>  | 0.456 $\pm$ 0.006 <sup>tsrqD</sup>   | 0.2618 $\pm$ 0.0104 <sup>onmD</sup>    | 0.99           |
|        | 6 | 3.93 $\pm$ 0.05 <sup>srqB</sup>   | 0.507 $\pm$ 0.002 <sup>uB</sup>      | 0.570 $\pm$ 0.005 <sup>rqB</sup>       | 0.99           |
|        | 7 | 6.40 $\pm$ 0.04 <sup>utA</sup>    | 0.442 $\pm$ 0.002 <sup>rqE</sup>     | 0.723 $\pm$ 0.008 <sup>tsA</sup>       | 0.99           |
| FPE    | 4 | 0.31 $\pm$ 0.06 <sup>jkC</sup>    | 0.46 $\pm$ 0.04 <sup>IB</sup>        | 0.037 $\pm$ 0.009 <sup>kC</sup>        | 0.94           |
|        | 5 | 0.28 $\pm$ 0.03 <sup>jkC</sup>    | 0.5870 $\pm$ 0.0106 <sup>cA</sup>    | 0.056 $\pm$ 0.003 <sup>kC</sup>        | 0.96           |
|        | 6 | 0.54 $\pm$ 0.17 <sup>ijkBC</sup>  | 0.57 $\pm$ 0.05 <sup>cA</sup>        | 0.098 $\pm$ 0.017 <sup>jkBC</sup>      | 0.98           |
|        | 7 | 6 $\pm$ 2 <sup>eA</sup>           | 0.264 $\pm$ 0.007 <sup>nD</sup>      | 0.35 $\pm$ 0.12 <sup>fgA</sup>         | 0.95           |
| 65FPE  | 4 | 0.21 $\pm$ 0.03 <sup>nC</sup>     | 0.58 $\pm$ 0.03 <sup>xA</sup>        | 0.040 $\pm$ 0.003 <sup>kC</sup>        | 0.97           |
|        | 5 | 0.55 $\pm$ 0.16 <sup>nmBC</sup>   | 0.56 $\pm$ 0.03 <sup>xwA</sup>       | 0.098 $\pm$ 0.019 <sup>klBC</sup>      | 0.98           |
|        | 6 | 2.0 $\pm$ 0.6 <sup>poB</sup>      | 0.39 $\pm$ 0.05 <sup>pC</sup>        | 0.18 $\pm$ 0.03 <sup>mIB</sup>         | 0.98           |
|        | 7 | 5.16 $\pm$ 1.04 <sup>tsA</sup>    | 0.3019 $\pm$ 0.0004 <sup>oD</sup>    | 0.34 $\pm$ 0.07 <sup>poA</sup>         | 0.97           |
| FP     | 4 | 1.5 $\pm$ 0.3 <sup>hijkD</sup>    | 0.34 $\pm$ 0.03 <sup>klAB</sup>      | 0.1117 $\pm$ 0.0106 <sup>ijkD</sup>    | 0.98           |
|        | 5 | 2.8 $\pm$ 0.3 <sup>ghd</sup>      | 0.34 $\pm$ 0.03 <sup>kA</sup>        | 0.216 $\pm$ 0.019 <sup>hijD</sup>      | 0.97           |
|        | 6 | 6.8 $\pm$ 0.9 <sup>eC</sup>       | 0.3115 $\pm$ 0.0115 <sup>lmABC</sup> | 0.46 $\pm$ 0.04 <sup>efC</sup>         | 0.97           |
|        | 7 | 13 $\pm$ 3 <sup>dB</sup>          | 0.30 $\pm$ 0.02 <sup>mBCD</sup>      | 0.81 $\pm$ 0.09 <sup>dB</sup>          | 0.98           |
| 65FP   | 4 | 3.052 $\pm$ 0.105 <sup>spD</sup>  | 0.277 $\pm$ 0.005 <sup>oCD</sup>     | 0.180 $\pm$ 0.003 <sup>mlD</sup>       | 0.99           |
|        | 5 | 6.6 $\pm$ 0.3 <sup>uC</sup>       | 0.29 $\pm$ 0.02 <sup>onCD</sup>      | 0.41 $\pm$ 0.04 <sup>pC</sup>          | 0.98           |
|        | 6 | 12.3 $\pm$ 1.5 <sup>wvB</sup>     | 0.302 $\pm$ 0.013 <sup>oBCD</sup>    | 0.80 $\pm$ 0.07 <sup>IB</sup>          | 0.98           |
|        | 7 | 22 $\pm$ 2 <sup>xA</sup>          | 0.275 $\pm$ 0.005 <sup>nD</sup>      | 1.27 $\pm$ 0.13 <sup>uA</sup>          | 0.98           |

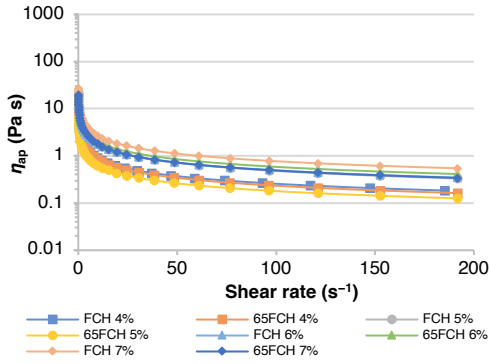
For the samples with the same conditions, the same lowercase letter in superscript in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ) (a–k for unheated, z–k for heated samples). To compare the same sample with the temperature effect, the same capital superscript letter in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ). C: Concentration (%).

Table 6. (Continued)

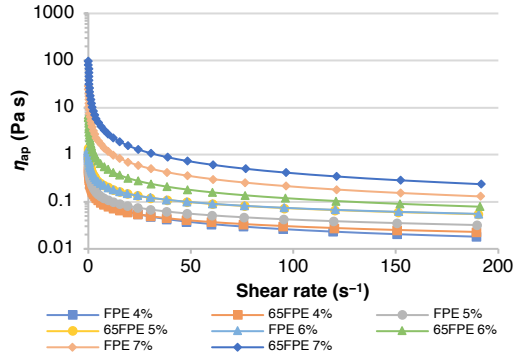
| Ostwald-De Waele Model |   |                               |                                   |                                 |       |
|------------------------|---|-------------------------------|-----------------------------------|---------------------------------|-------|
| Sample                 | C | $k$ (Pa s <sup>n</sup> )      | $n$                               | $\eta_{ap}$ (Pa s)              | $R^2$ |
| FC                     | 4 | 0.1017 ± 0.0009 <sup>kF</sup> | 0.7151 ± 0.0014 <sup>aA</sup>     | 0.03338 ± 0.00018 <sup>kF</sup> | 0.99  |
|                        | 5 | 0.338 ± 0.009 <sup>jkEF</sup> | 0.651 ± 0.002 <sup>bb</sup>       | 0.086 ± 0.002 <sup>jkE</sup>    | 0.99  |
|                        | 6 | 0.99 ± 0.04 <sup>ijkD</sup>   | 0.602 ± 0.006 <sup>cc</sup>       | 0.209 ± 0.005 <sup>hijD</sup>   | 0.99  |
|                        | 7 | 2.631 ± 0.019 <sup>fghB</sup> | 0.578 ± 0.013 <sup>cd</sup>       | 0.51 ± 0.03 <sup>eb</sup>       | 0.99  |
| 65FC                   | 4 | 0.130 ± 0.005 <sup>mF</sup>   | 0.716 ± 0.005 <sup>zA</sup>       | 0.0427 ± 0.0008 <sup>kF</sup>   | 0.99  |
|                        | 5 | 0.48 ± 0.03 <sup>nmE</sup>    | 0.642 ± 0.008 <sup>yB</sup>       | 0.118 ± 0.003 <sup>lkE</sup>    | 0.99  |
|                        | 6 | 1.51 ± 0.06 <sup>onC</sup>    | 0.585 ± 0.005 <sup>xD</sup>       | 0.297 ± 0.006 <sup>onC</sup>    | 0.99  |
|                        | 7 | 3.6 ± 0.4 <sup>rqA</sup>      | 0.55393 ± 0.01019 <sup>wE</sup>   | 0.63 ± 0.04 <sup>rqA</sup>      | 0.99  |
| FBPC                   | 4 | 1.98 ± 0.04 <sup>ghif</sup>   | 0.458 ± 0.013 <sup>hiA</sup>      | 0.2385 ± 0.0115 <sup>ghif</sup> | 0.99  |
|                        | 5 | 5.9 ± 0.6 <sup>eE</sup>       | 0.4682 ± 0.0015 <sup>ghia</sup>   | 0.74 ± 0.08 <sup>dE</sup>       | 0.99  |
|                        | 6 | 17.2 ± 1.6 <sup>cc</sup>      | 0.462 ± 0.002 <sup>hiA</sup>      | 2.10 ± 0.18 <sup>bc</sup>       | 0.99  |
|                        | 7 | 26.5 ± 1.5 <sup>aA</sup>      | 0.424 ± 0.009 <sup>ib</sup>       | 2.8 ± 0.2 <sup>aA</sup>         | 0.99  |
| 65FBPC                 | 4 | 5.2 ± 0.2 <sup>tsE</sup>      | 0.467 ± 0.013 <sup>tsA</sup>      | 0.64 ± 0.06 <sup>srE</sup>      | 0.99  |
|                        | 5 | 12.0 ± 0.8 <sup>vD</sup>      | 0.46360 ± 0.01013 <sup>tsrA</sup> | 1.47 ± 0.06 <sup>vD</sup>       | 0.99  |
|                        | 6 | 22.9 ± 1.9 <sup>yB</sup>      | 0.432 ± 0.005 <sup>qb</sup>       | 2.48 ± 0.17 <sup>xB</sup>       | 0.99  |
| FPESB                  | 4 | 2.09 ± 0.09 <sup>ghif</sup>   | 0.496 ± 0.004 <sup>defgA</sup>    | 0.290 ± 0.009 <sup>ghG</sup>    | 0.99  |
|                        | 5 | 5.4 ± 0.4 <sup>eE</sup>       | 0.487 ± 0.006 <sup>efghAB</sup>   | 0.72 ± 0.04 <sup>dE</sup>       | 0.99  |
|                        | 6 | 11.1 ± 1.3 <sup>cd</sup>      | 0.473 ± 0.014 <sup>ghicD</sup>    | 1.41 ± 0.09 <sup>cd</sup>       | 0.99  |
|                        | 7 | 22.87 ± 1.13 <sup>bb</sup>    | 0.462 ± 0.006 <sup>hiDEF</sup>    | 2.78 ± 0.09 <sup>aB</sup>       | 0.99  |
| 65FPESB                | 4 | 4.68 ± 0.16 <sup>srE</sup>    | 0.450 ± 0.005 <sup>srqF</sup>     | 0.54384 ± 0.01009 <sup>qF</sup> | 0.99  |
|                        | 5 | 13.5 ± 0.3 <sup>wC</sup>      | 0.459 ± 0.006 <sup>tsrEF</sup>    | 1.629 ± 0.016 <sup>wC</sup>     | 0.99  |
|                        | 6 | 21.8 ± 0.5 <sup>yxB</sup>     | 0.476 ± 0.003 <sup>tsrBC</sup>    | 2.81 ± 0.06 <sup>yB</sup>       | 0.99  |
|                        | 7 | 36.7 ± 0.7 <sup>zA</sup>      | 0.466 ± 0.003 <sup>tcDE</sup>     | 4.55 ± 0.04 <sup>zA</sup>       | 0.99  |

For the samples with the same conditions, the same lowercase letter in superscript in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ) (a–k for unheated, z–k for heated samples). To compare the same sample with the temperature effect, the same capital superscript letter in columns indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ). C: Concentration (%).

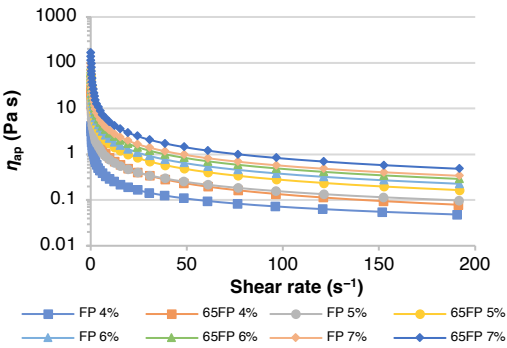
The effect of both concentration and temperature treatment on the  $\eta_{ap}$  trend is seen in Figure 6. Wang et al. (2018) indicated that apparent viscosity increased with a rising fiber concentration, which also happened in the results herein shown. Moreover, the apparent viscosity trend increased in the heated samples for all the samples, except for those formulated with FCH and FC, which seemed to follow the same trend in both heated and unheated concentration samples (Figure 6a,d).



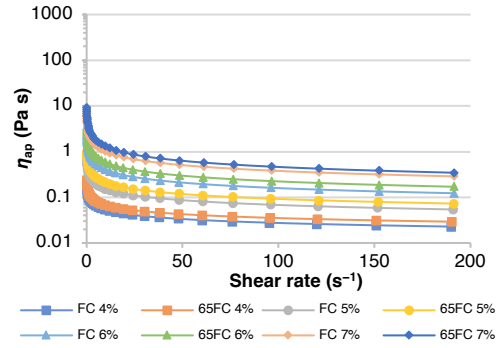
(a)



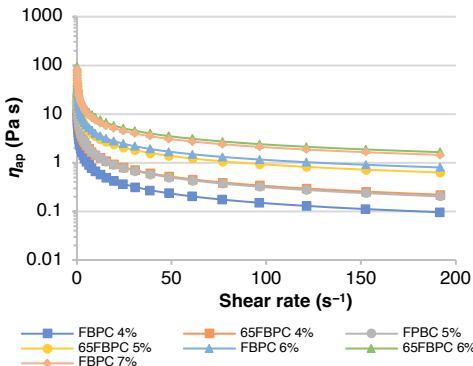
(b)



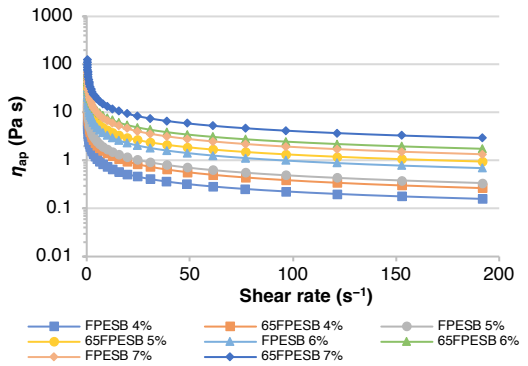
(c)



(d)



(e)



(f)

**Figure 6.** Apparent viscosity ( $\eta_{ap}$ ) vs. the shear rate of samples, where (a) shows the FCH, (b) FPE, (c) FP, (d) FC, (e) FBPC and (f) FPESB sample gels.

Pearson correlations were observed between hydration properties of gels (WHC, WRC, SWC, FAC and SWI) and mechanical properties (back extrusion and rheological properties) depending on how they have been formed (with or without heat treatment). When gels were formed without heating, SWC was negatively related to  $\eta_{ap}$  and  $k$  ( $-0.6688$  and  $-0.6383$ , respectively). Moreover, FAC and WRC were also negatively related to  $n$  ( $-0.8853$  and  $-0.6038$ , respectively); however, SWI showed positive correlation with  $n$  ( $0.6881$ ). On the other hand, for heated gels, Pearson correlations were presented between WHC and back extrusion parameters, negative correlation with consistency ( $-0.6381$ ) and firmness ( $-0.6011$ ) and positive correlation with viscosity ( $0.6901$ ) and cohesiveness ( $0.6194$ ). Furthermore, an increase in the WHC was associated with less  $\eta_{ap}$  ( $-0.7472$ ). Additionally, an increase in the  $n$  value was related to less FAC but high WSI ( $-0.8843$  and  $0.7713$ , respectively). Hence, the hydration properties of DFs are associated with gels structure; however, the type of DF and the treatment used to form the gel play an important role.

In order to finish characterization, the sample with consistency higher than 100 N s in the back extrusion analysis (65FBPC 7%) was analyzed by a texture profile analysis (TPA) because it presented a stable structure gel at this concentration. A resilience value of  $0.17 \pm 0.02$  and a chewiness value of  $1.5 \pm 0.2$  N were provided by the TPA analysis. Gel adhesiveness was negative ( $-1.6 \pm 0.2$  N s), which indicates that it forms a sticky gel. This result is comparable to those reported by Sharma, Kristo, Corredig and Duizer (2017) for carrot puréed using modified corn starch and ThickenUp®. Adhesiveness is defined as a gel's ability to adhere to a probe when it is withdrawn after the first compression. Therefore, this should be taken into account when making products because this texture might prove unpleasant for consumers because it adheres to palates, which requires making more effort to separate it (Velasco,



2010). Similar results for springiness ( $0.9105 \pm 0.0119$  mm) and cohesiveness ( $0.9105 \pm 0.0119$ ) have also been reported by Sharma et al. (2017), but gel hardness ( $2.0 \pm 0.4$  N) was similar to the potato starch gels (PS54) reported by Torres, Chenlo and Moreira (2017). As a result, this fiber can be used as a gelling agent and to also adapt the texture of different foods due to its water-holding and water retention capacities, and also to its ability to form stable gels.

### **4. Conclusions**

The results of this study revealed that all the DF samples had different physicochemical, hydrational, nutritional and mechanical properties. This is of great importance for the future use of this type of DF for the development of new food products such as meat analogue products, either to replace ingredients such as fat or to achieve new textures or improve/modify existing ones. In general, FPE and FCH showed good water-binding capacities and FP displayed good oil retention. We highlight the functional level values obtained for the FCH and FC samples. Moreover, in nutritional terms, FCH was a source of important minerals such as Fe, Mn and Cu. However, in suspension or gels, significant differences among samples in textural and rheological properties were found due to the employed treatment; as a result, FCH, FPE, FP and FC were more appropriate for thickening and FP at a high concentration, and both combinations of fiber (FBPC and FPESB) were more suitable to be used as gelling agents given their ability to form stable gels. In addition, the capacity of these DFs to change the texture without high temperatures can be highlighted. This enables them to modify the texture of different foods and to provide benefits of consuming DF.

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## Segunda parte

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Developing psyllium fibre gel-based foods: Physicochemical, nutritional, optical and mechanical properties.

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### **Abstract**

*Psyllium* fibre has known health benefits, which are highly related to its gelling properties, so it is important to know the functional properties and its gelling capacity to its possible incorporation as a new source of DF in foods. Therefore, the purpose of this research was to evaluate these properties of two *Psyllium* fibres. Physicochemical (particle size distribution, pH, water content, water activity, hygroscopicity and bulk density and porosity), hydration (water holding, water retention and swelling capacity, fat absorption capacity and solubility), and nutritional properties (mineral content, total phenolics compounds and antioxidant capacity) of dietary fibre (DF) samples were recorded. Moreover, physicochemical (water content, pH and colour) and mechanical properties (back extrusion test, rheological measurement and texture profile analysis) of gel samples were performed before and after heat treatment (20 min at 65 °C). The hydration properties of Plantago Husk (PH) were higher than those in Plantago Powder (PP), but in functional terms, high values of phenols and antioxidant activity were found in PP. However, both samples displayed similar gelling properties. As a result of the rheological and textural analysis, both fibres showed good gelling properties at both high (65 °C) and cool (5 °C) temperatures, at concentrations 4% to 7%. Moreover, the results indicate that PH and PP have suitable characteristics to be functional ingredients, which can help to avoid possible negative effects on food sensory and structural characteristics.

## 1. Introduction

There is a growing awareness about diet and health, which has brought about changes in consumer eating habits and the demand for healthier foods has increased (de Moraes Crizel, Jablonski, de Oliveira Rios, Rech, & Flôres, 2013). Fibre was one of the first ingredients to be associated with health in the 1980s and has been used by the food industry ever since (Dervisoglu, & Yazici, 2006; de Moraes Crizel et al., 2013). Plants high in dietary fibre (DF) and natural antioxidants have drawn increasing attention in recent years (Zhu, Huang, Peng, Qian, & Zhou, 2010). DF consumption offers several health benefits, including body weight control, reduced serum lipid and cholesterol, controlled postprandial glucose responses and colon cancer prevention, which are attributed to the physicochemical and functional properties of DF (Ma, & Mu, 2016). DFs also have well-known technological functions, such as water absorption and water retention and can, thus, reduce shrinkage, cooking loss and drip loss during storage, and can minimise production costs without affecting final products' sensory properties (Han, & Bertram, 2017). Thus the emergence of new fibre sources and processing methods to improve fibre's functionality has extended its applications in the food industry. Further, they have opened new possibilities to design new fibre-enriched products and generate new textures for a variety of applications (Rosell, Santos, & Collar, 2009).

*Psyllium* seed husk (*Plantago ovata*) is a source of natural DF that is of interest for food and pharmaceutical sciences as a functional ingredient (Ren, Linter, & Foster, 2020a). It comprises arabinoxylan, a polymer rich in arabinose and xylose, whose digestibility in humans is limited (Jalanka et al., 2019). *Psyllium* has been used in traditional medicine worldwide (Fradinho, Soares, Niccolai, Sousa, & Raymundo, 2020; Ren, Yakubov, Linter, MacNaughtan, & Foster, 2020b). Ren et al. (2020b) and Franco, Sanches-Silva, Ribeiro-Santos,

and Ramos de Melo (2020) have reported that *Psyllium* husk seed has good water absorbability with gelling properties, and can be used as a hydrocolloid for functional applications in food production. Hydrogels in food are mostly applied for structuring purposes to gain desirable rheological or textural properties, and for stabilising foams, dispersions, emulsions and particles. In recent years, hydrogels have drawn attention for their applications to deliver food bioactives (Mao, Lu, Cui, Miao, & Gao, 2020). Much research has been conducted into *Psyllium* health benefits for diabetes, constipation, colon cancer prevention, diarrhoea, inflammatory bowel disease (ulcerative colitis), irritable bowel syndrome symptoms, abdominal pain, obesity and hypercholesterolaemia (Guillon & Champ, 2000; Jane, McKay, & Pal, 2019; Franco et al., 2020; Belorio, Marcondes, & Gómez, 2020, Ren et al., 2020b). It also contributes to satiety, hypocholesterolaemia and prebiotics (Jalanka et al., 2019; Fradinho et al., 2020; Franco et al., 2020).

It is important to point out that the purpose of this research was to carry out a simpler and more practical characterisation of different commercial *Psyllium* than those found in the literature (Fischer et al., 2004; Yin et al., 2012; Benaoun et al., 2017; Patel, Tanna, Mishra & Jha, 2018; Patel et al., 2019) to facilitate the choice and its possible application by companies in the development of new products and modification of existing ones.

In order to determine *Psyllium* fibre applications, it is vital to characterise and evaluate them according to their functional properties. So this study aimed to characterise two commercial *Psyllium* fibres to know their physicochemical properties, phenol content, antioxidant properties and mineral contents, and to characterise the physicochemical, textural and rheological gels that form to elucidate their potential use in food products. This characterisation could help to avoid possible negative effects on food sensory and structural characteristics, and also to provide new textures to develop new foods.

## 2. Materials and Methods

### 2.1. Samples

Two *P. ovata* samples were herein used: Plantago Powder (PP) and Plantago Husk (PH). All these samples were supplied by Productos Pilarica S.A. (Paterna, Spain). The names, ingredients and proximate composition of the samples are shown in Table 1 and Figure 1.



PH: Plantago Husk, PP: Plantago Powder.

**Figure 1.** Image of *Psyllium* samples used in this study.

**Table 1.** Name, ingredients and proximate sample composition. Data provided by suppliers.

|               | Samples                  |                             |
|---------------|--------------------------|-----------------------------|
|               | PH                       | PP                          |
| Name          | Plantago Husk            | Plantago Powder             |
| Ingredients   | <i>Psyllium</i> Husk 95% | <i>Plantago ovata</i> seeds |
| Protein       | 2.5                      | 6.55                        |
| Lipids        | 0.5                      | 1.8                         |
| Carbohydrates | 4                        | 8.53                        |
| TDF           | 78                       | 70.5                        |
| IDF           | 77.1                     | 28.4                        |
| SDF           | 0.9                      | 42.1                        |

TDF (Total dietary fibre); IDF (Insoluble dietary fibre); SDF (Soluble dietary fibre).

### 2.2. *Psyllium* samples analysis

All the *Psyllium* samples were analysed in triplicate for each analysis.

### *2.2.1. Particle size distribution*

Sample particle size distribution was determined by applying the laser diffraction method and Mie theory following the ISO13320 regulation (AENOR 2009) in a particle size analyser (Malvern Instruments Ltd., Mastersizer 2000, UK) equipped with a dry sample dispersion unit (Malvern Instruments Ltd., Scirocco 2000). The volume (in percent) against particle size (in micrometres) was obtained and the size distribution was characterised by the volume mean diameter ( $D[4,3]$ ). The standard percentiles  $d(0.1)$ ,  $d(0.5)$ , and  $d(0.9)$  represent the particle size below which 10%, 50% and 90% of the sample lies, respectively. These parameters were estimated by the Mastersizer 2000 software (version 5.6) considering the particle diameter.

### *2.2.2. Water content and water activity*

Water content ( $x_w$ ) (g water/100 g sample) was determined by vacuum oven drying (Vaciotem, J.P. Selecta, Spain) at 70 °C until constant weight (AOAC, 2000). Samples' water activity ( $a_w$ ) was analysed by the AquaLab PRE LabFerrer equipment (Pullman, USA).

### *2.2.3. pH*

Samples' pH was measured upon dispersions (10% w/v) of samples in distilled water following described methods (Bender et al., 2020).

### *2.2.4. Hygroscopicity*

Samples (about 0.2 g in a Petri dish) were placed at 25 °C in an airtight plastic container with  $\text{Na}_2\text{SO}_4$  saturated solution (81% RH). After 1, 5, and 7 days, each sample was weighed and hygroscopicity (Hg) was expressed as g of water gained per 100 g dry solids (Cai & Corke, 2000).

### *2.2.5. Bulk density and porosity*

The porosity ( $\epsilon$ ), or percentage of air volume related to the total volume,

was calculated from the true ( $\rho$ ) and bulk ( $\rho_b$ ) densities using  $\varepsilon = (\rho - \rho_b) / \rho$  according to Igual, García-Segovia and Martínez-Monzó (2021). Samples' real density was determined by a helium pycnometer (AccPyc 1330, Micromeritics, Norcross, USA). For the bulk density ( $\rho_b$ ) determination, about 2 g of the powder were placed inside a 10 mL graduated test tube and the occupied volume was noted. Bulk density was calculated by dividing the powder mass by occupied volume, expressed as g/L.

#### *2.2.6. Determination of hydration properties*

Water-holding capacity (WHC) and water retention capacity (WRC) were determined as described by Raghavendra, Rastogi, Raghavarao, and Tharanathan (2004) and by Chantaro, Devahastin, and Chiewchan (2007). WHC and WRC were determined by placing 1 g of sample in a calibrated cylinder and then adding 30 mL of distilled water. Samples were hydrated for 18 h at 25 °C. WRC tubes were centrifuged at 3,000  $\times g$  for 20 min. Finally for both determinations, supernatants were removed, and the hydrated residues were weighed and dried at 100 °C for 3 h until constant weight. The results were expressed as g water/g dry sample.

Swelling water capacity (SWC) was determined as described by Navarro-González, García-Valverde, García-Alonso, & Periago (2011) with slight modifications. One gram of sample was placed in a graduated test tube and hydrated with 20 mL of distilled water. The sample was stored for 18 h at 25 °C, and then the bed volume was recorded. SWC was expressed as volume mL/g sample.

The water solubility index (WSI) was established by the method of Mahdavi, Jafari, Assadpour, and Ghorbani, (2016) with slight modifications. Samples (approx. 1 g) were mixed in centrifuge tubes with 30 mL of distilled water for 5 min until mixtures became homogeneous. Solutions were then



incubated at 37 °C in a water bath for 30 min. Afterwards, tubes were centrifuged at 17,640  $\times g$  for 20 min at 4 °C. Supernatants were collected and dried in an oven at 100 °C until constant weight. The results were expressed as a percentage.

Following the method reported by Navarro-González et al. (2011) with minor modifications, fat adsorption capacity (FAC) was determined. Samples (4 g) were placed in a centrifuge tube with 24 g of sunflower oil. Contents were stirred for 30 sec every 5 min for 30 min. Later samples were centrifuged at 1,600  $\times g$  for 25 min. Free oil was decanted and FAC was expressed as g oil/g sample.

### *2.2.7. Ash and mineral content*

The method 930.05 of AOAC procedures (Horwith, & Latimer, 2005) was used to determine the total ash content. A sample (500 mg) was incinerated at high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 24 h at 550 °C, and ash was gravimetrically quantified.

The multimineral determination was made in an inductively coupled plasma optical emission spectrometer, model 700 Series ICP-OES of Agilent Technologies (Santa Clara, USA), with axial viewing and a charge coupled device detector as described in García-Segovia, Igual, Noguerol, and Martínez-Monzo (2020). Mineral compositions (macro- and microelements) were expressed as mg/100 g sample.

### *2.2.8. Total phenolic compounds*

The extraction of total phenols (TP) comprised homogenising a sample with methanol and HCL (6N), and then centrifuging according to Tomás-Barberán, et al. (2001). TP were quantified by the method of Selvendran, and Ryden (1990) and Benzie, and Strain (1999) based on the Folin-Ciocalteu method. Absorbance was measured at 765 nm in a UV-visible

spectrophotometer (Thermo Electron Corporation, USA). Total phenolic content was expressed as mg of gallic acid equivalents (GAE) (Sigma-Aldrich, Germany) per 100 grams of sample using a standard curve 0-800 mg range of gallic acid /mL.

### *2.2.9. Antioxidant capacity*

Antioxidant capacity (AC) was assessed using the free radical scavenging activity of the samples evaluated with stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sánchez-Moreno et al., 2003) following the methodology of Igual et al. (2016). A UV-visible spectrophotometer (Thermo Electron Corporation, USA) was used to measure absorbance at 515 nm. The percentage of DPPH was calculated in the same way as other authors (Igual et al., 2019). The final results were expressed as milligram Trolox equivalents (TE) per 100 grams of sample (mg TE/100 g) using a Trolox calibration curve within the 10-500 mg/L range (Sigma-Aldrich, Germany).

### *2.3. Preparation of gels*

In order to prepare the gel samples, fibre powders were dissolved in cold water (5 °C) for 30 min at the 1, 2, 3, 4, 5, 6 and 7% concentrations. Then samples were divided into two batches. One of them was directly stored under cold conditions (24 h at 5 °C) until gel stabilisation. However, the other batch was subjected to heat treatment at 65 °C for 20 min before being stored at 5 °C for 24 h. After storing them, samples were tempered at 25 °C before carrying out the analysis.

### *2.4. Gel analysis*

#### *2.4.1. Water content and pH*

Water content ( $x_w$ ) (g water/100 g sample) was performed as in the powder fibres.

The gel samples' pH determined with a pH-meter Crison MultiMeter MM 41 (Hach Lange, Spain).

### *2.4.2. Colour*

In order to determine gel colour, the CIE\*L\*a\*b\* colours were measured according to García-Segovia et al. (2020) with a Konica Minolta CM-700d colorimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan). Measurements were taken on white and black backgrounds by taking D65 as a standard light and 10° as a standard observer. In order to determine samples' translucency, the Kubelka–Munk theory for multiple scattering to the reflection spectra was applied (Talens, Martínez-Navarrete, Fito, & Chiralt, 2002). The calculated reflectance of an infinitely thick layer of a material was used to obtain samples' coordinate CIE\*L\*a\*b\* parameters that presented translucency.

### *2.4.3. Back extrusion test*

A back extrusion test was performed according to the method described by Cevoli et al. (2013) with minor modifications. Textural characteristics were evaluated at all the sample concentrations (1-7%) by a TA-XT2 Texture Analyser (Stable Micro Systems Ltd, Godalming, UK) equipped with an extrusion disc (25.4 mm in diameter) positioned centrally over the sample plastic container (diameter: 50 mm, height: 75 mm) with 40 g of sample (approximately 30 mm height). The test was performed at a depth of 50% at the 1 mm/s test speed. The attributes calculated from the force-deformation curve were consistency (N s) (area under the curve up), firmness (N) (maximum force), cohesiveness (N) (maximum negative force) and viscosity (N s) (resistance to flow off the disc).

### *2.4.4. Rheological measurements*

Flow curves were performed only at the 4, 5, 6, and 7% concentrations, at which the consistency results of the back extrusion test were lower than 100 N

s, as measured by a Kinexus pro+rotational rheometer (Malvern Instruments, Worcestershire, UK) and the rSpace software, equipped with a system of coaxial cylinders (C25/PC25). A sample (20 mL) was loaded in the geometry and rested to equilibrate for 3 min to achieve a temperature equilibrium (25 °C) and stress relaxation in a heat-controlled sample stage (Peltier Cylinder Cartridge, Malvern Instruments, Worcestershire, UK). According to the method described by Cevoli et al. (2013) with slight modifications, samples were exposed to a logarithmically increase shear rate from 0 to 200 s<sup>-1</sup> in 3 min. Flow curves were evaluated using the *Ostwald -de Waele* (Eq. (1)) rheological model, where  $\sigma$  is the shear stress (Pa),  $\gamma$  is the shear rate (s<sup>-1</sup>),  $k$  is the consistency coefficient (Pa s<sup>n</sup>) and  $n$  the flow behaviour index (dimensionless). This model is used to describe flows of rheologically complex fluids (Shapovalov, 2017). The apparent viscosity at the 50 s<sup>-1</sup> shear rate ( $\eta_{ap}$ ) were calculated (Ribes, Peña, Fuentes, Talens, & Barat, 2020). Each flow curve was performed in triplicate at each sample concentration.

$$\sigma = k \gamma^n \quad \text{Eq. (1)}$$

#### 2.4.5. Texture profile analysis (TPA)

The texture profile analysis (TPA) was performed only on the sample concentrations from 4% to 7%, which presented consistency over 100 N s. Forty grams of each gel preparation concentration were weighed inside a cylindrical plastic container (diameter: 50 mm, height: 75 mm). Gel structures were removed from the container before the analysis, which was performed by a TA-XT2 Texture Analyser (Stable Micro Systems Ltd, Godalming, UK) and the Texture Exponent software (version 6.1.12.0). Following the method described by Ađar et al. (2016), a double compression cycle test was run up to

50% strain compression of the original portion height using an aluminium cylinder probe (diameter: 75 mm).

### *2.5. Statistical analysis*

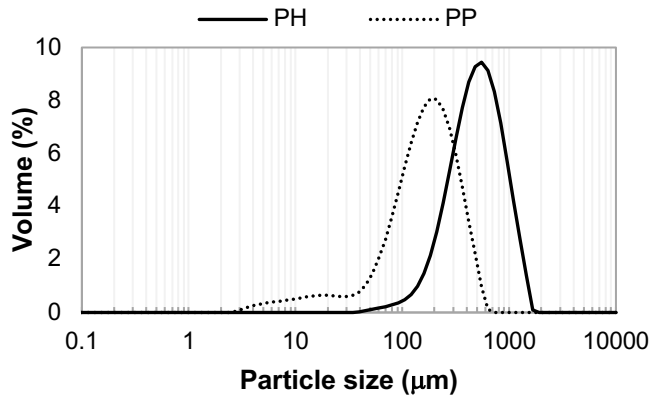
An analysis of variance (ANOVA), with a confidence 95% level ( $p < 0.05$ ), by the Statgraphics Centurion XVII Software, version 17.2.04, was applied to evaluate differences in samples. A correlation analysis of all the studied parameters with a 95% significance level was carried out (Statgraphics Centurion XVII).

## **3. Results and Discussion**

### *3.1. Psyllium fibres analysis*

#### *3.1.1. Physicochemical properties*

Particle size distribution is an important parameter that determines fibre functionality and role in the digestive tract (transit time, fermentation, and faecal excretion) (Rosell, Santos, & Collar, 2009). Figure 2 shows the particle size distribution in all the samples; as we can see, these samples vastly differed. PH presented the highest particle size, between 40 and 1,660  $\mu\text{m}$ , but with a narrow range of particles between 40 and 105  $\mu\text{m}$ . The particle size for PP was lower, and was distributed from 3 to 631  $\mu\text{m}$ , and concentrated mostly between 40–631  $\mu\text{m}$ . Table 2 shows the volume mean diameter ( $D[4,3]$ ) and the standard percentiles  $d(0.1)$ ,  $d(0.5)$ , and  $d(0.9)$  of samples' particle size. These differences in the samples' particle size distribution implied that the differences in  $D[4,3]$ ,  $d(0.1)$ ,  $d(0.5)$  and  $d(0.9)$  were significant ( $p < 0.05$ ).



PH: Plantago Husk, PP: Plantago Powder.

**Figure 2.** Volume particle size distributions (representative curves) of fibre samples.

Table 2 shows the physicochemical properties ( $a_w$ ,  $x_w$ , pH, and Hg); significant differences in  $a_w$  and  $x_w$  were found between samples ( $p < 0.05$ ). According to the  $a_w$  values of by the tested samples, PP presented the lowest  $a_w$ , which are similar to those found by de Moraes Crizel et al. (2013). According to Fernández-López et al. (2009), the ideal  $a_w$  to avoid microorganism growth and degradation reactions in products with a low water content is between 0.11 and 0.40. Both our tested samples fell within this range. PH showed the highest  $x_w$ , with a value of 5.93 (0.14) g<sub>w</sub>/100 g sample. These values are like some commercial DF of the different sources found by Rosell, Santos, and Collar (2009), but were lower than those shown by Chong, Ball, McRae, and Packer (2019) for *Psyllium* husk (10.53 g/100 g). The samples' pH values were similar, but significant differences were seen between them ( $p < 0.05$ ). PP had a significantly higher pH than PH ( $p < 0.05$ ). The hygroscopicity (Hg) of PH was significantly higher than PP ( $p < 0.05$ ). In storage terms, lower hygroscopicity could be more positive given the importance of its flowability factor (Moghbeli, Jafari, Maghsoudlou, & Dehnad, 2020).

**Table 2.** Physicochemical and hydration properties of Plantago husk and Plantago powder.

|  | <i>Samples</i>             |                            |
|--|----------------------------|----------------------------|
|  | <b>PH</b>                  | <b>PP</b>                  |
| <i>D</i> [4,3] ( $\mu\text{m}$ )                       | 597 (28) <sup>a</sup>      | 198 (4) <sup>b</sup>       |
| <i>d</i> (0.1) ( $\mu\text{m}$ )                       | 241 (20) <sup>a</sup>      | 48.2 (1.5) <sup>b</sup>    |
| <i>d</i> (0.5) ( $\mu\text{m}$ )                       | 535 (30) <sup>a</sup>      | 176 (2) <sup>b</sup>       |
| <i>d</i> (0.9) ( $\mu\text{m}$ )                       | 1051 (35) <sup>a</sup>     | 378 (8) <sup>b</sup>       |
| $a_w$  | 0.324 (0.002) <sup>a</sup> | 0.313 (0.005) <sup>b</sup> |
| $x_w$ ( $\text{g water}/100 \text{ g sample}$ )        | 5.97 (0.14) <sup>a</sup>   | 5.1 (0.1) <sup>b</sup>     |
| <i>pH</i> (dispersions 10% w/v)                        | 5.93 (0.02) <sup>b</sup>   | 6.14 (0.03) <sup>a</sup>   |
| <i>Hg</i> ( $\text{g water}/100 \text{ g dry solid}$ ) | 34.8 (0.2) <sup>a</sup>    | 28.4 (0.2) <sup>b</sup>    |
| $\rho_b$ (g/L)   | 354 (7) <sup>b</sup>       | 472 (15) <sup>a</sup>      |
| $\epsilon$   | 76.9 (0.7) <sup>a</sup>    | 69 (1) <sup>b</sup>        |
| <i>WHC</i> ( $\text{g water}/\text{g dry sample}$ )    | 27 (2) <sup>a</sup>        | 16.5 (1.9) <sup>b</sup>    |
| <i>WRC</i> ( $\text{g water}/\text{g dry sample}$ )    | 25.8 (0.7) <sup>a</sup>    | 8.1 (1.3) <sup>b</sup>     |
| <i>SWC</i> ( $\text{mL water}/\text{g sample}$ )       | 11.7 (0.7) <sup>a</sup>    | 10.0 (0.9) <sup>b</sup>    |
| <i>WSI</i> (%)   | 9.6 (0.5) <sup>b</sup>     | 37 (2) <sup>a</sup>        |
| <i>FAC</i> ( $\text{g oil}/\text{g sample}$ )          | 2.03 (0.06) <sup>a</sup>   | 1.01 (0.05) <sup>b</sup>   |

Mean values (and standard deviations).

The same letter in superscript in the line indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ).

PH: Plantago Husk, PP: Plantago Powder, *D*[4,3]: volume mean diameter, *d*(0.1), *d*(0.5), and *d*(0.9): standard percentiles,  $a_w$ : water activity,  $x_w$ : water content, *Hg*: hygroscopicity,  $\rho_b$ : bulk density,  $\epsilon$ : porosity, *WHC*: water holding capacity, *WRC*: water retention capacity, *SWC*: swelling capacity, *WSI*: solubility, *FAC*: fat adsorption capacity.

The bulk density ( $\rho_b$ ) and porosity ( $\epsilon$ ) of the samples are presented in Table 2. The samples with a lower  $\rho_b$  had a higher  $\epsilon$ , and *vice versa*. In addition, small, but significant differences were found for these parameters ( $p < 0.05$ ). The sample with the lowest  $\rho_b$  was PH. Lan et al. (2012) reported lower  $\rho_b$  values for *Polygonatum odoratum*, but they were similar to cellulose (0.38 g/mL). In addition, there were significant Pearson correlations among  $\rho_b$  with *D*[3,4], *d*(0.1), *d*(0.5), and *d*(0.9) ( $p < 0.05$ ) being -0.9888, -0.9855, -0.9876, and -0.9905, respectively. Therefore, the lower *D*[4,3], *d*(0.1), *d*(0.5), and *d*(0.9) the higher  $\rho_b$  because a fibre with a low bulk density should show more surface

area and polar groups, which could make both its swelling capacity and oil-binding capacity increase (Lan et al., 2012; Tejada-Ortigoza, Garcia-Amezquita, Serna-Saldívar, & Welte-Chanes, 2016). Uronic acid was detected in polysaccharides extracted from leaves or seeds of *Psyllium* (Zhang et al., 2019), this acid together with the polar groups and a more surface area to the surrounding water leads to an increase in the swelling volume (Tejada-Ortigoza et al., 2016).

### 3.1.2. Mineral content

Table 3 shows the ash content, total minerals and individual mineral content of both samples (Figs. S1 and S2 original equipment graphs). For both ash and total mineral contents, significant differences were found between PP and PH ( $p < 0.05$ ), where PP had the highest ash and mineral contents, which could be associated because the PP sample came from seed (Table 1). Regarding the individual mineral content, no significant differences ( $p > 0.05$ ) were observed between both DF samples (PP and PH) in Se, Zn and Cu contents. For the other analysed minerals, significant differences ( $p < 0.05$ ) were present between the PP and PH DF samples, and the P, K, Ca, Na, Fe and Mn values were higher for PP. The results of Ca, Fe and Cu in *Plantago psyllium* and *Plantago ovata* obtained by Ziemichód, Wójcik and Różyło (2019) were comparable to those presented for the PH sample. In addition, Bukhsh, Malik, and Ahmad (2007) showed similar results in *Psyllium* seed husk to K, Ca and Mn found in the PH sample. Chong et al. (2019) obtained similar values to the PH sample for both ash content and Ca, Mg, and Fe minerals in *Psyllium* husk. However, the Fe value for the PP sample, and also for other trace elements like Mn, Zn and Cu of both samples, should be highlighted because, as Rousseau et al. (2020) pointed out, Fe and Zn deficiency poses global health problems with about 30-33% of the world's population at risk, predominantly in underdeveloped countries.



3.1.3. Total phenolic compounds and antioxidant capacity

Table 3 shows the studied samples' TP content and antioxidant capacity (AC). The PP TP and AC values were significantly higher than for the other sample ( $p < 0.05$ ). This was probably due to this sample coming from seed compared to PH, which came from *Psyllium* husk. The TP and AC values obtained for PH were like those presented by Chong et al. (2019) for *Psyllium* husk. However, the TP content for PP was higher than that found by Navarro-González et al. (2011) for tomato peel fibre. According to our results, the TP content of the *Psyllium* samples was positively and significant compared to the studied samples' AC ( $p < 0.05$ ). The Pearson correlation between TP and AC was 0.9458. Thus, TP strongly affected AC, which coincides with other authors for Lulo (*Solanum quitoense*) (Iguar et al., 2014) and grapefruit (Iguar et al., 2019). Given the high TP content of PP, we highlight the remarkable attributes and quality as an alternative source of DF. The TP content presented positive correlations with ash, total mineral content, P, K, Ca, Na, Mg, Fe and Mn (0.9693, 0.9782, 0.9633, 0.9927, 0.9645, 0.9178, 0.9260, 0.9347 and 0.9474, respectively) ( $p < 0.05$ ), and also with AC (0.8944, 0.9701, 0.9815, 0.9563, 0.9434, 0.9135, 0.9637, 0.9671 and 0.9972, respectively). However, this could be negative as DF and components like polyphenols are related to lower mineral absorption in the small intestine due to binding and/or physical entrapment. Nevertheless, this is believed to be balanced for DF fermentation in the colon by gut microflora as the short-chain fatty acids that form can release trapped minerals to increase the absorptive surface area and their absorption, which is significant in the event of deficiency (Baye, Guyot, & Mouquet-Rivier, 2017).

**Table 3.** Total phenols (TP) (mg GAE/100 g sample), antioxidant capacity (AC) (mg TE/ 100 g sample), ash (%), total mineral content (mg/100 g sample) and individual mineral content (mg/100 g sample).

|                       | Samples                    |                          |
|-----------------------|----------------------------|--------------------------|
|                       | PH                         | PP                       |
| Ash                   | 2.104 (0.107) <sup>b</sup> | 3.1 (0.2) <sup>a</sup>   |
| Total mineral content | 473 (19) <sup>b</sup>      | 792 (17) <sup>a</sup>    |
| P                     | 45 (6) <sup>b</sup>        | 123 (10) <sup>a</sup>    |
| K                     | 77.2 (1.3) <sup>b</sup>    | 200 (8) <sup>a</sup>     |
| Ca                    | 165 (6) <sup>b</sup>       | 204.2 (1.8) <sup>a</sup> |
| Se                    | 0.06 (0.02) <sup>a</sup>   | 0.07 (0.03) <sup>a</sup> |
| Na                    | 156 (6) <sup>b</sup>       | 193 (9) <sup>a</sup>     |
| Mg                    | 20 (5) <sup>b</sup>        | 50 (5) <sup>a</sup>      |
| Zn                    | 0.81 (0.06) <sup>a</sup>   | 1.1 (0.5) <sup>a</sup>   |
| Fe                    | 8 (2) <sup>b</sup>         | 21 (2) <sup>a</sup>      |
| Cu                    | 0.8 (0.2) <sup>a</sup>     | 0.49 (0.18) <sup>a</sup> |
| Mn                    | 0.063 (0.005) <sup>b</sup> | 0.74 (0.15) <sup>a</sup> |
| TP                    | 55 (15) <sup>b</sup>       | 200 (9) <sup>a</sup>     |
| AC                    | 52 (7) <sup>b</sup>        | 126 (18) <sup>a</sup>    |

Mean values (and standard deviations).

The same letter in superscript in the line indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ). PH: Plantago Husk, PP: Plantago Powder.

#### 3.1.4. Hydration properties

The hydration properties of DF are related to the chemical structure of component polysaccharides, and other factors porosity, particle size, ionic forms, pH, temperature, ionic strength, type of ions in solution and stresses on fibres (Elleuch et al., 2011). These properties partly determine the fate of DF in the digestive tract and account for some of their physiological effects (Guillon, & Champ, 2000). The definition of WHC is the amount of water retained by a sample without being subjected to stress (Rosell, Santos, & Collar, 2009). PH and PP showed significant differences for WHC ( $p < 0.05$ ) (Table 2), and PH had the highest and PP the lowest WHC values. The WHC values of these DF

were higher than the values found by Rosell, Santos, and Collar (2009) for all the tested commercial fibres, except for inulin (11.05 g/g), and those observed by Kwindu, Onipe, and Jideani (2018) (3.83 g/g) for *Psyllium* fibre, but were markedly lower than those reported by Chong et al., (2019) (45 g/g) for *Psyllium* husk fibre. As in the results by Zhu et al. (2010) revealed for wheat bran DF before and after grinding, WHC increases with a larger particle size because a reduction in particle size may alter the fibre matrix structure.

WRC has DF's ability to retain water when subjected to an external force, such as centrifugation (Ma, & Mu, 2016). Table 2 shows the WRC values of the tested samples and the significant differences appeared between PH and PP ( $p < 0.05$ ). These results were notably lower than those of Kale, Yadav, and Hanah (2016) (45.7 g/g), but were higher than those reported by Kwindu, Onipe, and Jideani (2018) (4 g/g) for *Psyllium* fibre. The WRC of PH was higher than the value of Lan et al. (2012) for the DF isolated from *P. odoratum* by being left to dry in the sun (23.94 g/g), but the PP value was lower than that for cellulose (12.42 g/g). According to de Moraes Crizel et al. (2013) and Grigelmo-Miguel and Martin-Belloso (1999), DF with high WRC values can be used as a functional food ingredient to reduce calories, avoid syneresis and modify both the viscosity and texture of processed food. SWC is the volume occupied by a known fibre weight under the employed condition. In addition, SWC and WRC provide not only an overview of fibre hydration, but also useful information for fibre-supplemented foods (Guillon, & Champ, 2000). The SWC of PH was significantly higher than that of PP ( $p < 0.05$ ) (Table 2). Guillon, and Champ (2000) reported a list of fibres' hydration properties with different particle sizes where the range of SWC values was from 5.5 to 11.9 mL/g. Accordingly, the SWC values of both the tested samples fell within this range.

The nature of the glycidyl component and the structural characteristics of fibre are involved in the WSI, which is expressed as the percentage of the

fraction that is solubilised under defined conditions (de Moraes Crizel et al., 2013). PP had higher solubility, and significant ( $p < 0.05$ ) differences appeared between samples (Table 2). The high solubility of PP could be due to it being a sample with a high soluble fibre (SDF) (data from suppliers in Table 1). It is also well-known that high solubility can inhibit the digestion and absorption of nutrients from the gut (Guillon, & Champ, 2000), such as glucose and cholesterol (Belorio, Marcondes, & Gómez, 2020). Besides, soluble fibres have shown a better ability to provide viscosity and to form gels or act as emulsifiers (Elleuch et al., 2011). The WSI results for PP was higher than those reported by de Moraes Crizel et al. (2013) for DF from orange (28.95%), but lower than shown by Femenia et al. (1997) for DF from cauliflower florets dried at 75 °C (48.1%). However, the WSI value was lower for PH.

FAC is fibre's ability to absorb fat or oil, which is important in nutrition for preventing fat loss while cooking because fat is absorbed in the intestinal lumen, which lowers cholesterol (Navarro-González et al., 2011; Ma, & Mu, 2016) and retains food flavours (de Moraes Crizel et al., 2013). Table 2 shows the FAC of the tested samples. The FAC of PH was higher than that of PP, and significant differences were found between samples ( $p < 0.05$ ). The PH value was higher than the values shown for fibre from tomato peel (1.46 g oil/g) by Navarro-González et al. (2011), and was similar to that found by Femenia et al. (1997) for cauliflower stem fibre dried at 40 °C (2.1 g oil/g). However, the PP value was similar for the tomato peel fibre value indicated by Navarro-González et al. (2011).

The ability of *Psyllium* to absorb water and oil is by the interaction between the hydroxyl groups of water and those of the polysaccharide macromolecules present in the mucilage (Chaplin, 2003; Dikeman and Fahey, 2006; Beikzadeh et al., 2017). Polysaccharides obtained from seeds and husk of *Psyllium* are comprised of xylose, galactose, rhamnose, arabinose (Patel et al., 2019); then,

molecules such as arabinose and xylose create the hydrogen bonds in the mucilage (Beikzadeh et al., 2017). The hydrophilic feature of mucilage causes interaction between water and increases water retention capacity during cooking and storage (Beikzadeh et al., 2017).

The physicochemical parameters and hydration properties are related, a correlation analysis between parameters was conducted (Table 4). All the hydration properties showed statistically significant Pearson's correlation coefficients when related to D[4,3], d(0.1), d(0.5), d(0.9), Hg and pH ( $p < 0.05$ ). Hygroscopicity showed positive correlations with hydration properties, except for the WSI, where higher Hg led to a better ability to absorb water and oil. Moreover,  $a_w$  and  $x_w$  correlated positively with WHC, WRC and FAC, but negative correlations with the WSI, and no correlations with SWC were found. However, pH correlated positively with WHC and the WSI, but negatively with SWC, WRC and FAC. Bulk density ( $\rho_b$ ) correlated negatively with WHC, WRC and FAC, but positively with the WSI. When  $\rho_b$  had high values, the capacity of DF to bind water and oil decreased, but high  $\rho_b$  values meant increased DF solubility. However, particle size (D[4,3], d(0.1), d(0.5), d(0.9)) was positively related to hydration properties, except for the WSI. When samples' particle size grew, the absorption capacity of water and oil increased, but samples' solubility decreased. It can be generally stated that a reduced particle size is related to a diminished ability to retain water and oil (Lan, Chen, Chen, & Tian, 2012), but this effect cannot be generalised because both the chemical structure and shape of DF also play an essential role (Rosell, Santos, & Collar, 2009). Besides, authors like Zhu et al. (2010), Lan et al. (2012), and Ma, and Mu (2016) supported this correlation by reporting similar results. Conversely, other authors like Chantaro et al. (2008) and Rosell et al. (2009) showed that for a smaller particle size, fibres were better able to bind water. These authors also showed the importance of not generalising and analysing each fibre type

because, as Tejada-Ortigoza et al. (2016) stated, these properties are related to environmental conditions, the chemical structure of the DF polysaccharides, and to treatments and/or extraction conditions.

**Table 4.** Pearson correlation coefficients among hydration properties and physicochemical parameters and mineral content.

|  | SWC<br>(mL <sub>water</sub> /g <sub>sample</sub> ) | WHC<br>(g <sub>water</sub> /g <sub>drysample</sub> ) | WSI (%)  | WRC<br>(g <sub>water</sub> /g <sub>drysample</sub> ) | FAC<br>(g <sub>oil</sub> /g <sub>sample</sub> ) |
|--|--|--|----------|--|---|
| <i>D</i> [4,3] (μm)  | 0.8654*  | 0.9590*  | -0.9936* | 0.9900*  | 0.9925*   |
| <i>d</i> (0.1) (μm)  | 0.8774*  | 0.9630*  | -0.9909* | 0.9857*  | 0.9882*   |
| <i>d</i> (0.5) (μm)  | 0.8711*  | 0.9605*  | -0.9926* | 0.9884*  | 0.9909*   |
| <i>d</i> (0.9) (μm)  | 0.8568*  | 0.9565*  | -0.9946* | 0.9923*  | 0.9947*   |
| <i>x<sub>w</sub></i> (g <sub>water</sub> /100g <sub>sample</sub> ) | 0.7456   | 0.8698*  | -0.9772* | 0.9729*  | 0.9866*   |
| <i>a<sub>w</sub></i>   | 0.7233   | 0.8912*  | -0.8313* | 0.9084*  | 0.8774*   |
| <i>Hg</i> (g <sub>water</sub> /100g <sub>drysolid</sub> )          | 0.8281*  | 0.9604*  | -0.9905* | 0.9967*  | 0.9949*   |
| <i>pH</i>  | -0.8647*   | 0.9303*  | 0.9949*  | -0.9633*   | -0.9732*  |
| □ <sub>b</sub> (g/L)   | -0.7913  | -0.9420*   | 0.9761*  | -0.9957*   | -0.9971*  |
| □  | 0.7668   | 0.9234*  | -0.9845* | 0.9843*  | 0.9894*   |
| Total mineral<br>(mg/100g <sub>sample</sub> )                      | -0.8364*   | -0.9496*   | 0.9960*  | -0.9860*   | -0.9883*  |
| <i>P</i> (mg/100g <sub>sample</sub> )                              | -0.7994  | -0.9094*   | 0.9929*  | -0.9729*   | -0.9811*  |
| <i>K</i> (mg/100g <sub>sample</sub> )                              | -0.8722*   | -0.9573*   | 0.9944*  | -0.9898*   | -0.9883*  |
| <i>Ca</i> (mg/100g <sub>sample</sub> )                             | -0.8593*   | -0.9758*   | 0.9783*  | -0.9729*   | -0.9702*  |
| <i>Se</i> (mg/100g <sub>sample</sub> )                             | -0.2798  | -0.4119  | 0.2273   | -0.3423  | -0.3258   |
| <i>Na</i> (mg/100g <sub>sample</sub> )                             | -0.7515  | -0.9307*   | 0.9332*  | -0.9401*   | -0.9435*  |
| <i>Mg</i> (mg/100g <sub>sample</sub> )                             | -0.7578  | -0.8848*   | 0.9727*  | -0.9478*   | -0.9548*  |
| <i>Zn</i> (mg/100g <sub>sample</sub> )                             | -0.5249  | -0.6036  | 0.3601   | -0.4710  | -0.4036   |
| <i>Fe</i> (mg/100g <sub>sample</sub> )                             | -0.7968  | -0.9088*   | 0.9794*  | -0.9518*   | -0.9572*  |
| <i>Cu</i> (mg/100g <sub>sample</sub> )                             | 0.4314   | 0.5450   | -0.6276  | 0.6515   | 0.6889  |
| <i>Mn</i> (mg/100g <sub>sample</sub> )                             | -0.8047  | -0.8805*   | 0.9862*  | -0.9472*   | -0.9646*  |

\*Correlation is significant at the 0.05 level

Volume mean diameter *D*[4,3], standard percentiles *d*(0.1), *d*(0.5) and *d*(0.9), water activity (*a<sub>w</sub>*), water content (*x<sub>w</sub>*), pH, hygroscopicity (*Hg*), bulk density (□<sub>b</sub>), and porosity (□); SWC: swelling capacity; WHC: water-holding capacity; WRC: water retention capacity; FAC: fat absorption.

Moreover, a correlation analysis between the multimineral content and hydration properties was performed (Table 4). The total mineral content showed statistically significant negative Pearson's correlation coefficients when related to all the hydration properties (SWC, WHC, WRC and FAC),

except for the WSI, which had a positive correlation ( $p < 0.05$ ). Additionally, minerals P, K, Ca, Na, Mg, Fe and Mn correlated negatively with WHC, WRC and FAC, but positively with the WSI. This situation could be related to the valence and ratio of the adsorbed ions, because the ion valence increased or the ratio decreased, and the hydration force magnitude increased (Li et al., 2020). For this reason, these minerals must be divalent ions because they are adsorbed in completely hydrated states (Li et al., 2020). However, SWC was negatively associated with K and Ca.

### *3.2. Gel analysis*

#### *3.2.1. Physicochemical and optical properties*

Solutions of both DF samples were prepared at the 1, 2, 3, 4, 5, 6, and 7% concentrations (heated and unheated) to know the physicochemical and mechanical properties of each concentration, and their possible different uses in food. Table 5 shows both samples'  $x_w$ , pH and solution colour at each concentration. The  $x_w$  of both samples lowered when the DF concentration rose in both (with and without heat treatment). An interaction occurred between samples' concentration and the employed DF sample (PP or PH) when  $x_w$  lowered as the concentration of both DF samples increased. With PP, the drop in the 6% and 7% concentrations was significantly more marked ( $p < 0.05$ ) regardless of the temperature at which structures formed.

**Table 5.** Results of  $x_w$  (g/g sample), the pH and colour parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) of the formulated *Psyllium* gels.

| Samples | C | $x_w$                              | pH                             | $L^*$                         | $a^*$                            | $b^*$                            |
|---------|---|------------------------------------|--------------------------------|-------------------------------|----------------------------------|----------------------------------|
| PH      | 1 | 0.9906 (0.0007) <sup>aA</sup>      | 6.88 (0.06) <sup>aB</sup>      | 6.5 (0.9) <sup>iG</sup>       | 0.60 (0.04) <sup>deBC</sup>      | 1.4 (0.4) <sup>hEF</sup>         |
|         | 2 | 0.9790 (0.0005) <sup>bC</sup>      | 6.586<br>(0.109) <sup>cC</sup> | 11.4 (0.4) <sup>jiD</sup>     | 1.64 (0.15) <sup>aA</sup>        | 3.6 (0.3) <sup>dA</sup>          |
|         | 3 | 0.9697 (0.0009) <sup>dD</sup>      | 6.39 (0.03) <sup>eD</sup>      | 8.8 (0.7) <sup>kF</sup>       | 0.72 (0.12) <sup>deCD</sup>      | 0.83 (0.17) <sup>hF</sup>        |
|         | 4 | 0.9648 (0.0004) <sup>eE</sup>      | 6.28 (0.08) <sup>fEF</sup>     | 11.9 (0.9) <sup>iD</sup>      | 0.77 (0.09) <sup>deBC</sup>      | 1.1 (0.3) <sup>hEF</sup>         |
|         | 5 | 0.9522 (0.0009) <sup>fGH</sup>     | 6.14 (0.02) <sup>ghH</sup>     | 13.8 (1.3) <sup>hC</sup>      | 0.96 (0.13) <sup>cdBC</sup>      | 2.1 (0.5) <sup>gCD</sup>         |
|         | 6 | 0.9458 (0.0005) <sup>gl</sup>      | 6.10 (0.02) <sup>hH</sup>      | 26.1 (0.8) <sup>ba</sup>      | 1.16 (0.07) <sup>cb</sup>        | 3.2 (0.5) <sup>deAB</sup>        |
|         | 7 | 0.9412 (0.0003) <sup>hJ</sup>      | 6.0 (0.3) <sup>il</sup>        | 25.1 (0.7) <sup>ca</sup>      | 1.2 (0.4) <sup>cb</sup>          | 2.8 (0.3) <sup>efB</sup>         |
| 65PH    | 1 | 0.9879 (0.0018) <sup>yB</sup>      | 7.01 (0.03) <sup>zA</sup>      | 7 (2) <sup>sG</sup>           | 0.49 (0.09) <sup>vE</sup>        | 1.0 (0.2) <sup>veF</sup>         |
|         | 2 | 0.9792 (0.0013) <sup>xC</sup>      | 6.57 (0.12) <sup>xC</sup>      | 9.92 (1.04) <sup>fEF</sup>    | 0.516<br>(0.115) <sup>vDE</sup>  | 0.8 (0.4) <sup>veF</sup>         |
|         | 3 | 0.9689 (0.0006) <sup>wD</sup>      | 6.32 (0.02) <sup>VE</sup>      | 8.9 (0.7) <sup>uIF</sup>      | 0.42 (0.04) <sup>VE</sup>        | -0.24 (0.14) <sup>uG</sup>       |
|         | 4 | 0.96026<br>(0.00116) <sup>vF</sup> | 6.27 (0.04) <sup>uIF</sup>     | 10.8 (0.9) <sup>uDE</sup>     | 0.78 (0.07) <sup>wBC</sup>       | 1.3 (0.4) <sup>veF</sup>         |
|         | 5 | 0.95384<br>(0.00115) <sup>uG</sup> | 6.188<br>(0.008) <sup>sG</sup> | 21.3 (0.7) <sup>xB</sup>      | 0.82 (0.14) <sup>xwBC</sup>      | 1.4 (0.6) <sup>veF</sup>         |
|         | 6 | 0.95161<br>(0.00015) <sup>uH</sup> | 6.11 (0.03) <sup>qH</sup>      | 22.5 (1.4) <sup>yxB</sup>     | 0.87 (0.16) <sup>xwBC</sup>      | 1.5 (0.5) <sup>wvDE</sup>        |
|         | 7 | 0.945 (0.002) <sup>tsI</sup>       | 6.0 (0.3) <sup>pl</sup>        | 21.5 (1.7) <sup>xB</sup>      | 0.83 (0.15) <sup>xwBC</sup>      | 2.1 (0.5) <sup>xwC</sup>         |
| PP      | 1 | 0.9898 (0.0012) <sup>aA</sup>      | 6.71 (0.07) <sup>ba</sup>      | 10.9 (0.8) <sup>ji</sup>      | 0.45 (0.15) <sup>fFG</sup>       | 1.2 (0.6) <sup>hG</sup>          |
|         | 2 | 0.9803 (0.0003) <sup>bB</sup>      | 6.564<br>(0.009) <sup>cC</sup> | 15.0 (0.3) <sup>gH</sup>      | 0.64 (0.09) <sup>efEF</sup>      | 2.4 (0.4) <sup>fgEF</sup>        |
|         | 3 | 0.9734 (0.0004) <sup>cC</sup>      | 6.51 (0.05) <sup>dD</sup>      | 16.8 (0.3) <sup>fG</sup>      | 1.03 (0.05) <sup>cC</sup>        | 3.60 (0.13) <sup>dC</sup>        |
|         | 4 | 0.9644 (0.0002) <sup>eE</sup>      | 6.40 (0.03) <sup>eE</sup>      | 19.25<br>(0.13) <sup>eE</sup> | 1.40 (0.06) <sup>bb</sup>        | 5.32 (0.19) <sup>cb</sup>        |
|         | 5 | 0.9547 (0.0002) <sup>fF</sup>      | 6.3 (0.4) <sup>fFG</sup>       | 21.5 (0.2) <sup>dD</sup>      | 1.7600<br>(0.1114) <sup>aA</sup> | 6.6 (0.2) <sup>ba</sup>          |
|         | 6 | 0.9438 (0.0003) <sup>ghG</sup>     | 6.16 (0.02) <sup>gl</sup>      | 20.9 (0.4) <sup>dD</sup>      | 1.76 (0.08) <sup>aA</sup>        | 6.5 (0.2) <sup>ba</sup>          |
|         | 7 | 0.935 (0.007) <sup>hH</sup>        | 6.2 (0.7) <sup>gl</sup>        | 27.1 (0.6) <sup>aA</sup>      | 1.6 (0.2) <sup>abAB</sup>        | 7.1 (0.8) <sup>aA</sup>          |
| 65PP    | 1 | 0.99060<br>(0.00013) <sup>zA</sup> | 6.64 (0.09) <sup>yB</sup>      | 10.1 (0.9) <sup>uJ</sup>      | 0.10 (0.05) <sup>uH</sup>        | -0.98 (0.16) <sup>th</sup>       |
|         | 2 | 0.9794 (0.0005) <sup>xB</sup>      | 6.404<br>(0.013) <sup>wE</sup> | 14.2 (0.8) <sup>vH</sup>      | 0.33 (0.08) <sup>VG</sup>        | 0.89 (0.08) <sup>VG</sup>        |
|         | 3 | 0.969 (0.003) <sup>wD</sup>        | 6.29 (0.03) <sup>vuF</sup>     | 16.7 (0.7) <sup>wG</sup>      | 0.78 (0.07) <sup>wDE</sup>       | 2.9 (0.5) <sup>zyDE</sup>        |
|         | 4 | 0.9620 (0.0003) <sup>VE</sup>      | 6.244<br>(0.009) <sup>tG</sup> | 17.8 (0.3) <sup>wF</sup>      | 1.02 (0.05) <sup>xC</sup>        | 3.42 (0.15) <sup>zCD</sup>       |
|         | 5 | 0.9533 (0.0006) <sup>uF</sup>      | 6.2 (0.4) <sup>sH</sup>        | 26.1 (0.2) <sup>zB</sup>      | 1.0 (0.2) <sup>xwCD</sup>        | 2.3 (0.4) <sup>xF</sup>          |
|         | 6 | 0.9453 (0.0008) <sup>tG</sup>      | 6.148 (0.015) <sup>rl</sup>    | 23.06<br>(1.08) <sup>yC</sup> | 1.5 (0.2) <sup>yB</sup>          | 2.8 (0.8) <sup>zyDEF</sup>       |
|         | 7 | 0.9426 (0.0003) <sup>sG</sup>      | 6.1 (0.7) <sup>qJ</sup>        | 25.6 (0.6) <sup>zB</sup>      | 1.7 (0.4) <sup>zA</sup>          | 2.660<br>(1.114) <sup>yxEF</sup> |

Mean values (and standard deviations).

For the samples under the same conditions, the letter in superscript in the column indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ) (<sup>a-i</sup> for unheated, and <sup>z-q</sup> heated samples). To compare the same sample to the temperature effect, the same capital letter in superscript in the column indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ). C: Concentration (%); 65 indicates the samples heated at 65 °C, 20 min; PH: Plantago Husk; PP: Plantago Powder.



The pH of the tested gels was similar for both samples, and the pH went from 6 to 7. However, significant differences were observed in the pH values of both samples with and without heat treatment ( $p < 0.05$ ) (Table 5). An interaction between concentration and used DF sample (PP or PH) was observed, and the pH values slight dropped for both DF samples when concentration rose ( $p < 0.05$ ). For pH, the most marked decrease was observed in the PH sample up to the 3% fibre concentration (with and without heat treatment). The sample with the highest pH was 65PH at the 1% concentration. No significant effect on the pH values was observed in the solution concentrations for the PH fibre because of heat treatment ( $p > 0.05$ ), whereas the solutions made with the PP fibre had slightly lower pH values in the heated samples ( $p < 0.05$ ).

With both samples, when the DF samples' concentration went below 4%, the solid content precipitated. When the concentration rose, dispersion homogeneity markedly increased because samples were mixtures of whole cells and dispersed cell wall materials of different particle sizes, as shown in Fig. 2. Therefore, the bigger material absorbed water until it formed a stabler gel structure. Table 5 depicts the colorimetric parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) of the formulated samples. To illustrate the colour samples, the images of gels are presented in Fig. 3 (with and without heat treatment). It can be seen that, although both samples came from *Psyllium*, gel colours significantly differed ( $p < 0.05$ ), which could be due to the fact that PP came from *Plantago ovata* seed and PH came mostly from husk (Table 1). Similar behaviour was observed in the gels made with both samples, and parameters  $L^*$ ,  $a^*$  and  $b^*$  significantly increased with a rising fibre concentration both with and without heat treatment ( $p < 0.05$ ). As observed in this study, Wang et al. (2018) reported that the colour parameters increased in the homogenised suspension and became more saturated with red and yellow. The colour of gel samples depended mainly on

the colour of solids as seen in Fig. 1, where PP was markedly darker. The increase observed in the colour parameters was also related to the used DF, and the PP gels generally had higher  $L^*$  and  $a^*$  values in the unheated and heated samples ( $p < 0.05$ ), but lower  $b^*$  values. Moreover, no significant differences were observed in either luminosity ( $L^*$ ) or redness ( $a^*$ ) for the DF sample (PP or PH) and the applied treatment ( $p > 0.05$ ). Both samples displayed the same behaviour, but yellowness ( $b^*$ ) significantly decreased, which was observed when samples were prepared with heat treatment in the gels formulated with PP ( $p < 0.05$ ). The colour of these samples was darker than the gels made with hydrocolloids like xanthan gum (Chong et al., 2019). Other authors have indicated that the incorporating *Psyllium* into different food products can increase colour darkness in products (Ahmadi, Kalbasi-Ashtari, Oromiehie, Yarmand, & Jahandideh, 2012; Figueroa, & Genovese, 2019). Gel colour is related to mineral content because positive Pearson correlations were found. The increase in the luminosity ( $L^*$ ) of both samples (heated and unheated) can be associated with a higher total mineral content (0.8699), and Ca and Na showed a higher correlation (0.8898 and 0.8877, respectively). However, an increase in redness ( $a^*$ ) was more related to the total mineral content when samples were heated (0.8507.; Na, Ca, Mg, K, P and Fe were the minerals that were the most related to a rise in  $a^*$  when gels were formed with heat (0.8233, 0.8218, 0.8128, 0.8115, 0.8062 and 0.8022, respectively). For yellowness ( $b^*$ ), a higher and positive correlation was also found with the total mineral content when gels formed without heat treatment (0.8463). In this case, K, P, Fe, Mg and Mn were the minerals that most strongly influenced the increase in  $b^*$  (0.8976, 0.8923, 0.8874, 0.8801 and 0.8559, respectively).

### 3.2.2. Mechanical properties

The back extrusion assay was performed at all the gel concentrations (1-7%), similarly to a previous study in order to know the mechanical properties of

both *Psyllium* DFs (Fig. S3 original graphs). Table 6 depicts the back extrusion parameter results. This table indicates that not only the consistency and firmness of *Psyllium* gels increased as the DF concentration rose, but so did viscosity and cohesiveness as results showed by Noguerol, Igual and Pagán-Moreno (2021) for different DFs. However, the results of this study showed that both *Psyllium* fibres had greater values of all back extrusion parameters than DFs showed by Noguerol, Igual and Pagán-Moreno (2021). Moreover, a significant interaction took place between concentration and temperature ( $p < 0.05$ ), and all the back extrusion parameters increased when the gels were heated. This increase in consistency was similar in the gels with both the *Psyllium* DF samples.

The gelling process begins with the formation of junction zones, these junctions grow and join the polysaccharide molecules to form the gel network (Yu, Perret, Parker, & Allen, 2003). However, the interaction between treatment and the employed DF sample was observed for firmness, cohesiveness and viscosity where the gels formulated with the PP sample obtained significantly higher results for these parameters from the 5% concentration ( $p < 0.05$ ), except for viscosity, which presented significant differences only in the 7% concentration. It is a well-known fact that *Psyllium* fibre has positive gelling properties (Yu et al., 2003) and Askari et al. (2018) indicated that the gels formulated with *Psyllium*-maize starch films had a more compact and homogeneous structure than the films made only with starch. Therefore as this study indicates, both *Psyllium* fibres can be used as gelling agents in food products with or without starch addition. In line with the result shown in this study, Ren et al. (2020b) indicated that hydrated whole *Psyllium* husk powder exhibited a gel-like property and this gel became stronger when it was formed by applying heating. Authors like Igual et al 2013 and Igual et al., 2014 indicated that some parameters like consistency and viscosity are very important

because they are related to coverage in mouth with gelled products.

**Table 6.** Back extrusion parameters of the formulated *Psyllium* gels.

| Samples | C | Consistency (N s)          | Firmness (N)                 | Viscosity (N s)              | Cohesiveness (N)             |
|---------|---|----------------------------|------------------------------|------------------------------|------------------------------|
| PH      | 1 | 1.24 (0.08) <sup>gJ</sup>  | 0.22 (0.02) <sup>gI</sup>    | 0.08 (0.02) <sup>hG</sup>    | 0.171 (0.012) <sup>iI</sup>  |
|         | 2 | 7 (3) <sup>fgJ</sup>       | 1.4 (0.5) <sup>gI</sup>      | 1.00 (0.02) <sup>ghFG</sup>  | 1.3 (0.3) <sup>hHI</sup>     |
|         | 3 | 23 (8) <sup>ehI</sup>      | 3.8 (1.4) <sup>ehI</sup>     | 2.6 (0.7) <sup>IE</sup>      | 3.3 (0.9) <sup>gG</sup>      |
|         | 4 | 43 (2) <sup>dGH</sup>      | 6.8 (0.3) <sup>dGH</sup>     | 4.64 (0.15) <sup>eD</sup>    | 5.677 (0.109) <sup>fF</sup>  |
|         | 5 | 72 (5) <sup>cF</sup>       | 11.7 (0.9) <sup>cF</sup>     | 7.7 (0.7) <sup>cdC</sup>     | 9.3 (0.4) <sup>eE</sup>      |
|         | 6 | 105 (6) <sup>bE</sup>      | 16.9 (0.9) <sup>bE</sup>     | 8.6 (0.5) <sup>cC</sup>      | 13.2 (0.4) <sup>cD</sup>     |
|         | 7 | 142 (14) <sup>aD</sup>     | 23.8 (1.7) <sup>aD</sup>     | 10.1 (0.6) <sup>bB</sup>     | 16.5 (0.8) <sup>bC</sup>     |
| 65PH    | 1 | 8 (2) <sup>uJ</sup>        | 1.6 (0.5) <sup>rI</sup>      | 0.62 (0.15) <sup>IFG</sup>   | 1.5 (0.7) <sup>sGHI</sup>    |
|         | 2 | 24 (2) <sup>uHI</sup>      | 3.6 (0.3) <sup>rHI</sup>     | 2.0 (0.4) <sup>uEF</sup>     | 2.6 (0.3) <sup>sGH</sup>     |
|         | 3 | 49 (14) <sup>vG</sup>      | 9 (2) <sup>sFG</sup>         | 4.2 (0.7) <sup>vD</sup>      | 5.6 (1.2) <sup>IF</sup>      |
|         | 4 | 117 (15) <sup>wE</sup>     | 21 (2) <sup>tDE</sup>        | 8.2 (0.9) <sup>xwC</sup>     | 9.6 (0.8) <sup>uE</sup>      |
|         | 5 | 168 (21) <sup>xC</sup>     | 29 (3) <sup>uC</sup>         | 10 (2) <sup>xB</sup>         | 14.7 (1.7) <sup>wCD</sup>    |
|         | 6 | 234 (33) <sup>yB</sup>     | 41 (8) <sup>wB</sup>         | 13 (2) <sup>yA</sup>         | 20 (3) <sup>xB</sup>         |
|         | 7 | 307 (42) <sup>zA</sup>     | 53 (9) <sup>yA</sup>         | 14 (3) <sup>zyA</sup>        | 24 (4) <sup>yA</sup>         |
| PP      | 1 | 1.04 (0.02) <sup>gK</sup>  | 0.16 (0.04) <sup>gJ</sup>    | 0.056(0.003) <sup>hHI</sup>  | 0.148 (0.005) <sup>iHI</sup> |
|         | 2 | 5.5 (0.5) <sup>gJK</sup>   | 1.049 (0.109) <sup>gJ</sup>  | 0.71 (0.05) <sup>hGH</sup>   | 1.04 (0.05) <sup>hiGH</sup>  |
|         | 3 | 16 (3) <sup>efIJ</sup>     | 2.8 (0.6) <sup>efHI</sup>    | 2.16 (0.18) <sup>fgFG</sup>  | 3.1 (0.3) <sup>gG</sup>      |
|         | 4 | 36 (8) <sup>dH</sup>       | 6.1 (1.6) <sup>dG</sup>      | 4.5 (0.6) <sup>eE</sup>      | 6.14 (1.06) <sup>fF</sup>    |
|         | 5 | 68 (5) <sup>cF</sup>       | 11.8 (0.8) <sup>cF</sup>     | 6.992 (1.112) <sup>dD</sup>  | 10.8 (0.4) <sup>dE</sup>     |
|         | 6 | 106 (7) <sup>bE</sup>      | 18.22 (1.09) <sup>aE</sup>   | 8.87 (1.18) <sup>bcCD</sup>  | 14.0 (1.5) <sup>cD</sup>     |
|         | 7 | 145 (15) <sup>aD</sup>     | 25 (3) <sup>bD</sup>         | 13 (3) <sup>aB</sup>         | 18.8 (1.9) <sup>aC</sup>     |
| 65PP    | 1 | 2.68 (0.09) <sup>uJK</sup> | 0.57 (0.07) <sup>rJ</sup>    | 0.176 (0.006) <sup>IGH</sup> | 0.23 (0.02) <sup>sH</sup>    |
|         | 2 | 23 (3) <sup>uHI</sup>      | 3.8 (0.5) <sup>rHI</sup>     | 1.47 (0.09) <sup>IGH</sup>   | 2.08 (0.09) <sup>sGH</sup>   |
|         | 3 | 52 (6) <sup>vG</sup>       | 9.73 (1.04) <sup>sF</sup>    | 4.0 (0.4) <sup>vuEF</sup>    | 6.6 (0.6) <sup>IF</sup>      |
|         | 4 | 102 (7) <sup>wE</sup>      | 19.8 (1.7) <sup>tE</sup>     | 7.7 (0.7) <sup>wCD</sup>     | 11.0 (0.7) <sup>uE</sup>     |
|         | 5 | 178 (9) <sup>xC</sup>      | 34.362 (1.009) <sup>wC</sup> | 9.1 (1.4) <sup>xwC</sup>     | 17.5 (0.7) <sup>wC</sup>     |
|         | 6 | 247 (7) <sup>yB</sup>      | 47 (2) <sup>xB</sup>         | 14.1 (1.4) <sup>zyAB</sup>   | 24.71 (1.08) <sup>yB</sup>   |
|         | 7 | 298 (30) <sup>zA</sup>     | 61 (4) <sup>zA</sup>         | 15 (5) <sup>zB</sup>         | 28 (5) <sup>zA</sup>         |

Mean values (and standard deviations).

For the samples under the same conditions, the letter in superscript in the column indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ) (<sup>a-i</sup> for unheated, and <sup>z-r</sup> heated samples). To compare the same sample to the temperature effect, the same capital letter in superscript in the column indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ). C: Concentration (%); 65 indicates the samples heated at 65 °C, 20 min; PH: Plantago Husk; PP: Plantago Powder.

The Pearson correlations indicated that all the back extrusion parameters (consistency, firmness, viscosity, cohesiveness) were associated with samples'  $x_w$ , pH, and mineral composition. A negative correlation between  $x_w$  and pH with consistency (-0.8044 and -0.7445, respectively) and firmness (-0.7777 and

-0.7141) was found, and a positive correlation appeared between  $x_w$  and pH with viscosity (0.9035 and 0.8081, respectively) and cohesiveness (0.8923 and 0.7992, respectively). The total mineral content was positively related to consistency and firmness (0.7494 and 0.7636, respectively), and Ca (0.8267 for consistency and 0.8176 for firmness) and Na (0.8247 for consistency and 0.8154 for firmness) were the most influential minerals. However, viscosity and cohesivity negatively correlated with total mineral content (-0.8149 and -0.8609, respectively). In this case, Ca (-0.8939 for viscosity and -0.9213 for cohesiveness) and Na (-0.8859 for viscosity and -0.9223 for cohesiveness) were the most influential minerals. In practice, Li et al. (2018) and Liu et al. (2018) indicated that the gelation of food biopolymers could be induced by different approaches, including temperature, pressure, acids, salts, enzymes, ethanol, among others. From the results of this study, the consistency and firmness of the *Psyllium* gels increased with a lower pH. It can also be stated that gel formation is due to ionic interactions with Ca and Na (Mao et al., 2020). The consistency, firmness and viscosity parameters of the gels formed at cold temperature (5 °C) were also associated with DF samples' SWC, and negatively with firmness and consistency (-0.8740 and -0.8698, respectively) and positively with viscosity (0.8886). However, consistency was positively related to the WSI (0.8143). When gels were formed with heating, a negative Pearson correlation with WHC was found (-0.8187).

As the 1%, 2% and 3% concentrations were not homogeneous and did not form a stable hydrogel, a decision was made to perform flow curves and TPA from only concentrations 4% to 7%. Hence the samples with a consistency lower than 100 N s as a result of the back extrusion assay were characterised by the flow curve analysis (Fig. S4 original graphs). Consistency ( $k$ ), flow behaviour ( $n$ ) and goodness of fit ( $R^2$ ) are shown in Table 7. All the data were fitted with Ostwald's power law. The PP gels had the highest  $k$  and an increase

in  $k$  was related to a rising DF concentration, Su, Zhu, Adhikari, Li, and Wang (2020) indicated the same results for the citrus fibre-oil dispersions. On the contrary, no significant differences were observed between the gels made with PH fibre ( $p > 0.05$ ). All the formulated gels had a  $k$  higher than 20 Pa s. So their gelling properties can be identified with semisolid or spoonable foods (Aguayo-Mendoza et al., 2019), and can be used as fat replacers and/or texture modifiers of meat analogues, among others.  $n$  was lower than 1 in all the samples, which means that our samples behaved as pseudoplastic fluids. As no changes were observed in  $n$  due to increasing PH fibre content ( $p > 0.05$ ), the rising PP concentration led to a significant decrease in this parameter ( $p < 0.05$ ).

**Table 7.** Flow curve parameters  $k$  (consistency index),  $n$  (flow index) and  $\eta_{ap}$  (shear viscosity at 50 s<sup>-1</sup> shear stress) of the formulated *Psyllium* gels.

| Ostwald-de Waele model |       |                          |                            |                          |       |
|------------------------|-------|--------------------------|----------------------------|--------------------------|-------|
| Samples                | C (%) | $k$ (Pa s <sup>n</sup> ) | $n$                        | $\eta_{ap}$ (Pa s)       | $R^2$ |
| PH                     | 4     | 21.5 (0.3) <sup>c</sup>  | 0.468 (0.006) <sup>a</sup> | 2.7 (0.04) <sup>b</sup>  | 0.99  |
|                        | 5     | 24 (3) <sup>c</sup>      | 0.464 (0.016) <sup>a</sup> | 3.0 (0.2) <sup>b</sup>   | 0.99  |
| PP                     | 4     | 42 (2) <sup>b</sup>      | 0.404 (0.007) <sup>b</sup> | 4.10 (0.09) <sup>a</sup> | 0.99  |
|                        | 5     | 69.4 (0.5) <sup>a</sup>  | 0.32 (0.05) <sup>c</sup>   | 4.91 (1.02) <sup>a</sup> | 0.99  |

Mean values (and standard deviations).

The same letter in superscript in the line indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ). C: concentration (%); PH: Plantago Husk; PP: Plantago Powder.

Both  $k$  and  $n$  were related to particle size (D[4,3] and d (0.9)). For  $k$ , a negative Pearson correlation was presented, which meant that a drop in  $k$  was associated with a bigger particle size (-0.9211 for D[4,3] and -0.9757 for d (0.9)). However, positive correlations were found between D[4,3] and d (0.9) with  $n$  (0.8822 and 0.9545, respectively). Wang et al. (2018) have also reported that this parameter depends on particle size distribution. Pearson correlations were found between the flow curve parameters and hydration properties,

except for SWC.  $k$  and  $\eta_{ap}$  were negatively related to WHC (-0.9427 and -0.8861, respectively), WRC (-0.9939 and -0.8766, respectively) and FAC (-0.9933 and -0.8394, respectively), but positively with the WSI (0.9914 and 0.8123, respectively).  $n$  was positively correlated with WRC and FAC, and negatively with the WSI (0.8862, 0.9111 and -0.9252, respectively).

Dikeman and Fahey (2006) (p. 652) indicated that “*viscosity as related to DF refers to the ability of some polysaccharides to thicken or form gels when mixed with fluids resulting from physical entanglements among the polysaccharides constituents within the fluid or solution*” and apparent viscosity is the most common term in the literature related to DF. The trend of samples’ apparent viscosity is shown in Fig. 4, where it can be observed that the tendency of all the gels was similar, although the PH samples seemed to have lower apparent viscosity. Table 7 confirms this. It shows the samples’  $\eta_{ap}$  and that no concentration dependences were found with either sample ( $p > 0.05$ ), but both the  $\eta_{ap}$  of the PP gels was significantly higher than it was for the PH gels ( $p < 0.05$ ).

These results agree with the reports by Agarwal, Hewson, and Foster (2018), and shear viscosity was related to fibres’ source, processing and microstructure. Therefore in line with Ren et al. (2020b), freshly prepared *Psyllium* husk dispersion can be described as the concentrated suspension of gel particles and its rheological properties can be ascribed to particles’ viscoelastic properties and to the physical contacts and friction between them. It is noteworthy that authors like Niknam, Ghanbarzadeh, Ayaseh, and Rezagholi (2018) have reported that the addition of *Plantago major* seed to emulsions can enhance the stability of samples during storage by increasing continuous phase viscosity. Therefore, this could also be achieved by adding PP and/or PH. The present study confirms both fibres can be used as texturizing agents to modify the food texture to pudding-like consistency (>

1750 mPa s) for patients with oropharyngeal dysphagia (Ribes, Estarriaga, Grau, Talens, 2021).

When characterisation ended, a TPA was performed in those samples that presented a higher back extrusion consistency than 100 N s (Fig. S5 original graphs). The TPA parameter results are shown in Table 8. Regarding the hardness results for the gels formed without heat, it can be seen that the different concentrations of each DF sample ( $p > 0.05$ ), but the hardness of the gels formulated with PP was significantly higher ( $p < 0.05$ ). When gels were formed by heating, concentration fibre dependence was found for both samples, as was a high DF concentration and marked hardness. This increased hardness was greater for the PH gels than for PP one. The highest results were for the 65PH sample at 7% ( $p < 0.05$ ). A heat treatment effect appeared for the PH sample, and the hardness of the gels formed by heating with the DF sample was higher, high temperature fractions have stronger gel properties (Ren et al., 2020b). Comparing these results with gels formed with other vegetable fibres (Noguerol, Igual, Pagán-Moreno, 2021), *Psyllium* gels form more stable and firmer gels.

All the results for adhesiveness were negatives, which indicated that it formed a sticky gel. The PH samples with no heat treatment presented lower adhesiveness than the gels formed at 65 °C, but the adhesiveness for the PP gels decreased when gels were formed with heating. It could be related to the different composition of the *Psyllium* fibre, because the PP fibre is from the seed and has more proteins and carbohydrates (Table 1) which can modify its conformation with heating. In addition, Ren et al. (2020b) indicated that during heating, the gel phase gradually expanded and disappeared, which might play the role of junction zone formation and responsible for the thermoreversible gel-like properties. An interaction was observed between adhesiveness and the increased DF concentration with both samples. The importance of this



parameter lies in food texture becoming unpleasant for consumers when it adheres to the palate and requires much effort to separate it. It can be noted that springiness was similar for all the samples and did not depend on the used fibre or concentration. Although, formulations with high adhesiveness caused prolonged retention in buccal cavity (Bhatia & Ahuja, 2013). Regarding cohesiveness, the gel samples with heat treatment generally obtained significantly higher results ( $p < 0.05$ ). It also depended on the DF used because the cohesiveness of PP was greater ( $p < 0.05$ ) at the highest concentration tested in this study.

**Table 8.** TPA parameters of the formulated *Psyllium* gels.

| Samples | C | Hardness                     | Adhesiveness                     | Springiness                        | Cohesiveness                     | Resilience                        | Chewability                    |
|---------|---|------------------------------|----------------------------------|------------------------------------|----------------------------------|-----------------------------------|--------------------------------|
| PH      | 6 | 2.7<br>(0.4) <sup>bCD</sup>  | -3.2<br>(0.3) <sup>cC</sup>      | 0.90<br>(0.03) <sup>aAB</sup>      | 0.69<br>(0.02) <sup>bC</sup>     | 0.12<br>(0.02) <sup>cD</sup>      | 1.69<br>(0.19) <sup>cCD</sup>  |
|         | 7 | 3.2<br>(0.3) <sup>bC</sup>   | -3.89<br>(0.19) <sup>bB</sup>    | 0.89<br>(0.02) <sup>aB</sup>       | 0.72<br>(0.03) <sup>bBC</sup>    | 0.142<br>(0.016) <sup>bC</sup>    | 2.0<br>(0.2) <sup>bC</sup>     |
| 65PH    | 4 | 2.37<br>(0.14) <sup>wD</sup> | -1.839<br>(0.117) <sup>vuE</sup> | 0.914<br>(0.008) <sup>zA</sup>     | 0.708<br>(0.007) <sup>wC</sup>   | 0.185<br>(0.018) <sup>vuB</sup>   | 1.535<br>(0.104) <sup>vD</sup> |
|         | 5 | 3.0<br>(0.3) <sup>xC</sup>   | -2.4<br>(0.3) <sup>wD</sup>      | 0.91523<br>(0.01107) <sup>zA</sup> | 0.7337<br>(0.0104) <sup>wB</sup> | 0.206<br>(0.005) <sup>xwvAB</sup> | 2.0<br>(0.2) <sup>wC</sup>     |
|         | 6 | 4.7<br>(0.3) <sup>yB</sup>   | -4.0<br>(0.3) <sup>yB</sup>      | 0.898<br>(0.013) <sup>zAB</sup>    | 0.777<br>(0.008) <sup>yxA</sup>  | 0.200<br>(0.009) <sup>wvAB</sup>  | 3.3<br>(0.3) <sup>yB</sup>     |
|         | 7 | 6.2<br>(0.7) <sup>zA</sup>   | -4.9<br>(0.6) <sup>zA</sup>      | 0.9021<br>(0.0103) <sup>zAB</sup>  | 0.7678<br>(0.0115) <sup>xA</sup> | 0.217<br>(0.016) <sup>yxwA</sup>  | 4.3<br>(0.6) <sup>zA</sup>     |
|         | 6 | 4.0<br>(0.4) <sup>aB</sup>   | -3.95<br>(0.14) <sup>bAB</sup>   | 0.916<br>(0.013) <sup>aA</sup>     | 0.760<br>(0.017) <sup>aD</sup>   | 0.141<br>(0.012) <sup>bC</sup>    | 2.8<br>(0.3) <sup>aBC</sup>    |
| 65PP    | 4 | 2.3<br>(0.3) <sup>wD</sup>   | -1.99<br>(0.18) <sup>vD</sup>    | 0.90<br>(0.02) <sup>zAB</sup>      | 0.83<br>(0.04) <sup>zAB</sup>    | 0.19<br>(0.03) <sup>vuB</sup>     | 1.70<br>(0.19) <sup>wvD</sup>  |
|         | 5 | 3.5<br>(0.2) <sup>xC</sup>   | -2.20<br>(0.09) <sup>wvuD</sup>  | 0.909<br>(0.013) <sup>zAB</sup>    | 0.793<br>(0.013) <sup>yxC</sup>  | 0.242<br>(0.014) <sup>zyA</sup>   | 2.51<br>(0.14) <sup>xC</sup>   |
|         | 6 | 4.3<br>(0.6) <sup>yAB</sup>  | -3.0<br>(0.6) <sup>xC</sup>      | 0.902<br>(0.019) <sup>zAB</sup>    | 0.80<br>(0.03) <sup>yBC</sup>    | 0.250<br>(0.019) <sup>zA</sup>    | 3.1<br>(0.4) <sup>yB</sup>     |
|         | 7 | 4.6<br>(0.4) <sup>yA</sup>   | -3.7<br>(0.5) <sup>yB</sup>      | 0.9077<br>(0.0104) <sup>zAB</sup>  | 0.833<br>(0.016) <sup>zA</sup>   | 0.23<br>(0.02) <sup>zyxA</sup>    | 3.5<br>(0.3) <sup>yA</sup>     |

Mean values (and standard deviations).

For the samples under the same conditions, the same letter in superscript in the column indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ) (a-c for unheated, and z-u heated samples). To compare the same sample to the temperature effect, the same capital letter in superscript in the column indicates the homogeneous groups established by the ANOVA ( $p < 0.05$ ). C: Concentration (%); 65 indicates the samples heated at 65 °C, 20 min; PH: Plantago Husk; PP: Plantago Powder.

The chewability of both *Psyllium* samples was higher than the gel formed with a combination of bamboo, psyllium and citric fibre presented by Noguerol, Igual and Pagán-Moreno (2021). The highest chewability value was for the 65PH 7% gel. This parameter also depended on fibre concentration for the gels formed at 65 °C. Authors like Fradinho, Soares, Niccolai, Sousa, and Raymundo (2020) have indicated that both hardness and adhesiveness are dependent on the *Psyllium* concentration in pasta, and that pasta with *Psyllium* is less adhesive due to this material's gelling properties, which favour a more cohesive structure with less cooking loss. As in the back extrusion assay, positive correlations were found between hardness and Ca and Na minerals (0.6720 and 0.6607, respectively), but a negative correlation was observed when adhesiveness was related to these minerals (-0.7258 and -0.7143, respectively). With the cohesiveness Pearson correlations, this was associated with minerals K and Mn (0.6078 and 0.6056, respectively) but, as with hardness, chewability showed a relation with Ca and Na (0.6893 and 0.6789, respectively). As a result, both fibres PP and PH displayed gelling properties at not only lower temperatures (65 °C), but also at cool temperatures (5 °C). This should be highlighted because these samples can be used to adapt the texture of different foods, while supplementation with *Psyllium* fibres implies potential health benefits (Jalanka et al., 2019; Jane, McKay, & Pal, 2019). As with back extrusion and flow curves, the TPA parameters were also related to the hydration properties of the DF samples. When samples were formed without heat treatment, hardness correlated negatively with WHC, WRC and FAC (-0.8564, -0.9114 and -0.9069, respectively), and positively with the WSI (0.9247). On the contrary, adhesiveness was positively related to WHC, WRC and FAC (0.9443, 0.9150 and 0.9101, respectively) and negatively to the WSI (-0.8839). The resilience and chewability of the cold gels were associated with all the hydration properties: negatively with WHC, WRC, SWC and FAC and positively with the WSI (respectively -0.8996, -0.8917, -0.9415, -0.9034 and

0.9534 for resilience; -0.9015, -0.9521, -0.7637, -0.9518 and 0.9673 for chewability). However, when gels were formed with heating, relations were noted only between the cohesiveness and hydration properties, with negative correlations with WHC, WRC and FAC (-0.9099, -0.9758 and -0.9714, respectively), and a positive correlation with the WSI (0.9671). Therefore, gel structure formation (heating or not) depended on the hydration properties of the DF samples.

### *3.3. Practical implications of this study*

The clean label trend has emerged due to the concern of consumers about healthiness and sustainability of food products (Euromonitor International, 2016). Moreover, the COVID-19 health crisis has increased the concern for a healthy lifestyle (Academia Española de Nutrición y Dietética, 2020). Thus, the importance to include DF in our diet, since it offers health benefits for body weight control, cholesterol, diabetes, constipation, diarrhoea, inflammatory bowel disease, irritable bowel syndrome symptoms, and colon cancer prevention (Ma, & Mu, 2016; Franco et al., 2020; Belorio, Marcondes, & Gómez, 2020).

For these reasons, according to the results obtained in this study, these two Psyllium fibres could be used as new clean label texturizers, as well as to avoid the negative effects in foods such as cooking loss, drip loss, syneresis, and fat loss. In addition, they could also be used as fat replacers or to develop new plant-based products, since one of the main problems for their development is texture. Furthermore, one of the main reasons for the lower consumption of plant-based products by omnivores is because they do not like the texture and flavour (Fiestas-Flores & Pyhälä, 2018; Noguerol, Pagán, García-Segovia & Varela, 2021). This DF could also be used to adapt food products to patients with oropharyngeal dysphagia, because one of the most employed strategies to overcome this problem is the use of texturing agents

that modifies foods texture (Ribes et al., 2021).

#### **4. Conclusions**

This study revealed that PH had the highest values for hydration properties, thus could be a functional ingredient to avoid physical food properties, such as syneresis and fat loss during cooking, and to improve textural and sensory characteristics. However, the AC, the TP and the mineral content for PP were higher, which is a highlighted finding in quality terms as an alternative source of DF. As a result of the rheological and textural analysis, both fibres showed good gelling properties from concentration 4% to 7% at both high (65 °C) and cool (5 °C) temperatures. These properties generally depended on the DF concentration. According to these results, both PH and PP could lead to different gel types that would allow their use as new sources of DF in food with different characteristics, a use that could promote health benefits for human health. In addition, they could also be used as fat replacers, to develop new products, to modified textures and to adapt food products to elderly people. However, further studies in different foods are required to check behaviour with other ingredients and to adjust the suitable concentration to each food type.

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6. Supplementary material

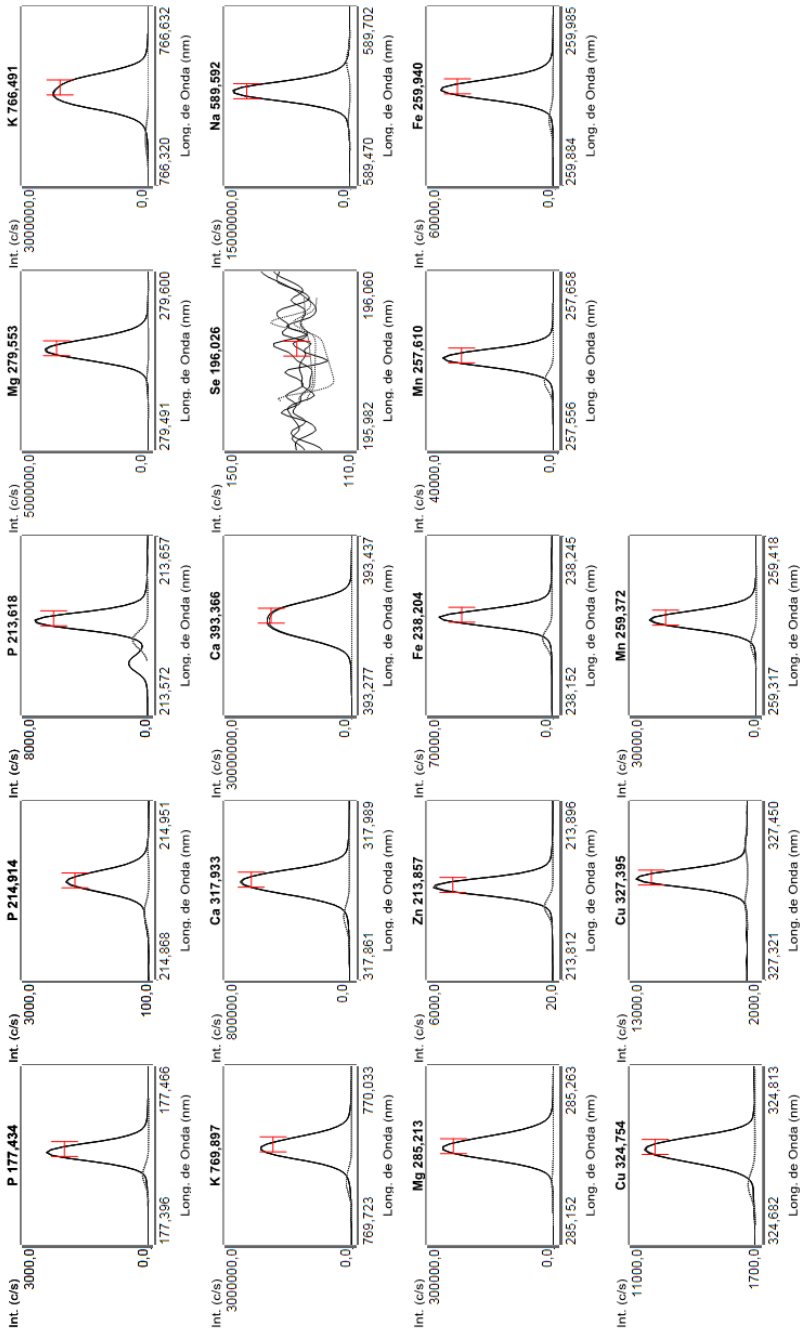


Figure S1. PH real graph obtained from plasma optical emission spectrometer for mineral determination.

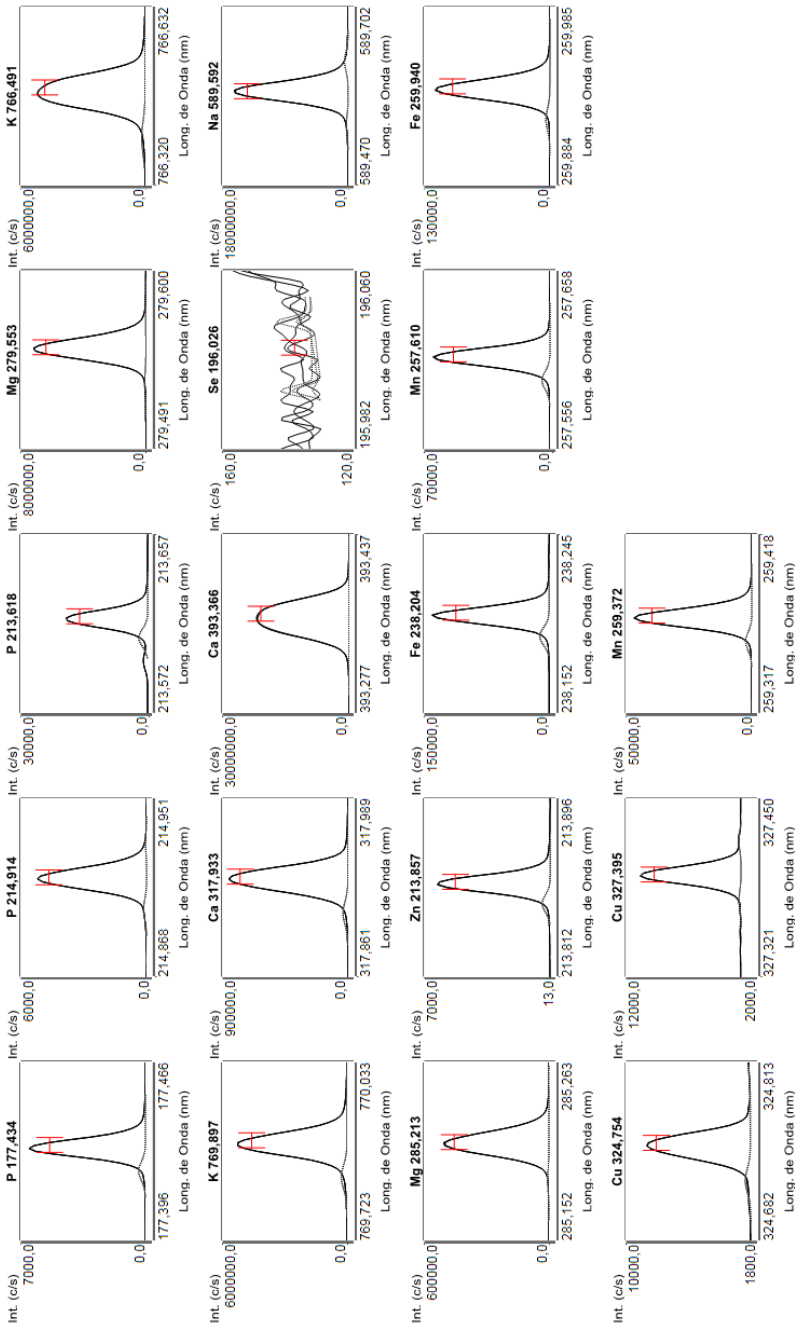


Figure S2. PP real graph obtained from plasma optical emission spectrometer for mineral determination.

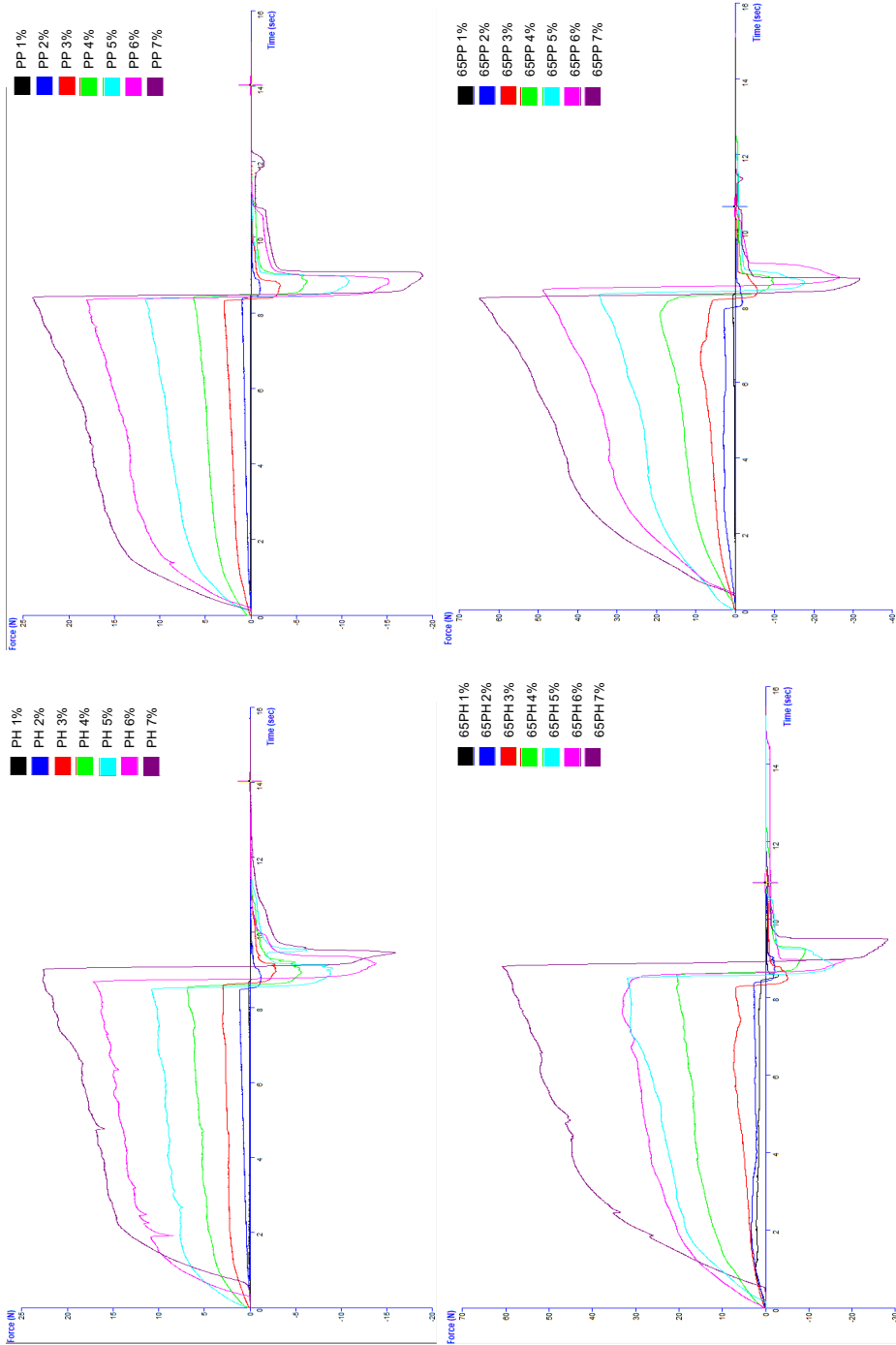


Figure S3. Original back extrusion graphs of *Psyllium* gel samples.

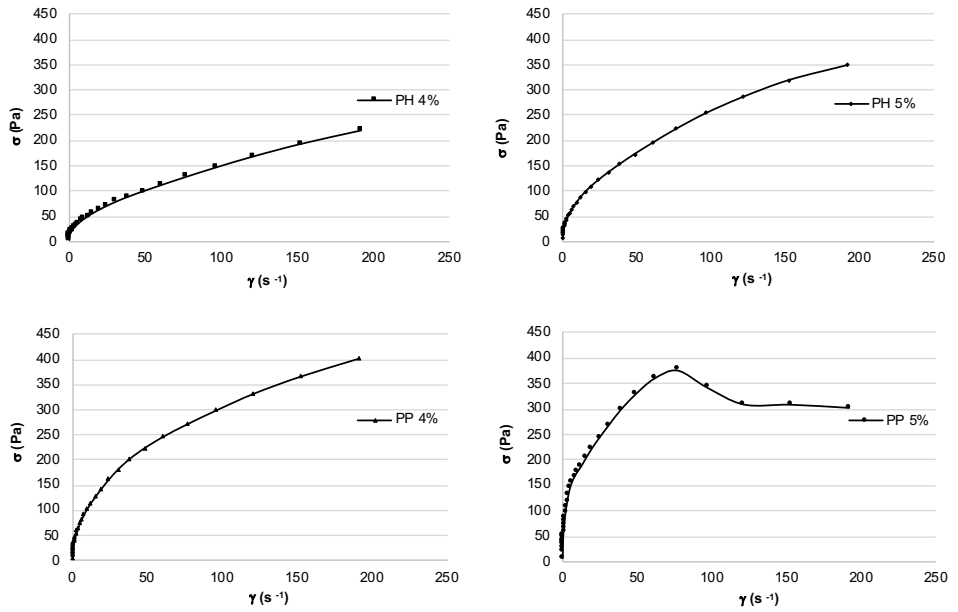


Figure S4. Original flow curves of *Psyllium* gel samples.



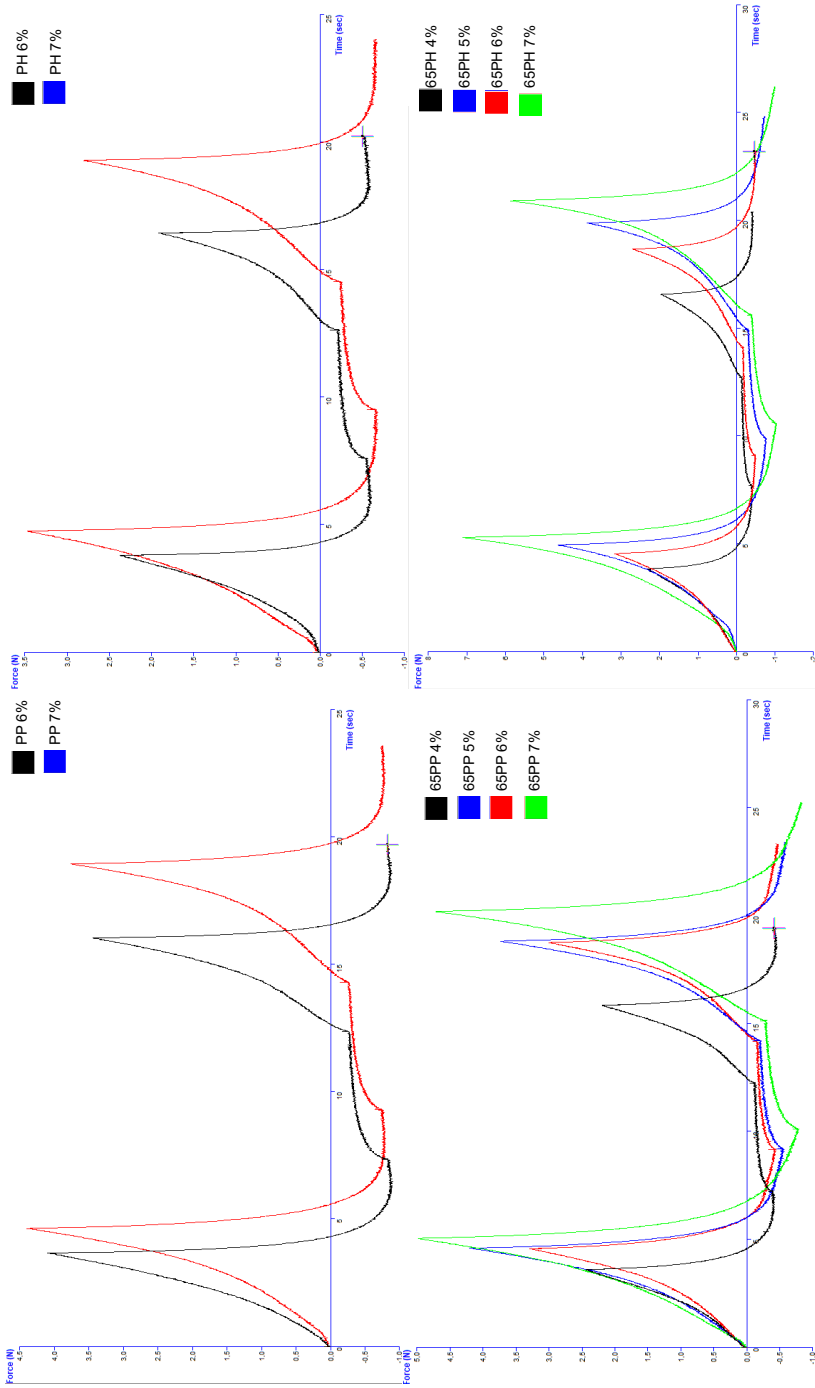


Figure S5. Original TPA graphs of *Psyllium* gel samples.



# **Capítulo 5**

## **Productos**



## Primera parte

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Development of plant-based spread with psyllium: effect on physicochemical, nutritional, functional and mechanical properties.

Este artículo ha sido enviado como:

Noguerol, A. T., Gozábez, A. & Pagán, M. J. Development of a plant-based spread with psyllium: effect on physicochemical, nutritional, functional and mechanical properties. *Food & Function* (Submitted, November 2021).



### **Abstract**

Consumer interest in health and sustainability has grown in recent years. Fibre has been one of the first ingredients to be associated with health. There has also been a trend to increase the intake of plant-based proteins and fresh fruit and vegetables, which means that the population has paid increasing attention to plants high in dietary fibre (DF). Psyllium husk has shown good gelling properties due to its water absorbability and can be used as a functional ingredient. Therefore, to optimise the psyllium fibre level to produce a plant-based spread as a source of protein and fibre and low in saturated fat, physicochemical, nutritional, hydrational and functional properties were determined to characterise the psyllium fibre sample. After the formulation of plant-based spreads, proximate composition, physicochemical, functional and mechanical properties were determined. The results show that psyllium fibre had good hydration properties, greater antioxidant capacity, and higher phenol and mineral contents. The results obtained with these spreads indicated that they were a source of fibre and protein, and were low in saturated fat. PW addition influenced neither samples' proximate composition nor their physicochemical properties. PW fibre addition increased samples' firmness and consistency, and the 0.5% PW sample was the stickiest and most difficult to spread. Thus as PW fibre addition improved product texture, this fibre can be used as a functional ingredient and a new source of DF in food.

## 1. Introduction

Given the plasticity and elasticity properties of solid fats and their role in particular food products, the complete replacement of hard fats with liquid vegetable oils is difficult and improving texture is necessary (Nasirpour-Tabrizi et al., 2020). Hydrocolloids like maltodextrin, xanthan gum, carrageenan, locust bean gum (Somogyi, 2006; Nasirpour-Tabrizi et al., 2020), and also dietary fibres like psyllium, can be used as fat replacers (Belorio, Moralejo & Gómez, 2020) thanks to their ability to modify rheology, and to improve physical stability and overall mouth-feel properties by increasing desirable overall characteristics (Rubel et al., 2019).

However, consumer interest in the health and sustainability of their diets has grown in recent years (Noguerol, Igual, Pagán, 2021b). One of the first ingredients associated with health has been fibre (de Moraes Crizel et al., 2013), which is why plants high in dietary fibre (DF) have drawn much attention in recent times (Noguerol, Igual, Pagán, 2021a). It is well-known that eating DF offers several health benefits, such as body weight control, reduced serum lipid and cholesterol, controlled postprandial glucose responses and colon cancer prevention (Ma & Mu, 2016; Noguerol et al., 2021b). These effects are related to the technological functions of DF, such as water absorption and water retention, which can reduce products' shrinkage, cooking loss and drip loss, and also minimise production costs without affecting final products' sensory properties (Han & Bertram, 2017). Ren, Yakubov, et al. (2020), Franco, Sanches-Silva, Ribeiro-Santos, and Ramos de Melo (2020), Ren, Linter, and Foster (2020) and Noguerol et al. (2021a) have reported that psyllium husk (*Plantago ovata*) has good gelling properties due to its water absorbability and can be used as a functional ingredient.

There is also a trend to reduce meat consumption and to increase the intake of plant-based proteins and fresh fruit and vegetables (Kumar, 2016;



Noguerol, Pagán, García-Segovia & Varela, 2021). Aschemann-Witzel et al., (2020) have indicated that the term “plant-based” is used to describe a recent consumer trend of avoiding animal-based products and choosing plant-based alternatives. For this reason, increasingly more companies are launching plant-based products, whose presence on markets is increasing, but they still focus on convenience (ready-to-eat) food (Lantern study, 2019). The Spanish Ministry of Agriculture, Fisheries and Food has indicated that the growing trend of eating ready-to-eat food continued in 2018.

Spreadable products are a convenience-type product eaten by spreading it on sandwiches on a base like bread (Arya et al., 2017). The aim of this study was to optimise the psyllium fibre level to produce a plant-based spreadable product as a source of protein and fibre that is low in saturated fats.

## **2. Material and Methods**

### *2.1. Materials*

The DF sample of *Plantago ovata* white (PW) was supplied by the company Productos Pilarica S.A., Paterna, Spain. Table 1 depicts the name, ingredients and proximate compositions of PW fibre as indicated by its producers.

The pea protein, whole chickpea flour, cheese flavour, tomato powder, salt and seasonings to make the spreadable samples were also supplied by the same company. Sunflower oil was purchased from a local supermarket.

### *2.2. Fibre characterisation*

#### *2.2.1. Physicochemical properties*

Water content ( $x_w$ ) (g water/100 g sample) was determined by vacuum oven drying (Vaciotem, J.P. Selecta, Spain) at 70 °C until a constant weight (AOAC, 2000).

**Table 1.** Name, ingredients and proximate composition of *Plantago ovata* white (PW). Data from suppliers.

|                      |                             |
|----------------------|-----------------------------|
| <b>Name</b>          | <i>Plantago ovata</i> white |
| <b>Ingredients</b>   | <i>Psyllium</i> husk powder |
| <b>Protein</b>       | 2.03                        |
| <b>Lipids</b>        | 1.6                         |
| <b>Ash</b>           | 1.4                         |
| <b>Carbohydrates</b> | 94.97                       |
| <b>TDF</b>           | >60.0                       |
| <b>IDF</b>           | >60.0                       |
| <b>SDF</b>           | <1.0                        |

TDF (Total dietary fibre); IDF (Insoluble dietary fibre); SDF (Soluble dietary fibre).

Samples' water activity ( $a_w$ ) was analysed by AquaLab PRE LabFerrer equipment (Pullman, USA).

In order to determine samples' hygroscopicity (Hg), about 0.2 g of sample were placed in a Petri dish at 25 °C in an airtight plastic container containing an Na<sub>2</sub>SO<sub>4</sub>-saturated solution (81% RH). After 1, 5, and 7 days, each sample was weighed and Hg was expressed as g of water gained per 100 g of dry solids (Noguerol et al., 2021b).

The pH of samples was measured on dispersions (10% w/v) of samples in distilled water following methods described elsewhere (Bender et al., 2020).

Particle size distribution was determined by applying the laser diffraction method and Mie theory following Standard ISO13320 (AENOR 2009) using a particle size analyser (Malvern Instruments Ltd., Mastersizer 2000, UK) equipped with a dry sample dispersion unit (Malvern Instruments Ltd., Scirocco 2000). Volume (as a percentage) against particle size (in micrometres) was obtained and size distribution was characterised by the volume mean diameter (D[4,3]), standard percentiles d(0.1), d(0.5), and d(0.9) the particle size below

which 10%, 50% and 90% of the sample lay, respectively. These parameters were estimated using the Mastersizer 2000 software (version 5.6) by considering particle diameter.

The porosity ( $\epsilon$ ), or percentage of air volume related to total volume was calculated from the true ( $\rho$ ) and bulk ( $\rho_b$ ) densities using  $\epsilon = (\rho - \rho_b) / \rho$  according to Noguero et al., (2021b). The real density of samples was determined by a helium pycnometer (AccPyc 1330, Micromeritics, Norcross, USA). For bulk density ( $\rho_b$ ) determinations, about 2 g of powder were placed inside a 10 mL graduated test tube and the occupied volume was noted. Bulk density was calculated by dividing powder mass by occupied volume, expressed as g/L.

### *2.2.2. Hydration properties*

All the hydration properties were determined as described by Noguero et al. (2021a). Water-holding capacity (WHC) and water-retention capacity (WRC) were determined by placing 1 g of sample in a calibrated cylinder and adding 30 mL of distilled water. Samples were hydrated for 18 h at 25 °C. WRC tubes were centrifuged at 3,000  $\times g$  for 20 min. Finally, supernatants were removed, and hydrated residues were weighed and dried at 100 °C for 3 h until constant weight. The results were expressed as g water/g dry sample.

One gram of sample was placed inside a graduated test tube and hydrated with 20 mL of distilled water for swelling water capacity (SWC). This sample was stored for 18 h at 25 °C after recording the bed volume. SWC was expressed as volume mL/g sample.

To determine the water solubility index (WSI), samples (approximately 1 g) were mixed in centrifuge tubes with 30 mL of distilled water for 5 min until mixtures became homogeneous. Next solutions were incubated at 37 °C in a water bath for 30 min and then tubes were centrifuged at 17,640  $\times g$  for 20 min

at 4 °C. Supernatants were collected and dried in an oven at 100 °C until constant weight. The results were expressed as a percentage.

Fat adsorption capacity (FAC) was determined by placing 4 g inside a centrifuge tube with 24 g of sunflower oil. Contents were stirred for 30 sec every 5 min for 30 min. Later samples were centrifuged at 1,600  $\times g$  for 25 min. Free oil was decanted and FAC was expressed as g oil/g sample.

### *2.2.3. Nutritional and functional properties*

Method 930.05 of the AOAC procedures (Horwith, & Latimer, 2005) was used to determine total ash content. A sample (500 mg) was incinerated at high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 24 h at 550 °C, and ash was gravimetrically quantified.

The multimineral determination was performed using an inductively coupled plasma optical emission spectrometer, model 700 Series ICP-OES from Agilent Technologies (Santa Clara, USA), with axial viewing and a charge coupled device detector as described by García-Segovia, Igual, Noguerol, and Martínez-Monzo (2020). Mineral composition (macro- and microelements) was expressed as mg/100 g of sample.

Antioxidant capacity (AC) was assessed by the DPPH method following the methodology of Igual et al. (2016). A UV-visible spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) was used for absorbance at 515 nm. The final results were expressed as milligram trolox equivalents (TE) per 100 g (mg TE/100 g).

Total phenol content (TP) was determined according to Noguerol et al. (2021b). Absorbance was measured at 765 nm in a UV-visible spectrophotometer (Thermo Scientific, Helios Zeta UV-Vis, Loughborough, UK). Total phenolic content was expressed as mg of gallic acid equivalents (GAE) (Sigma-Aldrich, Steinheim, Germany) per 100 g of sample.

### *2.3. Spread preparation and experimental design*

Spreadable products were prepared by mixing 45% water, 37% pea protein previously hydrated for 20 min, 10% sunflower oil, 3.5% whole chickpea flour, 1.5% cheese flavour, 1% tomato powder, 0.65% salt and 1.35% seasonings in a Thermomix TM 31 (Vorwerk, Wuppertal, Germany). To study the effect of adding psyllium, PW was added at 0%, 0.5%, 1%, 1.5% w/w. All the ingredients were mixed at 80 °C for 15 min. Then 40 g (approximately 30 mm in height) of each sample were placed inside plastic bottles (height 75 mm and diameter 50 mm). Finally, samples were stored at 5 °C for 24 h before further analyses. Two batches were made to run all the analyses.

### *2.4. Spread analysis*

#### *2.4.1. Proximate composition of the spreadable*

Samples' moisture (g water/100 g sample) was determined according to AOAC (2000). One gram of each sample was placed inside a vacuum oven to dry (Vaciotem, J.P. Selecta, Spain) at 70 °C until constant weight. Crude fat quantification was performed by ether extraction by an Ankom XT10 Extraction System (NY, USA) (AOCS, 2005). The Dumas method was followed in a Leco CN628 Elemental Analyzer (Leco Corporation, St. Joseph, MI, USA) to determine crude protein (nitrogen content  $\times 6.25$ ) according to Method 990.03 of AOAC International (2002). Crude ash content was determined following Method 923.03 (AOAC, 2002). A 1-gram sample was incinerated at high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 3 h at 550 °C, and ash was gravimetrically quantified. Carbohydrates were calculated by difference, and fibre content was calculated using the values from the technical data sheets of ingredients.

### *2.4.2. Physicochemical properties*

Samples' water activity ( $a_w$ ) was analysed by the AquaLab PRE LabFerrer equipment (Pullman, USA).

Vegetable spreads' pH values were measured by a pH meter MM41 (Crison Instruments S.A., Barcelona, Spain).

Sample colour was measured with a Konica Minolta CM-700d colorimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan) with standard D65 illuminate and the 10° visual angle. Samples were placed inside a reflectance glass container (3.7 x 2.2 x 5 cm). The measurement window was 6 mm in diameter. The results were expressed in the CIE Lab system (CIE, 1986). In order to observe colour difference due to PW addition, total colour difference ( $\Delta E$ ) was calculated according to:  $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ .

### *2.4.3. Functional properties*

Total phenols extraction (TP) and antioxidant capacity (AC) were measured as for the PW sample (Section 2.2.3.)

### *2.4.4. Mechanical properties*

Sample texture was measured by a TA-XT2 Texture Analyser (Stable Micro Systems Ltd, Godalming, UK) and the Texture Exponent software (version 6.1.12.0). The back extrusion analysis was performed following the methodology described by Cevoli et al. (2013). For this determination, a TA-XT2 Texture Analyser (Stable Micro Systems Ltd, Godalming, UK), equipped with a 50 kg load cell and the Texture Exponent software (version 6.1.12.0), was used. The analysis was carried out with a cylindrical extrusion disc (25.4 mm in diameter). The test was run at a depth of 50% (approx. 15 mm) at the 1 mm/s test speed. The attributes calculated from the force-deformation curve were area under the curve (consistency) (N s), maximum force (firmness) (N),

maximum negative force (cohesiveness) (N) and resistance to flow off the disc (work of cohesion) (N s) (Makroo et al., 2019).

Samples' viscoelastic properties were measured following the methodology described by Pluta-Kubica et al. (2021) using a dynamic oscillatory Kinexus pro+ rotational rheometer (Malvern Instruments, Worcestershire, UK), equipped with the rSpace software and a 35 mm-diameter parallel-plate geometry (DSR II, Upper Plate) with a 1-mm gap between the plate and the heat-controlled sample stage (Peltier Cylinder Cartridge, Malvern Instruments, Worcestershire, UK).

All the tested samples were measured using oscillatory sweeps within the frequency range from 0.05 to 100 Hz (at  $25 \pm 0.1$  °C) at 0.25% strain. This value was selected in the linear region of viscoelasticity by performing an amplitude sweep test. Samples were loaded onto the geometry plate and were left to rest for 5 min before taking each measurement.

In the oscillatory shear tests, the overall sample response was characterised by the elastic modulus ( $G'$  (Pa)) and the viscous modulus ( $G''$  (Pa)). The loss angle values ( $\tan \delta$ ) according to frequency, and defined as the  $G''$  to  $G'$  ratio, were also calculated (Uribe-Wandurraga et al., 2021; Pluta-Kubica et al., 2021).

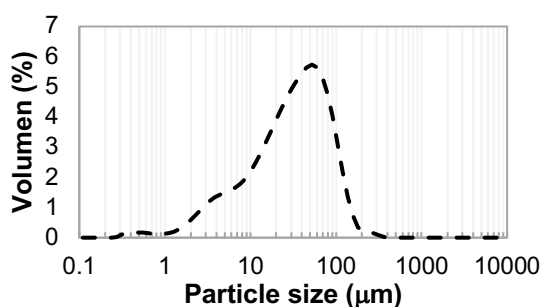
### *2.5. Statistical analysis*

All the analytical determinations were performed at least in quadruplicate. Version 17.2.04 of the Statgraphics Centurion XVII software was applied to carry out the analysis of variance (ANOVA). The LSD test was run to evaluate differences between samples ( $p < 0.05$ ).

### 3. Results and Discussion

#### 3.1. DF analysis

Figure 1 shows the particle size distribution of PW. It presented a wide peak, and the more concentrated particle size range went from 7.6 to 363  $\mu\text{m}$ , with small particles between 0.3 and 7.6  $\mu\text{m}$ . Table 2 shows the volume mean diameter ( $D[4.3]$ ) and the standard percentiles  $d(0.1)$ ,  $d(0.5)$ , and  $d(0.9)$  of the particle size for PW. PW obtained lower values in the particle size distribution ( $D[4.3]$ ,  $d(0.1)$ ,  $d(0.5)$  and  $d(0.9)$ ) compared to other psyllium fibres reported by Noguero et al. (2021a) and other vegetable fibres indicated by Noguero et al. (2021b). Rosell, Santos and Collar (2009) stated that particle size distribution is an important parameter for determining fibre functionality and its role in the digestive tract (transit time, fermentation, faecal excretion). A small particle can travel unhindered through the spaces between a large hydrocolloid molecule, but large molecules or particles' movement can be hindered (McClements, 2021).



**Fig. 1.** Volume particle size distribution (representative curve) of *Plantago ovata* white (PW).

Table 2 presents the mean values of the studied physicochemical properties ( $a_w$ ,  $x_w$ , pH, Hg,  $\rho_b$  and  $\varepsilon$ ). According to the  $a_w$  value, PW presented



a similar  $a_w$  to those found by Nogueroles et al. (2021a) for psyllium fibres and by de Moraes Crizel et al. (2013) for DF from orange by-products. Fernández-López et al. (2009) indicated that the ideal  $a_w$  to avoid microorganism growth and degradation reactions in products with low water content lies between 0.11 and 0.4. Therefore, the PW value should fall within this range. PW also showed a higher  $x_w$  compared to the psyllium (PP and PH) fibres (Nogueroles et al., 2021), but a lower value than that set out by Chong, Ball, McRae and Packer (2019) for psyllium husk. The PW pH value was strikingly similar to that found by Nogueroles et al. (2021a). The Hg of PW was similar to PP fibre and lower than PH fibre, both reported by Nogueroles et al. (2021a). PW had a  $\rho_b$  similar to PP fibre, which falls in line with Nogueroles et al. (2021a), but was higher than those indicated by Lan et al. (2012) for *Polygonatum odoratum*. Their samples with lower D[4,3], d(0.1), d(0.5), and d(0.9) generally presented a higher  $\rho_b$ , like the sample in the present study. Lan et al., 2012 and Tejada-Ortigoza, Garcia-Amezquita, Serna-Saldivar and Welti-Chanes (2016) indicated that this was because a fibre with a low bulk density should have a bigger surface area and more polar groups, which would increase its swelling capacity and oil-binding capacity.

PW fibre's hydration properties are found in Table 2. PW had a lower WHC compared to the PP and PH fibres according to Nogueroles et al. (2021a) and to the psyllium fibre reported by Chong et al., (2019), but higher WHC than the values of Rosell, Santos and Collar (2009) for the commercial fibres that they tested. This can be explained by a reduction in particle size possibly altering the fibre matrix structure. Thus WHC decreases with a smaller particle size, as indicated by Zhu et al. (2010). PW fibre's WRC was notably higher than the values reported by Kwindu, Onipe and Jideani (2018) and Nogueroles et al. (2021a) for another psyllium fibre. However, SWC was lower than that of fibres PP and PH reported by Nogueroles et al. (2021a). A high WRC indicates that DF

can be used as a functional food ingredient to reduce calories, avoid syneresis, and modify processed food's viscosity and texture (de Moraes Crizel et al., 2013). Moreover, SWC and WRC offer a fibre hydration overview and will provide useful information for fibre-supplemented foods (Guillon & Champ, 2000).

**Table 2.** *Plantago ovata* white (PW) values of volume mean diameter D[4,3], standard percentiles d(0.1), d(0.5), and d(0.9), water activity ( $a_w$ ), water content ( $x_w$ ), pH, hygroscopicity (Hg), bulk density ( $\rho_b$ ), porosity ( $\epsilon$ ) and hydration properties (WHC, WRC, SWC, WSI and FAC).

| Parameters                   | Values          |
|------------------------------|-----------------|
| D[4,3] ( $\mu\text{m}$ )     | 45.6 (1.3)      |
| d(0.1) ( $\mu\text{m}$ )     | 5.3 (0.2)       |
| d(0.5) ( $\mu\text{m}$ )     | 35.0 (0.6)      |
| d(0.9) ( $\mu\text{m}$ )     | 99 (2)          |
| $a_w$                        | 0.367 (0.003)   |
| $x_w$ (g water/100 g sample) | 6.22 (0.06)     |
| pH (dispersions 10% w/v)     | 5.91 (0.03)     |
| Hg (g water/100 g dry solid) | 28.19 (0.14)    |
| $\rho_b$ (g/L)               | 474 (13)        |
| $\epsilon$                   | 69.28 (0.13)    |
| WHC (g water/g dry sample)   | 10.53 (0.13)    |
| WRC (g water/g dry sample)   | 12 (3)          |
| SWC (mL water/g sample)      | 7.4 (0.6)       |
| WSI (%)                      | 30.7 (1.7)      |
| FAC (g oil/g sample)         | 1.1438 (0.0018) |

WHC: water holding capacity; WRC: water retention capacity; SWC: swelling water capacity; WSI: water solubility index; FAC: fat absorption capacity.

PW showed lower solubility than the PP fibre found by Nogueroles et al. (2021a). As high solubility can inhibit the digestion and absorption of nutrients from the gut (Guillon & Champ, 2000), FAC is important for preventing fat loss

during cooking and in nutrition because of absorbed fat in the intestinal lumen, which lowers cholesterol and retains food flavours (Noguerol et al., 2021a). The PW value was similar to the values obtained by Navarro-González et al. (2011) for fibre from tomato peel. Knowing the values of these properties for each DF is important because they are related to the chemical structure of component polysaccharides, and also for other factors like porosity, particle size, ionic forms, pH, temperature, ionic strength, type of ions in solution and stresses on fibres (Elleuch et al., 2011).

Table 3 shows the ash content, total minerals and individual mineral content for PW. Some studies have reported that *P. ovata* is a source of trace elements like Fe, Zn, Mn, Cu, K, Mg and Ca (Ziemichód et al., 2019; Noguerol et al., 2021a). About PW's fibre, Fe, Ca and Cu contents can be highlighted because the nutrient reference values (NRVs) indicated in Regulation (EU) No. 1169/2011 of the European Parliament and Council were high. Rousseau et al. (2020) stated that Fe deficiency causes global health problems for 30-33% of the world's population, but mostly in underdeveloped countries.

**Table 3.** *Plantago ovata* white (PW) values of ash, total mineral content, individual mineral content, nutrient reference values (NRVs) of minerals, total phenols (TP) and antioxidant capacity (AC).

|                     | Values/100 g PW | %NRVs/100 g |
|---------------------|-----------------|-------------|
| Ash (%)             | 1.4 (0.5)       | -           |
| Mineral totals (mg) | 459 (25)        | -           |
| P (mg)              | 32 (6)          | 4.6         |
| K (mg)              | 152 (11)        | 7.6         |
| Ca (mg)             | 130 (4)         | 16.3        |
| Na (mg)             | 126 (8)         | -           |
| Mg (mg)             | 13 (2)          | 3.5         |
| Zn (mg)             | 0.36 (0.08)     | 3.6         |
| Fe (mg)             | 5.8 (0.9)       | 41.4        |
| Cu (mg)             | 0.17 (0.07)     | 17          |
| Mn (mg)             | 0.059 (0.005)   | 2.3         |

**Table 3.** (Continued).

|             | Values/100 g PW | %NRVs/100 g |
|-------------|-----------------|-------------|
| TP (mg GAE) | 68.5 (0.9)      | -           |
| AC (mg TE)  | 57 (4)          | -           |

The TP content and AC values for the PW sample are shown in Table 3. PW presents a higher TP content and a similar AC to the PH fibre reported by Noguero et al. (2021a). This is probably due to PW also coming from psyllium husk (Table 1) like PH (Noguero et al., 2021a).

### 3.2. Spreadable analysis

#### Proximate composition

The spread samples' proximate composition appears in Table 4. The moisture results obtained for the different plant-based spread samples fell within the range from 71.5 to 72.8 g/100g of product, with no significant differences among them ( $p > 0.05$ ). Higher moisture values were observed than those found by Rodrigues, Maldonado and de Alvarenga Freire (2021) for partially replacing milk protein with vegetable proteins on Requeijão, but were similar to those of fresh sheep cheese with different DF types (bamboo, wheat, inulin, psyllium) reported by Peralta (2018). Knowing water content is important because this parameter provides information about foods' shelf life as it influences the growth of microorganisms and the development of chemical or enzymatic reactions that can negatively affect it. Lipid content was similar for all the samples at around 11%, but significant differences were observed in them all ( $p < 0.05$ ). The samples with a higher lipid content were the PW 0.5% and PW 1.5% spreads ( $p > 0.05$ ), while the sample with the lowest value was the control (PW 0%). Peralta (2018) obtained similar results in cheese with fibres, but Farahat et al. (2021) reported lower lipid content values for vegetable-processed cheese. The lipid content of the samples herein

formulated was lower than that found by Pluta-Kubica et al. (2021) for spreadable processed cheese. Savell and Cross (1988) indicated that fat improves texture and appearance, and contributes to product palatability. Making food more palatable improves consumer acceptance rates. However, excess fat intake, especially saturated fat, can increase the risk of cardiovascular and liver diseases, among others (Baird & Rivera, 2021), but eating mono- and polyunsaturated fats can prevent these diseases (Ros et al., 2015). The oil used in this work was sunflower oil, which is considered a low saturated fat product as the mono- and polyunsaturated fats in it represent almost 90% (Ros et al., 2015). Like lipid content, protein content was similar for all the samples (approx. 6%), but significant differences were observed among them ( $p < 0.05$ ). The sample with the lowest protein content was PW 1.5%. The samples with the highest content were the control and the PW 0.5% sample ( $p > 0.05$ ). The results obtained in this study can be related to the dilution effect for PW fibre addition. However, the protein content in all the samples exceeded 12% of the product's total calorie content and, according to Regulation (EC) No. 1924/2006 of the European Parliament and Council, the nutritional declaration of "protein source" can be indicated on labelling. This nutrient comes principally from pea protein, which has good nutritional and functional properties given its low content of antinutritional substances compared to other vegetable proteins like soya (Zhan et al., 2020). Significant differences were found in samples' ash content ( $p < 0.05$ ). Like other nutrients, ash content was similar for all the samples. However, Farahat et al. (2021) obtained similar ash values in vegetable-processed cheese. As with protein content, lower ash content was found in the samples containing PW fibre, and this decrease could also be due to the dilution effect. Food ash content represents mineral content (Morillas-Ruiz & Delgado-Alarcón, 2012). These micronutrients or trace elements perform essential and specific structural and metabolic functions (Shi et al., 2020). The ingredients providing our samples

with minerals were pea protein and chickpea flour, which are legumes. As mentioned in Section 3.1, this fibre presented high Fe, Ca and Cu levels, which may imply that our spread samples could contribute a substantial quantity of minerals. Carbohydrates and fibre content slightly increased in those samples containing PW fibre. This may be related to psyllium addition as it is a carbohydrate with high soluble fibre content (see Table 1), and as also indicated by Franco et al. (2020). Similar carbohydrate content results have been reported by Peralta (2018) in sheep's cheese with fibre. However, Farahat et al. (2021) reported lower DF results for vegetable-processed cheese. It should be highlighted that all the samples in this study can be labelled with the nutritional claim "source of fibre" as their content exceeds 3 g/100 g of product (Regulation (EC) No. 1924/2006). The population's fibre consumption is generally below that recommended, despite it being a well-known essential component of a healthy diet thanks to its many benefits, such as improving gastrointestinal disorders, controlling body weight and preventing cardiovascular diseases and diabetes (Ma & Mu, 2016). So the vegan spread herein formulated would be a healthy functional product to help to increase fibre consumption.

#### Physicochemical and functional properties

Our samples' physicochemical properties are depicted in Table 4. The addition of these PW concentrations to the spread samples did not affect the pH,  $a_w$  and colour parameters  $a^*$  and  $b^*$  because no significant differences were found ( $p > 0.05$ ). However,  $L^*$  decreased with PW fibre addition, and PW addition made samples slightly darker ( $p < 0.05$ ). Krystyan et al. (2018) indicated that the luminosity of biscuits containing *P. ovata* decreased and displayed a predominant greyish fibre powder colour. Our samples' pH values were considered adequate as microorganisms grow at a pH close to 6.5 and 7.5 (Fang & Tsai, 2003). Ghods Rohani and Rashidi (2019) reported similar pH

results for spreadable cheese made with other hydrocolloids, such as guar gum and xanthan. The  $a_w$  results indicated that this spread was a fresh product (Fontana, 2007) predisposed to the growth of many pathogenic microorganisms, but not to lipid oxidation reactions ( $a_w < 0.30$ ) or Maillard's reactions ( $a_w = 0.65$ ) (Mathlouthi, 2001). Raymundo et al. (2014) reported that adding psyllium fibre at 3-15% concentrations to biscuits lowered  $a_w$ . Therefore, the PW concentration applied in the present study did not affect the product's  $a_w$ .

**Table 4.** Proximate composition, physicochemical, optical, and functional properties of plant-based spread with different concentrations of *Plantago ovata* white (PW).

|                                | Samples                    |                            |                            |                            |
|--------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                | Control                    | PW 0.5%                    | PW 1%                      | PW 1.5%                    |
| Moisture (%)                   | 72.8 (0.8) <sup>a</sup>    | 71.5 (0.5) <sup>a</sup>    | 72.1 (0.4) <sup>a</sup>    | 71.7 (0.2) <sup>a</sup>    |
| Lipids (%)                     | 11.12 (0.09) <sup>c</sup>  | 11.79 (0.15) <sup>a</sup>  | 11.36 (0.02) <sup>b</sup>  | 11.64 (0.07) <sup>a</sup>  |
| Proteins (%)                   | 5.96 (0.04) <sup>a</sup>   | 6.022 (0.008) <sup>a</sup> | 5.84 (0.03) <sup>b</sup>   | 5.75 (0.03) <sup>c</sup>   |
| Ash (%)                        | 5.06 (0.06) <sup>a</sup>   | 4.97 (0.02) <sup>bc</sup>  | 4.92 (0.03) <sup>c</sup>   | 4.70 (0.05) <sup>c</sup>   |
| Carbohydrates (%) <sup>1</sup> | 5.06                       | 5.72                       | 5.78                       | 6.21                       |
| Fibre (%) <sup>2</sup>         | 3.93                       | 4.35                       | 4.78                       | 5.2                        |
| pH                             | 5.17 (0.05) <sup>a</sup>   | 5.19 (0.09) <sup>a</sup>   | 5.13 (0.04) <sup>a</sup>   | 5.14 (0.05) <sup>a</sup>   |
| $a_w$                          | 0.962 (0.002) <sup>a</sup> | 0.962 (0.003) <sup>a</sup> | 0.962 (0.003) <sup>a</sup> | 0.964 (0.003) <sup>a</sup> |
| $L^*$                          | 57.3 (1.4) <sup>a</sup>    | 56.1 (1.8) <sup>bc</sup>   | 55.4 (1.4) <sup>bc</sup>   | 54 (2) <sup>c</sup>        |
| $a^*$                          | 14.7 (0.7) <sup>a</sup>    | 15.2 (0.9) <sup>a</sup>    | 14.7 (0.9) <sup>a</sup>    | 14.5 (1.8) <sup>a</sup>    |
| $b^*$                          | 29.8 (1.3) <sup>a</sup>    | 30.2 (1.5) <sup>a</sup>    | 29.3 (1.7) <sup>a</sup>    | 29 (3) <sup>a</sup>        |
| $\Delta E$                     |                            | 3.2 (1.9) <sup>a</sup>     | 2.6 (1.7) <sup>a</sup>     | 4 (3) <sup>a</sup>         |
| TP (mg GAE/100 g)              | 71 (0.8) <sup>c</sup>      | 86 (7) <sup>b</sup>        | 105 (9) <sup>a</sup>       | 111 (2) <sup>a</sup>       |
| AC (mg TE/100 g)               | 51 (4) <sup>b</sup>        | 59 (4) <sup>a</sup>        | 59 (1) <sup>a</sup>        | 54 (7) <sup>ab</sup>       |

Means values and standard deviations.

<sup>1</sup> Calculated by difference.

<sup>2</sup> Calculated using the values from the technical data sheets of the ingredients.

TP: total phenols; AC: antioxidant capacity.

The same letter in superscript within row indicates homogeneous groups established by ANOVA ( $p < 0.05$ ).

Regarding colour, all the samples displayed a red-brown colour because tomato was employed in the formulation. Furthermore, PW addition brought about a colour change perceived by the human eye as  $\Delta E$  came close to or went over 3 (Bodart et al., 2008), although Noguerol, Larrea and Pagán (2021) indicated that fewer colour changes were observed with the PW sample.

Regarding functional properties, total phenols content (TP) increased by using PW in sample formulation ( $p < 0.05$ ) (Table 4). Similar values to PW 1% and 1.5% have been by Lucera et al. (2018) for artichoke spreadable cheese. Theuwissen and Mensink (2008) indicated that psyllium bioactive fractions are polysaccharides called hemicelluloses, which are responsible for functional properties. However, the antioxidant capacity (AC) values were similar for all the samples despite similar differences being found ( $p < 0.05$ ). All these results were lower than those reported by Lucera et al. (2008) for spreadable cheese fortified with fruit and vegetable by-products.

#### Mechanical properties

The obtained back extrusion analysis results (firmness, consistency, cohesiveness, work of cohesion) are shown in Table 5. As the PW concentration rose, samples' firmness increased, and the PW 1.5% sample had the highest firmness value ( $p < 0.05$ ). Consistency increased at the PW 1 and 1.5% concentrations, but no significant differences between them were found ( $p > 0.05$ ). The spread sample with the highest cohesiveness was PW 0.5%, while the least cohesive one was PW 1% ( $p < 0.05$ ), although values were similar for all the samples. Work of cohesion was similar for all the samples, but slightly significant differences were observed among them ( $p < 0.05$ ) where the PW 0.5% sample obtained the highest work of cohesion value and was, consequently, the most difficult sample to spread (Makroo et al., 2019). Therefore, PW addition increased the spread's firmness and consistency, but PW 0.5% was the stickiest and the most difficult sample to



spread. Similar results have been found by Nguyen et al. (2017) in skim yogurt after adding different hydrocolloids, and Frandinho et al. (2015) who reported how psyllium fibre increased biscuit firmness. Marcotte et al. (2001) observed using hydrocolloids to increase consistency.

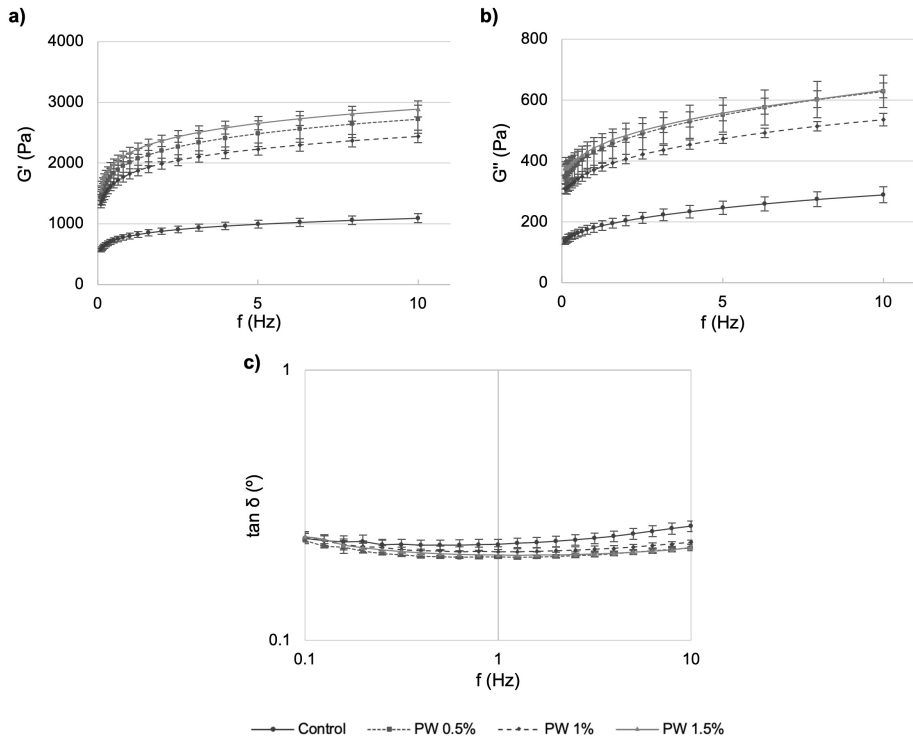
**Table 5.** Mechanical properties of plant-based spread with different concentrations of *Plantago ovata* white (PW).

|                        | Samples                    |                            |                            |                            |
|------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                        | Control                    | PW 0.5%                    | PW 1%                      | PW 1.5%                    |
| Firmness (N)           | 13 (2) <sup>d</sup>        | 20.2 (1.5) <sup>c</sup>    | 27 (3) <sup>b</sup>        | 36 (5) <sup>a</sup>        |
| Consistency (N.s)      | 95 (6) <sup>b</sup>        | 129 (14) <sup>b</sup>      | 167 (36) <sup>a</sup>      | 197 (61) <sup>a</sup>      |
| Cohesivity (N)         | -5.5 (0.7) <sup>ab</sup>   | -7 (2) <sup>c</sup>        | -4.2 (1.7) <sup>a</sup>    | -5.8 (0.9) <sup>bc</sup>   |
| Work of cohesion (N.s) | -0.13 (0.03) <sup>a</sup>  | -0.18 (0.08) <sup>b</sup>  | -0.09 (0.04) <sup>a</sup>  | -0.14 (0.03) <sup>ab</sup> |
| G' (Pa)                | 797 (48) <sup>c</sup>      | 2007 (158) <sup>a</sup>    | 1821 (82) <sup>b</sup>     | 2145 (88) <sup>a</sup>     |
| G'' (Pa)               | 181 (15) <sup>c</sup>      | 428 (49) <sup>a</sup>      | 371 (15) <sup>b</sup>      | 444 (17) <sup>a</sup>      |
| tan $\delta$           | 0.227 (0.009) <sup>a</sup> | 0.213 (0.009) <sup>b</sup> | 0.204 (0.002) <sup>b</sup> | 0.207 (0.006) <sup>b</sup> |

The same letter in superscript within row indicates homogeneous groups established by ANOVA ( $p < 0.05$ ).

Figure 2 shows the rheology parameters and Table 5 offers the values of these parameters. All the samples showed solid behaviour because the  $G'$  values were higher than the  $G''$  ones (Pluta-Kubica et al. 2021). Adding PW to formulations increased the value of both modules ( $G'$  and  $G''$ ), which indicated greater resistance for the formed fibre gel (Yuliarti et al., 2021).  $G'$  increased when a higher PW concentration was added, except for PW 1% with lower  $G'$  values than PW 0.5% ( $p < 0.05$ ).  $G''$  had equal values for the PW 0.5% and PW 1.5% concentrations ( $p > 0.05$ ). However, the PW 1% sample obtained a lower  $G''$  value than the other tested PW concentrations ( $p < 0.05$ ) at a frequency of 1 Hz (Table 5). This might be related to the obtained back extrusion test results (Table 5), which pointed out that the PW 1% sample had less work of cohesion

and a lower cohesiveness value compared to the other tested PW concentrations.



**Fig. 2.** Rheology parameters of plant-based spread samples with different concentrations of *Plantago ovata* white (PW).

Rubel et al. (2019) indicated a significant increase for  $G'$  and  $G''$  in ricotta cheese when carrageenan was used as a hydrocolloid, while neither  $G'$  nor  $G''$  showed significant differences with the control sample for xanthana and guar gum. Câmara et al. (2020) reported higher elastic and viscous modulus values using chia mucilage. Figure 2c represents the  $\tan \delta$  of the different spreadable samples. All the samples obtained  $\tan \delta$  values of  $< 1$  (Table 5), which indicate that they displayed elastic behaviour (Yuliarti et al., 2021). The values for the spread samples were lower than 0.5, which denotes that elasticity predominated viscosity (Bigne et al., 2017). As in the  $G'$  and  $G''$  moduli (Figure

2a and 2b), PW addition also affected  $\tan \delta$ , and this addition significantly increased samples' elasticity compared to the control sample ( $p < 0.05$ ). However, the different tested PW concentrations showed no significant differences ( $p > 0.05$ ).

### 3.3. Limitations

The present study shows the effect of psyllium fibre addition as gum modifies texture. However due to COVID restrictions, we were unable to assess these products' sensory acceptability. Further studies with consumers who wish to reduce or avoid meat consumption and omnivore consumers are highly recommended as Noguero et al. (2021) indicated that texture food is appreciated differently by these consumers, and is less important for vegan and vegetarian than for omnivore consumers.

## 4. Conclusions

This work revealed that *P. ovata* white fibre (PW) has good hydration properties, with higher antioxidant capacity, and phenol and mineral contents. The results of the spreads herein developed indicate that it is a source of fibre (> 3%) and protein (> 12%), and is low in saturated fat. PW addition does not influence samples' proximate composition. They all present high moisture and water activity, and can be considered fresh products. All the samples are red-yellow in colour, but luminosity is slightly lower after PW addition. Samples have increased firmness and consistency due to PW fibre addition. The 0.5% PW sample is the stickiest and most difficult one to spread, while the 1% PW is the least sticky and easiest to spread. PW fibre addition minimally affects product texture and confers it added value. Therefore, the herein presented product can be considered suitable for consumption in diet. We conclude that PW can be used as a functional ingredient and as new source of DF in food.

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## Segunda parte

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The effect of psyllium (*Plantago ovata* Forsk) fibres on the mechanical and physicochemical characteristics of plant-based sausages.

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### **Abstract**

Psyllium is a source of natural dietary fibre with recognised health benefits that can be used as a hydrocolloid with functional food applications. The purpose of this study was to determine the effect of different levels of *Plantago ovata* fibres in plant-based sausages on their composition, physicochemical and mechanical properties. Proximate composition was studied. Water activity ( $a_w$ ), water release, pH, colour measurement, texture profile analysis (TPA) and Warner-Bratzler Shear Force (WBSF) were determined to establish the physicochemical and textural properties of sausages. A plant-based sausages microstructure study and a sensory study were carried out to better understand conformation and to determine their acceptance. The results showed that sausages had high ash and carbohydrate contents but, above all, a low-fat content. The use of psyllium increased water-holding capacity. The results also indicated that employing *Plantago ovata* white (PW) fibre can minimise mechanical problems and reduce colour changes. However, PW fibre showed less retained water, which was why chickpea starch further developed and was more gelatinised. At the same time, the plant-based sausages with PW fibre obtained the best overall score with the fewest colour changes in the sensory evaluation. Nevertheless, further studies are recommended to improve the texture and acceptability of these plant-based sausages.

## 1. Introduction

Eating *veggie* diets is currently on the rise. The three diets forming part of the *veggie* world are flexitarian (predominantly plant-based diets with occasional portions of meat or fish), vegetarian (can include egg and dairy products) and vegan diets (excludes all animal-sourced foods) (Chen, Chaudhary, & Mathys, 2019; Lantern study, 2019). As the Lantern study (2019) indicated, 7.8% of the Spanish population followed one of these diet types in 2017, and the consumption of these diets increased by 27% in 2019. However, this percentage is lower than it is in countries like Germany and England (Noguerol, Pagán, García-Segovia & Varela, 2021), and these diets are on the increase in Europe and other Western countries (Müller, 2020). Between 2017 and 2018, a report on food consumption in Spain (Ministry of Agriculture, Fisheries and Food, 2018) recorded a 2.6% reduction in consumed meat. One of the main reasons for choosing this diet type could be because vegan and vegetarian diets are associated with lower risks of chronic diseases for adults (Segovia-Siapco, Burkholder-Cooley, Tabrizi, & Sabaté, 2019), and a wide range of health benefits like improving glycaemic control, blood lipids, body weight and blood pressure (Viguiliouk et al., 2019). Vegetarians and vegans describe their motives as "ideological" and primarily mention environmental concerns, animal welfare and other ethical considerations as their reasons for choosing to eat these diet types (Sneijder, & te Molder, 2009; Noguerol, Pagán, García-Segovia & Varela, 2021).

Meat production is mainly responsible for environmental pressures, such as pollution and unsustainable resources use (Elzerman, Hoek, van Boekel, & Luning, 2011; Smetana, Mathys, Knoch, & Heinz, 2015). According to a FAO report (1992), meat is not essential in diet, as proven by a large number of vegetarians who eat a nutritionally adequate diet. Kamani, Meera, Bhaskar, and Modi, (2019) concluded that meat should be substituted for totally

vegetable origin food so that the resulting product would be more similar to the original product in sensorial and textural acceptability terms with an adequate nutritional value. This makes replacing meat with plant-based meat substitutes an interesting alternative (Elzerman et al., 2011). However, the low success rate of meat analogues lies in poor sensory quality (Hoek, van Boekel, Voordouw, & Luning, 2011). Consequently, one formidable challenge for the food industry is to preserve the sensory and texture quality of analogous meat products. Various plant proteins can be used to help to overcome this problem, such as the group of proteins from (textured, flour, concentrated and isolated) legumes, cereals, oilseeds and soya because these ingredients have functional properties, such as emulsifiers, water and oil absorption capacity, and high nutritional values (Malav, Talukder, Gokulakrishnan, & Chand, 2015). Another problem with today's meat substitutes is that they are 3 to 4 times more expensive than meat products (Hoek et al., 2011).

Some studies have shown that certain hydrocolloids are capable of improving the physical and sensory food properties. It is a well-known fact that dietary fibres (DFs) possess technological functions, such as water absorption and water retention, and minimise production costs without affecting final products' sensory properties (Han, & Bertram, 2017). They have opened up possibilities to design novel fibre-enriched products and to generate new textures for a variety of applications (Rosell, Santos, & Collar, 2009). Psyllium seed husk (*Plantago ovata*) is a source of natural DF (Ren, Linter, & Foster, 2020) with good water absorbability and gelling properties (Noguerol, Igual & Pagán, 2021). This means that it can be used as a hydrocolloid with functional applications in new food production (Franco, Sanches-Silva, Ribeiro-Santos, & Ramos de Melo, 2020).

Thus the main purpose of this study was to determine and compare the effect of the using three different commercial DFs from *P. ovata* at

concentrations from 0% to 6% on the physicochemical and textural characteristics of plant-based sausages.

## **2. Materials and Methods**

### *2.1. Materials*

All the ingredients used to prepare samples were supplied by the company Productos Pilarica S.A., Paterna, Spain.

### *2.2. Preparation of plant-based sausages*

The control sausage formulation was that determined by Majzooobi, Talebanfar, Eskandari, and Farahnaky (2017) with minor modifications. The plant-based sausages were prepared by mixing 37% cold water, 32% hydrated texturised pea protein, 13.2% whole chickpea flour, 10.5% olive oil, 4.2% potato starch, 1.3% salt and 1.8% seasonings in a food mincer (Moulinex Multimoulinette, AT714G32, Moulinex, SEB Group, France) for 10 min. The texturised pea protein was properly hydrated by soaking in water for 30 min. To study the effect of adding psyllium, three different *Plantago ovata* fibres were used: Plantago Husk (PH), Plantago Powder (PP) and Plantago White (PW) at 0%, 3%, 4%, 5% and 6% w/w. The mixture was stuffed inside a 2.5 cm-diameter previously hydrated artificial casing (Productos Pilarica S.A., Paterna, Spain). Samples were cooked in a water bath at 80 °C for 20 min before being left to cool in cold water (8 °C) for 15 min. Finally, samples were stored at 4 °C for 24 h before running further experiments. Figure 1 shows all the plant-based sausage samples herein formulated.

### *2.3. Plant-based sausages analysis*

#### *2.3.1. Proximate composition of the plant-based sausages*

Samples' moisture (g water/100 g sample) was determined according to AOAC (2000). One gram of each sample was placed in a vacuum oven for



drying (Vaciotem, J.P. Selecta, Spain) at 70 °C until constant weight. Crude fat quantification was performed by ether extraction with an Ankom XT10 Extraction System (NY, USA) (AOCS, 2005). The Dumas method in a Leco CN628 Elemental Analyzer (Leco Corporation, St. Joseph, MI, USA) determined the crude protein (nitrogen content  $\times 6.25$ ) according to Method 990.03 of AOAC International (2002). The crude ash content was determined by Method 923.03 (AOAC, 2002). A 1-gram sample was incinerated at high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 3 h at 550 °C, and ash was gravimetrically quantified. Carbohydrates were calculated by difference.

### *2.3.2. Water activity ( $a_w$ )*

Samples' water activity ( $a_w$ ) was analysed by the AquaLab PRE LabFerrer equipment (Pullman, USA).

### *2.3.3. Water release*

Plant-based sausages' water release was determined according to Majzoobi et al., (2017). One gram of each sample was cut into a thin slice and placed between two filter papers (Whatman No. 1) of known weight. Then the samples between filters were pressed with a 1-kilogram weight for 20 min at 25 °C. The results were expressed as a percentage of water release.

### *2.3.4. pH*

The pH of the plant-based sausages was measured using a Crison Basic 20+ pH meter (Crison S.A., Barcelona, Spain) with a puncture probe (Crison 5231).

### *2.3.5. Colour measurement*

Samples' colour was measured using a Konica Minolta CM-700d colorimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan) with a

standard D65 illuminate and a 10° visual angle. A measurement was taken for both the powders of the previously ground *P. ovata* fibre samples (Minimoka GR-020, Coffemotion S.L., Lérida, Spain) to present the same granulometry and plant-based sausages. A reflectance glass (CR-A51, Minolta Camera, Japan) was placed between the sample and the colorimeter lens. The measurement window was 6 mm in diameter. For both powders and plant-based sausages, the results were expressed as in the CIELab system (CIE, 1986).

The total colour difference ( $\Delta E$ ) was calculated according to:  $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ . For the *P. ovata* fibre samples, the powder with the same granulometry was placed inside a circular aluminium sample holder (17.7 mm diameter x 9.53 mm high).  $\Delta E_1$  was determined to observe the colour differences between PH and the other fibres (PP and PW).  $\Delta E_2$  was performed for the differences between PP and PW.

For plant-based sausage colour, two sausages of each formulation were measured on both the internal and external sides.  $\Delta E_1$  was used to observe the differences between the internal and external colours at each concentration. To observe the colour difference due to the addition of *P. ovata* fibres (PH, PP and PW),  $\Delta E_2$  (internal and external) was determined in relation to the control sample.

### 2.3.6. Texture analysis

The plant-based sausages' texture was measured using a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) with the Texture Exponent software (version 6.1.12.0). A texture profile analysis (TPA) was performed as described in Kamani et al., (2019). Samples (2 cm long x 2.5 mm diameter) were compressed to 50% strain of their original height using a steel probe (45 mm diameter). A time of 5 s was allowed between the two

compression cycles and the test speed was 50 mm/min. The attributes calculated from the force-deformation curve were hardness (N), adhesiveness (N.s), springiness (mm), cohesiveness (dimensionless), resilience (dimensionless) and chewiness (N).

With the same texture analyser, Warner-Bratzler Shear Force (WBSF) was performed according to Jin, Kim, Choi, and Yim (2019) using a shearing V-shaped blade. The plant-based sausage samples (4 cm long × 2.5 mm diameter) were sheared at a crosshead speed of 100 mm/min. Firmness (N) was measured as the maximum peak force of shearing on the deformation curve.

### *2.3.7. Microstructure*

The microstructural study was carried out by Cryo-field emission scanning electron microscopy (Ultra 55 FESEM, ZEISS, Oberkochen, Germany) (Cryo-FESEM). Cubes (3 mm<sup>3</sup>) were cut by a stainless-steel cutter before being immersed in slush nitrogen (-210 °C) and transferred to a cryo-trans GeminiSEM 500 (ZEISS, Oberkochen, Germany) linked with a field emission scanning electron microscope that operated below -130 °C. Samples were cryofractured at -180 °C and etched at -90 °C.

Confocal scanning laser microscopy (CLSM) was conducted using a ZEISS 780 microscope coupled to an Axio Observer Z1 inverted microscope (Carl Zeiss, Germany). To visualise samples, the C-Apochromat 40X/1.2 W water immersion objective was used. Images were obtained and stored at a resolution of 1,024 x 1,024 pixels by the microscope software (ZEN). The employed stains were Rhodamine B and Calcofluor White (Fluka, Sigma-Aldrich, Missouri, USA).

Rhodamine B stained proteins and carbohydrates (starch granules) and was excited with diode line 488 and detected at 580 nm. Calcofluor White

stained polysaccharides and was excited with diode line 405 and detected at between 410 - 477 nm.

In order to observe and study samples, tissue sections (20 µm thick) were obtained using a cryostat (CM 1950, Leica Biosystems, Nussloch, Germany). The portion of tissue was placed on a slide. Then 20 µL of Rhodamine solution were added and left to rest for 5 min. The same procedure was followed for Calcofluor White, and samples were covered with a glass coverslip.

### *2.3.8. Sensory analysis*

The sensory evaluation of the plant-based sausages was carried out by 10 trained panellists. Sensory tests were run to describe differences in the addition of psyllium fibres, and to select the best valued fibre and concentration. Water was served as 'flavour cleaners' to avoid the sense-adaptation effect. Seven attributes of the cooked plant-based sausages were evaluated: general texture, chewiness, juiciness, gumminess, colour, visual aspect and overall acceptability. Each sausage was cut into 2 cm after removing the casing. The trained panellists were separated by at least 2 m following COVID-19 regulations. The design involved randomising the order that samples were served in. Each sensory attribute was represented with a 9-box scale, where the general texture was labelled from "I do not like" to "I like very much", chewiness from "tender" to "leathery", juiciness from "dry" to "juicy", gumminess from "gritty" to "rubbery", colour and the visual aspect from "unappetizing" to "very appetizing", and overall acceptability from "totally rejectable" to "totally acceptable". Panellists marked the box at the intensity level that they believed best characterised each sample.

### *2.4. Statistical analysis*

All the analytical determinations were made in at least triplicate. Version 17.2.04 of the Statgraphics Centurion XVII Software was applied to perform the

analysis of variance (ANOVA). The LSD test was followed to evaluate differences between samples ( $p < 0.05$ ). A correlation analysis was run at the 95% significance level among all the studied parameters (Statgraphics Centurion XVII).

### **3. Results and Discussion**

#### Proximate composition

Samples' proximate composition is presented in Table 1. The addition of the various levels of psyllium fibres had a significant effect on moisture ( $p < 0.05$ ). The samples with the highest moisture were control and PH 3%, with no significant differences between them ( $p > 0.05$ ). A general drop in moisture was shown as the concentration of psyllium fibres rose, and this decrease became more intense for fibres PP and PW. According to Stephan, Ahlborn, Zajul, and Zorn (2018), the moisture of vegan meat analogues is higher because more water is required to produce them than conventional meat products, and also due to the hydration of dried proteins and hydrocolloid powders. For this reason, the moisture values of all the studied meat-free sausages were higher than those found by Stephan et al., (2018) for German sausages (56.5%). However, the water content of vegan sausages made with isolated pea protein, as reported by Stephan et al. (2018), was higher than for all the samples tested in this study. The moisture in these plant-based sausages was similar to that of meat sausages in which animal fat had been replaced, like those studied by Grasso et al. (2020), who used sunflower seed flour as a fat replacer in frankfurters.

The highest protein content was obtained for samples PW 6% and, PP 5% and 6%. A fibre-concentration interaction was observed: when concentration rose, so did the protein content for fibres PP and PW, and this increase was intenser with the PP fibre. Nonetheless, no significant differences were found

at the 6% concentration for PP and PW ( $p > 0.05$ ). When adding the PH fibre, protein content significantly lowered ( $p < 0.05$ ) and protein content lowered as the concentration rose. These results are similar to those by Wang et al. (2019) for sausages made with *Lentinula edodes* as a replacer of pork lean meat at the 50% and 75% replacement levels.

**Table 1.** Mean values (and standard deviation) of the proximate composition of plant-based sausages.

| Sample  | Moisture (%)                   | Protein (%)                   | Fat (%)                          | Ash (%)                       | Carbohydrates* (%)           |
|---------|--------------------------------|-------------------------------|----------------------------------|-------------------------------|------------------------------|
| Control | 64.69 (0.14) <sup>a</sup>      | 7.25 (0.03) <sup>b</sup>      | 11.37 (0.16) <sup>a</sup>        | 4.56 (0.08) <sup>e</sup>      | 12.1 (0.3) <sup>g</sup>      |
| PH 3%   | 64.0 (0.4) <sup>ab</sup>       | 7.028<br>(0.008) <sup>c</sup> | 10.33 (0.08) <sup>h</sup>        | 4.74 (0.03) <sup>d</sup>      | 13.88 (0.05) <sup>f</sup>    |
| PH 4%   | 62.6 (0.8) <sup>cde</sup>      | 7.226<br>(0.003) <sup>b</sup> | 10.65 (0.03) <sup>defg</sup>     | 4.732<br>(0.007) <sup>d</sup> | 14.83 (0.04) <sup>d</sup>    |
| PH 5%   | 62.6 (0.9) <sup>cde</sup>      | 6.982<br>(0.007) <sup>c</sup> | 10.75 (0.15) <sup>cde</sup>      | 4.73 (0.04) <sup>d</sup>      | 14.940 (0.109) <sup>cd</sup> |
| PH 6%   | 62.14<br>(0.05) <sup>def</sup> | 7.02 (0.03) <sup>c</sup>      | 10.70 (0.08) <sup>cdef</sup>     | 4.74 (0.09) <sup>d</sup>      | 15.4 (0.2) <sup>b</sup>      |
| PP 3%   | 62.87 (0.16) <sup>cd</sup>     | 6.95 (0.05) <sup>c</sup>      | 10.750<br>(0.014) <sup>cde</sup> | 4.932<br>(0.004) <sup>b</sup> | 14.50 (0.06) <sup>e</sup>    |
| PP 4%   | 63.47 (0.14) <sup>bc</sup>     | 7.27 (0.13) <sup>b</sup>      | 10.72 (0.03) <sup>cdef</sup>     | 4.92 (0.03) <sup>bc</sup>     | 13.62 (0.07) <sup>f</sup>    |
| PP 5%   | 61.2 (0.8) <sup>f</sup>        | 7.52 (0.07) <sup>a</sup>      | 10.97 (0.03) <sup>b</sup>        | 5.18 (0.03) <sup>a</sup>      | 15.18 (0.07) <sup>bc</sup>   |
| PP 6%   | 61.6 (0.3) <sup>ef</sup>       | 7.50 (0.04) <sup>a</sup>      | 10.79 (0.14) <sup>bcd</sup>      | 5.16 (0.09) <sup>a</sup>      | 15.0 (0.3) <sup>cd</sup>     |
| PW 3%   | 63.0 (0.9) <sup>bcd</sup>      | 7.17 (0.05) <sup>b</sup>      | 10.83 (0.06) <sup>bc</sup>       | 4.704<br>(0.009) <sup>d</sup> | 14.30 (0.12) <sup>e</sup>    |
| PW 4%   | 63.5 (0.3) <sup>bc</sup>       | 7.04 (0.06) <sup>c</sup>      | 10.469<br>(0.002) <sup>gh</sup>  | 4.81 (0.08) <sup>cd</sup>     | 14.21 (0.02) <sup>e</sup>    |
| PW 5%   | 61.4 (1.2) <sup>f</sup>        | 7.20 (0.03) <sup>b</sup>      | 10.58 (0.04) <sup>efg</sup>      | 4.962<br>(0.013) <sup>b</sup> | 15.90 (0.08) <sup>a</sup>    |
| PW 6%   | 61.2 (0.3) <sup>f</sup>        | 7.54 (0.04) <sup>a</sup>      | 10.56 (0.03) <sup>fg</sup>       | 4.80 (0.05) <sup>d</sup>      | 15.90 (0.12) <sup>a</sup>    |

The same letter in superscript in a column indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ).

PH: Plantago Husk, PP: Plantago Powder, PW: Plantago White.

The control exhibited the highest fat content. However, this content was lower than in the meat-free sausages with different hydrocolloids made by Majzoobi et al. (2017), but higher than the fat content in the meat emulsion formulated with guar-xanthan gum mixture by Rather et al. (2016). In all the

studied samples, fat content dropped as the concentration of psyllium fibres rose, and the fat content of the plant-based sausages was very low.

The lowest ash content was for the control sample, although all these values were higher than not only all the vegan and vegetarian recipes (Majzooobi et al., 2017; Stephan et al., 2018), but also for meat products (Sousa et al., 2017; Grasso et al., 2020). It is worth noting that the ash content of these sausages was due to the contribution of the minerals that they could represent. It could also be related by the addition of psyllium fibres because a rise in the fibre concentration resulted in higher ash content. This effect was stronger from the 5% concentration with PP fibre addition because significant differences were observed between 5% and 6% of the PP fibres, and between 5% and 6% of fibres PH and PW ( $p < 0.05$ ).

A concentration-used fibre interaction was observed for carbohydrates content. The sample with the lowest carbohydrates content was the control, and carbohydrate content rose as the fibre concentration increased. This increase was greater when PW fibre was added. Therefore, the 5% and 6% PW samples had the highest carbohydrate content, and with no significant differences between them ( $p > 0.05$ ). Nevertheless, the 5% and 6% concentrations of samples PH and PW obtained significantly lower values than the PW samples ( $p < 0.05$ ).

A significant ( $p < 0.05$ ) correlation with moisture was found (-0.7999), which means that a higher carbohydrates content implies lower moisture, and this might be due to the water retention capacity of the added fibre samples (Noguerol, Igual & Pagán, 2021).

### Water activity, water release and pH

The water activity, water release and pH of the plant-based sausages are listed in Table 2. Nasonova and Tunieva (2019) indicated that using fat replacer

did not significantly affect  $a_w$ . The results observed in this study also affirmed that a meat-free sausage can display a similar water activity to both meat sausages and sausages formulated with fat replacers. This parameter is vital for food microbial stability. One good result was that the  $a_w$  of all the studied samples was slightly lower than the results reported by Stephan et al. (2018) for different vegan and vegetarian sausages, and those in Wang et al. (2019) for sausages formulated with *Lentinula edodes* as a fat replacer.

**Table 2.** Mean values (and standard deviations) of the water activity ( $a_w$ ), water release and pH of the studied samples.

| Sample  | $a_w$                          | Water release (%)         | pH                          |
|---------|--------------------------------|---------------------------|-----------------------------|
| Control | 0.9526 (0.0004) <sup>bcd</sup> | 10.4 (1.2) <sup>a</sup>   | 5.98 (0.02) <sup>de</sup>   |
| PH 3%   | 0.9513 (0.0013) <sup>cd</sup>  | 5.7 (0.4) <sup>b</sup>    | 5.95 (0.03) <sup>ef</sup>   |
| PH 4%   | 0.955 (0.003) <sup>a</sup>     | 4.8 (0.7) <sup>bcd</sup>  | 5.89 (0.03) <sup>i</sup>    |
| PH 5%   | 0.950 (0.002) <sup>d</sup>     | 3.8 (0.5) <sup>ef</sup>   | 6.02 (0.02) <sup>d</sup>    |
| PH 6%   | 0.9538 (0.0007) <sup>abc</sup> | 4.5 (0.4) <sup>cde</sup>  | 6.56 (0.02) <sup>a</sup>    |
| PP 3%   | 0.951 (0.002) <sup>cd</sup>    | 5.0 (0.6) <sup>bc</sup>   | 5.96 (0.07) <sup>e</sup>    |
| PP 4%   | 0.954 (0.002) <sup>ab</sup>    | 4.0 (0.2) <sup>def</sup>  | 5.91 (0.02) <sup>ghi</sup>  |
| PP 5%   | 0.9551 (0.0009) <sup>ab</sup>  | 3.5 (0.2) <sup>f</sup>    | 6.13 (0.02) <sup>b</sup>    |
| PP 6%   | 0.9506 (0.0013) <sup>d</sup>   | 3.9 (0.6) <sup>def</sup>  | 6.557 (0.006) <sup>a</sup>  |
| PW 3%   | 0.951 (0.002) <sup>d</sup>     | 3.6 (0.4) <sup>f</sup>    | 5.94 (0.02) <sup>efg</sup>  |
| PW 4%   | 0.956 (0.002) <sup>a</sup>     | 4.2 (0.8) <sup>cdef</sup> | 5.900 (0.002) <sup>hi</sup> |
| PW 5%   | 0.9548 (0.0013) <sup>ab</sup>  | 3.8 (0.4) <sup>ef</sup>   | 6.083 (0.012) <sup>c</sup>  |
| PW 6%   | 0.9526 (0.0007) <sup>bcd</sup> | 4.3 (0.4) <sup>cdef</sup> | 6.13 (0.03) <sup>bc</sup>   |

The same letter in superscript in a column indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ). PH: Plantago Husk, PP: Plantago Powder, PW: Plantago White.

The water release of the samples with added psyllium fibres came close to those obtained by Majzoubi et al. (2017) for meat-free sausages with different hydrocolloid levels. The water release value was significantly for the control sample ( $p < 0.05$ ). As water release correlates negatively with water-



holding capacity, a lower water release value is considered a desirable characteristic in sausages (Majzoobi et al., 2017) because it can result in a product's better texture and quality. The present study found an increase in the concentration of the three tested psyllium fibres (PH, PP, PW), which resulted in decreased water release, which means better water-holding capacity. Furthermore, significant negative correlations between carbohydrate content and water release (-0.7353) were observed when carbohydrates increased, water release decreased and water holding-capacity improved. It is a well-known fact that dietary fibres possess technological functions, such as water absorption and water retention (Han, & Bertram, 2017; Noguerol, Igual & Pagán, 2021).

The range of the pH values went from 5.89 to 6.56 (Table 2). A concentration-added fibre interaction took place, and increasing the concentration of all the tested added fibres led to a higher pH for sausages. This increase was greater for fibres PH and PP because of the significant differences between the 6% PP and PH samples and the PW 6% sample ( $p < 0.05$ ), and the PW 6% pH value was lower. The pH values in the sausages with the added psyllium fibres were comparable to not only those obtained by Majzoobi et al. (2017) for meat-free sausages with different hydrocolloid levels, but to those found by Stephan et al. (2018) for vegetarian sausage analogues and meat sausages like German boiled sausages. Jridi et al. (2015) and Majzoobi et al. (2017) reported slightly lower pH when adding hydrocolloids. On the contrary, pH increased in our study, which could be due to the pH of the psyllium fibres being between 5.10 and 6.14 (Noguerol, Igual & Pagán, 2021).

### Colour measurement

Table 3 depicts the colour parameters of the psyllium fibre samples. The sample with the significantly highest lightness ( $L^*$ ) value was the PW fibre ( $p < 0.05$ ). Redness ( $a^*$ ) and yellowness ( $b^*$ ) also significantly differed among the

three samples, and the PP fibre obtained the highest  $a^*$  and  $b^*$  ( $p < 0.05$ ). According to Bodart, Peñaranda, Deneayer, and Flamant (2008), colour differences ( $\Delta E_1$  and  $\Delta E_2$ ) among all samples were humanly appreciable, over 3, and the biggest colour difference was between fibre samples PP and PW.

**Table 3.** Mean values (and standard deviation) of the colour parameters of the fibre samples.

|              | Sample                  |                          |                           |
|--------------|-------------------------|--------------------------|---------------------------|
|              | PH                      | PP                       | PW                        |
| $L^*$        | 73.5 (0.4) <sup>b</sup> | 69.1 (0.4) <sup>c</sup>  | 80.9 (0.2) <sup>a</sup>   |
| $a^*$        | 2.3 (0.3) <sup>b</sup>  | 3.67 (0.09) <sup>a</sup> | 1.66 (0.04) <sup>c</sup>  |
| $b^*$        | 13.9 (0.3) <sup>b</sup> | 19.2 (0.2) <sup>a</sup>  | 11.96 (0.09) <sup>c</sup> |
| $\Delta E_1$ |                         | 7.0 (0.4) <sup>b</sup>   | 7.7 (0.4) <sup>b</sup>    |
| $\Delta E_2$ |                         |                          | 14.0 (0.5) <sup>a</sup>   |

The same letter in superscript in a line indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ).

$\Delta E_1$  was performed on the colour differences between PH and other fibres (PP and PW);  $\Delta E_2$  colour differences between PP and PW.

PH: Plantago Husk, PP: Plantago Powder, PW: Plantago White.

The colour parameters of our sausage samples appear in Table 4. Figure 1 shows the sausage samples herein prepared. On external colour, the samples with the highest lightness ( $L^*$ ) values were the control and the samples with less added fibre; more specifically, the 3% concentration of fibres PH and PW, PW at 4%, with no significant differences among them ( $p > 0.05$ ). Adding fibres influenced colour, and the  $L^*$  values significantly lowered with increasing fibre content. A fibre addition-concentration interaction took place internally and externally as the PP fibre addition more intensely lowered  $L^*$ . This could be related to the colour of the PP fibre sample which had the highest darkness value (Table 3). These results are comparable to those obtained by Grasso et al. (2020), who added sunflower seeds to frankfurters as a fat replacer. Those authors also reported a drop in  $L^*$  when adding sunflower seeds.

Our redness ( $a^*$ ) values were lower than those reported by Stephan et al. (2018) for the vegetarian sausage analogue, but similar to the meat-free sausages made with different gums by Majzoobi et al. (2017). The plant-based sausages with less  $a^*$  were those samples to which PP fibre was added, but with small differences between samples and the control for all the tested samples for both external and internal colours. In this case, the fibre with the highest  $a^*$  was PP (Table 3). This result in sausages could be due to a colour change that occurred during cooking and could also be related to the interaction with other sausage ingredients.

The sample with the highest external yellowness ( $b^*$ ), value was the control sausage. Both internally and externally, a fibre-concentration interaction was observed, and a  $b^*$  lowered when fibre concentration increased. The drop in the  $b^*$  value was significantly more marked at the PP fibre concentrations of 4%, 5% and 6% ( $p < 0.05$ ). However, the  $b^*$  value of the PP fibre was the highest (Table 3), which was also the case with the  $a^*$  value. No significant differences were observed between the samples with 4% and 5% of fibres PW and PH ( $p > 0.05$ ), but significant differences appeared between the samples with 6% of fibres PW and PH ( $p < 0.05$ ). These results were slightly higher than those reported by Stephan et al. (2018) for vegan sausage analogues, but are comparable to those obtained by Wang et al. (2019) for sausages with pork lean meat replaced with *Lentinula edodes*.

$\Delta E_1$  was performed to observe the differences between internal and external colours at each concentration (Table 4). These results generally showed that  $\Delta E_1$  was higher with a rising fibre concentration, but only for PH 3%, PH 6% and PP 6% (Bodart et al., 2008).

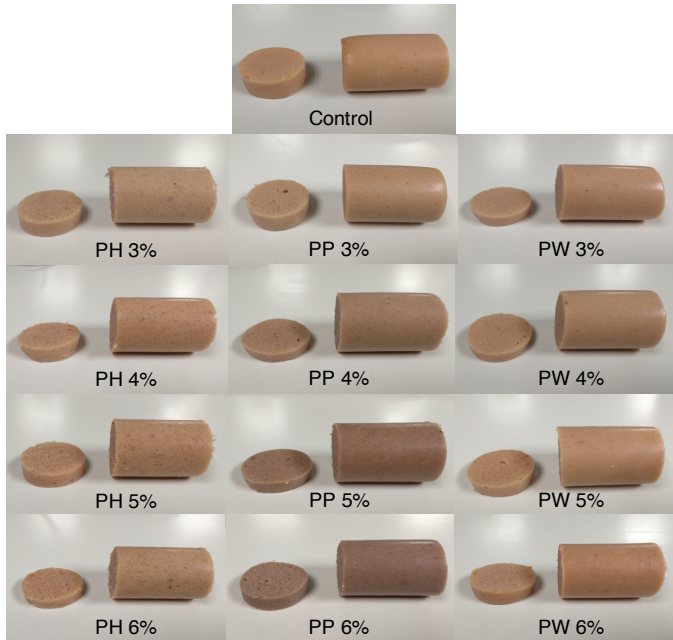
**Table 4.** Mean values (and standard deviation) of the colour parameters of the plant-based sausages.

| Sample  | External                  |                          |                             |                           |
|---------|---------------------------|--------------------------|-----------------------------|---------------------------|
|         | <i>L</i> *                | <i>a</i> *               | <i>b</i> *                  |                           |
| Control | 57.0 (0.4) <sup>a</sup>   | 7.7 (0.3) <sup>ab</sup>  | 19.2 (0.5) <sup>a</sup>     |                           |
| PH 3%   | 57.1 (0.6) <sup>a</sup>   | 6.6 (0.4) <sup>de</sup>  | 16.6 (0.4) <sup>d</sup>     |                           |
| PH 4%   | 54.6 (1.3) <sup>b</sup>   | 7.3 (0.7) <sup>abc</sup> | 17.2 (0.5) <sup>bcd</sup>   |                           |
| PH 5%   | 54.3 (0.9) <sup>bc</sup>  | 7.1 (0.5) <sup>cd</sup>  | 17.1 (0.8) <sup>bcd</sup>   |                           |
| PH 6%   | 52.7 (1.7) <sup>d</sup>   | 7.0 (0.8) <sup>cd</sup>  | 17.17 (1.18) <sup>bcd</sup> |                           |
| PP 3%   | 54.3 (0.9) <sup>bc</sup>  | 6.4 (0.3) <sup>e</sup>   | 16.5 (0.4) <sup>d</sup>     |                           |
| PP 4%   | 53.5 (0.9) <sup>bcd</sup> | 6.1 (0.6) <sup>e</sup>   | 14.14 (0.12) <sup>e</sup>   |                           |
| PP 5%   | 45.9 (1.6) <sup>e</sup>   | 7.1 (0.6) <sup>cd</sup>  | 13.4 (0.6) <sup>f</sup>     |                           |
| PP 6%   | 44 (2) <sup>f</sup>       | 6.2 (0.3) <sup>e</sup>   | 10.6 (0.6) <sup>g</sup>     |                           |
| PW 3%   | 57.2 (0.5) <sup>a</sup>   | 7.5 (0.3) <sup>abc</sup> | 17.4 (0.5) <sup>bc</sup>    |                           |
| PW 4%   | 56.3 (1.3) <sup>a</sup>   | 7.2 (0.5) <sup>bc</sup>  | 17.7 (0.8) <sup>b</sup>     |                           |
| PW 5%   | 53.2 (0.7) <sup>cd</sup>  | 7.8 (0.5) <sup>a</sup>   | 16.9 (0.5) <sup>cd</sup>    |                           |
| PW 6%   | 54.4 (0.6) <sup>bc</sup>  | 7.2 (0.2) <sup>bc</sup>  | 17.2 (0.6) <sup>bcd</sup>   |                           |
| Sample  | Internal                  |                          |                             |                           |
|         | <i>L</i> *                | <i>a</i> *               | <i>b</i> *                  | $\Delta E_1$              |
| Control | 57.0 (0.5) <sup>a</sup>   | 8.9 (0.3) <sup>c</sup>   | 18.3 (0.6) <sup>a</sup>     | 1.8 (0.5) <sup>e</sup>    |
| PH 3%   | 55.2 (0.4) <sup>b</sup>   | 8.4 (0.3) <sup>d</sup>   | 17.7 (0.9) <sup>abc</sup>   | 3.1 (0.8) <sup>bc</sup>   |
| PH 4%   | 54.5 (0.6) <sup>b</sup>   | 9.1 (0.2) <sup>bc</sup>  | 17.6 (0.7) <sup>abcd</sup>  | 2.3 (0.8) <sup>cde</sup>  |
| PH 5%   | 51.3 (1.2) <sup>e</sup>   | 9.6 (0.7) <sup>ab</sup>  | 16.9 (0.7) <sup>de</sup>    | 3.70 (1.13) <sup>ab</sup> |
| PH 6%   | 50.9 (0.7) <sup>e</sup>   | 9.1 (0.4) <sup>c</sup>   | 17.5 (0.8) <sup>bcd</sup>   | 2.8 (0.9) <sup>bcd</sup>  |
| PP 3%   | 53.4 (0.5) <sup>c</sup>   | 7.85 (0.16) <sup>e</sup> | 17.0 (0.5) <sup>cde</sup>   | 1.9 (0.6) <sup>de</sup>   |
| PP 4%   | 51.79(1.14) <sup>de</sup> | 7.7 (0.4) <sup>ef</sup>  | 14.7 (0.4) <sup>f</sup>     | 2.5 (0.9) <sup>cde</sup>  |
| PP 5%   | 48.2 (0.3) <sup>f</sup>   | 7.6 (0.3) <sup>ef</sup>  | 13.65 (0.13) <sup>g</sup>   | 2.8 (0.9) <sup>bc</sup>   |
| PP 6%   | 47.2 (0.6) <sup>g</sup>   | 7.3 (0.2) <sup>f</sup>   | 13.2 (0.3) <sup>g</sup>     | 4.4 (0.9) <sup>a</sup>    |
| PW 3%   | 57.3 (0.9) <sup>a</sup>   | 9.1 (0.6) <sup>c</sup>   | 17.7 (0.3) <sup>abc</sup>   | 2.2 (0.8) <sup>cde</sup>  |
| PW 4%   | 55.2 (0.7) <sup>b</sup>   | 9.0 (0.2) <sup>c</sup>   | 17.6 (0.4) <sup>abcd</sup>  | 2.6 (0.7) <sup>cde</sup>  |
| PW 5%   | 52.3 (1.5) <sup>c</sup>   | 9.7 (0.6) <sup>a</sup>   | 16.5 (0.8) <sup>e</sup>     | 2.2 (0.4) <sup>cde</sup>  |
| PW 6%   | 53.4 (0.4) <sup>d</sup>   | 9.1 (0.2) <sup>c</sup>   | 17.9 (1.2) <sup>ab</sup>    | 2.6 (0.6) <sup>cde</sup>  |

The same small letter in superscript in a column indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ).

PH: Plantago Husk, PP: Plantago Powder, PW: Plantago White.

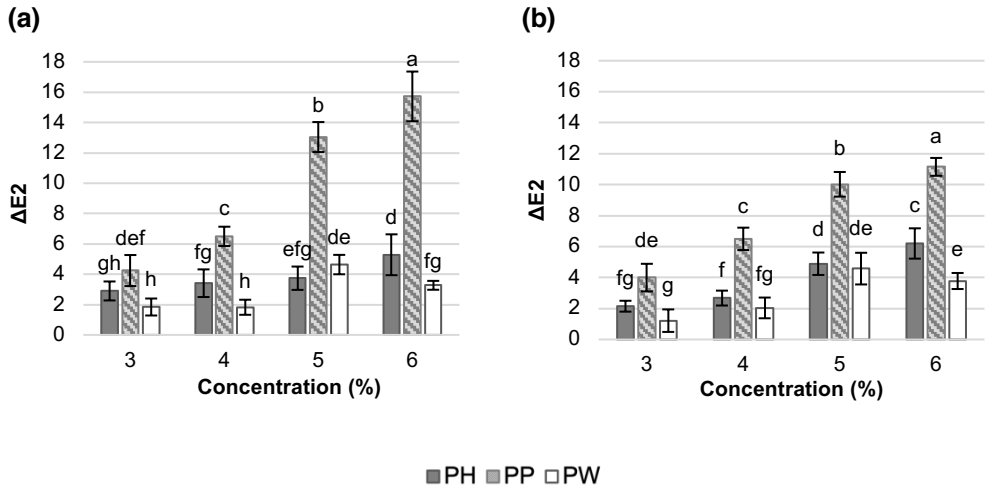
$\Delta E_1$ : colour differences between the internal and external colour at each concentration.



**Figure 1.** Plant-based sausages formulated with the addition of psyllium fibres. (PH: Plantago Husk, PP: Plantago Powder and PW: Plantago White).

Figure 2a and 2b depict the colour differences ( $\Delta E_2$ ) between addition of fibres and the control (internal and external colours). They show that  $\Delta E_2$  generally increased as the fibre concentration rose. Fibre samples PP had a higher  $\Delta E_2$  from the 3% concentration (see Figure 1). However, the samples with a lower  $\Delta E_2$  value were those formulated with the PW fibre, which was perceptible only at the 5% and 6% concentrations because the  $\Delta E_2$  values were above 3 (Bodart et al., 2008) (see Figure 1). It should be noted that colour differences were bigger superficially than internally, except for the plant-based sausages with 5% and 6% PH. This could be due to the shape and particle size of the husk fibre (PH), as reported by Noguero, Igual and Pagán (2021). Authors like Grasso et al. (2020), Pintado, Herrero, Jiménez-Colmenero, and Ruiz-Capillas (2016) and Henning, Tshalibe, and Hoffman (2016) have also stated colour alteration when adding different fibres and fat replacers in

frankfurters. For all these reasons, and according to Deliza et al. (2002), adding fibre minimises colour differences in the food matrix, which is important for avoiding possible consumer rejection because liking food with an appropriate appearance could favour consumers' healthier product consumption.



**Figure 2.** (a) External and (b) internal colour differences between the control sample and the plant-based sausages with psyllium fibres.

The same letter indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ). PH: Plantago Husk, PP: Plantago Powder, PW: Plantago White.

### Textural properties

Table 5 indicates the effect of adding different *P. ovata* fibres levels on the textural attributes of the plant-based sausages. The control sausage obtained the lowest values for all the studied parameters, except adhesiveness. This finding implies that adding psyllium fibres modifies products' mechanical properties. As for the force required to cut a sausage, the samples with the highest firmness and hardness values were those made with the PW fibre. A concentration-fibre interaction occurred for hardness, cohesiveness, resilience and chewiness as a higher content of the fibre samples assumedly increases these parameters. With hardness, the increase was significantly greater when

PW fibre was added at all the concentrations ( $p < 0.05$ ), and no significant differences were found when adding all the tested concentrations of fibres PH and PP ( $p > 0.05$ ). Significant differences were observed for cohesiveness, resilience and chewiness among all the plant-based sausages ( $p < 0.05$ ), and adding the PW fibre resulted in significantly higher values for all the studied concentrations of these parameters ( $p < 0.05$ ), followed by the plant-based sausages with PP fibre and, finally, by the lowest values when adding PH fibre ( $p < 0.05$ ). Significant ( $p < 0.05$ ) Pearson correlations were observed among carbohydrate content and firmness, hardness, cohesiveness, resilience and chewiness, with 0.8107, 0.8518, 0.6682, 0.6936 and 0.7583 respectively. This result indicates that adding psyllium fibres modifies the textural parameters of sausages. Significant negative ( $p < 0.05$ ) correlations were found between the water release and TPA parameters (hardness, cohesiveness, resilience, chewiness), with -0.6380, -0.7505, -0.7194, -0.6007, respectively. Hence plant-based sausage texture is related to the water-holding capacity of added fibres.

All the samples obtained similar values for springiness, although significant differences were found among the plant-based sausages ( $p < 0.05$ ). Stephan et al. (2018) reported similar elasticity values for both meat and meat-free sausages, whereas Kamani et al. (2019) indicated that non-meat proteins could hold more water and fat, which reduces springiness. So fibre content could also be included in this statement based on the results herein obtained. However, the elasticity values of Majzoobi et al. (2017) for meat-free sausages were slightly higher, but the hardness of meat-free sausages with xanthan were similar to samples with PW fibre at the 4%, 5% and 6% concentrations. According to Grasso et al. (2020), sausages' textural behaviour can be related to composition (mainly protein and fibre content) but, in this case, we examined the mechanical properties of plant-based sausages.

**Table 5.** Mean values (and standard deviation) of the textural parameters of the samples.

| Sample  | Firmness*<br>(N)            | Hardness<br>(N)                   | Adhesiveness<br>(N.s)            | Springiness<br>(mm)           | Cohesiveness                     | Resilience                     | Chewiness<br>(N)              |
|---------|-----------------------------|-----------------------------------|----------------------------------|-------------------------------|----------------------------------|--------------------------------|-------------------------------|
| Control | 2.2 (0.5) <sup>a</sup>      | 6.3 (0.2) <sup>h</sup>            | -2.9 (0.9) <sup>abcde</sup>      | 0.39 (0.02) <sup>c</sup>      | 0.26 (0.02) <sup>f</sup>         | 0.051 (0.003) <sup>h</sup>     | 0.65 (0.06) <sup>j</sup>      |
| PH 3%   | 3.11<br>(0.14) <sup>f</sup> | 10.3 (0.5) <sup>a</sup>           | -1.9 (0.5) <sup>ab</sup>         | 0.45 (0.03) <sup>c</sup>      | 0.311 (0.009) <sup>e</sup>       | 0.071 (0.006) <sup>g</sup>     | 1.425<br>(0.112) <sup>h</sup> |
| PH 4%   | 3.81<br>(0.04) <sup>e</sup> | 13.17<br>(0.16) <sup>f</sup>      | -3.4 (1.2) <sup>bcdef</sup>      | 0.50 (0.03) <sup>c</sup>      | 0.377 (0.014) <sup>d</sup>       | 0.098<br>(0.008) <sup>ef</sup> | 2.46 (0.12) <sup>a</sup>      |
| PH 5%   | 4.3 (0.2) <sup>d</sup>      | 16.2 (0.9) <sup>a</sup>           | -1.5 (0.8) <sup>a</sup>          | 1.1 (0.8) <sup>a</sup>        | 0.386 (0.017) <sup>d</sup>       | 0.110(0.007) <sup>cd</sup>     | 3.0 (0.2) <sup>f</sup>        |
| PH 6%   | 4.8 (0.2) <sup>c</sup>      | 19.3186<br>(1.0003) <sup>bc</sup> | -4 (2) <sup>cd</sup>             | 0.476<br>(0.014) <sup>c</sup> | 0.384 (0.012) <sup>d</sup>       | 0.101<br>(0.007) <sup>de</sup> | 3.5 (0.2) <sup>e</sup>        |
| PP 3%   | 3.0 (0.2) <sup>f</sup>      | 10.5 (0.6) <sup>a</sup>           | -3.8 (1.3) <sup>def</sup>        | 0.53 (0.03) <sup>c</sup>      | 0.390 (0.017) <sup>d</sup>       | 0.087 (0.004) <sup>f</sup>     | 2.2 (0.2) <sup>a</sup>        |
| PP 4%   | 3.91<br>(0.06) <sup>e</sup> | 12.6 (0.8) <sup>f</sup>           | -4.8 (1.3) <sup>f</sup>          | 0.549<br>(0.014) <sup>c</sup> | 0.441 (0.013) <sup>c</sup>       | 0.111<br>(0.004) <sup>cd</sup> | 3.1 (0.3) <sup>f</sup>        |
| PP 5%   | 4.7 (0.2) <sup>c</sup>      | 15.0 (1.5) <sup>a</sup>           | -2.3 (1.6) <sup>abcd</sup>       | 1.0 (0.7) <sup>ab</sup>       | 0.46 (0.06) <sup>bc</sup>        | 0.13 (0.03) <sup>b</sup>       | 3.3 (0.2) <sup>ef</sup>       |
| PP 6%   | 6.1 (0.3) <sup>b</sup>      | 18.5<br>(0.8) <sup>cd</sup>       | -4.2 (1.5) <sup>ef</sup>         | 0.533<br>(0.014) <sup>c</sup> | 0.445 (0.012) <sup>c</sup>       | 0.118<br>(0.003) <sup>bc</sup> | 4.4 (0.2) <sup>d</sup>        |
| PW 3%   | 3.79<br>(0.12) <sup>e</sup> | 13.4 (0.6) <sup>f</sup>           | -3.27 (1.13) <sup>bcdef</sup>    | 0.60 (0.02) <sup>c</sup>      | 0.4566<br>(0.0108) <sup>bc</sup> | 0.125 (0.008) <sup>b</sup>     | 3.7 (0.2) <sup>e</sup>        |
| PW 4%   | 4.8 (0.2) <sup>c</sup>      | 17.92<br>(0.14) <sup>d</sup>      | -4.3 (1.5) <sup>ef</sup>         | 0.60 (0.02) <sup>bc</sup>     | 0.482 (0.009) <sup>ab</sup>      | 0.145 (0.006) <sup>a</sup>     | 5.2 (0.2) <sup>c</sup>        |
| PW 5%   | 6.2 (0.3) <sup>b</sup>      | 20.5 (0.6) <sup>a</sup>           | -3.23 (1.09) <sup>bcdef</sup>    | 0.581<br>(0.012) <sup>c</sup> | 0.484 (0.009) <sup>ab</sup>      | 0.143 (0.002) <sup>a</sup>     | 5.8 (0.2) <sup>b</sup>        |
| PW 6%   | 6.6 (0.5) <sup>a</sup>      | 22 (2) <sup>b</sup>               | -2.148<br>(1.005) <sup>abc</sup> | 0.584<br>(0.015) <sup>c</sup> | 0.493 (0.009) <sup>a</sup>       | 0.150 (0.004) <sup>a</sup>     | 6.3 (0.7) <sup>a</sup>        |

The same letter in superscript in a column indicates the homogeneous groups established by ANOVA ( $p < 0.05$ ).

PH: Plantago Husk, PP: Plantago Powder, PW: Plantago White.

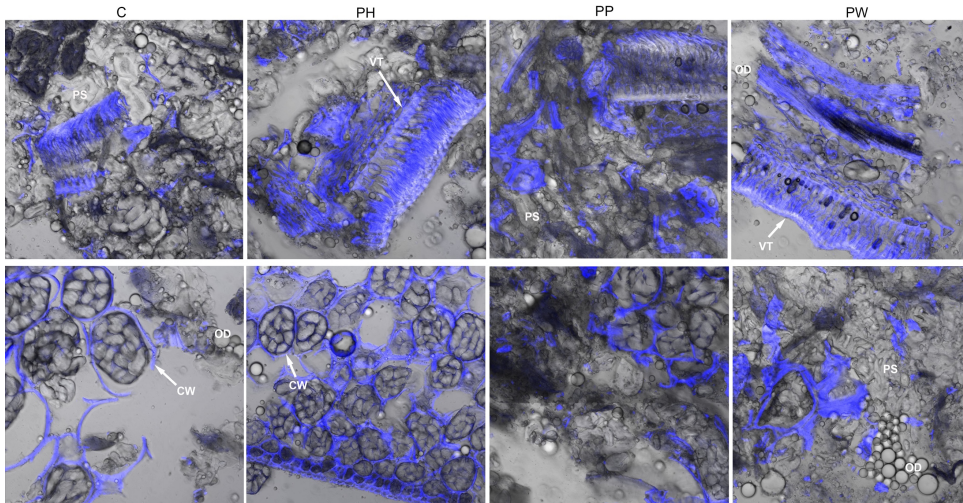
### Microstructure

In order to visualise the structure of the plant-based sausages, the lowest (3%) and highest (6%) concentrations of each psyllium fibre type were selected, as well as the control sample. No differences were found in the microstructural observation between both concentrations. Images correspond to the 6% concentration.

Figures 3 and 4 show the distribution of the ingredients in the plant-based sausages studied by CLSM (confocal scanning laser microscopy). Polysaccharides, such as vegetal walls and fibre, were observed in blue by staining agent Calcofluor (Figure 3). A very complex matrix is observed in the first row; vegetal tissue, probably from chickpea flour, and is dispersed among the matrix, together with partially gelatinised potato starch granules and oil



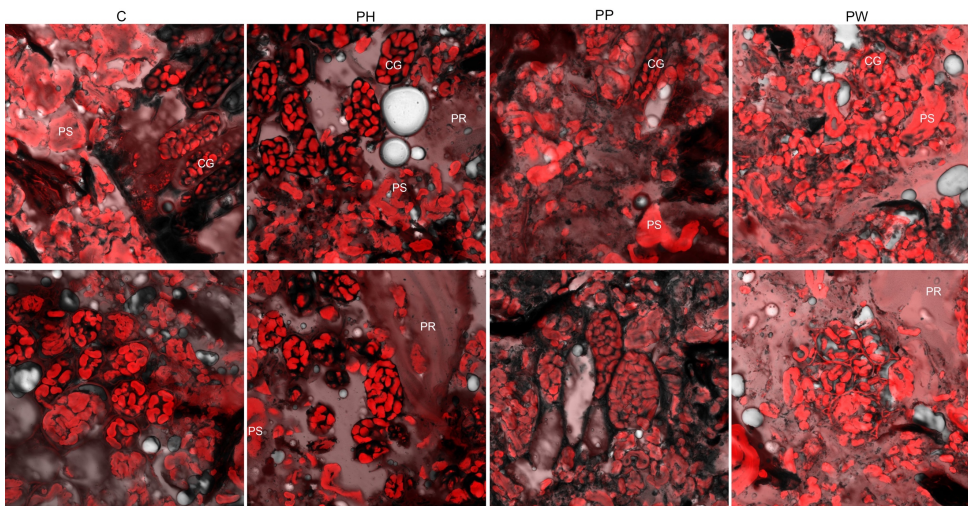
droplets. Although the different psyllium fibres were not clearly identified, probably because psyllium interacted with other components in the matrix, its presence increased the consistency of the continuous phase, as reflected by the different firmness and hardness values (Table 5). Stained cell walls surrounding chickpea starch granules are observed in Figure 3 (second row).



**Figure 3.** Images taken with a confocal scanning laser microscope (CLSM) of the different plant-based sausages stained with Calcofluor (20X). Potato starch granules (PS), vegetal tissue (VT), oil droplets (OD), cellular walls (CW). (PH: Plantago Husk, PP: Plantago Powder and PW: Plantago White).

With staining agent Rodamine, starch granules and protein were red-stained (Figure 4). Protein is observed in the background and is less intensely stained. In all the samples, potato starch granules are larger and more gelatinised than chickpea granules, which are smaller and surrounded by cell walls. The plant-based sausages with PH fibre showed the most packed and least gelatinised chickpea starch granules, perhaps because PH fibre had the highest WHC and WRC values (Noguerol, Igual & Pagán, 2021), which means less water available to hydrate starch. Beikzadeh, Peyghambaroust, Homayouni and Beikzadeh (2017) indicated that some gums prevent starch

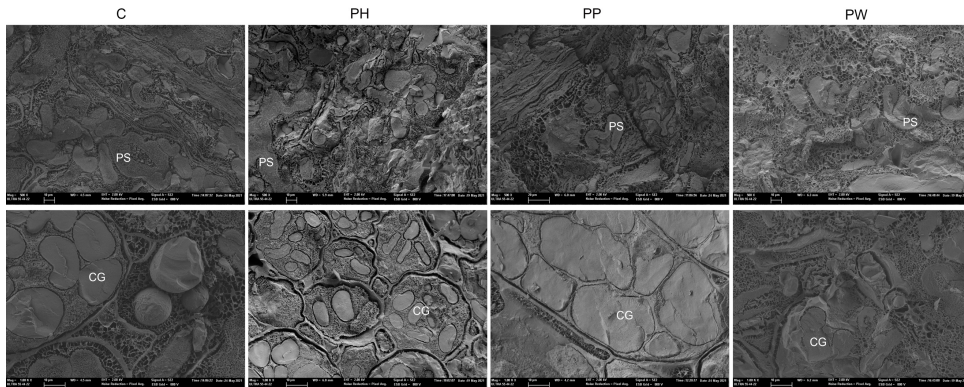
granules from swelling, chain gums avoid the interaction between starch polymers, and also between protein and starch, and results in product texture softening. This falls in line with the results obtained in the present study because the sausages made with PH fibre had lower firmness values (Table 5). The control and PP sausages presented a similar structure to the PH samples. However, the PW sausages showed the most deformed, broken and loose chickpea starch granules. In fact, chickpea tissue in the formulation with PW appeared disintegrated and, consequently, starch granules were more swollen and freely interacted with other matrix components. This could indicate that PW fibre retained less water and the PW samples would have more water available to interact with other components and, therefore, chickpea starch would develop more, which would make it more gelatinised.



**Figure 4.** Images taken with a confocal scanning laser microscope (CLSM) of the different plant-based sausages stained with Rodamine (20X). Potato starch granules (PS), chickpea granules (CG), protein (PR). (PH: Plantago Husk, PP: Plantago Powder and PW: Plantago White).

Consequently, this could correlate with samples' hardness, and the sausages that contained the PW fibre were those with the highest firmness, hardness and chewiness values (Table 5).

Figure 5 shows the structure of the plant-based sausages using Cryo-FESEM. Once again, a complex matrix was observed with gelatinised potato starch in the matrix (first row). Details of the chickpea starch granules (second row) confirm that granules were packed and almost intact in both the control and PH and PP samples. This was not the case with the PW samples, where the chickpea cell tissue breakdown and granules gelatinisation were once again observed.

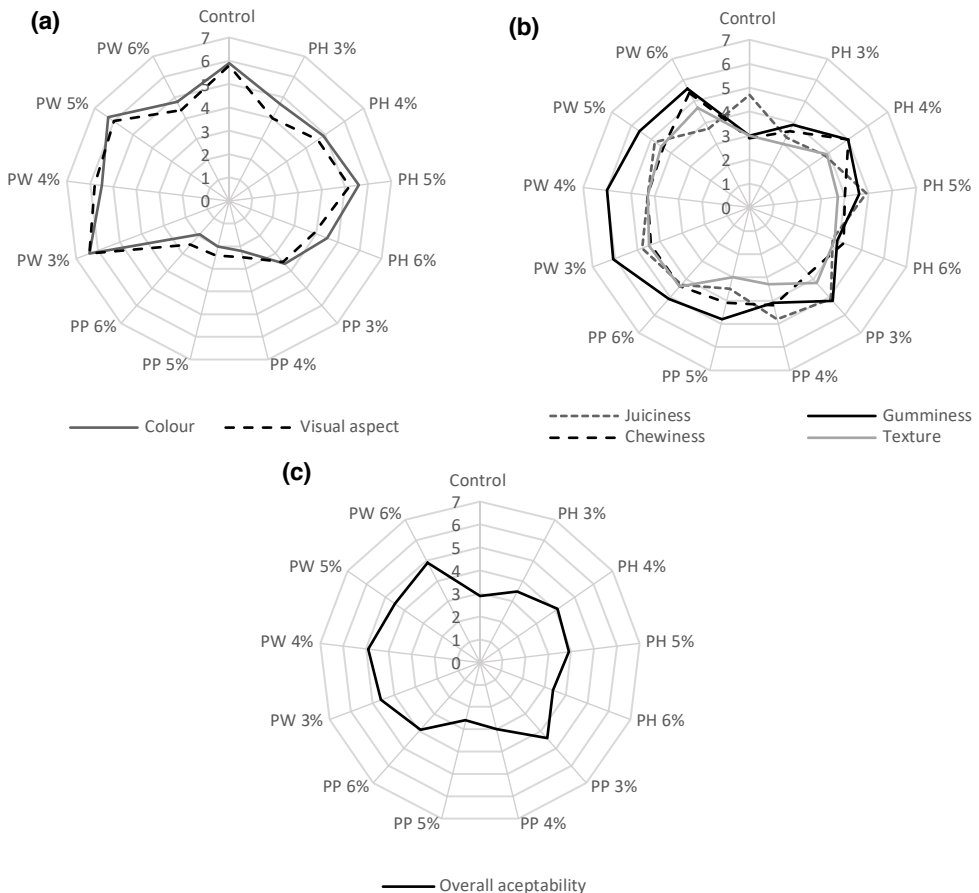


**Figure 5.** Images taken with Cryo-field emission scanning electron microscopy (Cryo-FESEM). Potato starch granules (PS), chickpea granules (CG). (PH: Plantago Husk, PP: Plantago Powder and PW: Plantago White).

### Sensory analysis

Figure 6 shows samples' sensory attributes. The sensory panel gave lower colour and visual aspect scores to the samples made with PP fibre than the samples with fibres PH and PW (Figure 6a). The samples with the PW fibre scored the best and were perceived to be more similar to the control sample. This scenario could be related to the colour parameters of the fibre and

sausage samples (Table 3 and 4, respectively), where the darkest fibre sample was PP, and the plant-based sausages with lower  $L^*$ ,  $a^*$  and  $b^*$  values were also those made with PP fibre. The highest  $\Delta E_2$  values were between the control sample and the sausages with PP, generally at high concentrations (5% and 6%) (Figure 2). This result was expected because they confirmed that marked colour changes can imply rejection, as indicated by Deliza et al. (2002).



**Figure 6.** (a) Colour and visual aspect, (b) juiciness, gumminess, chewiness and general texture and (c) overall acceptability of the plant-based sausages produced with 0%, 3%, 4%, 5% and 6% of Plantago Husk (PH), Plantago Powder (PP) and Plantago White (PW).

Regarding the texture of the plant-based sausages (Figure 6b), the samples with high juiciness values were the control, PW 3%, PW 5%, PH 5%, PP 3% and PP 4%. However, samples PW 3% and PW 4% were evaluated as the gummiest samples. The sample with the highest chewiness value was PW 6% because this parameter was analysed by a TPA (Table 5). Consequently, these results revealed that samples PW 3%, 4%, 5% and 6% and PP 3% and 6% offered a generally good texture. It was concluded that this texture must be improved to achieve a similar texture to meat products because it is known that meat replacers or avoiders do not improve product texture, and texture is one of the main reasons why omnivores reject such products (Noguerol, Pagán, García-Segovia & Varela, 2021).

Finally, the overall acceptability values of the 4% and 6% PW plant-based sausages were higher than for the other samples (Figure 6c). These PW fibre concentrations seemed suitably desirable, which indicates that this fibre would be acceptable for preparing plant-based sausages, although further research is recommended to improve texture.

### Limitations

As these are products mainly address those consumers who wish to reduce or avoid meat consumption, a sensory analysis should be carried out with this diet type. Noguerol et al. 2021 indicated that product texture is less important for vegan and vegetarian consumers than for omnivores.

Moreover in the present study, only psyllium fibres were used as gum to modify texture. General aspect, colour and gummies were the attributes with the highest scores (>6 on a scale from 0 to 9). Thus future studies should include a combination with other ingredients in an attempt to improve general texture.

#### 4. Conclusions

The plant-based sausages made in this study had high ash and carbohydrate contents due to the addition of *P. ovata* fibres. Above all, a lower fat content can be highlighted compared to other meat and meat-free frankfurters sausages. The use of psyllium fibres also increases the water-holding capacity which, as has herein observed, improves the texture of vegan sausages. However, chewability and colour changes could pose major problems for these sausages. The results of this study show that employing PW fibre can minimise these problems because hardness and chewiness increase and colour changes are almost imperceptible compared to the control. However, PP fibre is rejected above all by the colour it confers sausages. The sensory evaluation showed that these three fibres can be used to prepare plant-based sausages. Moreover, PW fibre can be highlighted for obtaining the overall best score and for the fewest colour changes. Nevertheless, it would be desirable for future studies to research with other fibres, gelling agents, or their combination, to improve the texture and acceptability of these plant-based sausages.

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# **Capítulo 6**

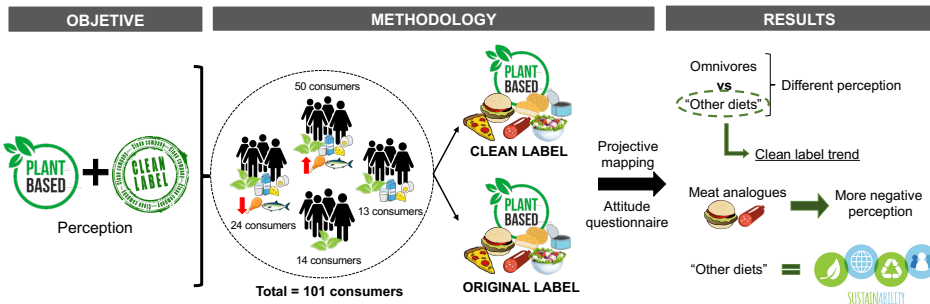
## **Discusión general**



En esta Tesis Doctoral se han querido abordar desde la percepción a la elaboración de productos con etiqueta *clean label*, dirigiendo estos productos a la población que decide restringir o eliminar por completo la carne y derivados cárnicos de su dieta, aunando así dos de las consideradas tendencias actuales (Aschemann-Witzel et al., 2019; Aschemann-Witzel et al., 2020).

Como se ha observado en el **Capítulo 3** (resumen gráfico Figura 1), estas dos tendencias están muy relacionadas. La popularidad de los productos *plant-based* está en continuo crecimiento, cada vez se lanzan al mercado más productos de este tipo (Lantern Study, 2019). Sin embargo, como resultado del **Capítulo 3**, se observó que la categorización y percepción de los productos *plant-based* y *clean label* fue diferente según la dieta de los consumidores. Para los omnívoros no había diferencias entre los productos veganos y vegetarianos *clean label* y con etiqueta original (**Figura 2, Capítulo 3**). Pero para los consumidores que reducen o eliminan el consumo de carne y derivados los productos *clean label* fueron percibidos de manera diferente a los productos con etiqueta original (**Figura 3, Capítulo 3**). De esta manera se puede confirmar que los flexitarianos, veganos y vegetarianos están más interesados en la tendencia actual *clean label*, ya que además indicaron que preferían consumir alimentos sin aditivos y sin procesar. Esta afirmación se puede relacionar con lo indicado por Clicerri et al. (2018), ya que en su estudio se observó que los consumidores que reducían o eliminaban el consumo de carne mostraban una actitud más positiva sobre la salud y los productos naturales que los consumidores omnívoros. Muchos estudios han afirmado que esta actitud hacia unos productos “más naturales” viene dada por una mayor preocupación por el bienestar animal y el medio ambiente (Díaz, 2016; Fiestas-Flores & Pyhälä, 2018; Asvatourian et al., 2018; Hopwood et al., 2021), lo que se confirma también el **Capítulo 3**. Por otro lado, se observó que uno

de los principales motivos por los que los omnívoros eran más reacios al consumo de productos *plant-based* es porque suelen ser menos sabrosos y más caros (Rosenfeld & Tomiyama, 2020).



**Figura 1.** Resumen gráfico Capítulo 3. (Fuente: Noguero et al., 2021).

Una vez conocida la percepción de los productos *plant-based* y *clean label*, y sabiendo que hay un creciente interés por la salud (de Moraes Crizel et al., 2013), se decidió seleccionar una serie de fibras alimentarias para estudiar su posible utilización como texturizantes. Es conocido que el consumo de fibra alimentaria ofrece beneficios para la salud, como el control de peso, la reducción del colesterol, el control de la glucosa postprandial lo que reduce la incidencia de diabetes tipo II, y la prevención de cáncer de colon (Ma & Mu, 2016). Además, la fibra alimentaria funciona como un hidrocoloide debido a sus funciones tecnológicas como la absorción y retención de agua, lo que reduce la pérdida de agua del producto, las pérdidas de peso durante el cocinado y/o almacenado, y afectando mínimamente a las propiedades sensoriales del producto (Han & Bertram, 2017). En el **Capítulo 4 Parte 1** (ver Figura 2) se estudiaron la características y propiedades gelificantes de diferentes fibras vegetales, dentro de las cuales las fibras FPE (Fibra de guisante) y FCH (Harina de chía) mostraron mejores propiedades de hidratación, y la fibra FP (Fibra de patata) una mejor retención de grasa o aceite. Sin embargo, de estas tres fibras la que presentó una mayor capacidad

antioxidante, mejor contenido en fenoles totales y un importante contenido en minerales como Fe, Cu y Mn fue la muestra FCH. Estas propiedades de la chía (*Salvia hispanica L.*) también han sido mencionadas por autores como Câmara et al. (2020) y, además, diferentes estudios han analizado el uso del mucílago de chía para la elaboración de productos cárnicos (Pintado et al., 2016; Ding et al., 2018; Câmara et al., 2020). Pero, en cuanto a propiedades gelificantes, se observó que las combinaciones de fibras (FBPC: fibra de bambú, psyllium y cítricos, y FPESB: fibra de guisante, caña de azúcar y bambú) eran más apropiadas, ya que formaban geles estables, a diferencia de las otras fibras, como la FCH, las cuales tenían mejores capacidades espesantes. No obstante, la muestra PH (*Psyllium Husk*) procedente de *Plantago ovata* (**Capítulo 4 Parte 2**, ver Figura 3) presentó valores significativamente mayores tanto en la capacidad de retención de agua como de absorción de agua ( $p < 0.05$ ). En cambio, la muestra PP (Plantago en polvo) fue la muestra con un índice de solubilidad más alto ( $p < 0.05$ ). Además, cabe destacar el contenido de Fe de la muestra PP, siendo este muy superior al encontrado en las demás muestras. Rousseau et al. (2020) destacaron que la deficiencia de Fe es un problema de salud global que afecta al 30–33% de la población, pero sobre todo de países en vías de desarrollo. En cuanto a las propiedades gelificantes, ambas muestras de *Psyllium* (PH y PP) mostraron tener una capacidad mayor que las otras fibras, ya que formaron geles estables que incluso podían desmoldarse (Figura 4). Debido a la heterogeneidad en los resultados de estas muestras, autores como Tejada-Ortigoza et al. (2016) han señalado la importancia de analizar cada tipo de fibra y no generalizar, ya que las propiedades de cada una están relacionadas con las condiciones ambientales, su estructura química de polisacáridos y los tratamientos y condiciones de extracción utilizados.

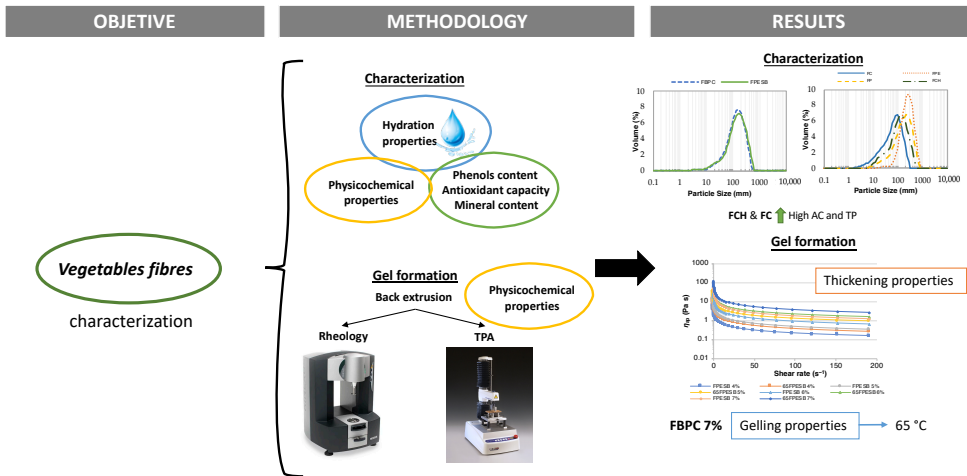


Figura 2. Resumen gráfico Capítulo 4 Parte 1. (Fuente: elaboración propia).

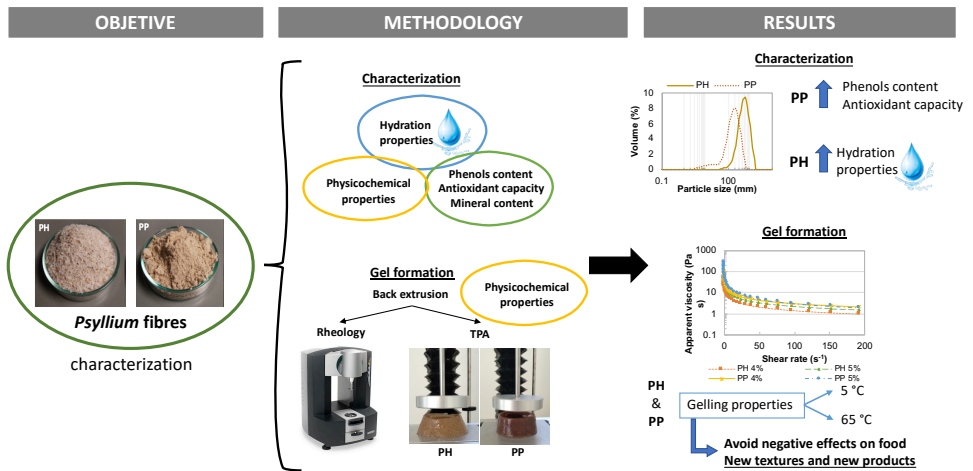
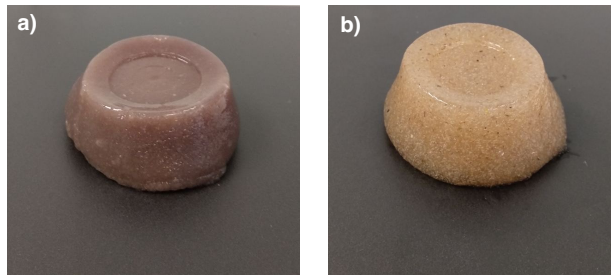


Figura 3. Resumen gráfico Capítulo 4 Parte 2. (Fuente: Noguero, Igual & Pagán, 2021).





**Figura 4.** Imágenes de los geles realizados con (a) PP al 7% y (b) PH al 7%, ambos con tratamiento de calor (65 °C, 20 min).

Una vez obtenida la caracterización de todas las fibras se decidió realizar productos para así probar estas muestras en una matriz alimentaria. Las matrices alimentarias finalmente seleccionadas fueron una emulsión tipo salchicha Frankfurt y un untable. Debido a esta elección, las fibras escogidas fueron las fibras procedentes de *P. ovata* (PP y PH), ya que el objetivo era conseguir una estructura gelificada estable. A estas fibras se añadió la fibra PW (Plantago White) también procedente de *P. ovata* (**Capítulo 5 Parte 1**, ver Figura 5). Esta fibra presentó un tamaño de partícula significativamente menor que las muestras PP y PH ( $p < 0.05$ ). Además, mostró una capacidad de retención de agua mayor que las fibras PH y PP, aunque la absorción de agua fue menor. El contenido en minerales fue ligeramente menor que el contenido de la fibra PH; sin embargo, presentó un contenido mayor de fenoles totales y similar de actividad antioxidante al de la fibra PH.

Los productos untables están considerados productos de conveniencia debido a que son rápidos de preparar (Arya et al., 2017). Además, la tendencia en el consumo de productos listos para comer sigue al alza (Ministerio de Agricultura, Pesca y Alimentación de España, 2018). Por ello, con la fibra PW se decidió realizar un producto untable *plant-based* para estudiar el efecto de esta en su composición y en sus propiedades mecánicas (**Capítulo 5 Parte 1**, ver Figura 5). Se observó que las muestras con adición de PW a las

concentraciones 0.5, 1 y 1,5% presentaban una composición nutricional similar, destacando que todas las muestras de untable realizadas podrían contener la declaración nutricional de “fuente de proteínas y fibra” de acuerdo con la Regulación (EC) N° 1924/2006 del Parlamento Europeo y del Consejo. Por otro lado, se observó una disminución de  $L^*$  (luminosidad) producida por la adición de fibra PW, lo que significó que las muestras con mayor contenido de fibra fueron ligeramente más oscuras. En cuanto a la textura de las muestras, la adición de PW incrementó la firmeza y la consistencia de estas, sin embargo, la muestra más pegajosa y la más difícil de untar fue la muestra con una concentración de PW al 0.5%, mientras que la concentración 1% de PW resultó ser la más fácil de untar.

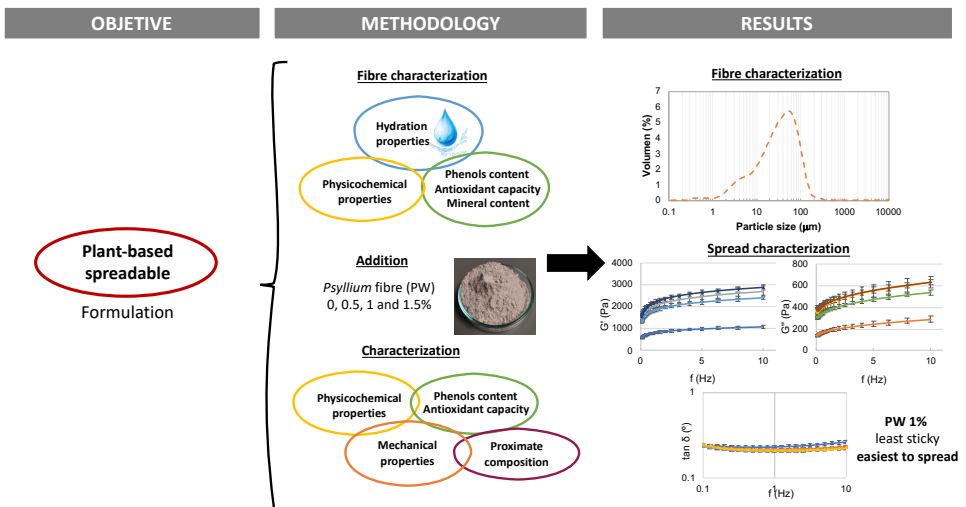
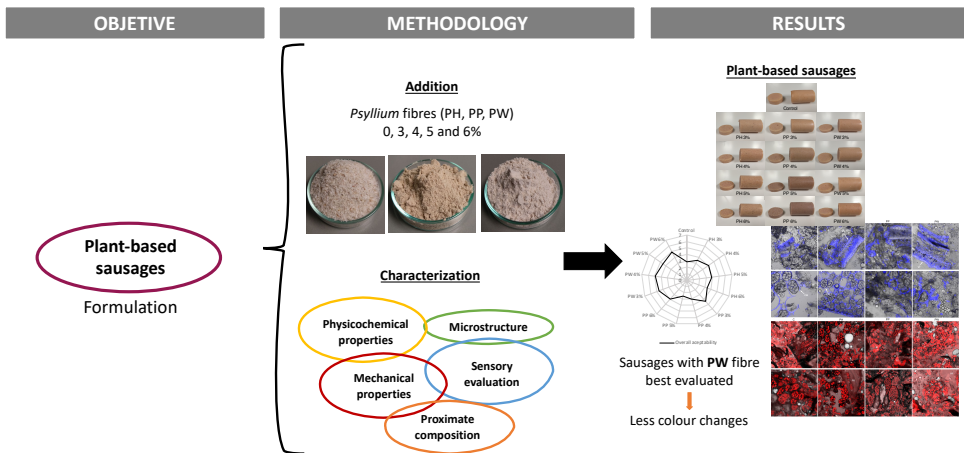


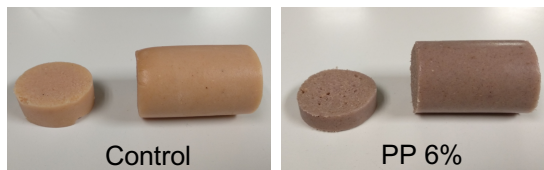
Figura 5. Resumen gráfico Capítulo 5 Parte 1. (Fuente: Elaboración propia).

Después de conocer el efecto de la fibra PW en la formulación de unttables *plant-based* se decidió probar esta fibra junto con las otras dos procedentes de *P. ovata* (PP y PH) en una matriz en la que el poder gelificante fuera el deseado, como en el caso de las salchichas tipo Frankfurt. En el **Capítulo 5 Parte 2** (ver Figura 6) se estudia el efecto de la adición de PP, PH y PW a las

concentración 0, 3, 4, 5 y 6% en salchichas *plant-based* y *clean label*. Estas muestras de salchichas presentaron un contenido de agua mayor que las salchichas tradicionales, realizadas con carne, ya que se requiere más agua para hidratar las proteínas secas y los hidrocoloides utilizados (Stephan et al., 2018). Sin embargo, estas salchichas veganas presentaron un contenido de grasa inferior en comparación con las salchichas realizadas con carne (Majzoobi et al., 2017). Por otro lado, a diferencia de los mostrado por Jridi et al. (2015) y Majzoobi et al. (2017), la adición de las fibras de *Psyllium* (PP, PH y PW) al pH incrementándolo ligeramente. Esto puede ser debido a que los valores de pH de estas fibras están entre 5.10 y 6.14 (**Capítulo 4 Parte 2 y Capítulo 5 Parte 1**). En cuanto al color de las salchichas veganas, se observó, al igual que lo ocurrido en los untables con PW (**Capítulo 5 Parte 1**), que la adición de las todas las fibras disminuyó la luminosidad, oscureciéndose el color de todas las salchichas con respecto a la muestra control. Sin embargo, lo más destacable en cuanto al color fue la diferencia de color producida por la adición de la fibra PP (Figura 7), produciendo este cambio de color el rechazo e inferior puntuación sobre el aspecto de las salchichas por parte de los catadores. Deliza et al. (2002) ya indicó que la adición de fibra tiene que minimizar los cambios de color con respecto a la muestra original para evitar el rechazo de los consumidores. Por ello, en este caso la fibra PW fue la que mostró menos cambio de color con respecto a la muestra control. Además, esta fibra fue la que presentó una mayor a dureza y masticabilidad en el análisis experimental, y la mejor valorada en el sensorial. Sin embargo, sería recomendable realizar más estudios con la adición de otros ingredientes texturizantes para conseguir una textura más parecida a la de las salchichas originales realizadas con carne, ya que, aunque a los consumidores veganos y vegetarianos no les importe la textura, para conseguir que los consumidores omnívoros consuman este tipo de productos la textura es un factor muy importante, como se ha indicado en el **Capítulo 3**.



**Figura 6.** Resumen gráfico Capítulo 5 Parte 2. (Fuente: Elaboración propia).



**Figura 7.** Diferencia de color entre la salchicha *plant-based* control y con adición de fibra PP al 6%.

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# **Capítulo 7**

## **Conclusions**



The conclusions of this Doctoral Thesis are:

- The categorization and perception of plant-based and clean label products is different depending on the diet of consumers. Omnivores do not differentiate between clean label and original label vegan and vegetarian products. However, for consumers who reduce or eliminate the meat and meat products consumption, clean label products are perceived differently than products with the original label. In this way, it can be confirmed that flexitarians, vegans and vegetarians are more interested in the current clean label trend, because they mention that they prefer to consume processed food without additives. Furthermore, the main motivation for this group for choosing this "more natural" products is concern for the environment and animal welfare.

- The vegetable fibres studied in this work have texturizing properties, both thickening and gelling. However, the behaviour is very different depending on the fibre analysed, which makes it necessary to characterize each one from the techno-functional point of view for its possible application in food. The fibres from *Plantago ovata*, also called psyllium fibres, are, among those studied, those that present better gelling properties at low concentrations, both at cold temperature and using a heat treatment, while the rest of the fibres analysed, even at high concentrations, have better thickening capacity.

- Depending on the concentration of vegetable fibre used, different textures are obtained, from thickener to gelling agent, so these could have a place in the development of new products and achieve new textures. In addition, these fibres have functional properties, such as the antioxidant capacity of chia flour and psyllium fibres, and the concentration of minerals, highlighting the iron content of psyllium dietary fibre (PP).

- Texturizing preparations made with psyllium fibres show similar results in both plant-based sausages and spreads. The addition of psyllium at different

concentrations increases the hardness and elasticity of the samples in both matrices. Therefore, these fibres can be used as gelling agents for this type of product. However, in the case of sausages and as a result of the evaluation obtained in the sensory analysis, it would be convenient to combine the psyllium fibres with other gelling agent, to improve their similarity to originalmeat sausages.

- In the two food matrices studied, the addition of PW fibre (*Plantago ovata* White) is the one that gives the least colour changes and the best textural properties to the final products. Therefore, it can be concluded that it is the most appropriate in products of this type.

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## Agradecimientos

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