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# PROGRAMA DE DOCTORADO EN CIENCIA E INGENIERÍA AGROALIMENTARIA Y DE BIOSISTEMAS

TESIS DOCTORAL

# EMPLEO DEL PSYLLIUM PARA EL DESARROLLO DE NUEVOS PRODUCTOS A BASE DE CEREALES

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**“El saber es el único espacio de libertad del ser.”**

*Michel Foucault*

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# Índice

# Table of contents

# Resumen

Los hidrocoloides son ingredientes muy utilizados en la industria alimentaria debido principalmente a dos características: su solubilidad y su viscosidad. Sin embargo, los hidrocoloides son considerados aditivos y, en vista de la demanda actual por productos *"clean label"*, es necesario buscar alternativas naturales que tengan propiedades similares.

El psyllium es un ingrediente natural, con propiedades funcionales similares a de la goma xantana, que presenta grandes ventajas nutricionales, debido a su alto contenido en fibras. Así se ha demostrado su acción positiva sobre diversas enfermedades o disfunciones del organismo, como el estreñimiento, la diarrea, el síndrome de intestino irritable (SII), el cáncer de colon, la diabetes y la hipercolesterolemia. Además, el psyllium es uno de los sustitutos del gluten más utilizados en la elaboración de panes comerciales sin gluten a nivel mundial, junto con el HPMC, la goma xantana y la goma guar. Sin embargo, los efectos de la incorporación del psyllium en productos horneados están poco estudiados.

Se sabe que el psyllium tiene interesantes propiedades gelificantes y espesantes, por ello, la presente tesis evalúa el efecto del psyllium en las propiedades del almidón de maíz, ya que el almidón es una de las alternativas a la harina de trigo más utilizadas en la elaboración de panes comerciales, y otros productos sin gluten. A partir de este estudio y mediante una mejor comprensión de las propiedades funcionales del psyllium, se observó que su uso en la elaboración de panes sin gluten podría afectar a la hidratación de dichas masas, lo que alteraría los efectos de este hidrocoloide en la masa y en la calidad final de los panes. Por lo tanto, esta tesis también evalúa el efecto del psyllium sobre la hidratación de los panes sin gluten, utilizándolo como único sustituto del gluten y comparando sus efectos con otros hidrocoloides.

La alta capacidad de absorción de agua del psyllium también hace que pueda funcionar como sustituto de la grasa. Este efecto puede ser útil en la fabricación de productos de bollería más saludables, ya que estos productos suelen tener grandes cantidades de grasa en su formulación. Así, en esta tesis también se estudian los efectos de la utilización de una mezcla de psyllium y agua para sustituir la grasa en las formulaciones de bizcochos y galletas para producir productos finales más sanos, empleándose diferentes niveles de sustitución de la grasa. Previamente se estudia la influencia del tamaño de partícula de harinas de maíz para la elaboración de galletas sin gluten. El estudio del efecto de la sustitución de la grasa en la formulación de galletas se realizó tanto en galletas con gluten como en galletas libres de gluten.

El empleo del psyllium en diferentes porcentajes de sustitución del almidón, mostró un aumento de las propiedades de hidratación del almidón y su efecto fue similar al de la goma xantana. Para los geles elaborados sin exceso de agua, ambos hidrocoloides disminuyeron su dureza. A pesar de presentar pequeñas diferencias en la temperatura de formación de pasta, el comportamiento viscoso del psyllium y de la xantana son distintos a altas concentraciones, en las cuales el psyllium aumenta el *setback* y la temperatura de formación de la pasta.

El efecto del psyllium sobre la hidratación de panes sin gluten fue analizado y comparado con otros hidrocoloides (HPMC y goma xantana), en masas elaboradas con almidón de maíz y harina de arroz. En todos los casos, las formulaciones con almidón de maíz tuvieron mayor volumen específico que las elaboradas con harina de arroz. Las formulaciones con psyllium mostraron un comportamiento similar con respecto al volumen específico y la pérdida de peso, en relación a la goma xantana. El HPMC produjo panes con mayor volumen específico, pero al compararse volúmenes específicos similares, los panes con HPMC presentaron mayor dureza que las formulaciones con psyllium o goma xantana.

Distintas mezclas de psyllium y agua fueron evaluadas como sustitutos del aceite en bizcochos, empleándose sustituciones del 25%, 50% y 75%. Las mayores sustituciones del aceite produjeron bizcochos finales con una corteza más clara y volumen específicos menores, pero sin diferencias significativas en comparación con el control. El test de aceptabilidad mostró que bizcochos con un 25% menos de aceite no tuvieron diferencia significativa frente al control y que los bizcochos con el 75% de sustitución obtuvieron buena aceptabilidad, con una puntuación de 7 sobre 9.

En la elaboración de galletas es necesario la presencia de un cierto porcentaje de partículas finas en la harina, pues ellas son las responsables por dar cohesividad a la masa y así se permite su correcto laminado. Tamaños de partículas mayores producen un mayor grado de expansión y menor dureza en las galletas finales. Al sustituir toda la grasa de galletas, con y sin gluten, por una pasta de psyllium y agua se obtuvieron galletas con menor grado de expansión, diámetros pequeños y mayor dureza. Las galletas con gluten presentaron una humedad final mayor que las sin gluten. Estas características hicieron que las galletas tuviesen baja aceptabilidad por los consumidores y este resultado fue más evidente en las galletas sin gluten.

# Abstract

Hydrocolloids are widely used as ingredients in the food industry mainly due to two characteristics: their solubility and their viscosity. However, hydrocolloids are considered food additives and, in view of the current demand for "clean label" products, it is necessary to look for natural alternatives that have similar properties.

Psyllium is a natural ingredient, with functional properties similar to xanthan gum, and presents great nutritional advantages, due to its high fibre content. Thus, it has been shown that psyllium has a positive effect in the treatment of diseases or body’s dysfunctions, such as constipation, diarrhoea, irritable bowel syndrome, colon cancer, diabetes and hypercholesterolemia. In addition, psyllium is one of the most widely used gluten substitute in formulations of worldwide commercial gluten-free breads, such as the HPMC, xanthan gum and guar gum. However, the effects of incorporating psyllium into baked products have been little studied.

It is known that psyllium has interesting gelling and thickening properties. Therefore, this thesis evaluates the effect of psyllium on the properties of maize starch, since this starch is one of the most used alternatives to wheat flour in the production of commercial gluten-free breads, and other gluten-free products. From this study and through a better understanding of the functional properties of psyllium, it was observed that its use in the production of gluten-free breads could affect the hydration of these doughs, which would change the effects of this hydrocolloid on the dough and on the final quality of the breads. Therefore, this thesis also evaluates the effect of psyllium on the hydration of gluten-free breads, using it as the only gluten substitute and comparing its effects with other hydrocolloids.

The high-water absorption capacity of psyllium also makes possible its use as a fat substitute. This effect can be useful in the manufacturing of healthier baked products, as these products often have great amount of fat in their formulation. Thus, this thesis also studies the effects of using a mixture of psyllium and water to replace fat in cakes and cookies formulations to produce healthier final products, using different levels of fat replacement. Previously, the influence of the particle size of maize flour on the production of gluten-free cookies was studied. The study about the effect of fat substitution in cookies formulation was carried out both in gluten-content and gluten-free cookies.

The use of psyllium in different percentages of starch substitution, showed an increase in the hydration properties of starch and this effect was similar to that of xanthan gum. For gels made without excess water, both hydrocolloids decreased their hardness. Despite small differences in pasting temperature, the viscous behaviour of psyllium and xanthan gum are different at high concentrations, where psyllium increases the setback and the pasting temperature. The effect of psyllium on the hydration of gluten-free breads was analysed and compared with other hydrocolloids (HPMC and xanthan gum), in doughs made with maize starch and rice flour. In all the cases, the formulations with maize starch had a higher specific volume than those made with rice flour. Psyllium formulations showed similar behaviour with respect to specific volume and weight loss, in relation to xanthan gum. The HPMC produced breads with higher specific volume, but when similar specific volumes were compared, HPMC breads were harder than formulations with psyllium or xanthan gum.

Different mixtures of psyllium and water were evaluated as oil substitutes in cakes, using substitutions of 25%, 50% and 75%. The higher oil replacements produced final cakes with lighter crust and lower specific volume, but they had no significant differences compared to the control. The test of acceptance showed that cakes with less 25% of oil did not differ significantly from the control and the cakes with 75% of oil substitution obtained good acceptability, with a score of 7 out of 9.

In cookies elaboration is necessary the presence of a certain percentage of fine particles in the flour, because they are responsible for giving cohesiveness to the dough and thus allows proper lamination. Large particle sizes produce a higher degree of expansion and lower hardness in the final cookies. The total replacement of fat in cookies, with and without gluten, through using a paste of psyllium and water, resulted in cookies with a lower degree of expansion, small diameters and great hardness. Cookies with gluten had a higher final moisture content than those without gluten. These characteristics were responsible for the low acceptability of these cookies by consumers and this result was most evident in the gluten-free cookies.

# Lista de artículos incluidos en la tesis

1. Belorio, M., Marcondes, G., & Gómez, M. (2020). Influence of psyllium versus xanthan gum in starch properties. *Food Hydrocolloids, 105*, 105843. https://doi.org/10.1016/j.foodhyd.2020.105843

2. Belorio, M., & Gómez, M. (2020). Effect of hydration on gluten-free breads made with hydroxypropyl methylcellulose in comparison with psyllium and xanthan gum. **Enviado a Journal of Food Engineering en 06 de julio de 2020*.

3. Belorio, M., Sahagún, M., & Gómez, M. (2019). Psyllium as a fat replacer in layer cakes: batter characteristics and cake quality. *Food and Bioprocess Technology, 12*, 2085-2092. https://doi.org/10.1007/s11947-019-02362-3.

4. Belorio, M., Sahagún, M., & Gómez, M. (2019). Influence of flour particle size distribution on the quality of maize gluten-free cookies. *Foods, 8*, 83. https://doi.org/ 10.3390/foods8020083.

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# INTRODUCCIÓN

# Introducción

## 1. HIDROCOLLOIDES: ASPECTOS GENERALES

Los hidrocoloides son un grupo grande y heterogéneo de sustancias poliméricas que incluyen principalmente algunas proteínas (gelatina) y polisacáridos cuya composición puede ser derivada de la unión de las mismas unidades de glucosa (celulosa y almidón), de dos monómeros distintos (alginato) o por un mayor número de monómeros (goma arábica) (BeMiller, 2008).

Los hidrocoloides poseen una gran cantidad de grupos hidroxilos, lo que aumenta su afinidad para unirse a las moléculas de agua, al convertirlos en compuestos hidrofílicos. Además, son capaces de producir una dispersión que es intermedia entre una solución verdadera y una suspensión, y así exhiben propiedades de un coloide. Teniendo en cuenta estas dos propiedades, se comprende mejor la nomenclatura de ***hidrocoloide*** (Saha y Bhattacharya, 2010).

El uso de los hidrocoloides en la industria de alimentos está muy relacionado con su ***solubilidad,*** debido a los grupos hidroxilos presentes en su estructura y a su efecto sobre la ***viscosidad***, que cambia de acuerdo con su concentración, peso molecular o estructura (lineal, muy ramificada, o ligeramente ramificada) (Al- Assaf y Phillips, 2015).

Los hidrocoloides pueden clasificarse de acuerdo con su origen (vegetal, animal o sintético) y son divididos en: gomas naturalmente encontradas en la naturaleza, gomas modificadas obtenidas por cambios químicos de las gomas naturales y gomas sintéticas que son obtenidas por síntesis química, o por el empleo de microorganismos (Nussinovitch y Hirashima, 2014). En general, entre los hidrocoloides es común utilizar el término "goma" en su nomenclatura, por ejemplo, la goma xantana o la goma garrofín. Este nombre está relacionado con la capacidad de estos hidrocoloides en disolverse o dispersarse en agua, generando una solución viscosa o una dispersión. A su vez, el termino mucilago también es muy utilizado y está asociado a los materiales viscosos encontrados en las cáscaras de las semillas de algunas plantas, como es el caso del psyllium (Li y Nie, 2016).

El uso de los hidrocoloides como ingredientes en la industria de alimentos suele estar muy relacionado con su influencia en las propiedades reológicas de los productos. Las distintas funcionalidades de los hidrocoloides se muestran en la figura 1.

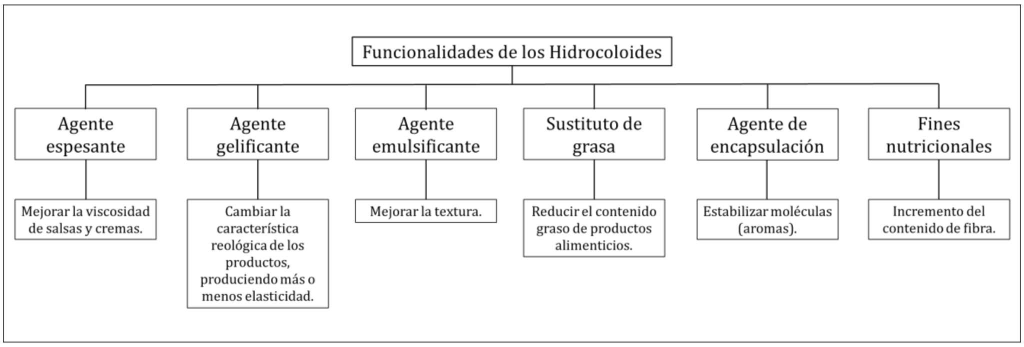

*Figura 1: Funcionalidades de los hidrocoloides. Adaptado de Li y Nie (2016).*

Varios hidrocoloides pertenecen a la categoría de aditivos alimentarios permitidos en muchos países. Diversas formulaciones de alimentos como sopas, caldos y salsas para ensaladas utilizan hidrocoloides como aditivos para lograr una viscosidad y "sensación en la boca" agradable al consumidor. Los hidrocoloides también son utilizados en otros productos para crear la textura deseada, como por ejemplo en panes, helados, mermeladas, postres lácteos, bizcochos y caramelos (Milani y Maleki, 2012).

En los productos de panadería, los hidrocoloides se utilizan para mejorar la tolerancia a un exceso de amasado, las características del producto final y la calidad sensorial. A menudo se añaden a las formulaciones para actuar sobre la textura de las migas y reducir el endurecimiento del pan durante el almacenamiento. También pueden utilizarse en la preparación de masas congeladas (para evitar daños por congelación) o con fines nutricionales, ya que son fibras (Saha y Bhattachrya, 2010).

Teniendo en cuenta su función en el ajuste de la viscosidad y la textura de los alimentos, se han realizado numerosos estudios sobre la aplicación de hidrocoloides en diversos sistemas alimentarios. Entre estos estudios destacan los centrados en la elaboración de productos sin gluten, pues en estos es necesario utilizar un "sustituto del gluten". Los panes elaborados con harina de trigo presentan una red de gluten, la cual está formada por las proteínas de la harina (principalmente la glutenina y la gliadina) y se desarrolla al hidratar las proteínas y someterlas a trabajo mecánico. Esta red es la responsable de las propiedades viscoelásticas y de la extensibilidad de la masa, así como de la retención del gas producido durante la fermentación. Algunos autores han evaluado la combinación de distintos hidrocoloides como sustitutos del gluten, analizando como modifican las características y la calidad de distintos productos sin gluten, como, panes, bizcochos o galletas (Anton y Artfield, 2008; Gao et al., 2017; Román et al., 2019).

## 2. PSYLLIUM: ORIGEN, OBTENCIÓN Y ESTRUCTURA

El psyllium es el ingrediente obtenido de la cáscara que recubre las semillas de la planta del género *Plantago genus* (figura 2). Las especies *Plantago ovata* y *Plantago psyllium (o Plantago asiática*) son las más importantes y son muy usadas en las industrias farmacéutica, cosmética y, de manera más reciente en la de los alimentos (Board, 2003; Gupta, 1991; Panda, 2002).

La cáscara de *Plantago psyllium* se obtiene al separarse de la semilla durante la molienda. La trituración de la semilla suele realizarse con un molino que dispone de un sistema de aspiración neumática. Así, la cáscara es separada del resto de las semillas mediante una ligera presión mecánica. Mientras las semillas siguen el proceso de molturación, el subproducto aspirado (cáscara y semillas residuales) es tamizado, para eliminar todas las semillas restantes. Las cáscaras, en este punto ya totalmente separadas, son molidas para la reducción del tamaño de partícula y obtención del psyllium en polvo (Ajit Patel, 2013).

La cáscara es una membrana mucilaginosa inodora, insípida, translúcida y de color marrón claro, que constituye aproximadamente el 30% del peso de la semilla (Gupta, 1991). Este producto es rico en fibras, las cuales son responsables de las ventajas nutricionales, medicinales y funcionales del psyllium.

*Figura 2: Planta de Plantago psyllium y sus semillas. Fuente: Nie et al. (2018).*

La mayor parte de la fibra soluble que compone el psyllium está compuesta por un polisacárido, del tipo arabinoxilano, similar a los que se encuentran en la mayoría de los cereales (Izydorczyk y Biliaderis, 1995). Diferentes estudios describieron el psyllium como un arabinoxilano compuesto por diferentes monosacáridos, como la xilosa, arabinosa, galactosa, ramnosa, glucosa y manosa. La diferente proporción molar y composición de los monosacáridos entre estos polisacáridos puede estar relacionada con los métodos de separación o purificación que pueden utilizarse para obtener el psyllium (Zangh et al., 2019; Nie et al., 2018). La figura 3 representa la estructura propuesta por Nie et al. (2018) para el polisacárido obtenido de las semillas de *Plantago asiática*, tras distintas cromatografías y procesos de caracterización.

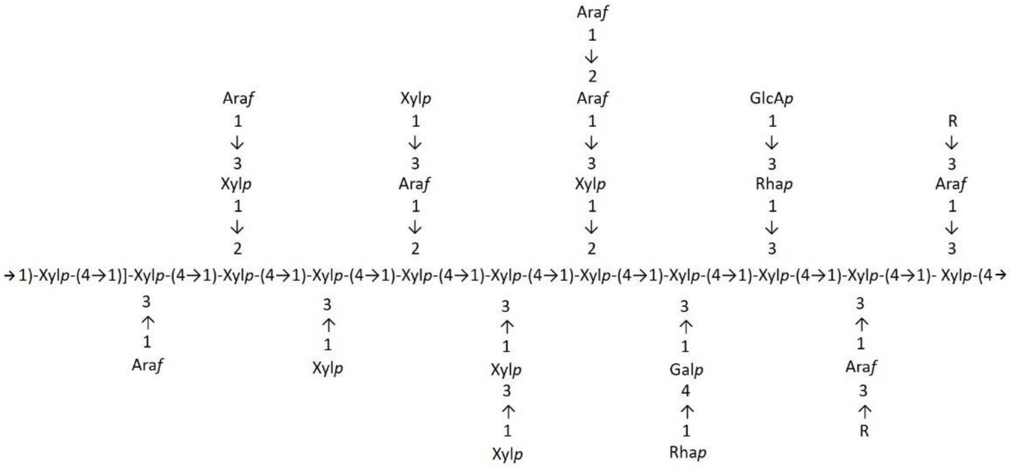

*Figura 3: Estructura primaria obtenida para el polisacárido obtenido de las semillas de Plantago asiática tras distintos procesos de separación y caracterización (Adaptado de Nie et al., 2018).*

La fibra tanto del psyllium como del salvado de trigo está compuesta en gran parte por arabinoxilanos muy ramificados, que consisten en espinas dorsales lineales de xilopiranosa (Xyl*p*) unidas por enlaces β-D-(1-->4). A estas cadenas se adhieren unidades de α-L-arabinofuranosa (Ara*f*) como residuos, a través de los enlaces α(1-3) y α(1-2). La fibra de psyllium posee una estructura más compleja que la fibra del salvado de trigo, con una amplia variación en las cadenas laterales y en los sustituyentes unidos en las posiciones O-2 y/o O-3 (Edwards et al., 2003; Fischer et al., 2004). En el psyllium es posible distinguir muchos tipos de arabinoxilanos, similares en su composición monomérica pero que varían en su conformación polimérica (Yin et al., 2012a; Yu et al., 2017). En la extracción del mucílago influirá la temperatura del agua. Así el mucílago extraído en agua fría tiene una alta proporción de ramnosa y ácido galacturónico, y un bajo contenido de arabinosa y xilosa, en comparación con el mucílago obtenido en condiciones extremas (calor y alcalinidad). El mucílago extraído en frío también tiene un mayor peso molecular y un radio hidrodinámico menor que el resto (Yu et al., 2017). Estas características son responsables de algunas ***propiedades de absorción*** que hacen del psyllium un ingrediente muy útil para ser empleado en la elaboración de una gran variedad de productos (Ziemichód et al., 2018).

La India es el mayor productor de *Plantago psyllium* y también el mayor exportador, con más del 90% de su producción exportada a Estados Unidos, Alemania y el Reino Unido (Golkar et al., 2017). El mercado del psyllium está en crecimiento y las ventas seguirán impulsadas por el aumento del uso de este ingrediente en productos medicinales como, por ejemplo, en laxantes de origen natural para el estreñimiento crónico.

Otro importante empleo del psyllium está relacionado a su inclusión en productos ricos en fibra, que presentan un gran potencial para la reducción del colesterol. El empleo del psyllium como aditivo alimentario es todavía minoritario frente a otros hidrocoloides. Sin embargo, se espera que se incremente en los próximos años debido a los estudios que se están desarrollando y por el mayor acceso de la industria agroalimentaria a este ingrediente (figura 4).

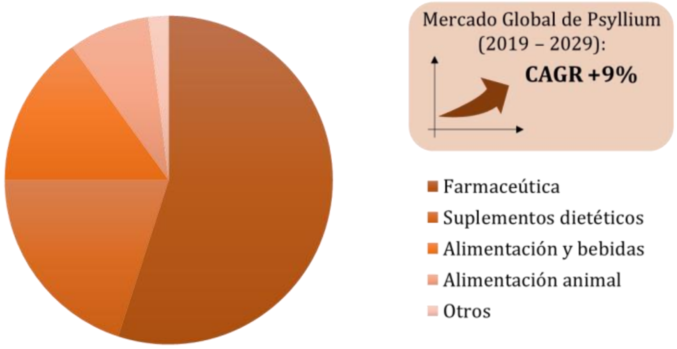

*Figura 4: Mercado global de produtos elaborados com psyllium. Adaptado de XPloreMR, 2019.*

## 3. VENTAJAS NUTRICIONALES Y BENEFICIOS PARA LA SALUD DEL PSYLLIUM

Los beneficios para la salud del psyllium están relacionados a su ***viscosidad*** y han sido discutidos en múltiples artículos científicos. Entre estos beneficios se incluyen sus efectos sobre el estreñimiento, la diarrea, el síndrome de intestino irritable (SII), la enfermedad inflamatoria intestinal (colitis ulcerosa), el cáncer de colon, la diabetes y el hipercolesterolemia (Singh, 2007; Wärnberg et al., 2009). Estos efectos y las ventajas nutricionales del psyllium están relacionados con algunas de sus propiedades que, por lo tanto, serán discutidas brevemente a continuación.

- *Control glucémico y del colesterol:*

El carácter viscoso del psyllium tiene una gran influencia en el transito gastrointestinal. Se sabe que el consumo de fibras viscosas puede alterar la viscosidad de la digestión en el tracto gastrointestinal, inhibiendo así la absorción de nutrientes, en particular de la glucosa y el colesterol (Dikeman y Fahey, 2006), lo que directamente influye en el control de estas sustancias en los organismos. Así, los efectos sobre la reducción del colesterol y la mejora del control glucémico están relacionados con las modificaciones que provoca el psyllium en el intestino delgado y, en general, la alta viscosidad que promueven algunas fibras solubles (fibras formadoras de gel como β-glucanos, psyllium y goma guar) es la responsable de estos efectos. Las fibras solubles con escaso poder espesante (inulina, oligosacáridos de frutas y dextrina de trigo) y las fibras insolubles (salvado de trigo) no proporcionan estos beneficios para la salud, ya que tales beneficios dependen de su poder espesante (McRorie y McKeown, 2017).

El psyllium puede disminuir el colesterol total y el de las lipoproteínas de baja densidad (LDL), como se ha analizado en profundidad en varias revisiones y metaanálisis (Anderson et al., 2000; Bernstein et al., 2013; Olson et al., 1997; Wei et al., 2009). En la actualidad, el psyllium está siendo considerado como un potencial agente de apoyo al tratamiento de la hiperlipidemia, ya que es un trastorno relacionado con la alta ingesta de colesterol y/o productos ricos en grasa. Este efecto ha sido comprobado en muchos casos debido a que la cáscara de *Plantago ovata* juega un papel clave en la reducción del colesterol sérico (Xing et al., 2017). Además de la capacidad del psyllium para reducir el colesterol, también es capaz de reducir el índice glucémico, tanto en pacientes diabéticos como no diabéticos (Abutair et al., 2016; Gibb et al., 2015). Este efecto es más evidente cuando el psyllium es ingerido junto con los alimentos y no antes de una comida (Wolever et al., 1991). Sin embargo, el consumo de psyllium antes de la ingesta de pan también reduce la respuesta glucémica (Munari et al., 1998).

- *Tratamiento del síndrome del intestino irritable (SII) y del estreñimiento:*

La SII o simplemente síndrome del colon irritable afecta el intestino grueso y, aunque no se han comprobado sus causas, se sabe que algunas contracciones vigorosas del intestino pueden llevar a su desarrollo. Según el Instituto Nacional de

Salud (NIH), los síntomas de la SII normalmente son: dolor y molestia abdominal, hinchazón abdominal, estreñimiento y/o diarrea (dependiendo del paciente).
El psyllium ayuda en el tratamiento de la SII debido a su efecto laxante. Sólo se conocen dos tipos de productos que presentan este efecto: las partículas de fibra insoluble de gran tamaño, como las del salvado de trigo, que irritan mecánicamente la mucosa intestinal y estimulan la secreción de agua y de mucosa en el intestino; y la alta capacidad de retención de agua y formación de gel del psyllium, que aumenta la resistencia a la deshidratación. Ambos mecanismos requieren que la fibra resista a la fermentación y permanezca relativamente intacta en todo el intestino grueso, es decir, la fibra debe estar presente en las heces. Estos dos procesos conducen a un mayor contenido de agua en las heces, lo que da lugar a heces voluminosas, blandas y fáciles de evacuar (McRorie y Mc Keown, 2017).

Por lo tanto, para el tratamiento del SII, un trastorno gastrointestinal crónico común, muchos médicos recomiendan que los pacientes aumenten su ingesta de fibra dietética. Sin embargo, la eficiencia de cada tipo de fibra para obtener estos resultados varía mucho. En general, se prefieren las fibras dietéticas de cadena larga, de viscosidad intermedia o alta, solubles y moderadamente fermentables, como es el caso del psyllium, pues generan poco gas y, por consiguiente, no causan los síntomas relacionados con una excesiva producción de gas. Por lo tanto, el psyllium es una de las fibras más recomendadas para este propósito (Bijkerk et al., 2004; El-Salhy et al., 2017).

Asimismo, y a diferencia de otros tipos de fibras, el psyllium, junto con el salvado de trigo, tiene un papel demostrado en la prevención del estreñimiento (Gelinas, 2013). En este caso, el efecto del psyllium se basa en su alta capacidad de absorción de agua y en su efecto lubricante, que ayuda a estimular el intestino. Así, el psyllium es indicado como una alternativa más natural para el tratamiento del estreñimiento, comparado con otros productos utilizados para este fin (Ramkumar y Rao, 2005).

- *Prevención del cáncer de colon:*

Además de todos los beneficios mencionados anteriormente, se ha demostrado que el psyllium ayuda a prevenir el cáncer de colon, especialmente cuando se combina con el salvado de trigo (Alabaster et al., 1993). Este efecto se ha asociado al hecho de que la fermentación anaeróbica del psyllium en el colon produce una cierta cantidad de ácidos grasos de cadena corta, los cuales juegan un papel importante en la prevención del cáncer (Wärnberg et al., 2009).

- *Ventajas nutricionales y efectos sobre la saciedad*:

El psyllium puede ser considerado un suplemento alimentario rico en fibras cuya principal ventaja, comparado con otros suplementos ricos en fibra, es su menor coste y su mejor tolerancia, por lo que es uno de los suplementos con más potencial (Pal y Radavelli-Bagatini, 2012).

Además del reconocimiento del psyllium como una importante fuente de fibras, este hidrocoloide también tiene efecto positivo sobre la saciedad, ya que se ha demostrado que su ingesta disminuye la sensación de hambre y reduce la ingesta calórica en voluntarios normales (sin enfermedades metabólicas previas). Este efecto se ha relacionado con un aumento del tiempo de absorción intestinal (Rigaud et al., 1998). Estos resultados sobre la saciedad disminuyen el riesgo de síndrome metabólico y de enfermedades cardiovasculares, por lo que se recomienda su inclusión en las dietas de adelgazamiento (Jane et al., 2019).

Basándose en las evidencias de los beneficios a la salud, debido a su ingestión, en los EE.UU., la *Food and Drug Administration* (FDA) (2018) permitió la inclusión de una alegación de salud en productos que contienen al menos 1,7 g de fibra soluble por cada cantidad de referencia habitualmente consumida. El texto de la alegación dice que *"las dietas que son bajas en grasas saturadas y colesterol y que incluyen fibra soluble de la cáscara de la semilla de psyllium "pueden" o "podrían" reducir el riesgo de enfermedades cardíacas"*. La FDA también especifica la ingesta diaria de fibra soluble que es necesaria para reducir el riesgo de enfermedades coronarias, que en este caso es de un mínimo de 7 g de psyllium al día.

El gobierno canadiense también autorizó el uso de un texto predefinido en las etiquetas de los productos que contienen al menos 1,75 g de psyllium, como fibra soluble, por cantidad de referencia y por porción de un tamaño establecido (Health Canada, 2011). El texto propuesto es el siguiente: *"[el tamaño de la porción del cuadro de Información Nutricional en medidas métricas o comunes para el hogar] de (Nombre de la marca) [nombre del alimento] con suministros de psyllium/proporciona X % de la cantidad diaria de las fibras (7g) que se ha demostrado ayudar a reducir/reduce el colesterol"*.

Aunque están claros los diferentes beneficios nutricionales que supone la ingesta de psyllium, su incorporación en los alimentos modifica sus propiedades organolépticas. Estas modificaciones, que pueden ser positivas o negativas, dependen de sus propiedades funcionales, que son analizadas en el siguiente apartado.

# 4. PROPIEDADES FUNCIONALES DEL PSYLLIUM

- *Capacidad de absorción de agua:*

La capacidad de absorción de agua está relacionada con la capacidad de la fibra para unir o retener el agua en su estructura. Robertson y Eastwood (1980) descubrieron que esta propiedad puede estar más relacionada con la estructura de la fibra que con su composición química.

La alta capacidad de absorción de agua del psyllium es una de sus características más importantes, principalmente cuando se trata de su empleo en la formulación de alimentos. Esta capacidad es diez veces mayor que la de la celulosa obtenida del trigo o del bambú, y cinco veces mayor que la de la fibra de la manzana (Dello et al., 2017). Este comportamiento es una ventaja del psyllium y contribuye a su efecto positivo en el organismo, así como en su capacidad para reducir el estreñimiento y el SII, como ya se ha comentado.

La capacidad del psyllium para absorber agua hace que tenga un comportamiento similar a otros hidrocoloides y permite su uso en la elaboración de alimentos. Esta capacidad también está relacionada con su habilidad para minimizar el endurecimiento de los panes y reducir el contenido graso en bizcochos y otros productos alimentarios.

- *Capacidad espesante y gelificante:*

El poder espesante de una fibra está relacionado con la propiedad de algunos polisacáridos de espesarse o formar gel cuando son mezclados con fluidos. El gel que se forma es el resultado de una modificación física en la que la disposición de los polisacáridos cambia, dando como resultado una estructura más sólida (Dikeman y Fahey, 2007). El gel puede clasificarse como una estructura semisólida, formada por una fase líquida retenida por una estructura polimérica tridimensional. Los geles son importantes porque son rígidos y elásticos, lo que confiere buenas propiedades de textura y reología cuando son empleados en la formulación de alimentos (Mao et al., 2019).

La capacidad del psyllium como agente gelificante ha sido estudiada por muchos autores. Por ejemplo, Haque et al. (1993) compararon la capacidad del psyllium para formar un gel débil con el comportamiento de la goma xantana, encontrando resultados similares con ambos productos. Guo et al. (2009) estudiaron la fracción obtenida por extracción alcalina de los polisacáridos del psyllium y confirmaron que esta fracción es capaz de formar un gel de estructura débil tras el calentamiento.

Los siguientes estudios evaluaron las propiedades del psyllium para formar geles a través de sus características reológicas, tras someter el psyllium a distintas condiciones, por ejemplo: cambios de la concentración, método de extracción (caliente, frío, medio alcalino), presencia de iones (calcio o sodio), combinación con otras gomas, cambios de pH y diferentes tamaños de partícula. Guo et al. (2009) mencionan la presencia de una estructura fibrilar con valores de G' mayores que G", lo que coincide con la investigación de Farahnaky et al. (2010). Estos investigadores también observaron que los valores de G' y G" aumentaron con la concentración de psyllium, al igual que la viscosidad aparente, que disminuyo cuando se incrementó la frecuencia, presentando un comportamiento de fluido pseudoplástico. Estos resultados sobre el comportamiento del psyllium coinciden con lo encontrado por otros autores. Por ejemplo, Yin et al. (2016) estudiaron las propiedades reológicas del polisacárido obtenido de las semillas de *Plantago asiática*, tras extracción alcalina y sin calentamiento previo. Estos autores demostraron que las concentraciones más altas del polisacárido redujeron la dependencia de la viscosidad aparente frente a la frecuencia, y los valores de G' se incrementaron. Al calentar las mezclas, la viscosidad aparente y su dependencia frente a la frecuencia disminuyeron, mientras que se promovió una reducción de los valores de G' y G". De hecho, a partir de 60 °C los valores de G" superan los de G'. No obstante, hay que tener en cuenta que el método de extracción de estos polisacáridos puede afectar a su funcionalidad, al igual que lo hace a su estructura.

Con una extracción en frío, el mucílago de psyllium presentó un comportamiento de fluido viscoelástico ($\text{tg}\ \delta > 1$) comparado con los resultados obtenidos en condiciones de extracción en caliente o alcalinas, en las cuales el comportamiento fue de gel ($\text{tg}\ \delta < 1$) (Yu et al., 2017). El mismo estudio demostró que la G' y la G" del mucílago extraído en frío eran altamente dependientes de la frecuencia, y este efecto se redujo al aumentar la fuerza de la extracción. Los valores de G' disminuyeron con el aumento de la temperatura y se acentuó alrededor de 70-80 °C, lo que coincide con la temperatura de termogelatinización de la hidroxipropilmetilcelulosa (HPMC). Por este motivo Haque et al. (1993) propusieron su uso combinado como sustituto del gluten en productos de panadería.

Se sabe que la presencia de iones de calcio influye en gran medida en las propiedades reológicas del psyllium. Así, Guo et al. (2009) confirmaron que la presencia de calcio aumentaba drásticamente los valores de G' de los geles debido a una estructura más densa y con capas lineales más espesas. De hecho, la eliminación del calcio de los polisacáridos obtenidos de *Plantago asiatica* generó una reducción de la viscosidad intrínseca, del radio hidrodinámico y del peso molecular (Yin et al., 2015), así como de la estabilidad térmica (Yin et al., 2012b). Los valores de G' de estos geles también aumentaron en presencia de iones de sodio, pero el efecto fue menos pronunciado que el obtenido con iones de calcio (Yin et al., 2016).

Las propiedades reológicas de los geles de psyllium también pueden verse afectadas por otros ingredientes presentes en la solución. Se ha encontrado que la presencia de goma arábiga o Z-trim (biofibra de maíz) reduce la viscosidad de las soluciones con psyllium, mientras que las maltodextrinas no producen ningún efecto. Sin embargo, la presencia de goma garrofín provoca una ligera reducción de la viscosidad inicial, seguida de un aumento no significativo (Kale et al., 2016).

La reología de los geles de psyllium también está influenciada por el pH. Los valores de G' se reducen a pH ácido (2,5), lo que Farahnaky et al. (2010) atribuyeron al hecho de que el psyllium es un polisacárido aniónico. La extensión e interacción entre las cadenas moleculares son influenciadas por repulsiones electrostáticas que generan enlaces intermoleculares, lo que provoca la gelificación. Sin embargo, las repulsiones electrostáticas y la interacción entre las moléculas disminuyen con la reducción del pH. Otra posible explicación para los cambios en el comportamiento reológico del psyllium en condiciones ácidas está relacionada con la reducción del área superficial de sus partículas (Cheng et al., 2009). De hecho, la capacidad de retención de agua y de hinchamiento del psyllium se reducen después de estos tratamientos, por lo que los geles obtenidos son más débiles y menos adhesivos. No obstante, las propiedades reológicas de los geles de psyllium no presentan diferencias en los pH habituales en las formulaciones de los alimentos (entre 4,7 y 10) o estas diferencias son muy limitadas (Farahnaky et al., 2010).

Un efecto similar al obtenido con tratamientos ácidos se puede lograr con tratamientos enzimáticos. Así, Yu et al. (2003) redujeron la capacidad de absorción de agua del psyllium y la dureza de sus geles al pretratarlos con una combinación enzimática que incluía celulasas, hemicelulasas, xilanasas, arabinosas y β-glucanasas. Estos autores observaron una reducción en el área superficial y una superficie menos espesa en las partículas de psyllium tras el tratamiento enzimático. Entre estas enzimas, las que actúan específicamente sobre los xilanos son las más eficaces, pero se podría obtener un mejor resultado con una combinación de ellas (Yu y Perret, 2003a, 2003b). Esta hidrólisis enzimática modifica las propiedades funcionales del psyllium, pero no reduce sus efectos hipolipidémicos (Allen et al., 2004).

Modificaciones químicas del psyllium, como la sulfatación, la hidroxipropilación y la succinilación, también redujeron al mínimo la dureza y la adhesividad de los geles y la capacidad de hinchamiento del psyllium *("swelling capacity")*, pero aumentaron su capacidad de unirse a los ácidos biliares. Esto es importante porque la unión de los ácidos biliares a los polímeros puede mejorar su eliminación, lo que promueve la conversión del colesterol existente en el hígado en ácidos biliares, y así, puede reducir los niveles de colesterol total y del LDL en el plasma y, por último, reducir el riesgo de enfermedades cardiovasculares (Niu et al., 2013).

## 5. APLICACIÓN DEL PSYLLIUM EN PRODUCTOS DE PANADERÍA

- *Panes con gluten:*

La calidad del pan con gluten está muy relacionada a la presencia de la red de gluten, que confiere características viscoelásticas importantes a la masa. Los hidrocoloides no juegan un papel tan importante en los panes con gluten como en los panes sin gluten, pero pueden ayudar a mejorar su calidad final.

En los panes con gluten, la incorporación de hidrocoloides permite un aumento de la hidratación de la masa y puede promover una mayor estabilidad durante la fermentación y una mejor retención de la humedad durante la cocción, así como mejorar el volumen específico de los panes finales (Rosell et al., 2001). Se ha comprobado que el uso de psyllium con otros hidrocoloides mejora la absorción de agua de la masa y el tiempo de amasado, lo que produce masas más fuertes (Czuchajowska et al., 1992). Farbo et al. 2020 evaluaron la incorporación del psyllium en masas elaboradas con una variedad antigua de trigo y compararon los resultados entre diferentes hidrocoloides. El estudio demostró que el psyllium aumenta la extensibilidad y la capacidad de retención de gases de la masa, cuyo efecto es similar al de la goma xantana. De la misma manera, el psyllium puede reducir la pérdida de humedad durante el almacenamiento, previniendo el envejecimiento y retrasando el endurecimiento de la miga (Davidou et al., 1996; Guarda et al., 2004). Este efecto antienvejecimiento también se observó en los panes precocidos (Bárcenas y Rosell, 2007) en los cuales para obtener estos resultados solo es necesaria una pequeña cantidad de hidrocoloides, menos del 0,5%, dependiendo del tipo de hidrocoloide empleado.

Aunque los efectos del psyllium en el volumen del pan no son uniformes y dependen de la formulación o del tipo de harina utilizada, diferentes estudios admiten que el uso de psyllium produce panes con mayor humedad, menos secos y con una textura más blanda (Czuchajowska et al., 1992; Park et al., 1997). Este efecto también ha sido comprobado en panes cocidos al vapor *("steamed breads")* (Sim et al., 2015). Por el contrario, Jensen et al. (2015) observaron que el psyllium redujo significativamente el volumen de los panes con harina de tapioca y aumentó su dureza. Es importante destacar que en este estudio no se modificó la hidratación de las masas en función de análisis previos con un farinógrafo o mixógrafo. Esto muestra la enorme importancia de hacer esta corrección, debido a la influencia que el psyllium tiene sobre la absorción de agua.

En general, los estudios sobre el uso de psyllium se ven limitados por su uso en pequeños porcentajes, no superiores al 1%. Es conveniente utilizar una mayor cantidad de psyllium para lograr sus beneficios nutricionales en los productos finales. Existen pocos estudios que hayan analizado la incorporación de altas cantidades de psyllium, aunque Man et al. (2017) comprobó que al aumentar el porcentaje de incorporación de psyllium (15%) se eleva la humedad de los panes con gluten en más de un 25%, pero se reduce el volumen específico en un 37%. Es importante mencionar que el estudio no especificó la formulación, ni las características de la harina de trigo utilizada. Por su parte, Pejcz et al. (2018) observaron que la incorporación de un 8% de psyllium llevó a un aumento en el volumen del pan sin diferencias significativas en la aceptabilidad entre estos panes y el control. Estos autores utilizaron masas con una consistencia de 300 FU Brabender, que es menor que la de otros estudios (500 FU), lo que indica la necesidad de realizar nuevas investigaciones para comprobar estas diferencias.

Otra oportunidad de investigación está relacionada al porcentaje correcto de psyllium que es necesario para lograr una reducción efectiva del índice glucémico de los panes. Ray et al. (2018) reemplazó harina de trigo del pan parotta (pan típico del Sur de India), por una combinación de psyllium (7,5% del peso total de la harina), harina de garbanzos y semilla de fenogreco *("fenugreek")* en polvo. Ellos demostraron que con el 25% de sustitución se obtiene un incremento del almidón de lenta digestión y del almidón resistente y, en consecuencia, reducción significativa del almidón de rápida digestión.

- *Panes sin gluten:*

En las formulaciones de panes sin gluten es común la incorporación de gomas e hidrocoloides como sustitutos del gluten, con el objetivo de obtener productos con volumen y textura similares a los de los panes de trigo (Anton y Artfield, 2008; Sciarini et al., 2010). Entre estos sustitutos el HPMC es el más utilizado, tanto en los panes comerciales como en la investigación científica, seguido de la goma xantana (Masure et al., 2016, Mir et al., 2016; Román et al., 2019). El HPMC tiene la ventaja de generar panes con mayor volumen que otros hidrocoloides (Sabanis y Tzia, 2011), pero estos panes tienen una textura más seca y más desmenuzable (Liu et al., 2018). Por esta razón, es común combinar el HPMC con otros hidrocoloides que poseen mayor capacidad de retención de agua, como las gomas guar o xantana (Horstmann et al., 2018).

El mayor empleo del psyllium en la industria alimentaria se da en los productos de panadería, pero especialmente en los panes sin gluten. Un estudio de 228 panes comerciales sin gluten, producidos en diferentes países, mostró que el 16% de esos productos utilizaban psyllium como principal sustituto del gluten, y en el 34% el psyllium estaba presente en las formulaciones, siendo el cuarto hidrocoloide más utilizado, sólo por detrás del HPMC, la goma xantana y la goma guar (Román et al., 2019).

El psyllium es una alternativa natural a otros hidrocoloides, y tiene beneficios nutricionales con propiedades reológicas similares a las de la goma xantana, como lo demuestran Haque et al. (1993). Estos autores propusieron el uso tanto del HPMC como del psyllium para la elaboración de panes sin gluten, porque la G' de las masas con psyllium disminuyen a 70-80 °C, lo que coincide con la termogelación del HPMC, por lo que ambos efectos podrían ser compensados durante la cocción. Sin embargo, el uso de psyllium en los panes sin gluten se ha estudiado en menor medida que otros hidrocoloides, y se limita a su combinación con el HPMC (Haque y Morris, 1994; Mancebo et al., 2015) o con otras gomas y fibras (Aprodu y Banu, 2015; Cappa et al., 2013; Collar et al., 2015; Tubili et al., 2016). Cuando se mezclan dos hidrocoloides, ellos no se comportan de la misma manera que lo harían en solitario, por lo que es importante estudiarlos individualmente para evaluar como sus interacciones pueden afectar en la elaboración de los productos (Gao et al., 2017).

Solo dos estudios han analizado el uso del psyllium como único sustituto del gluten. Así, Zandonadi et al. (2009) elaboraron panes sin gluten con psyllium y obtuvieron productos con buenas características organolépticas, pero otras características tecnológicas, como el volumen o la textura no fueron evaluadas. Fratelli et al. (2018) también utilizaron psyllium en panes sin gluten y demostraron su efecto en la reducción de la respuesta glucémica de estos productos. En general, la cantidad de psyllium utilizada en estos estudios no superó el 2%. Así, Fratelli et al. (2018) establecieron un óptimo cercano al 2% para la calidad organoléptica de los panes e incorporaron casi un 17% de psyllium para reducir la respuesta glucémica.

La adicción de psyllium a las masas de panes sin gluten también afecta a la reología como observaron Collar et al. (2015). Estos autores atribuyeron el aumento de los valores de G' y G" a la gran capacidad espesante y de absorción de agua del psyllium. Este efecto es muy importante, ya que se ha demostrado que cuanto menor es la consistencia de estas masas mayor es el volumen específico de los panes obtenidos, hasta cierto punto en el que las masas son excesivamente débiles y no pueden soportar su estructura durante la fermentación o la cocción (Mancebo et al., 2017). Algunos estudios también han considerado la hidratación de las masas con psyllium (Fratelli et al., 2018; Mancebo et al., 2015). Estos estudios demostraron que es necesario aumentar la hidratación de la masa de pan sin gluten, cuando se incorpora psyllium en la formulación, para lograr mejores resultados de volumen y una reducción de la dureza en los panes finales. Mariotti et al. (2009) propusieron un cambio en la hidratación de la masa a través del análisis con el farinográfo, al evaluar las masas de pan sin gluten con y sin psyllium. Estos autores demostraron que cuando se modificó la hidratación, en base al análisis farinográfico, los valores de G' se igualaron, pero los de G" seguían siendo más elevados en el caso de las masas con psyllium. En cualquier caso, los resultados sobre el efecto del psyllium en el volumen específico y la textura de los panes dependen de si se modifica la hidratación para cada formulación, y de cómo se realiza esta modificación.

Además del empleo del psyllium en polvo, que es la forma más habitual y disponible de este ingrediente, algunos estudios evaluaron el empleo de las semillas de psyllium en la elaboración de panes. Así, la incorporación de la semilla entera (natural o molida) de *Plantago psyllium* en la elaboración de panes sin gluten también ha sido estudiada. Los panes obtenidos con semilla de psyllium presentaron un volumen específico menor (menos de 2 $cm^3/g$) en todos los casos, pero el uso de estas semillas mejoró la textura del pan (menor dureza, mayor elasticidad y cohesividad) y al aumentar la hidratación de la masa se logró un mayor rendimiento (Ziemichód et al., 2018). En este caso, se utilizó un 5% de semillas, como proporción del total de la harina utilizada, siendo este valor mayor que el de otros estudios que utilizaron cáscara o goma de psyllium. Por su parte, Pejcz et al. (2018) demostraron que el uso de semillas de psyllium molidas, en lugar de la cáscara, redujo el volumen y la humedad final de los panes, al contrario de lo observado en estudios con fibra o cáscara de psyllium, ya que este efecto se relaciona con la alta capacidad de absorción de agua de la goma de psyllium que se encuentra en la cáscara. La mayor ventaja de las semillas de psyllium es su mayor nivel de polifenoles y capacidad antioxidante, por lo que al utilizar las semillas es posible mejorar estos factores en el producto final (Li et al., 2005).

- *Bizcochos:*

Los estudios sobre el uso de psyllium en masas batidas, como las de bizcochos, son escasos y, en general, afirman que el aire incorporado y el volumen específico de los bizcochos disminuye, las migas de los bizcochos se oscurecen y la dureza aumenta cuando se emplea más del 5% de psyllium en la formulación, lo que puede estar relacionado con una menor expansión durante la cocción (Beikzadeh et al., 2016; Bhise y Kaur, 2015).

El uso de gomas con características similares a las del psyllium, como la goma xantana, en pequeños porcentajes (1%), pueden mejorar las propiedades organolépticas de los bizcochos y minimizar la pérdida de humedad y la dureza durante el almacenamiento (Gómez et al., 2007). El uso de pequeños porcentajes (1%) de hidrocoloides, como la goma xantana, también puede reducir el enranciamiento de los bizcochos (Beikzadeh et al., 2017). Estos resultados, así como los estudios sobre el empleo de algunos hidrocoloides con propiedades similares al psyllium, como es el caso de la goma xantana o de los β-glucanos, para la reducción de grasa en bizcochos, indica el potencial del psyllium para la mejora de bizcochos (Kalinga y Mishra, 2009; Lee et al., 2005).

- *Galletas:*

El empleo del psyllium también ha sido considerado en formulaciones de galletas. Algunos autores han propuesto el uso de 3%-20% de psyllium en las recetas de galletas (Fradinho et al., 2015; Krystyjan et al., 2018; Raymundo et al., 2014). En general, cuanto más alto sea el contenido de psyllium en la fórmula, mayores serán los valores de G', G" y la dureza de la masa, lo cual está relacionado con el poder espesante del psyllium.

El uso de psyllium en galletas elaboradas con harina de trigo incrementa el índice de expansión *("spread factor")*, lo que puede estar relacionado con la competencia entre el psyllium y el gluten por la cantidad de agua disponible, reduciendo la formación de la red de gluten, ya que un gluten fuerte disminuye la expansión de las galletas durante la cocción (Pareyt y Delcour, 2008). También se ha observado que las galletas con psyllium son más oscuras, mientras que los resultados de textura son inconsistentes. Al incorporarse hasta 9% de psyllium a la formulación de galletas, Fradinho et al. (2015) encontraron un claro aumento de la dureza. Por su parte, Raymundo et al. (2014) confirmaron que hasta un 10% de incorporación de psyllium llevaría a una disminución de la dureza y obtuvieron valores similares al control con porcentajes más altos. Krystyjan et al. (2018) también encontraron una reducción en la dureza de las galletas con psyllium. Los diferentes resultados entre estos estudios pueden deberse a las diferentes formulaciones o formas de medir la dureza, que pueden influir en los resultados, porque, aunque todos estos estudios realizaron pruebas de penetración, las sondas utilizadas tenían diferentes diámetros. También hay que tener en cuenta que las pruebas de penetración no son las más indicadas para medir dureza en galletas, que suele medirse con un ensayo de rotura.

Por otro lado, el empleo del psyllium como sustito de grasa en galletas ha sido evaluado por Zbikowska et al. (2018) que propusieron el uso de una combinación de psyllium y celulosa microcristalina. Sin embargo, los resultados obtenidos no fueron positivos, ya que las galletas presentaron una mayor dureza y menor aceptabilidad que el control, y se encontraron resultados similares cuando se sustituyó el mismo porcentaje de grasa (25%) por harina de trigo.

- *Masas de pizzas:*

El empleo del psyllium para la elaboración de masas de pizzas también ha sido propuesto para sustituir la grasa hidrogenada empleada por el aceite de colza, en una masa de pizza enriquecida con proteínas (Sen Gupta et al., 2015). Para ello se utilizó un 5% de psyllium, lo que llevó a una disminución de la tasa de expansión *("spread ratio")* y de la calidad organoléptica de las pizzas. Los autores sugieren mejorar la formulación con aditivos (SSL y gluten) y enzimas (amilasas y proteasas).

## 6. APLICACIÓN DEL PSYLLIUM EN OTROS PRODUCTOS

- *Mayonesas:*

Como se ha mencionado anteriormente, los hidrocoloides se han utilizado como sustitutos de la grasa en diferentes formulaciones debido a su capacidad de retener agua (Peng y Yao, 2017). En el caso de las mayonesas, se ha propuesto el uso de diferentes hidrocoloides con propiedades similares a las del psyllium, como la goma xantana, para sustituir el aceite (Ma y Barbosa-Canovas, 1995; Su et al., 2010). De la misma manera, el psyllium también se puede utilizar como sustituto del aceite en la elaboración de mayonesa. En este caso es posible obtener productos con propiedades reológicas similares al original, y sin diferencias en cuanto a la aceptabilidad, siempre que no se supere un cierto porcentaje de sustitución (hasta el 50%) (Amiri et al., 2014).

- *Pastas:*

La influencia del psyllium a la respuesta glucémica por la ingestión de pastas también ha sido evaluada. Peressini et al. (2020) comprobaron que el uso del psyllium en la formulación de pastas produce una reducción del índice glucémico. Otro estudio demostró que con la adición del 15% de psyllium se logra una reducción superior al 50%, similar a los resultados obtenidos con salvado de avena (Foschia et al., 2015b). Esta incorporación da lugar a pastas más oscuras y a un aumento del tiempo de cocción, así como de las pérdidas en la cocción, pero reduce la resistencia a la rotura, además de mejorar la absorción de agua durante la cocción (Foschia et al., 2015a). Estos cambios están relacionados con el color oscuro del psyllium, su mayor absorción de agua y el menor contenido de gluten en las pastas. Combinaciones de psyllium con inulina de baja polimerización han sido utilizadas para lograr pastas con porcentajes de fibra similares, minimizando los cambios debidos al uso del psyllium. De esta manera, se reduce la influencia del psyllium sobre la respuesta glucémica prevista. Para mantener el efecto sobre la respuesta glucémica, es necesario utilizar una inulina con un mayor número de polimerización, pero en este caso los cambios en la pasta se mantienen.

- *Postres lácteos:*

Los hidrocoloides también se utilizan en diversos productos lácteos, especialmente en aquellos con bajo contenido graso, donde pueden actuar como estabilizantes y agentes espesantes o gelificantes (Hansen, 1994).

El uso de psyllium se ha propuesto en estos productos con fines específicos. En los postres lácteos gelificados se ha utilizado para mejorar la textura de los productos sin azúcar, generando una textura final similar a la de la muestra de referencia (sensación cremosa y carácter gelatinoso), pero con peor evaluación en cuanto al sabor y la aceptabilidad global, cuando se comparan con otras fibras (Dello-Staffolo et al., 2017). También se ha estudiado la adición de psyllium a los yogures para reducir el contenido graso (Ladjevardi et al., 2015), como en el caso de las mayonesas, ya que el psyllium aumenta la viscosidad y reduce la sinéresis de esos productos. Del mismo modo, cantidades de psyllium inferiores al 1% han sido empleadas para mejorar la calidad del Kash, un producto lácteo fermentado de origen iraní. Se sabe que un porcentaje correcto de psyllium puede promover mejores características organolépticas al aumentar la viscosidad, reducir la sinéresis y la separación de fases (Amini et al., 2018). Por el contrario, la adición de una cantidad excesiva de psyllium podría producir efectos indeseables en la calidad organoléptica de los yogures, como han demostrado Bhat et al. (2008). De esta manera, si el propósito es mejorar las características nutricionales del producto, y es necesario añadir una mayor cantidad de psyllium, es mejor utilizar psyllium hidrolizado, que tiene un menor efecto espesante (Yadav et al., 2016). Estos autores han demostrado que esta hidrólisis no disminuye el potencial hipocolesterolémico de los yogures enriquecidos con psyllium y aumenta su efecto prebiótico.

- *Mermeladas:*

Se ha estudiado la adición de psyllium junto con pectina para mejorar las propiedades nutricionales de las mermeladas, lo que reduce enormemente la sinéresis, pero genera una textura excesivamente gomosa (Figueroa y Genovese, 2018). Resultados similares fueron obtenidos con la incorporación de psyllium en la formulación de mermeladas de manzana (Figueroa y Genovese, 2020). Por lo tanto, se recomienda combinar el psyllium con otras fibras celulósicas para mejorar el contenido de fibra de estos productos.

- *Otros:*

El efecto prebiótico del psyllium hace posible desarrollar nuevos productos con beneficios nutricionales. También se ha propuesto el uso del psyllium, en combinación con alginatos para "coencapsular" bacterias probióticas, generando productos con mayor rendimiento de encapsulación y mayor estabilidad que otros prebióticos (Peredo et al., 2016). Además, el psyllium mejora la estabilidad de los materiales encapsulados cuando se reduce el pH, lo que puede ser de interés para su aplicación en productos ácidos como los yogures (Nami et al., 2017). Por último, se ha propuesto la incorporación de psyllium en suplementos nutricionales con textura gomosa por su efecto prebiótico (Lele et al., 2018).

También es posible utilizar el psyllium para obtener películas biodegradables (Askari et al., 2018; Krystyjan et al., 2017; Sukhija et al., 2016) con mayor resistencia a la tracción, más termoestables y menos permeables y solubles al vapor de agua.

El uso de gomas y almidones es habitual para la obtención de líquidos texturizados, que son utilizados a menudo en el tratamiento de la disfagia. La disfagia es el término médico que define la dificultad para tragar líquidos. Estos líquidos texturizados mejoran el control del bolo y ayudan a prevenir la aspiración (Cichero, 2013). Entre las gomas utilizadas para estos productos destaca la goma xantana (Kim et al., 2017), que como se ha comentado tiene características similares a las del psyllium. De hecho, el psyllium se ha utilizado en mezclas comerciales, y se ha demostrado que la combinación de psyllium con la goma gellan genera productos con propiedades sensoriales mejores a los que solo usan goma gellan (Ishihara et al., 2011a). En estos casos, el psyllium aumenta la homogeneidad estructural del bolo y la miscibilidad con la saliva (Ishihara et al., 2011b). Este producto podría mezclarse con distintas cantidades de agua para lograr distintas consistencias y sensaciones en la boca (Yokoyama et al., 2014).

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# OBJETIVOS

# Objetivos

Esta tesis doctoral tiene como objetivo principal estudiar las propiedades funcionales básicas del psyllium y evaluar su empleo en la elaboración de productos a base de cereales (galletas, bizcochos y panes), y como afecta a sus características reológicas, físicas y aceptabilidad.

Así, para lograr el objetivo general de la tesis, se han marcado unos objetivos específicos:

I. Estudiar el efecto del uso del psyllium en las propiedades funcionales del almidón de maíz.

II. Analizar el efecto sobre la hidratación óptima y las características finales de panes sin gluten elaborados con diferentes fuentes de almidón (harina de arroz y almidón de maíz), comparando el uso de psyllium con otros hidrocoloides (HPMC y goma xantana).

III. Estudiar el uso del psyllium como sustituto del aceite en la formulación de bizcochos para la elaboración de productos más saludables.

IV. Analizar cómo influye la sustitución de grasa por pastas de psyllium en la calidad de galletas de trigo y libres de gluten.

# ESTRUCTURA

# Estructura

La presente tesis doctoral está estructurada en base a las diversas publicaciones científicas obtenidas a partir de las investigaciones desarrolladas a lo largo de la tesis. Los diferentes capítulos se distinguen en base al tipo de producto en el que se contempla la incorporación de psyllium.

**Capítulo 1: Propiedades funcionales del psyllium**

1. Belorio, M., Marcondes, G., & Gómez, M. (2020). Influence of psyllium versus xanthan gum in starch properties. *Food Hydrocolloids, 105*, 105843. https://doi.org/10.1016/j.foodhyd.2020.105843

**Capítulo 2: Influencia del psyllium en la elaboración de panes sin gluten.**

2. Belorio, M., & Gómez, M. (2020). Effect of hydration on gluten-free breads made with hydroxypropyl methylcellulose in comparison with psyllium and xanthan gum. *Enviado al *Journal of Food Engineering* en 06 de julio de 2020.

**Capítulo 3: Empleo del psyllium como sustituto de grasa en bizcochos.**

3. Belorio, M., Sahagún, M., & Gómez, M. (2019). Psyllium as a fat replacer in layer cakes: batter characteristics and cake quality. *Food and Bioprocess Technology, 12*, 2085-2092. https://doi.org/10.1007/s11947-019-02362-3.

**Capítulo 4: Estudio de la calidad y la reducción de grasa en galletas de maíz.**

4. Belorio, M., Sahagún, M., & Gómez, M. (2019). Influence of flour particle size distribution on the quality of maize gluten-free cookies. *Foods, 8*, 83. https://doi.org/ 10.3390/foods8020083.

5. Belorio, M., Moralejo, C., & Gómez, M. (2020). Assessing psyllium as a fat replacer in wheat and gluten-free cookies. *Enviado a *Food Science and Technology International* en 15 de junio de 2020.

# CAPÍTULO 1:

# Propiedades funcionales del psyllium

# INFLUENCE OF PSYLLIUM VERSUS XANTHAN GUM IN STARCH PROPERTIES

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# Influence of psyllium versus xanthan gum in starch properties

## Abstract

Psyllium is a natural polysaccharide with interesting nutritional properties and high potential to be used as an ingredient in formulations of cereal-based products. The objective of this study was to analyse how this hydrocolloid influences the hydration, pasting, rheology and gel properties of starch. The incorporation of psyllium increased the hydration properties of starch, and it was more notable when these properties were analysed under cold conditions. The viscosity and G′ values increased after gelatinisation, without changes in the G″ of the gels, but the hardness decreased after cooling. In general, psyllium promoted similar changes to the properties of starch as those obtained with xanthan gum. Thus, psyllium can be an alternative to xanthan gum in multiple applications.

## 1. Introduction

Psyllium is a soluble fibre found in the husks of *Plantago ovata* seeds. This fibre is mainly composed of arabinoxylans, similar to those found in cereals (Izydorczyk & Biliaderis, 1995). The functional properties of psyllium have been compared with those of xanthan gum (Haque et al., 1993). After heating, this fibre can form a weak gel (G′ > G″) with fibrillar structure (Guo et al., 2009; Farahnaky et al., 2010).

Psyllium has proven health benefits for diabetes, constipation, colon cancer, diarrhoea, inflammatory bowel disease (ulcerative colitis), irritable bowel syndrome, colon cancer, diabetes and hypercholesterolaemia (Singh, 2007; Wärnberg et al., 2009). Some of these properties have been related to the viscous character of psyllium, which has a water retention capacity 10-fold greater than that of cellulose obtained from wheat or bamboo, and 5-fold greater than that of apple fibre (Dello Staffolo, Sato, & Cunha, 2017). It is well established that viscous fibres, like psyllium, gums, pectins and β-glucans, can alter the viscosity of digesta in the gastrointestinal tract, inhibiting the absorption of nutrients, such as glucose and cholesterol (Dikeman & Fahey, 2006).

Psyllium was proposed for use in gluten-free bread formulations because of its functional properties or nutritious advantages, either as a single gluten substitute (Zandonadi, Assunçao-Botelho, & Coelho-Araujo, 2009) or combined with other hydrocolloids (Aprodu & Banu, 2015; Mancebo, San Miguel, Martinez, & Gomez, 2015). Nowadays, psyllium is the worlds' fourth most used hydrocolloid after hydroxypropyl methylcellulose (HPMC), xanthan gum and guar gum, in formulations of this type of bread (Román, Belorio, & Gómez, 2019). It has also been proposed for use in wheat bread elaborations to produce softer bread with more moisture and less staling (Czuchajowska, Paszczynska, & Pomeranz, 1992; Pejcz et al., 2018) or to slow the digestion of these types of bread (Ray et al., 2018). When elaborating bakery products using new ingredients, they must interact with starch. Accordingly, numerous studies have analysed starch–hydrocolloid interactions (BeMiller, 2011; Mahmood et al., 2017). Nevertheless, the interactions between psyllium and starch are less documented. In particular, the gel properties of xanthan gum have been analysed in other studies about formulations of gluten-free breads (Mir, Shah, Naik, & Zargar, 2016; Román et al., 2019). Psyllium was also evaluated with respect of its rheology in elaborations of different products, such as: bread (Haque & Morris, 1994; Mancebo et al., 2015), cookies (Fradinho, Nunes, & Raymundo, 2015; Raymundo, Fradinho, & Nunes, 2014), and nutritional improvement of cakes (Beikzadeh et al. 2016; Bhise and Kaur 2015). However, the gelling properties of psyllium have not been addressed in any studies.

This study compared the gel properties from composites made with maize starch and psyllium or xanthan gum. Maize starch is the most common raw material in gluten-free bread formulations (Román et al., 2019) and among the most popular starch ingredients used in the food industry. Combinations of maize starch and different percentages of psyllium (2, 5 and 10%) as starch substitute were evaluated for hydration properties and pasting properties (Rapid Visco Analyser, RVA), and the gels were tested for rheology, texture and colour. These properties were compared with those obtained from combinations of maize starch and xanthan gum, one of the most used hydrocolloids, which also presents similar properties to psyllium.

## 2. Materials and methods

### 2.1. Materials

Psyllium (Vitacel P95) was supplied by Rettenmaier Ibérica (Barcelona, Spain), and xanthan gum was supplied by Roko (Galicia, Spain). The maize starch (Tereos, Syral Iberia SAU, Zaragoza, Spain) was bought locally.

### 2.2. Methods

All the measurements (hydration properties, pasting properties and gel properties) were performed on samples containing solids (starch and hydrocolloids), with different percentages of starch weigh substitution (0%, 2%, 5%, 10% and 100%) using hydrocolloids (psyllium or xanthan gum). The rheology tests were carried out using substitutions of 0%, 2%, 5% and 10%, with respect to the starch weight. All the tests were carried out in duplicate.

### *2.2.1. Hydration properties*

Water-binding capacity (WBC), which is the amount of water retained by the sample after centrifugation, was evaluated according to the American Association of Cereal Chemists (AACC) method 56-30.01(AACC, 2012). Water-holding capacity (WHC) and swelling volume (SV) were obtained using a total of 5 g of solids to which 100 mL of distilled water was added. The samples were kept at room temperature (25 °C) for 24 h. After the amount of water in excess was carefully removed, the amount of water retained by the sample, without any stress, was obtained from the ratio of the difference between the hydrated sample and the dried sample relative to the weight of the hydrated sample. The SV was calculated by dividing the total volume of the swollen sample by the original dry weight of the sample.

The water absorbance index (WAI) evaluates the amount of water retained by the sample after heating and centrifugation. A total of 2.5 g of solids was dispersed in 30 mL of water and centrifuged (600 rpm) at 90 °C for 15 min, followed by centrifugation at 3000 rpm for 10 min, without heating. The water in excess was carefully removed, and the hydrated sample was weighed to calculate the WAI as the ratio between the hydrated sample and the dried sample.

### *2.2.2. Pasting properties*

The pasting properties of maize starch and maize starch with different percentages of weight substitution (0%, 2%, 5%, 10% and 100%) using psyllium and xanthan gum, respectively, were evaluated using an RVA (RVA-4C, Newport Scientific Pty.

Ltd., Warriewood, Australia). A suspension was prepared by dispersing 3.5 g of solids in 25 g of distilled water. The sample was maintained at 50 °C for 1 min, then heated until 95 °C and held at this temperature for 2.5 min. Afterwards, it was cooled to 50 °C and held at this temperature for 2 min under a rotation of 160 rpm, then cooled to 30 °C and held at 35 °C for 2.5 min to assist the gelation process of the hydrocolloids. The curve for each analysis was obtained.

### *2.2.3. Rheological properties*

Pastes obtained from the RVA were analysed in a rheometer (Haake RheoStress 1, Thermo Fischer Scientific, Scheverte, Germany) installed with a titanium, parallel, serrated plate geometry sensor PP60 Ti (60 mm diameter) and a 3 mm gap. Once the rheometer was stabilised at 30 °C, the sample was placed on the plate and covered with Vaseline oil to avoid drying. Before initiating the test, the sample rested for 500 s. A dynamic oscillatory test was performed through first executing deformation sweeps (0.1–100 Pa) at a constant frequency (1 Hz) to determine the maximum deformation achieved by the sample in the linear viscoelastic range. Subsequently, a frequency sweep test, was commenced using a strain value within the linear viscoelastic region over a frequency range of 10–0.1 Hz. The parameters obtained were storage modulus ($G'$ [Pa]), loss modulus ($G''$ [Pa]) and loss tangent ($\tan \delta = G''/G'$) as a function of frequency.

### *2.2.4. Gel texture*

Gels were prepared using the pastes obtained from the RVA, which were placed into cylindric plastic recipients (100 mm in diameter by 20 mm height) and cooled in a fridge at 4 °C for 24 h. After, the gels rested at room temperature (25 °C) for 30 min before carrying out the texture analysis using a TA.XT2i texture analyser (Stable

Micro Systems Ltd., Surrey, UK) equipped with Texture Expert version 1 software for Windows. A 5 kg load cell was applied to force calibration and a 50 mm-diameter cylindrical probe was used for compression cycle which was conducted at a constant velocity of 10 mm $s^{-1}$ to a sample depth of 10 mm, followed by a return to the original position. A curve force versus time was obtained and used to calculate the values of the peak force obtained in the compression cycle (hardness).

### *2.2.5. Gel colour*

The gel colour was measured in the CIE L*a*b* colour space using a Minolta CN-508i chromameter (Minolta Co., Ltd., Osaka, Japan) under a standard D65 lamp and 2° standard observer.

### *2.2.6. Statistical analysis*

A one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) test ($p < 0.05$) was performed to differentiate between the medians. Statistical analyses were completed using Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton, VA, USA).

## 3. Results and discussion

### 3.1. Hydration properties

As observed in Table 1, as single ingredients, both psyllium and xanthan gum presented hydration properties (WHC, WBC and SV) under cold conditions that were very similar to each other but markedly higher than those of maize starch.

*Table 1. Hydration properties.*

| Sample | WHC (g water/g solid) | SV (mL/g) | WBC (g water/g solid) | WAI (g water/g solid) |
|---|---|---|---|---|
| Control | 0.90 ± 0.16 a | 1.60 ± 0.00 a | 0.75 ± 0.01 a | 5.13 ± 0.04 b |
| PSY 2% | 1.49 ± 0.15 b | 2.80 ± 0.01 b | 1.51 ± 0.01 b | 6.23 ± 0.04 c |
| PSY 5% | 1.79 ± 0.02 bc | 3.19 ± 0.00 c | 2.73 ± 0.01 c | 5.21 ± 0.07 b |
| PSY 10% | 1.63 ± 0.10 b | 3.20 ± 0.00 c | 3.96 ± 0.07 e | 7.08 ± 0.04 d |
| PSY 100% | 3.42 ± 0.06 d | 5.20 ± 0.01 e | 4.82 ± 0.01 f | 11.99 ± 0.02 e |
| XAN 2% | 1.81 ± 0.01 bc | 2.80 ± 0.01 b | 1.41 ± 0.16 b | 2.59 ± 0.88 a |
| XAN 5% | 2.08 ± 0.16 c | 3.20 ± 0.01 c | 3.07 ± 0.32 d | 2.73 ± 0.06 a |
| XAN 10% | 2.12 ± 0.16 c | 3.20 ± 0.01 c | 5.00 ± 0.00 f | 11.98 ± 0.01 e |
| XAN 100% | 3.64 ± 0.38 d | 4.00 ± 0.00 d | 4.98 ± 0.01 f | 11.99 ± 0.01 e |

*Data are expressed as means ± SD of duplicate assays. Values with the same letter in the same column do not present significant differences ($p < 0.05$). PSY: psyllium. XAN: xanthan gum. WHC: water-holding capacity. SV: swelling volume. WBC: water-binding capacity. WAI: water absorbance index.*

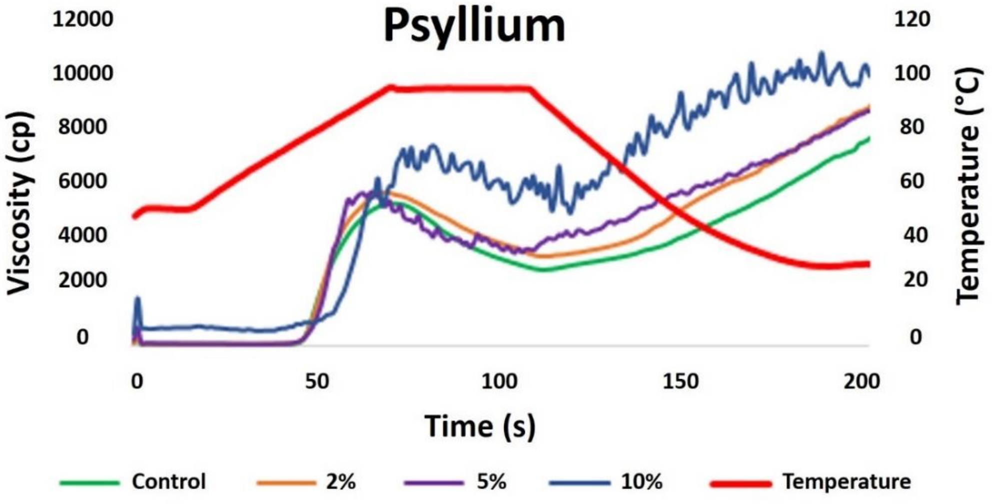
Similarly, Dello Staffolo et al. (2017) and Sandhu, Simsek, and Manthey (2015) observed a greater water absorption capacity of psyllium and xanthan gum, respectively, relative to starch. These comparatively high hydration and swelling capacity properties of psyllium and xanthan gum increased the hydration properties of their respective mixtures with starch. The effect of both hydrocolloids on the WHC and SV was very similar, although the WBC was slightly higher for xanthan gum than psyllium when greater percentages (5% and 10%) were used. For all the parameters evaluated, the mixes of starch and psyllium or xanthan exhibited greater values than those of the single ingredients, considering each percentage. Thus, a synergistic effect was observed, and this was most apparent for the WHC and SV at the smallest percentage of starch substitution (2%), and less so at the higher percentages.

The WBC increased progressively as the percentage of substitution was increased. For xanthan gum, the values obtained when it reached 10% of starch substitution were equal to those achieved with this hydrocolloid alone. This ability of hydrocolloids to increase starch hydration in the cold has already been proved in other studies with agar or carrageenan (Martínez et al., 2015), and with basil seed gum (Matia-Merino et al., 2019). Gularte and Rosell (2011) found this effect was more predominant for starch added with xanthan gum than with other hydrocolloids, such as guar gum, HPMC, carboxyl methylcellulose or pectin. According to Christianson, Hodge, Osborne, and Detroy (1981), the addition of hydrocolloids can considerably increase the shear forces exerted on the swollen granules in the centrifuged phase compared with the forces present in starch–water suspensions. These increased forces can enhance water uptake (increasing swelling), granule breakdown and the amount of material exuded into the continuous phase.

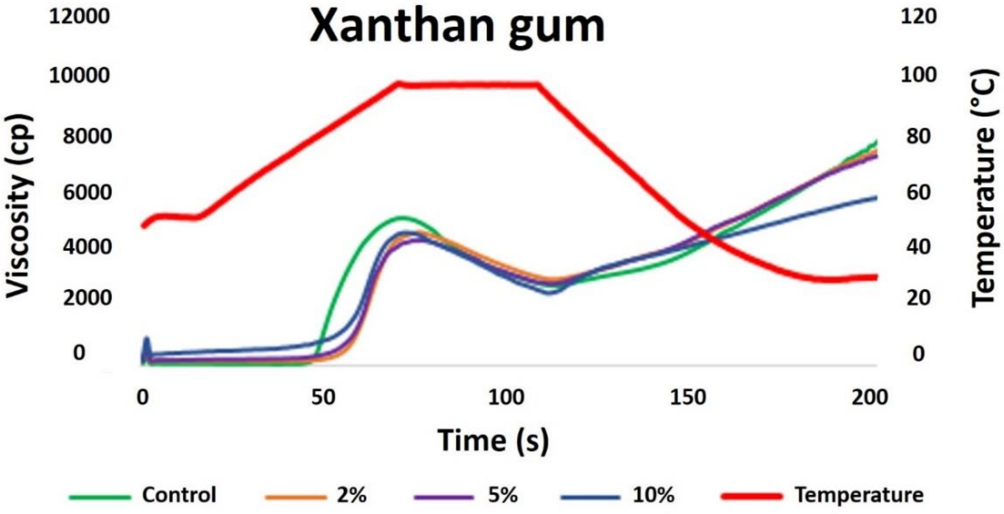
Unlike the results of the hydration properties in cold conditions, there were no great differences in this parameter between the starch and the hydrocolloids after heating, even if the WAI values of the hydrocolloids were more than double that of the starch. The reason is because of the starch gelatinisation during the process, which increases the water absorption capacity when compared with the ungelatinised starch. Accordingly, flours in which the starch is pre-gelatinised, have higher water absorption capacity relative to the non-treated flours (Martínez et al., 2015). Both hydrocolloids showed similar WAI values. However, psyllium progressively increased the WAI values of starch, except at 5% of starch substitution, while there was a marked difference in the WAI between the smallest percentages (2% and 5%) of added xanthan gum. Consequently, the addition of xanthan gum lowered the WAI values of starch, such that the 10% mixed suspension had a WAI equal to the value of xanthan gum alone. Matía-Merino et al. (2019) observed increasing values of WAI even with comparatively smaller doses of added hydrocolloid. In general, an increase in water retention is expected because of the presence of hydroxyl groups in the hydrocolloids, which bind to the water molecules. However, the competitive activity for water between starch and hydrocolloid, their interactions and the variations in the hydration conditions can generate the differences observed.

### 3.2. Pasting properties

Figure 1 shows how the pasting properties of starch are affected by the incorporation of psyllium (Fig. 1a) and xanthan gum (Fig. 1b). The smallest percentage of psyllium (2% of starch substitution) slightly increased the viscosity of the samples over the whole curve obtained from the starch gelatinisation, similarly to the behaviour with 5%. This effect coincides with the amylograph data published by Buksa and Krystyjan (2019), who incorporated arabinoxylans into a rye starch dough. It also corroborates the findings of Collar et al. (2015), who mixed psyllium with maize starch and rice flour, and those of Manceo et al. (2015) when psyllium was added to a mix of rice flour and HPMC. However, an irregular curve was generated for the mixture with 5% psyllium after starch gelatinisation, which indicates the formation of broken structures (gel-like) and a great setback.



(a)



(b)

*Figure 1: Pasting properties from starch due to different percentages of psyllium (a) and xanthan gum (b).*

This effect was higher with 10% psyllium, and this percentage also promoted a strong increase in the viscosity, and a delay in the gelatinisation temperature, which can be related to the low availability of water, such as found for other hydrocolloids (BeMiller, 2011). Although there are still far too few studies of psyllium, Mancebo et al. (2015) also observed a dramatic increase in the setback with an increasing amount of psyllium. This trend could be associated with arabinoxylans that increase starch retrogradation, as observed by Gudmundsson et al. (1991) with the derivates from wheat or rye. Likewise, it could explain the nature and activity of the complexes formed between these products and amylopectin (Michniewicz & Jankiewicz, 1988).

Xanthan gum delayed the gelatinisation temperature in all the samples evaluated, but there was no increase in the viscosity after heating, considering all the percentages studied. This increase in the gelatinisation temperature has been noticed by other authors using different starches (Chaisawang & Suphantharika, 2006; Zhang et al., 2018). The effect was attributed to the low availability of water to the starch because of the higher water absorption capacity of xanthan gum (BeMiller, 2011). However, the viscosity in cold conditions progressively increased as the amount of xanthan gum increased because of both its thickener property and its water absorption capacity. In earlier studies about the interaction between xanthan gum and other hydrocolloids, the peak viscosity results are contradictory. For instance, Chaisawang and Suphantharika (2006) showed this viscosity increased with cassava starch but decreased with anionic cassava starch. Kim and BeMiller (2012) observed differences to the viscosity analysis of pea starch when guar gum was used and not to the other hydrocolloids studied. Lee et al. (2002) found that hydrocolloids decreased the peak viscosity of sweet potato starch, and Korus et al. (2004) related an increase with triticale. Zhang et al. (2018) observed that the presence of xanthan gum reduced the extent of starch swelling and maintained the integrity of the grain, suggesting that it could stabilise the grains by acting as a lubricant and a barrier. Regarding the effects of hydrocolloids on maize starch, both an increase (Alloncle & Doublier, 1991) and a decrease in the peak viscosity (Song, Kim, & Shin, 2008; Weber et al., 2009) have been reported, which coincides with the results found in this study. However, the effect of the viscosity on the RVA curve depends on the type of starch used and the starch:hydrocolloid:water ratio, which usually differs between each test, as does the sample preparation method (Mandala & Bayas, 2004). Moreover, in this study, the concentration of the hydrocolloids is lower when compared with other studies. It must be emphasised that for great amounts of xanthan gum, differently from what happens with psyllium, a reduction in the setback is obtained. This phenomenon was previously observed by Chantaro and Pongsawatmanit (2010) with cassava starch, and by Weber et al. (2009) with maize starch.

### 3.3. Rheology

As shown in Table 2, both hydrocolloids influenced, in a slightly different way, the rheology of the gels obtained in the RVA. A significant correlation at 95% was observed between the values of WHC, WBC and $G'$, with r equals to 0.56 and 0.60 respectively. The small values of r, and the lack of correlation with the $G''$ values, indicate that the water absorption capacity cannot explain all the changes observed in the rheology of the pastes. Thus, the incorporation of psyllium did not modify the values of $G'$ but progressively increased $G''$ with increasing amounts of psyllium in the samples. On the contrary, the xanthan gum only increased $G'$ when added at the smallest percentage and, even it increased the values of $G''$, independently of the xanthan concentration. As a consequence of these changes, tan δ increased as the amount of added hydrocolloid increased, but the increase was greater with the addition of psyllium than xanthan gum. The results obtained with xanthan gum are surprising. Previous research affirmed that when xanthan gum is incorporated with distinct starches, it increases G′′ and, more so G′, and decreases the tan δ values, which are influenced by the increase in the amount of xanthan (Alloncle & Doublier, 1991; Kim & Yoo, 2006; Ptaszek et al., 2009). However, in these studies, pastes were elaborated with very small percentages of starch substitution by hydrocolloids, less than 5%. By contrast, when the concentration of solids increased, similarly as proposed in our study, the values of G′ and G′′ hardly varied (Biliaderis et al., 1997) or even reduced, in the case of G′′ (Aguirre-Cruz, Mendez-Montealvo, Solorza-Feria, & Bello-Perez, 2005). Thus, it seems that the increase in the values of the rheological parameters is related to the availability of free water. Our experiments confirmed this hypothesis because a slight increase in the rheological parameters occurred with the addition of the smallest amount of xanthan gum, but when the water absorption capacity of the samples was higher, with the largest concentration of this hydrocolloid, the rate of increase was reduced, and no differences were observed relative to the control. In the case of psyllium, despite only a few previous studies, the same behaviour could be expected. Interestingly, different from our study, Krystyjan et al. (2017) observed an increase in G′ and G′′ when psyllium was added to starch to obtain gels, but, as in the studies with xanthan gum, the concentration of solids used in the samples was much lower than in our study.

*Table 2. Rheological properties of maize starch gels containing different percentages of psyllium and xanthan gum obtained through frequency sweeps (10-0.1 Hz).*

| Sample | G' (Pa) | G'' (Pa) | Tan δ |
|---|---|---|---|
| Control | 1460 ± 0.07 a | 73 ± 0.45 a | 0.05 ± 0.00 ab |
| PSY 2% | 1635 ± 0.01 a | 75 ± 1.44 a | 0.04 ± 0.00 a |
| PSY 5% | 1575 ± 0.13 a | 101 ± 0.35 ab | 0.06 ± 0.00 bc |
| PSY 10% | 1800 ± 0.18 a | 177 ± 0.06 c | 0.10 ± 0.00 d |
| XAN 2% | 2550 ± 0.65 b | 131 ± 0.29 b | 0.05 ± 0.00 ab |
| XAN 5% | 1970 ± 0.14 ab | 98 ± 0.24 ab | 0.05 ± 0.00 ab |
| XAN 10% | 1640 ± 0.01 a | 121 ± 0.28 b | 0.07 ± 0.00 c |

*Data are expressed as means ± SD of duplicate assays. Values with the same letter in the same column do not present significant differences ($p < 0.05$). PSY: psyllium. XAN: xanthan gum. G′: storage modulus. G′′: loss modulus. Tan δ: loss factor.*

### 3.4. Gel properties

In general, both hydrocolloids decreased the hardness of the starch gel (Table 3), but psyllium had less effect than xanthan gum, yet there was no significant difference between the two hydrocolloids when analysing the highest percentages. With the smallest percentage of psyllium, it was even possible to increase the gel hardness, while it was decreased by xanthan gum. It is noteworthy that it was not possible to make a gel using only the hydrocolloids (without starch), so the hardness of these samples could not be measured because the gels collapsed easily.

*Table 3. Hardness and colour parameters of gels made with different percentages of maize starch substitutions by hydrocolloids (psyllium and xanthan gum).*

| **Sample** | **Hardness (N)** | ***L**** | ***a**** | ***b**** |
|---|---|---|---|---|
| Control | 7.05 ± 1.22 d | 60.68 ± 0.51 cde | -2.63 ± 0.15 a | -9.28 ± 0.54 ab |
| PSY 2% | 9.75 ± 1.27 e | 62.16 ± 0.56 e | -2.58 ± 0.09 a | -8.07 ± 0.99 bc |
| PSY 5% | 6.17 ± 0.13 cd | 60.67 ± 0.13 cde | -2.60 ± 0.30 a | -6.61 ± 0.37cd |
| PSY 10% | 3.83 ± 0.98 ab | 59.43 ± 0.25 c | -1.83 ± 0.23 b | -5.56 ± 0.59 d |
| PSY 100% | ND | 34.46 ± 0.71 a | 4.14 ± 0.45 d | 3.45 ± 0.65 f |
| XAN 2% | 5.63 ± 0.85 bcd | 59.69 ± 1.27 cde | -2.73 ± 0.53 a | -9.71 ± 0.31 a |
| XAN 5% | 4.76 ± 0.04 bc | 59.07 ± 1.43 c | -2.67 ± 0.21 a | -9.58 ± 0.27 ab |
| XAN 10% | 2.52 ± 0.72 a | 61.5 ± 1.53 de | -2.76 ± 0.38 a | -8.19 ±1.30 abc |
| XAN 100% | ND | 55.03 ± 0.29 b | -0.96 ± 0.20 c | 1.82 ± 0.46 e |

*Data are expressed as means ± SD of duplicate assays. Values with the same letter in the same column do not present significant differences ($p < 0.05$). PSY: psyllium. XAN: xanthan gum; ND: not developed.*

The decrease in gel hardness when using xanthan gum coincides with the results of gels of xanthan–wheat starch (Seetapan et al., 2013) and xanthan–maize starch (Matía-Merino et al., 2019). Both works described the gels as having an ordered honeycomb-like microstructure with bigger cells and very smooth cell walls in comparison to gels made with native starch, and these characteristics influenced the texture. These differences were also correlated with differences in water mobility and rearrangement of starch molecules under storage conditions. Tunnarut and Pongsawatmanit (2017) found that the presence of xanthan retarded the retrogradation phenomenon, which coincides with our analysis of the pasting properties at the highest percentages of added hydrocolloid. Furthermore, this effect prevented the formation of a strong structure from amylose molecule association during cold storage.

Despite no prior study about the interaction of psyllium with starch, it is known that this fibre presents positive gelling properties that can be reduced by enzymatic degradation (Yu et al., 2003). In addition, psyllium–maize starch films showed a more compact and homogeneous structure relative to the films made only with starch (Askari et al., 2018). These structural changes could explain the greatest hardness of gels made with the smallest percentages of added hydrocolloid. Likewise, for the highest percentages, the reduction in the amount of starch, and therefore, less retrogradation phenomenon, could explain the smallest hardness.

Analysing the colour, gels made with psyllium were darker and presented higher values of a* and b* (both positive) than those with xanthan gum. In turn, gels made with xanthan also presented small values of L* and high values of a* and b* when compared with the gels made only with starch. However, the small percentages of hydrocolloid in the samples only changed the gel colour (i.e. not luminosity). Psyllium did not alter the luminosity, and the value of parameter a* decreased with 10%, psyllium, as occurred with xanthan gum. Considering parameter b*, it decreased in gels with 5% psyllium, but no significant differences were observed between the samples with xanthan gum. Gels were not formed at temperatures higher than 100 °C, so Maillard reactions or sugar caramelisation did not occur, and the colour of the gels was mainly dependent on the colour of the solids. Thus, the incorporation of psyllium could increase the colour darkness in products, such as films (Ahmadi, Kalbasi-Ashtari, Oromiehie, Yarmand, & Jahandideh, 2012) or fruit jellies (Figueroa & Genovese, 2019), which coincides with our results.

## 4. Conclusion

Psyllium, which is a natural polysaccharide with important nutritional advantages, changes the functional properties of maize starch, in the same way as xanthan gum. Both hydrocolloids similarly increase the water absorption capacity of starch and promote changes in the rheology of gels when there is no water in excess, and both decrease the gel hardness to the same extent. Nevertheless, they present slight differences in pasting temperature, and while xanthan gum retards this temperature and, at its highest percentages evaluated, decreases the setback, psyllium increases the setback and promotes an increase in the pasting temperature only if added at high percentages. These similarities may help to find new applications for psyllium and to comprehend its functionalities in the already existing uses, especially in those that use xanthan gum, such as gluten-free breads.

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# CAPÍTULO 2:

# Influencia del psyllium en la elaboración de panes sin gluten

# EFFECT OF HYDRATION ON GLUTEN-FREE BREADS MADE WITH HYDROXYPROPYL METHYLCELLULOSE IN COMPARISON WITH PSYLLIUM AND XANTHAN GUM

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# Effect of hydration on gluten-free breads made with hydroxypropyl methylcellulose in comparison with psyllium and xanthan gum

## Abstract

The use of hydrocolloids in gluten-free breads is a strategy to improve their quality and obtain products with acceptable structural and textural properties. Hydration level (HL) optimization is important to maximize the hydrocolloids effects on dough and bread quality. This study evaluated the optimum hydration level (OHL) for gluten-free breads prepared with different starch sources (rice flour or maize starch) and hydroxypropyl methylcellulose (HPMC) in comparison with psyllium and xanthan gum. Breads with the same final volume and the maximum HL were evaluated. Breads made with HPMC had high specific volume and greater dependence on the HL, especially for elaborations with maize starch. Psyllium had similar behaviour to xanthan gum with respect to specific volume and weight loss. Breads elaborated with maize starch and HPMC had low hardness due to their specific volume; however, the combined decreased hydration and similar specific volume generated a harder bread than the use of psyllium or xanthan.

## 1. Introduction

Gluten plays an important role in bread formulation. The gluten network is formed by wheat proteins that with correct hydration and mechanical work, form a cohesive, extensible and elastic dough, which is able to retain the gas formed during fermentation and baking (Delcour et al., 2012). To elaborate gluten-free breads, it is necessary to resort to starches and gluten-free flours, but it is also important to replace gluten with another ingredient. However, a functionally equivalent ingredient has not yet been found that allows for the full replacement of gluten. The most often used ingredients for this purpose are hydrocolloids (Mir et al., 2016; Sabanis and Tzia, 2011; Sciarini et al., 2010; Anton and Artfield, 2008).

HPMC and xanthan gum are the hydrocolloids most often used as gluten substitutes in gluten-free breads, while rice flour and maize starch are the starchy ingredients most often employed in these formulations, both in scientific articles and in commercial products (Masure et al., 2016; Román et al., 2018). In commercial products, the use of psyllium is also prominent. In fact, Román et. al (2018) indicated that 16% of all evaluated breads included psyllium as the major gluten replacer and 34% incorporated it as a secondary replacer, mixed with another main hydrocolloid. The use of psyllium has some advantages because, besides being a natural product, it is responsible for health benefits such as the regulation of glucose in diabetic disease and decreased symptoms of constipation, diarrhoea, irritable bowel syndrome and others (Singh, 2007). Psyllium and xanthan gum present similar rheological behaviours, as both are responsible for weak gelling properties (Haque et al., 1993). Nevertheless, studies about the elaboration of gluten-free breads with psyllium are scarce, and this fibre has always been studied in mixtures with other hydrocolloids such as HPMC and xanthan gum (Cappa et al., 2013; Haque and Morris, 1994; Mancebo et al., 2015a) but never as a unique gluten replacer.

Dough hydration in gluten-free bread is a fundamental aspect of final product quality. In general, it is known that the greater the hydration, the higher the specific volume of a bread, until a maximum point at which the weak structure of the dough promotes collapse during the fermentation or baking process (Mancebo et al., 2017; McCarthyet al., 2005). However, these studies were based on doughs elaborated with HPMC, and there is little or no information about the effect of hydration on doughs made with other hydrocolloids. Generally, previous scientific studies applied the same hydration levels, despite possible changes in the formulation, or they modified hydration based on pre-proofs which were not detailed. Some authors have attempted to correct hydration by performing rheological analysis (rheometer or farinographic) (Martínez et al., 2014; Nunes et al., 2009; Ziobro et al., 2016; Ziobro et al., 2013). Nevertheless, Sahagún and Gómez (2018) proved that distinct gluten-free formulations achieve a maximum specific volume with differences in both hydration levels and rheological properties. Furthermore, hydration influences bread volume differently, depending on the formulation used.

Gluten-free breads prepared with the most used gluten-substitutes (HPMC or xanthan gum) were compared with breads made with psyllium in doughs with rice flour or maize starch. Therefore, the objective of this study was to evaluate the different effect of using psyllium as a gluten replacer in gluten-free breadmaking. The influence of these hydrocolloids was evaluated on dough hydration and on the specific volume of the final breads. For each case, breads with the highest specific volume were analysed in terms of crust colour and texture (hardness, springiness, cohesiveness, chewiness and resilience).

## 2. Materials and methods

### 2.1 Materials

Gluten-free breads were made with rice flour with a protein content of 7.54 g/100 g (Molendum Ingredients SL, Zamora, Spain) or maize starch (Tereos, Syral Iberia SAU, Zaragoza, Spain). Other ingredients used were refined sunflower oil (Urzante, Navarra, Spain), white sugar (AB Azucarera Iberica, Valladolid, Spain), instant dry baker's yeast (Dosu Maya Mayacilik A.S, Istanbul, Turkey), salt (Disal, Unión Salinera de España S.A, Madrid, Spain) and tap water. Hydroxypropyl methylcellulose (HPMC) (Vivapur K4M, J. Rettenmaier & Söhne, Rosenberg, Germany), xanthan gum (Industrias Roko S.A., Llanera, Asturias, Spain) and psyllium (Rettenmaier Ibérica, Barcelona, Spain) were studied as gluten replacers.

## 2.2 Methods

### *2.2.1 Gluten-free breadmaking*

A gluten-free bread recipe was composed as follows (per 100 g flour or starch): 100 g of maize starch (MS) or rice flour (RF), 6 g of sunflower oil, 5 g of sugar, 3 g of yeast powder, 1.8 g of salt and 2 g of hydrocolloid (HPMC, xanthan gum or psyllium). The amount of water was defined according to topic 2.2.2.

All the ingredients were mixed by using a Kitchen Aid Professional mixer (Kitchen Aid, St. Joseph, Michigan, USA) with a dough hook (K45DH) at 58 rpm for 1 minute, except for the dry yeast and tap water. During this minute, the water was placed in a plastic recipient, the dry yeast was gentle laid on top of the water and it was carefully mixed with the use of a glass rod to guarantee the hydration of the whole yeast. Subsequently, the hydrated yeast was mixed (90 rpm for 8 minutes) into the dough at a blend. Portions of bread dough (150 g) were placed into aluminium pans (127 x 98 x 33 mm) previously coated with sunflower oil. The dough was fermented in a proofing chamber at 30 °C and 80% relative humidity for 60 minutes. The fermented doughs were baked at 190 °C for 40 minutes. The aluminium pans were removed, and the bread was allowed to cool for 60 minutes and placed in plastic bags, which were closed properly and stored at 20 °C for 24 h until subsequent analysis. All studied formulations were produced in duplicate.

### *2.2.2. Defining the optimum hydration level of breads*

The influence of hydration level was evaluated for each bread formulation considering the use of RF, MS and the different hydrocolloids (HPMC, xanthan gum and psyllium), similarly to a study by Sahagún and Gómez (2018). Breads were made with formulations containing 70, 80, 90, 100, 110 and 120 g/100 g. Their specific volumes were obtained, and those with the maximum specific volume were considered to have the optimum hydration level (OHL). The volume of all breads was measured by using a Volscan Profiler 300 (Stable Microsystems, Surrey, UK), and the specific volume was calculated as the relation between the final volume and weight of breads, 24 hours after baking. Four loaves of bread were evaluated for each formulation.

To evaluate the physical characteristics of the breads, the OHL was chosen. The amount of dough to be used in the aluminium pans was recalculated for each formulation with the aim of obtaining the same final bread volume (680 mL). However, this volume exceeded the mould capacity, and it was observed that while some doughs could grow beyond the moulds, others overgrew it and exceeded the upper edge of the pan, causing the dough drop outside the mould without increasing the bread volume. In these cases, dough hydration was reduced to the percentage at which the bread could rise without the dough dropping outside the mould.

### *2.2.3 Physical characteristics of breads*

The weight lost during baking was calculated as the difference between the bread and dough weights divided by the dough weight.

The texture of two central slices (20 mm thick) from two breads of each formulation was evaluated. A TPA (texture profile analysis) was performed by using a TA-XT2 texture analyser with a cylindrical probe 25 mm in diameter. The probe penetrated 50% of the depth of each slice, with a trigger force of 5g and a test speed of 1 mm/s. A delay of 10 seconds between the first and second compressions were applied. Hardness, springiness, cohesiveness and resilience were measured.

Crumb colour was measured by using a Minolta CM-508i spectrophotometer (Minolta Co., Ltd., Japan) with D65 as the standard illuminant and a 2º standard observer. The results were expressed in the CIE L*a*b* colour space. Measurements were made on two central slices of two breads from each formulation (2 x 2 x 2).

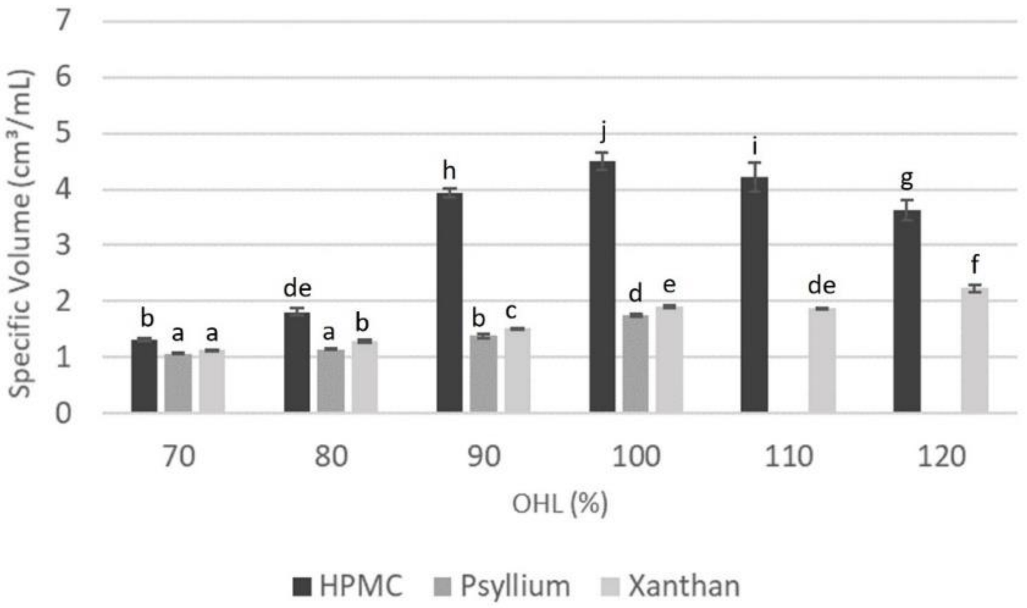
### *2.2.4 Statistical analysis*

Analysis of variance (ANOVA) was performed with Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, USA) to evaluate all the results obtained. The 95% confidence intervals were described by Fisher's least significant differences (LSD) test.

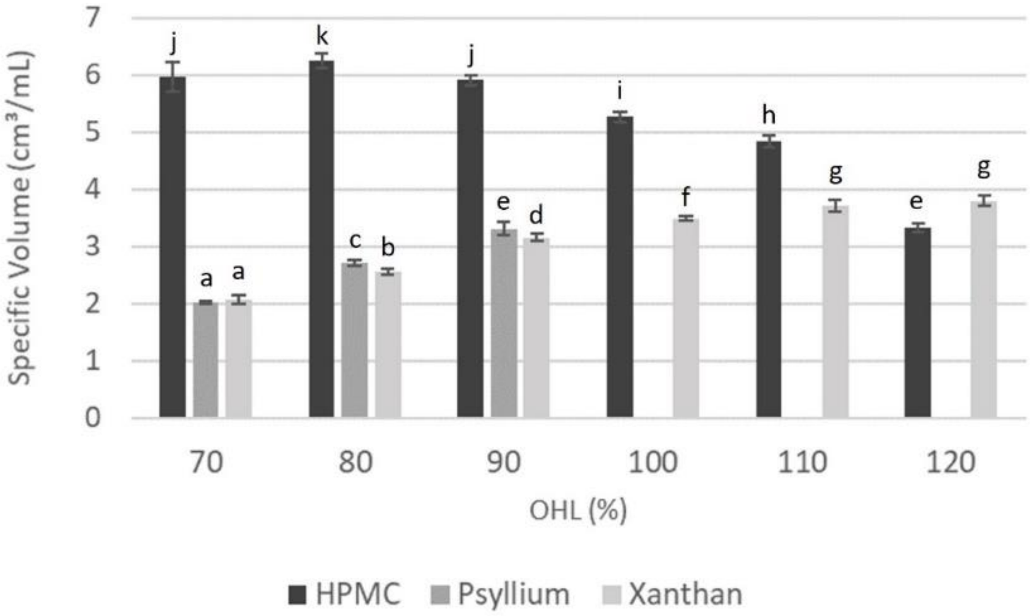
## 3. Results and Discussion

### 3.1 Optimum hydration level

Specific volumes for each hydration level of gluten-free breads made with different hydrocolloids are shown in Figure 1. Among breads made with RF (Figure 1a), the specific volume of all breads increased with high hydration levels up to 100 g water/100 g flour. This increase was much larger in breads with HPMC than in those with psyllium or xanthan gum. In fact, breads with HPMC had a specific volume more than double of those obtained with the other hydrocolloids at 100% hydration, whereas at 70% hydration, they had similar specific volumes. The highest specific volume observed in breads elaborated with HPMC coincides with the results of Sabanis and Tzia (2011). Nevertheless, that study showed smaller differences than those found in this research, because breads elaborated with HPMC were less hydrated than those formulated with xanthan, which reduced their volume. This is related to the water retention capacity of xanthan gum, which generates more viscous doughs. However, as indicated in this study, breads made with HPMC were similarly hydrated to those made with xanthan, providing breads with high specific volume. The behaviour of HPMC is related to its capacity to form a thermo-reversible gel during baking, which increases the viscosity and establishes gas cell walls, providing high volume by preventing moisture loss (Crockett et al., 2011). From the 100% hydration level, the specific volume of breads with HPMC decreased, while that of bread made with xanthan was reduced at 110% and slightly increased at 120%. However, at this hydration level the volume of the dough was reduced during fermentation, and a small increase was observed during baking. As the final volume of breads made with xanthan gum were not improved at 120% hydration, the OHL was defined as 100%, considering that at this level, the highest specific volume was obtained without the dough dropping during fermentation or baking. Increasing specific volume with increasing hydration up to a certain limit was also observed by Mancebo et al. (2017) in breads elaborated with RF and by Sahagún and Gómez (2018) in breads with MS. Both studies analysed breads with HPMC. Mancebo et al. (2017) reported the specific volume of breads with optimum values of G′ and G″. Sahagún and Gómez (2018) found that these rheological values depended on the bread formulation employed. Ziobro et al. (2016) evaluated breads made with starches, guar gum and pectin, and they showed that a viscosity limit value exists at which bread volume decreases during baking. In fact, Mir et al. (2016) affirmed that the internal viscosity of doughs should not be too low to avoid the release of bubbles during baking. Encina-Zelada et al. (2019) also observed that the specific volume of breads made with xanthan gum or guar gum increased with increasing hydration. In this case, a relation with dough rheology was also mentioned, and it was shown that for high levels of xanthan, it was necessary to add more water.



(a)



(b)

*Figure 1: Variation of specific volume at different hydration levels for each gluten-free bread formulation and hydrocolloid: a) Rice flour (RF) b) Maize starch (MS).*

In the case of psyllium, doughs with greater than 100% hydration, although they grew during fermentation, dropped over the edges of the moulds during baking. It is possible that the viscosity was reduced during the early stages of baking (before gelatinization) because of the increase in temperature, which promoted excessively liquid doughs with a weak structure that dropped over the edges of the mould. Thus, over 100% hydration, it was not possible to obtain properly baked rice bread containing 2% of psyllium (Figure 1). The specific volume of breads made with psyllium was similar to those made with xanthan gum. This could be related to the rheological properties of psyllium, which are very similar to those of xanthan gum (Haque et al., 1993).

Among breads made with MS, those with HPMC had the highest specific volume at the optimum hydration of 80% (Figure 1), and their volume gradually decreased with increasing hydration. This optimum is similar to the results obtained by Sahagún and Gómez (2018) using a very similar formulation. Breads with xanthan gum presented an OHL at 110% hydration, because at 120% the volume of the dough decreased during fermentation and increased again during baking, but it was not larger than that obtained with 110%, and there was no significant difference between the two hydration levels. MS breads with psyllium increased in specific volume up to 90% hydration; however, at this level, breads were completely hollow, which indicated that the dough structure was too weak, and during baking the interior matrix sunk, while at the external surface, a thin crust was formed because of drying that occurred at the beginning of baking. Thus, it was not possible to measure these breads because of their weak structures. MS breads behaved similarly to RF breads, because formulations with HPMC at the OHL had nearly double the specific volume of those breads elaborated with psyllium or xanthan gum; the differences between the latter two were small.

It is important to highlight that breads made with MS had a higher specific volume than those made with RF, considering all hydration levels and the different hydrocolloids used. All hydrocolloids (HPMC, psyllium and xanthan gum) increased the final specific volume of breads by almost 50%, considering the maximum specific volume obtained for each of them. The highest specific volumes were previously found with the use of HPMC in comparisons of breads elaborated with MS to those with RF (Martínez and Gómez, 2017; Mancebo et al., 2015a). Martínez and Gómez (2017) attributed these differences to the higher consistency of doughs made with RF, but as observed in Figure 1, all the doughs elaborated with MS had large volumes for all hydration levels and hydrocolloids. These authors also suggested another possible explanation, which is based on the presence of a protein layer that covers the starch grains of the flour, modifying the pasting behaviour and increasing the pasting temperature. With respect to the OHL, breads made with HPMC clearly had lower OHL in the presence of MS than with RF, but in the case of xanthan gum this value was higher with MS. In the case of psyllium, it was not possible to compare values of OHL, since they were not determined by considering the maximum specific volume but because of structural problems discovered in case of high hydration levels. However, considering the use of psyllium, the OHL was larger with MS (sinking of the internal structure) than RF (dough dropped outside of the mould during baking).

### 3.2 Gluten-free bread properties

Texture and volume were analysed by using breads with the same final volume to avoid the influence of volume on texture measurements. For this purpose, the amount of dough added to the moulds was re-calculated on the basis of specific volume. In this way, breads made with MS and HPMC were used as references, because they presented similar final volumes to commercial wheat-based breads. Thus, it was necessary to increase the amount of dough in the moulds for other formulations (with psyllium or xanthan). Nevertheless, in some optimally hydrated elaborations, the dough exceeded the height of the mould and dropped over the edges due to the weakness of the dough. These hydrations were disregarded and reduced for each formulation, until the level was reached at which the dough had a sufficiently strong structure to rise over the edges of the mould. Table 1 shows the optimum hydration, specific volume and weight loss for each formulation. The final breads are shown in Figure 2. Breads with MS allowed the same OHL (80%) for all the hydrocolloids, even though elaborations with psyllium or xanthan gum generated breads with high specific volumes at high hydration levels. However, breads made with RF and psyllium or xanthan were elaborated with 90% hydration, which was a similar level (100%) to that obtained for the largest specific volume for these hydrocolloids. On the other hand, breads with HPMC were elaborated with only 70% hydration to obtain a final dough with a desirable structure. This hydration was lower than the optimum, and the final specific volumes of these breads were less than one third of the maximum volume obtained, but similar to that of breads made with psyllium or xanthan.

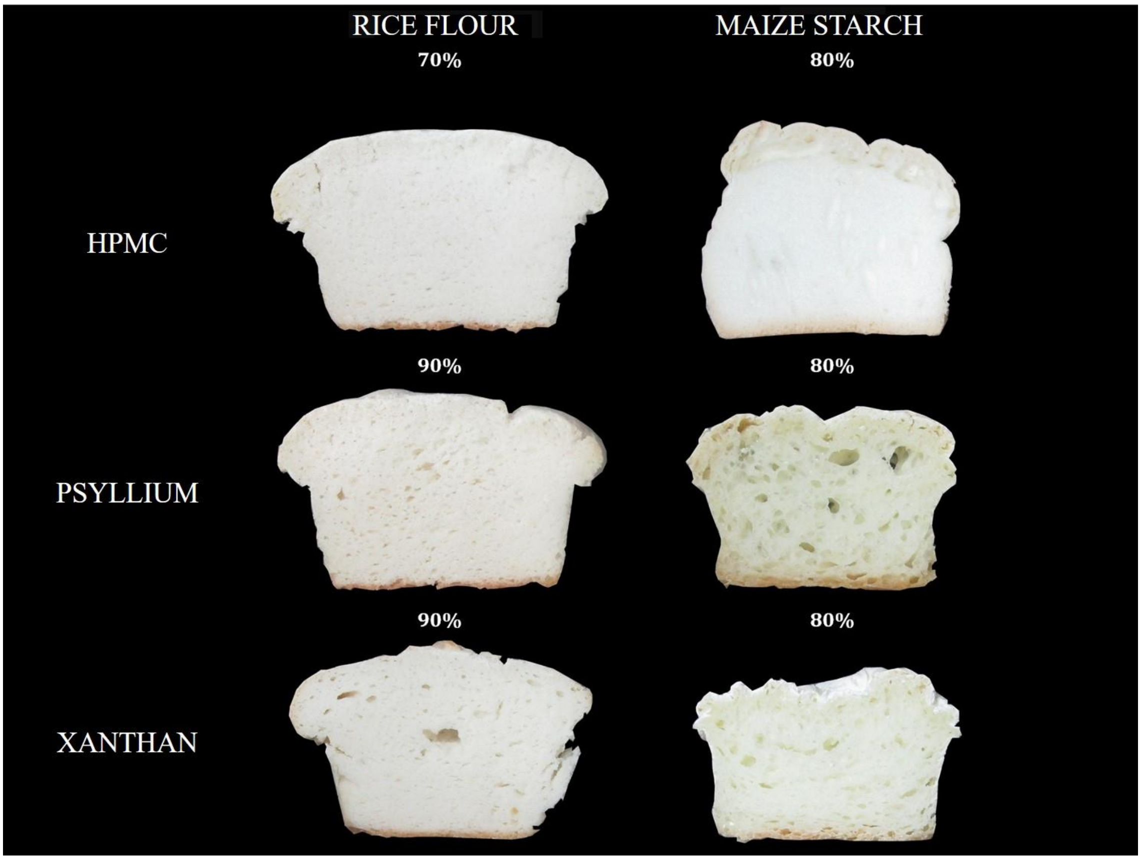


*Figure 2: Variation of specific volume at different hydration levels for each gluten-free bread formulation and hydrocolloid: a) Rice flour (RF) b) Maize starch (MS).*

*Table 1: Variation of specific volume at different hydration levels for each gluten-free bread formulation and hydrocolloid: a) Rice flour (RF) b) Maize starch (MS).*

| | **OHL (%)** | **Specific Volume ($cm^3/g$)** | **Weight Loss (g/100g)** |
|---|---|---|---|
| RF HPMC | 70 | 1.33 ± 0.01a | 0.0933 ± 0.0088a |
| RF Psyllium | 90 | 1.44 ± 0.02ab | 0.0989 ± 0.0018a |
| RF Xanthan | 90 | 1.48 ± 0.03b | 0.0976 ± 0.0018a |
| MS HPMC | 80 | 7.58 ± 0.04d | 0.2820 ± 0.0012c |
| MS Psyllium | 80 | 2.37 ± 0.08c | 0.1660 ± 0.0086b |
| MS Xanthan | 80 | 2.25 ± 0.08c | 0.1750 ± 0.0150b |

*Data are expressed as means ± SD of duplicate assays. Values with the same letter in the same column do not present significant differences ($p < 0.05$). OHL: optimum hydration level. RF: rice flour. MS: maize starch.*

Table 2 shows the values of texture parameters for the different formulations. Breads elaborated with psyllium or xanthan gum and MS presented greater hardness than those formulated with HPMC. This could be related to the high specific volume of these breads. In fact, this relation between specific volume and hardness was indicated in other studies (Gallagher et al., 2003; Mancebo et al., 2017; Martínez and Gómez, 2017). Xanthan gum and psyllium breads showed similar water losses during baking. However, breads with HPMC lost more water. Other studies found that breads with a high specific volume, such as those elaborated with HPMC, tended to lose more water during baking (Mancebo et al., 2017), in agreement with the results found in this study. Nevertheless, these papers considered breads of different final volumes, as they used the same amount of dough in each mould, and water loss is related to the exchange surface. In this study, it was not possible to justify changes in hydration with differences in external surface area, because all breads had the same final volume. Thus, the greater weight loss observed in breads made with HPMC may be related to the low retention capacity of this hydrocolloid compared with xanthan (Horstmann et al., 2018). With respect to the other parameters, there were no significant differences in springiness among the different hydrocolloids. Breads made with xanthan were less cohesive and resilient, although there were no significant differences in resilience between these breads and those made with HPMC.

*Table 2: Texture parameters of gluten-free breads made with RF or MS for each hydrocolloid.*

| | **Hardness (N)** | **Springiness** | **Cohesiveness** | **Resilience** |
|---|---|---|---|---|
| RF HPMC | 42.44 ± 0.21d | 0.796 ± 0.004a | 0.656 ± 0.023ab | 0.383 ± 0.009a |
| RF Psyllium | 14.98 ± 0.60c | 0.891 ± 0.025b | 0.748 ± 0.037c | 0.479 ± 0.041bc |
| RF Xanthan | 9.04 ± 3.00b | 0.922 ± 0.043bc | 0.807 ± 0.024c | 0.501 ± 0.013bc |
| MS HPMC | 1.44 ± 0.12a | 1.011 ± 0.023d | 0.754 ± 0.030c | 0.493 ± 0.034bc |
| MS Psyllium | 19.51 ± 3.40c | 0.974 ± 0.004cd | 0.733 ± 0.037bc | 0.550 ± 0.052c |
| MS Xanthan | 19.58 ± 1.55c | 0.964 ± 0.002cd | 0.606 ± 0.037a | 0.420 ± 0.047ab |

*Data are expressed as means ± SD of duplicate assays. Values with the same letter in the same column are not significantly different ($p < 0.05$). RF: rice flour. MS: maize starch.*

Breads made with the different hydrocolloids and RF did not differ significantly among them with respect to weight loss or specific volume. Breads made with HPMC were expected to lose the least amount of weight, because of its high capacity to retain water. However, this effect could be compensated by the high HPMC+flour/water ratio, since these breads had low hydration. We emphasize that breads made with RF lost less weight than those made with MS, which may be related to the lower water retention of starches compared with flours (Matia-Merino et al., 2019), which is probably due to the high protein content of the latter. In contrast to the results obtained for breads with MS, the use of HPMC in RF breads gave greater hardness than other hydrocolloids, while the use xanthan gum produced the softest breads.

Various studies found an increase in the hardness of gluten-free breads with the use of xanthan gum when compared with other hydrocolloids (Lazaridou et al., 2007; Schober et al., 2007). The high hardness of breads made with HPMC is a novel finding and is due to the correct hydration and the consequent specific volume of the final breads; in this respect, there were no differences between hydrocolloids. In previous studies, breads with HPMC presented high specific volumes and low hardness (Sabanis and Tzia, 2011). However, rather than being soft, those breads were drier and had a crumblier texture (Liu et al., 2018). Thus, the greater hardness of breads with HPMC found in this study may be attributed to the gels reverting to a weakly entangled form upon cooling, which reduced crumb firmness after baking (Crockett et al., 2011; Grover, 1982). With respect to the differences between breads elaborated with MS or RF, those made with HPMC presented the highest specific volumes and the lowest hardness. Nevertheless, despite the higher specific volume of breads elaborated with MS, breads made with psyllium showed a similar hardness, and those made with xanthan gum were even harder. These differences can be explained by the distinct effect of xanthan gum on the pasting properties of the starch, which includes retrogradation between native starches or flours (Matia-Merino et al., 2019). Thus, it seems that maize starch generates harder breads than rice flour. This coincides with the findings of Mancebo et al. (2015b), who reported that the texture of breads made with starch was inferior to that of breads made with rice flour. Regarding other texture parameters, formulations with RF and HPMC presented low values for springiness, cohesiveness and resilience, while those made with psyllium and xanthan had similar values between them. These differences confirmed the distinct behaviour of gels made with HPMC after baking.

In comparisons of crust colour (Table 3), breads made with xanthan gum were the darkest (small values of L*), and no significant differences were observed between breads made with HPMC and psyllium. Neither were significant differences observed between breads elaborated with RF and MS, despite of the higher protein content of RF, which could influence Maillard reactions. Values of a* and b* had small significant differences, and no clear tendency was observed. However, breads made with xanthan gum had the largest values of a* in elaborations containing RF and the smallest values of b* among those made with MS. Breads containing HPMC presented the highest values of a* and b* among elaborations with MS. The crust colour of breads is related to the Maillard reaction, which occurs between amino acids and reducing sugars, as well as sugar caramelization (Purlis and Salvadori, 2009). Originally, differences in sugar content and amino acids should not exist between breads elaborated with different hydrocolloids. However, water activity can vary depending on the hydrocolloid and hydration of the doughs; this in turn can affect Maillard reactions favouring the mobility of reactants (Gonzales et al., 2010). In fact, Sabanis and Tzia (2011) also found significant differences among the crust colours of breads made with distinct hydrocolloids.

*Table 3: Crust colour parameters of gluten-free breads.*

| | **L*** | **a*** | **b*** |
|---|---|---|---|
| RF HPMC | 81.68 ± 3.05c | 1.64 ± 0.25bc | 17.15 ± 0.68bc |
| RF Psyllium | 79.92 ± 4.67bc | 1.23 ± 0.39b | 15.36 ± 0.18b |
| RF Xanthan | 75.05 ± 0.83ab | 4.48 ± 0.08d | 20.25 ± 1.22c |
| MS HPMC | 82.09 ± 0.04c | 2.64 ± 0.14c | 19.32 ± 0.22c |
| MS Psyllium | 86.20 ± 2.13c | -0.05 ± 1.07a | 14.56 ± 2.64b |
| MS Xanthan | 71.26 ± 1.92a | 0.06 ± 0.09a | 9.72 ± 1.93a |

*Data are expressed as means ± SD of duplicate assays. Values with the same letter in the same column are not significantly different ($p < 0.05$). RF: rice flour. MS: maize starch.*

## 4. Conclusion

In general, breads elaborated with HPMC and maize starch showed higher specific volumes than elaborations with other hydrocolloids or rice flour. Nevertheless, the degree of hydration of the dough can change these results. The hydration effect is much more evident in breads prepared with HPMC than in those made with psyllium or xanthan gum. Thus, optimization of hydration is fundamental when different gluten-free breads are evaluated. This study also showed that even though breads with HPMC normally present low hardness, due to their high specific volume, when the hydration level changes and the specific volume equalizes, the obtained breads are much harder than those elaborated with other hydrocolloids.

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## Conflicts of interest

There are no conflicts of interest to declare.

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# CAPÍTULO 3:

# Empleo del psyllium como sustituto de grasa en bizcochos.

# PSYLLIUM AS A FAT REPLACER IN LAYER CAKES: BATTER CHARACTERISTICS AND CAKE QUALITY.

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# Psyllium as a fat replacer in layer cakes: batter characteristics and cake quality.

## Abstract

Consumers are demanding healthier and lower calorific products. In this study, oil was substituted in layer cakes using a combination of psyllium and water. Psyllium was used as oil replacer because of its gelling and emulsifying properties, as well as its beneficial health properties. Substitutions of 25, 50, 75 and 100% were carried out to evaluate batter (density and bubbles distribution) and cake characteristics (specific volume, weight loss, texture and colour). An acceptability test was also made. A higher substitution of oil increased bubbles size, but no significant difference was observed in batter density. Increasing the oil replacement decreased the specific volume of cakes, however there was no significant difference in hardness compared to control cake, even after seven days of storage. There was no significant difference in cohesiveness and springiness when 25% of the oil was replaced, but it did increase with higher substitution levels. Crust colour became lighter with increases in oil replacement, showing smaller values to a* and higher to b*. Cakes replaced with 25% of psyllium:water showed no significant differences in acceptability when compared to control cakes, but those with up to 75% of oil replacement were highly acceptable (7 out of 9 points).

## 1. Introduction

Obesity and heart disease affect many people, and in most cases, they are related to the consumption of large amounts of fat. People have adopted some changes to their eating habits because of both health and lifestyle reasons. In this way, a reduction of fat in food would provide new possibilities to those consumers who look for a decrease in fat.

Among bakery products, layer cakes have the highest fat content (Matz 1992). Fats or oils facilitate the incorporation of air in batter contributing to an increase in volume during baking and improving the final tenderness of the cake (Psimouli and Oreopoulou 2013). Distinct hydrocolloids and fibres have been used as fat replacers in cookies (Forker et al. 2011; Rodríguez-García et al. 2012a). Several oil and fat replacement ingredients has been proposed in muffins and cakes, such as green banana puree (Oliveira et al. 2018), avocado puree (Othman et al. 2018) or berry pomace (Quiles et al. 2018) and different fibres such as chia mucilage (Felisberto et al. 2015), functional ingredients derived from flaxseeds (Eslava-Zomeño et al. 2016), succinyl chitosan (Rios et al. 2018), cocoa fibres (Karp et al. 2017), resistant maltodextrin or potato fibre (Diez-Sánchez et al. 2018), inulin (Zahn et al. 2010; Rodríguez-García et al. 2012b, 2014; Majzoobi et al. 2018) and guar and xanthan gums (Zambrano et al. 2004) . All these studies found that batter with lower fat content showed changes in rheology and greater bubbles size, resulting in harder cakes and, in most cases, smaller volumes. Moreover, no one of these studies showed a fat reduction of more than 30% without changing the sensory properties and acceptability of the cakes. Psyllium is a natural fibre obtained from a tropical plant known as *Plantago* genus. This fibre has similar properties to xanthan gum (Haque 1993) exhibiting good gelling and emulsifying properties when applied as fat replacers (Yu 2008). Psyllium has also been used extensively both as a pharmacological supplement and in processed food to aid weight control, to regulate glucose control for diabetic patients and to reduce serum lipid levels in hyperlipidaemic (Singh 2007).

The objective of this work is to replace oil in layer cake formulations (oil substitutions of 25, 50, 75 and 100%) by hydrated psyllium (one tenth psyllium and nine tenths water). The density and microscopy of the cake batters were evaluated. Cake characteristics, such as specific volume, weight loss, texture (fresh cakes and seven-day old cakes) and colour (crust and crumb) were also measured. In addition, a sensory analysis was carried out to evaluate cake acceptability.

## 2. Materials and Methods

### 2.1 Materials

Wheat flour (10.9g/100g moisture; 8.98g/100g protein) and psyllium used in the layer cakes preparation was supplied by Harina Castellana S.A (Valladolid, Spain) and Rettenmaier Ibérica (Barcelona, Spain), respectively. The other ingredients used were liquid whole milk (Lactalis Food Service Iberia, Madrid, Spain), liquid pasteurised eggs (Alvarez Camacho Sevilla, Spain), refined sunflower oil (Urzante, Navarra, Spain), baking powder (Puratos, Gerona, Spain), and white sugar (AB Azucarera Iberia, Valladolid, Spain.

### 2.2 Methods

#### *2.2.1 Cake Elaboration*

Layer cakes were prepared according to the following formulation: 350g wheat flour, 315g white sugar, 210g milk, 175g liquid pasteurised eggs, 105g sunflower oil and 10.5g baking powder. All ingredients were mixed for 10 minutes (speed 4 for 1 minute and speed 6 for 9 minutes) using a professional mixer (Kitchen Aid, St. Joseph, Michigan, USA. The reduced fat cakes were made by replacing the sunflower oil by a mixture of psyllium and water (ratio of 1:9) in different proportions (25, 50, 75 and 100%). The proportion of psyllium:water was determined in preview tests to maintain batter viscosity similar to the final viscosity of the control batter. All the formulations are shown in Table 1.

The cake batter (185g) was placed into an oil-coated aluminium pan (127 x 98 x 33mm) and baked at 190 °C for 25 minutes. After baking, the cakes were left to cool at room temperature for 60 minutes, then placed in plastic bags to avoid drying out and put in a chamber at 20 °C until analysis. All the cakes were made in duplicate.

### *2.2.2 Batter Characteristics*

Batter density was evaluated after the mixing process using an Elcometer 1800 pycnometer (Manchester, UK). Batter microstructure was evaluated at 20 times magnification using a DM750 microscope (Leica Microsystems, Wetzlar, Germany) and the images were captured using LAS-EZ software (Leica Microsystems, Wetzlar, Germany). Before being submitted for analysis, a sample of the batter was placed on a glass slide covered with a lip. To obtain a uniform thickness, a weight of 1kg was supported on the cover. All the analyses were performed in duplicate.

*Table 1: Composition of cakes formulations (g/100g wheat flour) evaluated.*

| **Ingredients** | **Control** | **WF25** | **WF50** | **WF75** | **WF100** |
|---|---|---|---|---|---|
| WF | 100 | 100 | 100 | 100 | 100 |
| White sugar | 90 | 90 | 90 | 90 | 90 |
| Milk | 60 | 60 | 60 | 60 | 60 |
| Liquid pasteurized egg | 50 | 50 | 50 | 50 | 50 |
| Sunflower oil | 30 | 22.5 | 15 | 7.5 | - |
| Baking powder | 3 | 3 | 3 | 3 | 3 |
| Psyllium | - | 0.75 | 1.50 | 2.25 | 3 |
| Water | - | 6.75 | 13.50 | 20.25 | 27 |

*WF: wheat flour. WF25: layer cake with 25% of oil replacement. WF 50: layer cake with 50% of oil replacement. WF75: layer cake with 75% of oil replacement. WF100: layer cake with 100% of oil replacement.*

To maintain similar final viscosities, the viscosity of the batters was evaluated using a Rapid Visco Analyser (RVA-4) (Newport Scientific model 4-SA, Warriewood, Australia) with 25g of cake batter (Sahagún et al. 2018). The final viscosity obtained for all the samples was equal to the control (3257 cp) with a margin of ± 2%. Analysis was carried out at 30 °C after stirring for 3 minutes at 160 rpm.

### *2.2.3 Cake Specific Volume and Weight Loss*

Specific volume was obtained using the ratio between the volume of the cakes and their weight. Cake volume was determined by a Volscan Profiler volume analyser

(Stable Microsystems, Surrey, UK). Weight loss was calculated by considering the difference between the weight of the batter placed in the pan and the weight of the cakes after baking. The analyses were carried out in duplicate on each batch at 24 hours after baking, being cakes stored in plastic bags in a chamber at 20 °C until the measurement.

### *2.2.4. Texture Analysis*

Crumb texture was evaluated using a 25mm diameter cylindrical aluminium probe to execute a Texture Profile Analysis (TPA) with a double compression test at 50% of depth penetration, a test speed of 2 mm/s and a delay of 30 seconds between the first and second compression. The tests were carried out using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK). Two central slices (each 2cm thick) of two cakes from each batch (2x2x2) were evaluated. Hardness, cohesiveness and springiness values were obtained from the TPA graphic (Gómez et al. 2007). Texture parameters were evaluated on cakes stored after 24 hours and seven days in plastic bags in a chamber at 20 °C.

### *2.2.5 Crust and Crumb Colour*

Crust colour was measured at four different points of the cake surface in four cakes of each batch (4x4x2). Crumb colour was evaluated at the central point of four different slices obtained from two cakes of each batch (4x2x2). The analyses were carried out using a Minolta CN-508i spectrophotometer (Minolta Co., Ltd., Osaka, Japan) and a D65 illuminant with the 2° standard observer. Values were expressed in the CIE L* a* b* colour space and the parameter ΔE*, which refers to the total colour difference between the control and other cake samples with different levels of oil substitutions. It was calculated according to the equation:

$$\Delta E^{*} = \sqrt{(L - L_0)^2 + (a - \ a_0)^2 + (b - b_0)^2}$$

The values of L*, a* and b* are designed by L, a and b and the subscript “0” refers to the control.

### *2.2.7 Consumer Testing*

A sample of 88 volunteer cake consumers aged from 16 to 34 years old took part in a hedonic sensory evaluation. This evaluation was carried out in individual booths at the Sensory Science Laboratory of the Agricultural Engineering College at the University of Valladolid, Palencia (Spain). Cakes were evaluated using a nine-point hedonic scale, ranging from extreme dislike (score 1) to extreme like (score 9). Appearance, texture, taste, odour and the overall acceptability of the five cakes were evaluated. Cake portions of 2cm were presented on white plastic dishes coded with four-digit random numbers and served in random order. For the appearance evaluation, one entire cake was presented on the principal table. Water was available to drink it between samples. Cakes were elaborated 24 hours before and stored in plastic bags at 20ºC.

### *2.2.7 Statistical Analysis*

All the results obtained were analysed for variance (ANOVA) using Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, USA). Intervals with 95% confidence were described based on Fisher’s least significant differences (LSD).

# 3. Results and Discussion

## 3.1. Batter Characteristics

No significant differences were observed between the density of all batter formulations, as shown in Table 2. This fact indicates that the whole air volume incorporated in different batters was similar. In other studies, when fat was substituted by extruded flour (Román et al. 2015) or by hydrocolloids (Zambrano et al. 2004), a small increase in batter density with higher substitutions was found. However, when Zambrano et al. (2004) used a commercial fat substitute, no differences in batter density were observed, as seen in this study. This indicates that differences in the air incorporated into batters depends on the fat replacer used. In the case of Román et al. (2015), greater density of batter density was corrected by adding an emulsifier to the formulation. Therefore, the good emulsifying properties of psyllium (Yu et al. 2008) could explain the absence of increasing batter density in cakes formulated with psyllium. Nevertheless, as Román et al. (2015) found, psyllium does not have the same effect as a conventional emulsifier because it produced batters with the smallest density when an emulsifier was added to formulations with less oil content. Oils are responsible for stabilising bubbles in cake batter generating bubbles of a smaller size and regular distribution (Psimouli and Oreopoulou 2013). Replacing oil with psyllium in cake formulations allowed the formation of bigger bubbles with irregular distribution and decreased number, as shown in Figure 1. Similar results were found in other researches when replacing oil with inulin or extruded flour in cake formulations (Rodríguez-Garcia et al. 2012b, 2014; Román et al. 2015; Diez-Sánchez et al. 2018).

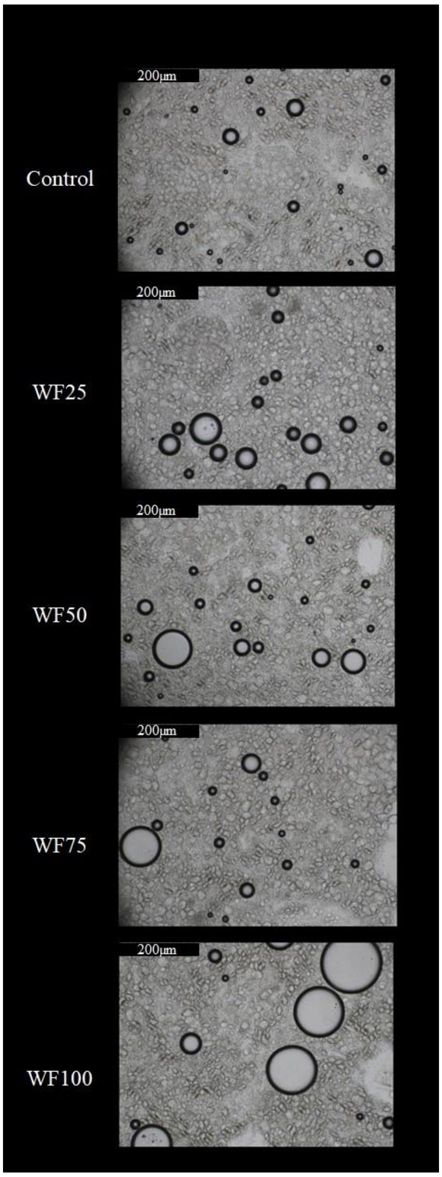

*Figure 1: Bubble size distribution of cake batter with fat replacement by psyllium and water.*

### 3.2. Cake Characteristics

An increase in oil replacement reduced the specific volume values (see Table 2), showing a reduction of 14% when the entire oil amount was substituted. The differences in specific volume were only significant in 50% and upwards of oil substitution. This tendency was also observed by other authors when substituting oil or fat in cakes or muffins (Zahn et al. 2010; Román et al. 2015). The decrease in specific volume could be related to an increase in bubble size in batters with low oil content as a bigger size of bubbles provides less stability and releases the bubbles into the atmosphere (Rodríguez-García et al. 2014). According to Zahn et al. (2010), specific volume does not depend only on the air incorporated into the batter, but also on the air retained after baking, which could explain why the specific volumes decreased. As previously mentioned, other authors minimised the changes in the specific volume of cakes with extruded flour or chia mucilage gel as fat replacers, by using an emulsifier in the formulation (Zambrano et al. 2004; Román et al. 2015).

*Table 2. Characteristics of batters and layer cakes with reduced fat content.*

| **Sample** | **Batter density (g/ml)** | **Specific volume (ml/g)** | **Weight loss (g/100g)** |
|---|---|---|---|
| CONTROL | 1,02 ± 0,05a | 2,74 ± 0,08d | 0,15 ± 0,01a |
| WF25 | 1,07 ± 0,00a | 2,62 ± 0,06cd | 0,16 ± 0,00a |
| WF50 | 1,08 ± 0,00a | 2,56 ± 0,10bc | 0,18 ± 0,02a |
| WF75 | 1,08 ± 0,00a | 2,45 ± 0,03ab | 0,17 ± 0,00a |
| WF100 | 1,07 ± 0,01a | 2,35 ± 0,03a | 0,18 ± 0,00a |

*The values with the same letter in the same column do not present significant differences (p<0.05). WF25: layer cake with 25% of oil replacement. WF 50: layer cake with 50% of oil replacement. WF75: layer cake with 75% of oil replacement. WF100: layer cake with 100% of oil replacement.*

No significant differences in weight loss were observed between the control samples and the formulations with oil replaced by psyllium. Cake weight loss depends mostly on the water maintained in the cake after baking. It is important to note that oil was replaced with a mix of psyllium and water, so these cakes had more water in the formulation than the control cakes, so a greater weight loss is expected. However, in general, cakes with a higher volume produce a greater weight loss (De La Hera et al. 2014).

Therefore, in the case of cakes where oil was substituted by psyllium and a lower specific volume was obtained, although a minor weight loss was expected due to the higher water amount, this loss seems to be compensated by the great water binding capacity of psyllium.

Rodríguez-García et al. (2012b) also found no significant differences in weight loss between cakes made with inulin as a fat replacement and the control cakes. In this case, cakes with a lower fat content also presented smaller values, as found in our study. However, the amount of water added together with the inulin was lower than in our study. This indicates that psyllium has a strong potential to bind water and retain almost all of the added water in the final cakes, as observed by Aprodu and Banu (2015). Increases in weight loss were found by Román et al. (2015) when the fat was replaced by extruded flour. They found that the cakes with emulsifier did not have any differences in specific volume, so the effect of higher water content could not be compensated for by the lower volume of cakes with the replaced oil. In the case of the cakes without emulsifier, those in which the specific volume was reduced with increasing oil substitution, were observed to have significant differences to those cakes with 100% of oil replacement. This might also suggest that extruded flour has a smaller capacity for water retention than psyllium.

Texture results of the cakes are presented in Table 3. No significant differences were found in the hardness of the cakes between the control sample and those formulations with increasing oil replacement. It is known that the lower the specific cake volume, the greater the hardness is. (Chung et al. 2010; Rodríguez-García et al. 2012b). Therefore, in our study, the greater amount of water added to the formulations could compensate the effect of the volume on the hardness. Other studies that substituted fat by fibre showed increasing values for hardness, from two-thirds (Román et al. 2015) or 70% (Rodríguez-García et al. 2012b) of substitution, with reduced specific volumes, but in these cases the water added was less than that added in our study. In fact, cakes made with psyllium instead of fat obtained lower hardness values than those made with other fat replacements.

*Table 3. Texture properties of layer cakes with reduced fat content.*

| | **CONTROL** | **WF25** | **WF50** | **WF75** | **WF100** |
|---|---|---|---|---|---|
| Hardness (N) | 7,33±0,55a | 7,23±0,44a | 7,10±0,26a | 7,39±0,15a | 7,32±0,4a |
| Δ Hardness (N) | 3,44±0,82a | 3,31±0,34a | 3,39±0,74a | 3,19±0,90a | 4,67±0,77a |
| Cohesiveness | 0,662±0,024a | 0,683±0,006ab | 0,700±0,001b | 0,732±0,009c | 0,762±0,004c |
| ΔCohesiveness | -0,106±0,014a | -0,108±0,007a | -0,110±0,030a | -0,125±0,045a | -0,098±0,082a |
| Springiness | 0,970±0,001b | 0,962±0,011ab | 0,955±0,002a | 0,985±0,004c | 0,974±0,001bc |
| Δ Springiness | -0,069±0,004a | -0,043±0,027a | -0,047±0,026a | 0,058±0,174a | -0,068±0,011a |

*The values with the same letter in the same column do not present significant differences (p<0.05). WF25: layer cake with 25% of oil replacement. WF 50: layer cake with 50% of oil replacement. WF75: layer cake with 75% of oil replacement. WF100: layer cake with 100% of oil replacement.*

Regarding the rest of parameters, there was an increase in cohesiveness in those cakes with higher levels of fat substitution, with significant differences in substitutions of above 50%. However, there was no clear trend in the springiness of the cake. Some authors did not find significant differences of cohesiveness when using various levels of fat replacements (Zahn et al. 2010; Rodríguez-García et al. 2012b; Román et al. 2015; Eslava-Zomeño et al. 2016; Diez-Sánchez et al. 2018). Some studies showed increasing springiness values with higher fat substitutions (Zahn et al. 2010; Rodríguez-García et al. 2014; Diez-Sanchéz et al. 2018) while others reported decreasing values (Román et al. 2015) or no differences (Eslava-Zomeño et al. 2016) when the fat was replaced. These results indicate that texture parameters differ depending on the fat substitute used.

During storage, hardness increased and cohesiveness was reduced for all cakes. These changes were independent of the differences in the percentage of oil replacement. As it was observed in a previous study, there was correlation between initial hardness and its evolution over time (Gómez 2008), which confirms our observations. The results obtained for the hardness evolution coincided with those reported by Román et al. (2015) for cakes with extruded wheat flour which indicated that these type of oil replacements do not modify the texture of the cakes during storage.

Colour parameters are shown in Table 4. The crust of the cakes made with oil replacer showed increasing L* values, which means they were lighter than the others, but significant differences were only found in substitution levels above 75%. Samples WF75 and WF100, besides showing significant differences to ΔE*, they also presented significant differences in a* and b* values, which decreased and increased, respectively. High darkness of the crust was expected because of caramelisation and Maillard reactions which occurs during the baking process (Purlis 2010). However, the quantities of sugar and protein were constant for all samples, so this does not explain the changes in crust colour. The influence of lipids on this type of reaction could explained the changes of colour observed, since a derivate substance coming from their oxidation modify products colour while present (Hidalgo and Zamora 2000).

*Table 4. Crust and crumb colour of layer cakes with reduced fat content.*

| | | **CONTROL** | **WF25** | **WF50** | **WF75** | **WF100** |
|---|---|---|---|---|---|---|
| Crust | ΔE* | 0,00 ± 0,00a | 2,47 ±1,78a | 3,25 ± 0,33a | 15,82 ± 0,33b | 17,89 ± 3,13b |
| | L* | 51,99 ± 0,83ab | 50,77 ± 1,05a | 53,92 ± 0,85b | 64,31 ± 0,86c | 65,30 ± 1,84c |
| | a* | 13,72 ± 0,47b | 13,36 ± 0,47b | 13,03 ± 0,47b | 8,21 ± 0,47a | 8,40 ± 0,47a |
| | b* | 15,45 ± 2,12a | 14,72 ± 0,59a | 17,96 ± 1,80a | 23,67 ± 1,29b | 26,13 ± 0,77b |
| Crumb | ΔE* | 0,00 ± 0,00a | 0,88 ± 0,88ab | 1,02 ± 0,63ab | 0,66 ± 0,11a | 2,97 ± 1,53b |
| | L* | 75,71 ± 1,46a | 76,36 ± 2,45a | 76,29 ± 2,34a | 75,37 ± 1,60a | 72,88 ± 2,98a |
| | a* | -0,135 ± 0,06a | -0,27 ± 0,28a | -0,11 ± 0,23a | -0,045 ± 0,26ab | 0,64 ± 0,40b |
| | b* | 15,74 ± 0,43a | 15,91 ± 1,01a | 15,86 ± 1,39a | 15,73 ± 1,19a | 15,755 ± 1,04a |

*The values with the same letter in the same column do not present significant differences ($p<0.05$). WF25: layer cake with 25% of oil replacement. WF 50: layer cake with 50% of oil replacement. WF75: layer cake with 75% of oil replacement. WF100: layer cake with 100% of oil replacement.*

No significant differences of L* and b* values were observed in the crumb colour of those cakes made with oil replacement compared with control cakes and Figure 2 shows the crumbs for each formulation. However, greater fat substitutions presented significant differences to ΔE* compared to the control. High a* values were only observed in cakes with total oil substitution, whereas some studies of fat or oil replacement found major differences. Felisberto et al. (2015) obtained darker crumbs when using chia mucilage gel and lighter ones when incorporating inulin in the cakes (Rodríguez-García et al. 2012b). The temperature inside the cakes does not exceed 100 °C, so Maillard reactions and caramelisation cannot occur. In this way, the crumb colour depends mainly on the colour of the ingredients. Therefore, the differences found in these studies depends on the oil employed and the oil replacer. Fradinho et al. (2015) observed that cookies with psyllium incorporated had increased values of L* which was attributed to the darker colour of the psyllium. However, it must be noted that in our study the quantity of water added to the cake formulations was important as it diluted this effect.

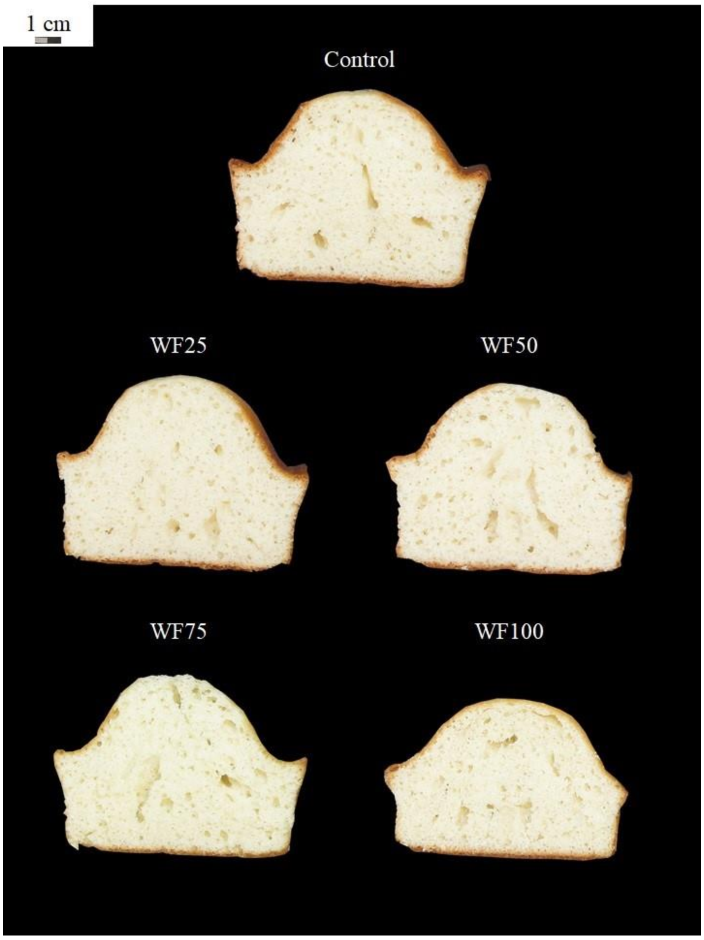

*Figure 2: Cellular structure from the crumb of the cakes.*

### 3.3. Consumer Test

Results of sensory evaluation are shown in Table 5. In general, the control and cakes with 25% of the oil replaced presented higher values for all the sensory parameters, with significant differences in taste, texture and overall acceptability compared to the rest samples. The cakes with 50 and 75% of oil replacement did not show any significant differences either in all sensory parameters but produced minor differences in the values for taste and texture compared to the control cakes, which reduced their global acceptability. However, all the cakes obtained a high evaluation (nearer to 7 and above 9) with differences that did not exceed 0.5 points between the control cakes and those with 75% of oil substitution. The cakes with 100% of oil replacement produced worse values than the control cakes in all aspects evaluated and were worse than all the other samples in aspects including odour, texture and overall acceptability. It must be considered that these cakes had a lower specific volume and lighter crust colour which could have negatively influenced the evaluations of the consumers. The texture of the cakes substituted with oil were given poor evaluations by the consumers, except to the cakes with 25% of substitution. Other studies also found differences in the acceptability of cakes with fat replacement. Cakes made with green banana puree were less acceptable due to their darker colour (Oliveira de Souza et al. 2018). Diez-Sánchez et al. (2018) found that using potato fibre as a substitute produced lower acceptability levels when 30% of fat was substituted which was attributed to a deterioration in their texture. When using inulin as a substitute, Rodríguez-García et al. (2012) observed minor levels of acceptability of cakes with 100% of fat replacement, which was explained by the crumbling and irregular crumb cell structure. In general, oil or fat substitution by fibres or other ingredients reduced consumers' acceptability of the cakes, but in the case of psyllium, this reduction was very small, and the products received a good global evaluation. Therefore, it might be possible to replace oil in cakes up to 25% with hydrated psyllium without reducing their acceptability and up to 75% with only a minimum decrease in sensorial evaluation.

*Table 5: Sensorial acceptability of layer cakes with reduced fat content.*

| Sample | Appearance | Odour | Taste | Texture | Overall acceptability |
|---|---|---|---|---|---|
| CONTROL | 7.27 ± 1.24b | 7.36 ± 1.30b | 7.33 ± 1.34 c | 7.15 ± 1.31c | 7.45 ± 1.10c |
| WF25 | 7.23 ± 1.25b | 7.27 ± 1.26b | 7.14 ± 1.41bc | 7.14 ± 1.28c | 7.39 ± 1.01c |
| WF50 | 7.41 ± 1.08b | 7.03 ± 1.32b | 6.78 ± 1.28b | 6.60 ± 1.33ab | 7.00 ± 1.08b |
| WF75 | 7.17 ± 1.35b | 6.97 ± 1.30b | 6.74 ± 1.56ab | 6.70 ± 1.41b | 6.99 ± 1.33b |
| WF100 | 6.08 ± 1.96a | 6.44 ± 1.54a | 6.32 ± 1.64a | 6.20 ± 1.52a | 6.34 ± 1.51a |

*The values with the same letter in the same column do not present significant differences (p<0.05). WF25: layer cake with 25% of oil replacement. WF 50: layer cake with 50% of oil replacement. WF75: layer cake with 75% of oil replacement. WF100: layer cake with 100% of oil replacement.*

## 4. Conclusion

The incorporation of pre-hydrated psyllium can be a convenient alternative for reducing the oil percentage in cake formulations. The higher oil replacement developed an irregular batter structure with greater bubbles and less stability, which results in cakes with lower specific volume. However, these volume changes did not produce changes in hardness for any substitution level although the cohesiveness increases slightly. For its part, crust colour gets lighter as the oil content in the formulation decreases. Despite these changes, cakes with 25% of oil substitution did not produce significant differences in consumer’s acceptability compared to the control cakes. In addition, cakes with 75% of replaced oil obtained acceptability scores higher than 6 points by consumers. Therefore, it is possible to produce cakes with a lower lipid content and higher consumer acceptability by using psyllium.

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# CAPÍTULO 4:

# Estudio de la calidad y la reducción de grasa en galletas de maíz.

# INFLUENCE OF FLOUR PARTICLE SIZE DISTRIBUTION ON THE QUALITY OF MAIZE GLUTEN-FREE COOKIES.

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# Influence of flour particle size distribution on the quality of maize gluten-free cookies.

## Abstract

The objective of the present study was to analyse the influence of particle size distribution of maize flour in the formulation of gluten-free cookies. Different cookie formulations were made with three distinct maize flour fractions obtained by sieving (less than 80 µm; between 80 and 180 µm; greater than 180 µm). Cookies dimension, texture and colour were evaluated. Flour hydration properties and cookie dough rheology were also measured. Overall, an increase in maize flour particle size decreases the values of water holding capacity (WHC), swelling volume and G' (elastic modulus) for the doughs. An increase in average particle size also increases diameter and spread factor of the cookies but decreases their hardness. A higher percentage of thick particles is more effective to reduce cookie hardness, but a certain percentage of thinner particles is necessary to give cohesion to the dough and to allow formation of the cookies without breaking. Cookies with a larger diameter also presented a darker colour after baking.

## 1. Introduction

Coeliac disease is an autoimmune disease related to an intolerance of gluten, which affects adults and children. It is treated by restricting gluten-containing food in the diet [1], which significantly increases the demand for gluten-free products. Therefore, to take advantage of this growth, many companies are looking to diversify and develop new products that meet this demand.

Most processed and pre-packaged bakery products, such as breads, cakes and cookies, are commonly produced with wheat flour. Although gluten plays an important role in bakery food processes, a glutenous structure is not developed in most types of cookies because of the high fat and sugar content in recipes and the scarce mechanical work imparted in the mixing process [2,3]. Thus, it is possible to obtain gluten-free cookies with characteristics very similar to those made with wheat flour [3–5]. Amongst cookies made with gluten-free flours, those formulated with maize flour are rated the highest by consumers [3].

In the case of cookies made with wheat flour, some characteristics, such as protein content or water absorption capacity, can justify its use in cookie preparation [2]. Maize, rice or legume flours are usually coarser than wheat flour due to their harder grains. It is already known that the particle size of wheat flour can influence cookie quality [6] but it could also be true for gluten-free flours. To investigate this, Rao et al. [7] observed that coarse sorghum flours produced cookies with lower hardness and better consumer acceptability. Mancebo et al. [8] also reported that coarse rice flours produced cookies with higher spread factors and lower hardness, which agrees with the observations by Ai et al. [9] with bean powders. However, Mancebo et al. [8] did not observe this effect in maize cookies, since both types of maize flour they used exhibited minor differences in particle size.

In some research papers, different particle size is obtained by forcing the grinding process until particles with a finer size are obtained [9,10]. However, this mechanical process causes an increase in damage starch that also affects how cookies develop during elaboration after elaboration [6,11]. There are few studies that achieve distinct fractions by sieving [3,7], nor are there any studies into blends of different flour fractions with different distributions of particle size.

Thus, the present research proposes studying how different fractions of sieved white maize flour, both alone and in combination, could influence gluten-free cookie dough properties (hydration and rheology) and cookie quality (physical properties, texture and colour).

## 2. Materials and Methods

### 2.1. Cookie Ingredients

The white maize flour (5.87 g/100 g protein) used in this study was produced by Molendum Ingredients S.L. (Zamora, Spain). A Bühler MLI 300B sifter (Milan, Italy) holding 80 and 180 µm sieves was used for 15 minutes to obtain three maize flour fractions: A (<80 µm), B (80–180 µm) and C (>180 µm).

Other ingredients used in the cookie recipe were white sugar (AB Azucarera Iberia, Valladolid, Spain), margarine (Argenta crema, Puratos, Barcelona, Spain), sodium bicarbonate (Manuel Riesgo S. A., Madrid, Spain) and local tap water.

### 2.2. Flour Characterisation

Flour particle size was evaluated using a Mastersizer 3000 particle size analyser (Malvern Instruments, Malvern, UK). Values of D[4,3], which represents the equivalent spherical diameter of the particles, and of D10), D(50) and D(90), which represent the maximum particle diameter below which 10%, 50% and 90% of the sample fall, respectively, were obtained. All the measurements were carried out in duplicate.

Regarding hydration, water binding capacity (WBC, i.e., the amount of water retained by the sample after it has been centrifuged) was measured as described in AACC International method 56-30.01 [12]. Water holding capacity (WHC, i.e., the amount of water retained by the sample without being subjected to any stress) and swelling volume (SV, i.e., the volume occupied by a known weight of sample) were measured as described by Mancebo et al. [3]. All hydration properties were analysed in duplicate.

### 2.3. Cookie Formulation

The original maize flour and the individual fractions were applied in different percentages giving rise to eleven combinations, as shown in Table 1. The mixing process was carried out as described by Mancebo et al. [8]. For the rheology test, the dough was rolled out to 3 mm thickness on a dough sheeter and then cut into round shape with a cutter of 60 mm diameter. For the baking test, samples were rolled out to 6 mm thickness and were cut with a 40 mm diameter cutter. The baking process was performed in a baking oven at 185 °C for 14 minutes. The cookies were cooled for one hour at room temperature and then placed in plastic bags, to avoid the interference of humidity. They were stored for seven days in a chamber with controlled temperature (25 °C) for further analysis. All cookie baking was done in duplicate.

*Table 1. Gluten-free cookie formulations presented in grams with different percentages of maize flour and fractions A (<80 µm), B (80–180 µm) and C (>180 µm).*

| **Ingredients** | **CF** | **100A** | **100B** | **100C** | **50A/50B** | **50A/50C** | **50B/50C** | **25A/75B** | **75A/25B** | **25A/75C** | **75A/25C** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| White maize flour | 173.2 | - | - | - | - | - | - | - | - | - | - |
| A (<80 µm) | - | 173.2 | - |  | 86.6 | 86.6 | - | 43.3 | 129.9 | 43.3 | 129.9 |
| B (80–180 µm) | - |  | 173.2 | - | 86.6 | - | 86.6 | 129.9 | 43.3 | - | - |
| C (>180 µm) | - |  | - | 173.2 | - | 86.6 | 86.6 | - | - | 129.9 | 43.3 |
| White sugar | 124.8 | 124.8 | 124.8 | 124.8 | 124.8 | 124.8 | 124.8 | 124.8 | 124.8 | 124.8 | 124.8 |
| Margarine | 77.6 | 77.6 | 77.6 | 77.6 | 77.6 | 77.6 | 77.6 | 77.6 | 77.6 | 77.6 | 77.6 |
| Sodium bicarbonate | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 |
| Tap water | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |

### 2.4. Dough Rheology

Rheological behaviour of the fresh cookie dough was evaluated using a rheometer (Haake RheoStress 1, Thermo Fisher Scientific, Scheverte, Germany). Each dough sample was placed on a titanium parallel-serrated plate geometry PP60 Ti (60 mm diameter, 3 mm gap) and covered with Panreac Vaseline oil (Panreac Química S.A., Castellar del Vallés, Spain) to avoid drying during the test. A Phoenix II P1-C25P water bath maintained the temperature at 25 °C.

In the first measurement, the cookie dough was subjected to a strain sweep (stress range of 0.1–100 Pa) at a constant temperature (25 °C) and frequency (1 Hz) to identify the linear viscoelastic region. Then, using these results, a stress value within the linear viscoelastic region was selected and applied in a frequency sweep test to obtain the values of the elastic modulus (G’, Pa), viscous modulus (G”, Pa), complex modulus (G*) and tan delta (G”/G’) over a range of frequency values (w, Hz). The measurements were made in duplicate.

### 2.5. Cookie Characteristics

Cookie moisture was evaluated as described by AACC method 44-15.02 [23] and the test was made in duplicate.

Cookie diameter was measured twice, in perpendicular directions, to achieve an average diameter (D). Cookie thickness (T) was also measured to obtain the spread factor (D/T).

Texture parameters of the cookies were analysed by using a Texture TA-XT2 texture analyser (Stable Micro Systems, Surrey, UK). The peak force, or hardness, (N) and the elastic modulus (N/mm2) were obtained by the compression of a ‘three-point bending' test with a three-point bending rig probe (HDP/3PB). The measurement conditions were: travel distance of 20 mm, trigger force of 5 g and test speed of 2.0 mm/s.

Cookie colour was measured at the centre of the surface crust with a Minolta CN-508i spectrophotometer (Minolta Co. Ltd., Osaka, Japan) using a D65 illuminant with a 2° standard observer angle. L*, a* and b* values were expressed in the colour space defined by the International Commission on Illuminaion (CIE).

All cookie characteristics were measured in six cookies of each batch, seven days after baking.

### 2.6. Statistical Analysis

Statistical analysis of the differences between the parameters of the different formulations were evaluated by analysis of variance (ANOVA) using Statgraphics Centurion XVI software (StatPoint Technologies Inc., Warrenton, DC, USA). Fisher's least significant difference (LSD) was used to describe means with 95% confidence intervals.

## 3. Results

### 3.1. Particle Size and Hydration Properties

As shown in Table 2, the different blends presented an average size D[4,3] within the range of particle sizes of the fractions that comprised it. As expected, the average particle size of blends formed from fractions A and B increased with the quantity of fraction B. This also occurred with the mixtures of fractions A and C. The control sample had an average particle size slightly higher than fraction B, but clearly lower than fraction C. D(10) and D(50) values decreased as the percentage of fraction A increased. However, for mixtures containing 50% or more of A, there were no further significant changes, regardless of whether they were mixed with B or C. This is logical if it is remembered that these values refer to 10% or 50% of the thinnest flours from the mixtures obtained from fraction A. In the case of mixtures with 25% of A, there was a significant difference in D(50) values and they were even smaller in mixtures with the fraction B. Control flour had relatively low D(10), which was similar to mixtures with 25% of A and smaller than mixtures without fraction A. For its part, the D(50) is minor in all mixtures with A, with just one exception (25A/75C), and no significant differences were observed with fraction B. In general, the average sizes of the flours used in this research were similar to those applied in other studies on gluten-free cookie formulations [3,4,7].

*Table 2. Maize flour, maize flour fractions and their combinations particle size measurements.*

| | **D[4,3]** | **D(10)** | **D(50)** | **D(90)** |
|---|---|---|---|---|
| Control flour | 199.9 ± 6.4g | 26.5 ± 1.4c | 178.8 ± 6.5e | 401.4 ± 8.8e |
| 100A (<80 µm) | 61.6 ± 3.9a | 14.6 ± 0.4a | 49.4 ± 4.7a | 128.3 ± 7.2a |
| 100B (80–180 µm) | 186.3 ± 9.2f | 88.3 ± 9.6d | 177.0 ± 8.5e | 304.7 ± 9.7d |
| 100C (>180 µm) | 354.2 ± 5.4i | 211.2 ± 1.4f | 337.4 ± 4.5g | 533.6 ± 12.2h |
| 50A/50B | 124.1 ± 1.3c | 19.1 ± 0.4ab | 105.0 ± 0.6c | 263.2 ± 4.2c |
| 50A/50C | 172.2 ± 4.8e | 18.9 ± 0.3ab | 105.6 ± 4.5c | 415.9 ± 8.6e |
| 50B/50C | 261.1 ± 3.8h | 113.1 ± 3.0e | 242.4 ± 3.7f | 444.7 ± 4.9f |
| 25A/75B | 151.9 ± 3.8d | 27.7 ± 1.3bc | 144.0 ± 5.4d | 287.3 ± 2.7cd |
| 75A/25B | 101.1 ± 10.0b | 16.9 ± 0.4a | 74.2 ± 8.2b | 230.7 ± 23.3b |
| 25A/75C | 252.8 ± 3.8h | 27.7 ± 0.1c | 250.8 ± 3.4f | 489.8 ± 9.0g |
| 75A/25C | 113.1±4.4bc | 16.5 ± 0.2a | 66.5 ± 1.6b | 299.5 ± 15.4d |

*D[4,3]: average particle size which constitutes the bulk of the sample volume. D(10): maximum particle diameter below which 10% of the sample falls. D(50): maximum particle diameter below which 50% of the sample falls. D(90): maximum particle diameter below which 90% of the sample falls. Data are expressed as means ± standard deviation (SD)SD of duplicate assays. The values with the same letter in the same column do not present significant differences (at a significant level of $p < 0.05$).*

According to Table 3, higher values of WHC are related to the compositions A and B, while lower values are found in mixtures with percentages of C higher than 50%

(100C, 25A/75C and 50A/50C). In fact, there is a negative correlation between the WHC and D[4,3] r = −0.74 (significant at 99%). The relationship between WHC and the particle size was observed by other authors [10] and can be explained by the large surface area presented by finer flours.

*Table 3. Maize flour, maize flour fractions and their combinations: hydration properties.*

| | **Hydration Properties** | | |
|---|---|---|---|
| | WHC | Swelling | WBC |
| Control flour | 1.57 ± 0.04bcd | 2.09 ± 0.16abc | 1.28 ± 0.04de |
| 100A (<80 μm) | 1.85 ± 0.07fg | 2.29 ± 0.16d | 1.30 ± 0.01ef |
| 100B (80–180 μm) | 1.87 ± 0.13g | 2.19 ± 0.01bcd | 1.47 ± 0.01g |
| 100C (>180 μm) | 1.48 ± 0.03bc | 2.20 ± 0.00cd | 1.30 ± 0.01ef |
| 50A/50B | 1.64 ± 0.10cde | 2.09 ± 0.14abc | 1.27 ± 0.04de |
| 50A/50C | 1.43 ± 0.09ab | 1.99 ± 0.01a | 1.19 ± 0.01bc |
| 50B/50C | 1.83 ± 0.05fg | 2.27 ± 0.11cd | 1.33 ± 0.01f |
| 25A/75B | 1.74 ± 0.11efg | 2.18 ± 0.01abcd | 1.15 ± 0.02b |
| 75A/25B | 1.69 ± 0.04def | 2.19 ± 0.02bcd | 1.05 ± 0.01a |
| 25A/75C | 1.41 ± 0.02a | 2.00 ± 0.01ab | 1.20 ± 0.01c |
| 75A/25C | 1.56 ± 0.04abcd | 2.20 ± 0.01cd | 1.24 ± 0.04cd |

*ND: no development. WHC: water holding capacity. WBC: water binding capacity. Data are expressed as means ± SD of duplicate assays. The values with the same letter in the same column do not present significant differences (at a significance level of $p < 0.05$).*

Nevertheless, other studies were either not able to establish a correlation between particle size and WHC, or no decrease in WHC was found with reductions in particle size [13]. These differences could be related to both different compositions of particles with distinct sizes and to the morphology and particle size distribution, which was not evaluated in other studies. In this case, it is important to stress that the correlation of WHC with D(90) values was higher than with D[4,3], r = −0.86 (significant at 99.9%). This fact may indicate that the percentage of coarse fractions is what most affects this property. This could be explained by the fact that the finer fractions present similar particle sizes while the thicker fractions have greater differences between them. The swelling volume is highly correlated with WHC, D[4,3], and D(90) (r = 0.80, significant at 99%). Nevertheless, no correlation between particle size and WBC values was found, as observed by De la Hera et al. [14]. Thus, the larger values of WBC are related with fraction B followed by other individual fractions (pure A and pure C) and mixtures with the coarser fractions (B/C). On the other hand, the smaller values of WBC were observed in mixtures comprising A and B, except 50A/50B, which showed no significant difference compared to the control. Combinations of A and C presented intermediate values with no significant differences. Intermediate values of WBC were also shown by the control flour. In this case, the percentage of different fractions, their packing capacity and particle morphology seems to have a higher influence on this behaviour than the average particle size when using fine and coarse flours together in the centrifugal process.

### 3.2. Dough Rheology Properties

Dough rheology is a fundamental characteristic for cookie formulation so that if the dough is very soft or firm it is not easy to manipulate. The dough must be sufficiently cohesive to remain united during the different phases of processing and to be easily cut by the moulds [15]. In fact, an important finding from our rheology measurements was that values were obtained only for samples containing fraction A. The other doughs were extremely brittle and were impossible to laminate without breaking. From this result, it is possible to conclude that to make a cookie dough that is cohesive and laminable, it is necessary for it to contain a minimum proportion of reduced size particles. This conclusion was not found in other studies because those only considered pure fractions with a unique size, bigger or smaller, obtained by screening or by another grinding process.

In general, G’ values were higher than G” values (Table 4), showing that the elastic component is dominant over the viscous one, which suggests a solid elastic-like behaviour of all the doughs studied. This result was confirmed in values of tan delta that were all lower than 1.0, in agreement with other studies about cookies [8,16,17]. Between different doughs, smaller values of G’ corresponded to those flours with a higher value of D[4,3], such as the control flour and the mixture 25A/75B. Meanwhile, the biggest values of G’ were found in doughs with finer flours (with lower D[4,3]) with a higher percentage of fraction A. This corresponded with low values of D(10) (100A, 75A/25B, 75A/25C). This indicates that finer flours have a better packing quality, which results in more cohesive doughs. However, it is also important to note the larger values of G’ obtained with mixtures of fractions A and C where C made up more than 50%. In those cases, it was very difficult to manipulate the doughs because of their weakness. These samples presented a different texture compared with other samples because they showed an anomalous rheological behaviour. These doughs were like those where it was not possible to measure the rheology parameters, but their breakability characteristics were less extreme.

*Table 4. Maize flour, maize flour fractions and their combinations: cookie dough rheology.*

| | **Dough Rheology** | | |
|---|---|---|---|
| | G' (×$10^6$) | G" (×$10^6$) | Tan Delta |
| Control flour | 0.42 ± 0.02a | 0.25 ± 0.27a | 0.59 ± 0.54b |
| 100A (<80 µm) | 1.36 ± 0.27c | 0.26 ± 0.18a | 0.15 ± 0.04 ab |
| 100B (80–180 µm) | ND | ND | ND |
| 100C (>180 µm) | ND | ND | ND |
| 50A/50B | 0.72 ± 0.02ab | 0.12 ± 0.17a | 0.24 ± 0.35ab |
| 50A/50C | 1.20 ± 0.22c | 0.10 ± 0.13a | 0.10 ± 0.14ab |
| 50B/50C | ND | ND | ND |
| 25A/75B | 0.27 ± 0.23a | 0.05 ± 0.30a | 0.25 ± 0.11ab |
| 75A/25B | 1.25 ±0.18c | 0.06 ± 0.88a | 0.05 ± 0.08ab |
| 25A/75C | 1.49 ± 0.17c | 0.08 ± 0.11a | 0.01 ± 0.00a |
| 75A/25C | 1.05 ± 0.15bc | 0.06 ± 0.90a | 0.14 ± 0.01ab |

*ND: no development. WHC: water holding capacity. WBC: water binding capacity. Data are expressed as means ± SD of duplicate assays. The values with the same letter in the same column do not present significant differences (at a significance level of $p < 0.05$).*

Regarding the viscous component (G"), it was not possible to find significant differences between the samples. Probably, this fact could be due to the excessive variability of the data, especially of the more fragile doughs. The same effect was observed in tan delta values, where some differences were found between control flour and the 25A/75C mixture, which were the most difficult samples to perform the rheology test on because of the cohesiveness. Even though previous studies reported correlations between rheological cookie values and hydration properties when flours or mixtures were used [17–19], in this case, no correlations were observed. This was due to the anomalous brittleness of samples with smaller proportions of fraction A and the importance between the particle size distribution and rheological behaviour.

### 3.2. Cookie Characteristics

Cookie characteristics are shown in Table 5. It is important to underline that it was not possible to make cookies with pure fractions B and C because the dough was very breakable (Figure 1). This brittle character was also observed in B/C mixtures (25B/75C, 50B/50C and 75B/25C) where, even though it was not possible to measure the rheological parameters, only the mixture 50B/50C was at least capable of being made. This fact could be explained because the rheological test required a thin layer of the dough, which was easily broken. These three flours (B, C, and B/C) were the ones that presented higher values of D(10), which means that it is necessary to add a small number of finer particles to the fractions. These particles take their place between the thick particles and increase the cohesiveness of the dough which allows the dough to be laminated. Furthermore, a mixture made using different particle sizes (B/C) seems to be more convenient than those made of only one size, which can be explained by the same effect of fine particles getting placed between coarse ones.

*Table 5. Physical properties of cookies.*

| | **Dimensions** | | **Texture** | | **Colour** | | |
|---|---|---|---|---|---|---|---|
| | **Diameter (mm)** | **Spread Factor** | **Hardness (N)** | **Cookie Elastic Modulus ($N/mm^2$)** | **L*** | **a*** | **b*** |
| Control flour | 56.4 ± 0.40c | 7.48 ± 0.31de | 34.10 ± 1.22cd | 26.90 ± 1.52ab | 55.33 ± 1.97c | 5.59 ± 2.79abc | 18.33 ± 0.92b |
| 100A (<80 μm) | 43.1 ± 1.22a | 4.05 ± 0.24a | 50.84 ± 1.97f | 54.48 ± 2.73d | 78.45 ± 0.95f | 3.70 ± 1.40a | 21.41 ± 1.02bcd |
| 100B (80–180 μm) | ND | ND | ND | ND | ND | ND | ND |
| 100C (>180 μm) | ND | ND | ND | ND | ND | ND | ND |
| 50A/50B | 48.4 ± 0.22bc | 5.54 ± 0.34bc | 34.43 ± 1.94cd | 33.41 ± 3.13bc | 69.44 ± 1.27e | 5.47 ± 1.12abc | 21.37 ± 0.40bcd |
| 50A/50C | 55.1 ± 0.20d | 8.47 ± 0.20ef | 24.25 ± 0.37b | 27.15 ± 3.97ab | 61.20 ± 0.11d | 7.19 ± 0.76bcd | 19.14 ± 1.04b |
| 50B/50C | 54.9 ± 2.53d | 9.47 ± 1.02f | 25.98 ± 0.30b | 19.83 ± 0.45a | 51.11 ± 1.23b | 7.85 ± 1.40cd | 13.37 ± 1.04a |
| 25A/75B | 50.7 ± 0.07c | 6.31 ± 0.13cd | 36.24 ± 0.05d | 38.54 ± 1.38c | 69.84 ± 1.89e | 5.98 ± 0.55abc | 25.23 ± 1.12d |
| 75A/25B | 43.9 ± 1.22a | 4.53 ± 0.19ab | 42.82 ± 1.98e | 38.71 ± 10.61c | 75.66 ± 1.80f | 4.68 ± 0.60ab | 24.42 ± 1.32cd |
| 25A/75C | 63.0 ± 0.71e | 14.01 ± 1.31g | 17.93 ± 0.8a | 29.70 ± 5.37abc | 46.52 ± 3.10a | 9.71 ± 1.00d | 12.93 ± 2.13a |
| 75A/25C | 47.14 ± 0.79b | 5.10 ± 0.01abc | 33.24 ± 0.80c | 34.81 ± 1.74bc | 74.34 ± 2.63f | 5.19 ± 0.08abc | 21.06 ± 4.24bc |

*ND: no development. Data are expressed as means ± SD of duplicate assays. The values with the same letter in the same column do not present significant differences (at a significance level of $p < 0.05$).*

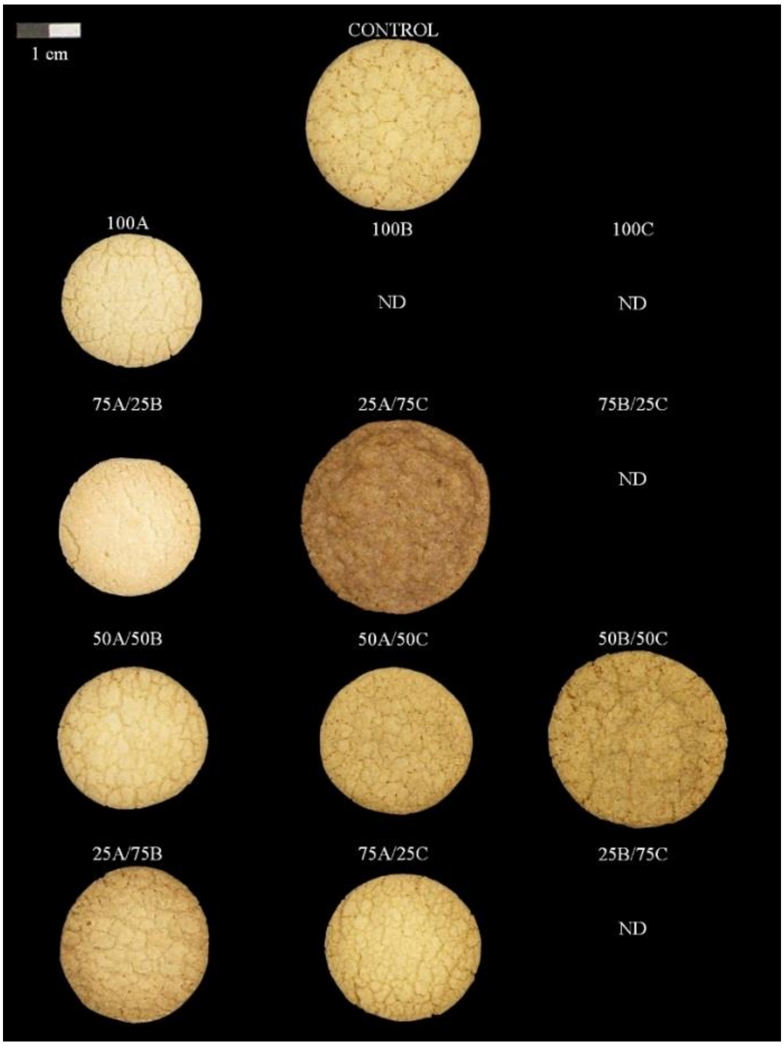

*Figure 1. Image from cookies made with different particles sizes of white maize flour.*

Among the obtained cookies, no significant differences were found in final product moisture, and in all cases the values were less than 1%. It is also important to stress that the 25A/75C cookies showed the highest diameter and spread factor followed by those made with 50% of C (A/C and B/C). This fact seems to indicate that a higher percentage of coarse flour is favourable to releasing and spreading during baking, generating cookies of a greater diameter. This was also observed by Mancebo et al. [3] with rice flours, Rao et al. [7] with sorghum flour and Ai et al. [9] with dry bean powders. For their part, fractions with a higher percentage of A and, therefore, a lower average size (A, 75A/25B and 75A/25C) showed small spread factors; the next lowest spread factor was 50A/50B (with larger average size). In fact, 99.9% significant correlations were found between the diameter and D[4,3] (r = 0.98) and between the spread factor and D[4,3] (r = 0.92). Some authors affirm that flours with a lower hydration capacity produced cookies with higher diameters because they allowed excess water to dissolve the sugar, to reduce the initial viscosity of the doughs and to allow more expansion during baking [20,21]. Thus, in this study, significant correlations of 99% were found between WHC and the values of diameter (r = −0.74) and spread factor (r = −0.72), as found in similar studies [3,8]. In this way, it seems that the value of D[4,3] is a better indicator of dough expansion during baking and the final diameter, but D(10) shows a better possibility of obtaining cohesive doughs that can be laminated and cut without breaking.

Regarding the texture of the cookies, a significant correlation coefficient of r = 0.88 was found in all cases but at differing significance levels: 99.9% significance between hardness, diameter and spread factor; 99% between the elastic modulus and diameter and 95% between the elastic modulus and the spread factor). Therefore, cookie dimensions showed a stronger correlation with hardness than with the gradient obtained by the curve. Thus, harder cookies presented both a lower diameter and spread factor (100A and 75A/25B). On the other hand, less hard cookies showed higher spread factors (25A/75C) followed by those mixtures with A/C and B/C which presented values greater than all the other samples. Mancebo et al. [3] described how cookies with higher spread factors showed a lower peak force in textural analysis, but we found no correlation whatsoever between these two parameters for any of the cookies we analysed. In addition to the dimensions of the cookies, these differences may be related to their internal structure, the particle size of the flours and their compaction capacity. Parameters of texture and indicators of flour particle size showed a better correlation than with cookie dimensions, which demonstrates that as the particle size decreases, the hardness of the cookies increases, in agreement with the observations of similar studies [7,9]. In the case of D[4,3], the correlation with hardness also presented a significant correlation of $r = -0.87$ (99% significance). One observed correlation, between hardness and D(90), stands out ($r = -0.93$, significant at 99.9%), as it is higher even than the ones observed between hardness and D(50) ($r = -0.72$, significant at 99%) and between hardness and D(10) ($r = -0.61$, significant at 95%). Therefore, it seems that what most reduced the hardness of the cookies was an increase in the percentage of thicker particles, even though it is necessary to remember the importance of having a certain percentage of smaller particles to allow the formation of the cookies.

Regarding colour, important differences were found among the values of L*. Cookies with a larger percentage of A (100% or 75%) were lighter in colour and presented lower spread factors. Meanwhile, cookies made with a larger percentage of C (25A/75C) showed darker colour (small values of L*) followed by those made with 50% of C. With regard to a* and b* values, only those samples with extreme mixes stand out: sample A, which had a lower particle size, diameter and spread factor, presented the smallest values of a*, while the sample 25A/75C, which had a higher particle size, spread factor and diameter, showed the highest values of a* and smallest of b*. Mancebo et al. [3] also observed the smallest values of L* and b* for cookies with higher spread. This could be because of a higher diameter and a lower thickness of cookies, which produces high temperatures in a short time within the dough. Thus, temperature increases led to more caramelization and Maillard reactions, which are the main causes of final cookie colour [22]. In fact, significant correlations of 99.9% were found between the spread factor of the cookies and the parameters L* (r = −0.91), a* (r = −0.87) and b* (r = −0.81).

## 4. Conclusions

The final characteristics of cookies can be influenced by the properties of the ingredients used in their elaboration such as the particle size of the flour, the sugar content and the fat used in formulation. There are no references about how both of these last factors can affect the quality of the cookies, which may be an opportunity for future studies. However, previous studies proved that a higher average particle size favours spread factor and decreases cookie hardness. Nevertheless, this study proves for the first time that it is necessary to also have a certain percentage of fine particles, which, being placed between coarser particles in the dough, give rise to a higher dough cohesiveness. Otherwise, doughs are excessively fragile in the laminating process and cookies are impossible to be made. These results must be considered in future studies about gluten-free cookies and in food development in the industry.

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# ASSESSING PSYLLIUM AS A FAT SUBSTITUTE IN WHEAT AND GLUTEN-FREE COOKIES.

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# Assessing psyllium as a fat substitute in wheat and gluten-free cookies

## Abstract

The potential of psyllium as a fat substitute was evaluated in cookies with gluten (wheat flour) and without gluten (maize flour). The elimination of fat and the addition of psyllium to the cookie formulation demands an increase in the amount of water, so the dough can be laminated. Doughs without fat had low values of G’, G” and G*, but high values of tg delta. Cookies made without fat had a small diameter and spread factor; however, they presented high hardness and final moisture content. The absence of fat produced lighter cookies. Variation of the water content in formulations, obeying the limits that allowed correct lamination, only influenced the final cookie characteristics. For the wheat cookies without fat, high moisture content and height were observed, but they were softer than the gluten-free cookies, which was related to the development of a gluten network because of the elimination of fat and the increased amount of water in the doughs. These changes produced cookies with low acceptability and this result was more evident in gluten-free cookies.

## 1. Introduction

Fat plays a fundamental role in cookie elaboration. It supports the incorporation of air and promotes changes in the rheology and stickiness of doughs. It also increases the spread factor in formulations with a sugar content of less than 50% of flour weight, and harder and more breakable cookies are produced when the fat percentage is decreased (Gómez, 2008). Nevertheless, cookies have a high caloric content, and the consumption of saturated fats, such as those used in cookies, is related to high cholesterol values and increased risk of atherosclerosis (Khosla and Sundram, 1996; Kritchevsky, 2000). The decrease in fat content in gluten-free products is especially interesting since gluten-free diets have a higher saturated fat content than traditional diets (Vici et al., 2016).

To minimize the effects of fat reduction in cookie formulations, different fat substitutes have been used, which are fundamentally carbohydrates, such as maltodextrins, inulin or polydextrose, oat-derived fibre products and hydrocolloid-based products (Conforti, Charles and Duncan, 1997; Lee and Inglett, 2006; Rodríguez-Garcia et al., 2013; Sanchez, Klopfenstein and Walker, 1995; Sudha et al., 2007; Zoulias, Oreopoulou and Tzia, 2000; Zoulias, Oreopoulou and Tzia, 2002), or okra gum (Romanchik-Cerpovicz, Tilmon and Baldree, 2002). All these studies focused on analysing cookies made with wheat flour.

Psyllium is a natural soluble fibre, originated from the husks of seeds from *Plantago ovata*, with some properties similar to those of xanthan gum (Haque et al., 1993), and has been successfully used as a fat substitute in cake formulations (Belorio, Sahagún and Gómez, 2020). This fibre is mainly composed of arabinoxylans (Edwards et al., 2007) and some authors have proved its action over constipation, diarrhoea, irritable bowel syndrome, inflammatory bowel disease including ulcerative colitis, colon cancer, diabetes and hypercholesterolaemia (Singh, 2007; Wärnberg et al., 2009).

The objective of the present work was to evaluate the use of pastes made with different ratios of psyllium/water to partially replace the fat content in cookies made with wheat flour or white maize flour. The measures of dough rheology, cookie dimensions, texture, colour and acceptance tests with potential consumers were carried out to evaluate the results.

## 2. Materials and Methods

### 2.1 Materials

The cookies were made with: wheat flour (WF) (moisture 10.32 g/100 g) (Comercial Gallo S.A.U., Barcelona, Spain), white maize flour (WMF) (moisture 8.87 g/100 g) which was supplied by Molendum Ingredients (Zamora, Spain), white sugar (AB Azucarera Iberia, Valladolid, Spain), 100% vegetable margarine (Argenta Puratos, Barcelona, Spain), sodium bicarbonate (Manuel Riesgo S.A., Madrid, Spain), VITACEL Psyllium P95 (Rettenmaier Ibérica, Barcelona, Spain) and local tap water.

### *2.2 Methods*

#### *2.2.1 Paste and cookie preparation*

The quantity of each ingredient is indicated in Table 1. Psyllium pastes were made using different combinations of psyllium (1/n) and water ((n − 1)/n) with n = 4, 5 and 6 which means the total parts of the psyllium + water mix. The pastes were prepared at room temperature and the psyllium and water were mixed in a plastic bowl.

*Table 1. Cookie and psyllium paste formulations.*

| | | | | | | **1/n** | **(n −1)/n** |
|---|---|---|---|---|---|---|---|
| **Sample** | **Flour (g)** | **White sugar (g)** | **Margarine (g)** | **Sodium bicarbonate (g)** | **Water (g)** | **Psyllium $^{P}$(g)** | **Water $^{P}$ (g)** |
| Control WMF | 173.2 | 124.8 | 77.6 | 3.6 | 15.0 | - | - |
| WMF n = 4 | 173.2 | 124.8 | - | 3.6 | 15.0 | 19.4 | 58.2 |
| WMF n = 5 | 173.2 | 124.8 | - | 3.6 | 15.0 | 15.5 | 62.1 |
| WMF n = 6 | 173.2 | 124.8 | - | 3.6 | 15.0 | 12.9 | 64.7 |
| Control WF | 173.2 | 124.8 | 77.6 | 3.6 | 25.0 | - | - |
| WF n = 4 | 173.2 | 124.8 | - | 3.6 | 25.0 | 19.4 | 58.2 |
| WF n = 5 | 173.2 | 124.8 | - | 3.6 | 25.0 | 15.5 | 62.1 |
| WF n = 6 | 173.2 | 124.8 | - | 3.6 | 25.0 | 12.9 | 64.7 |

*Values with the same letter in the same column are not significantly different ($p < 0.05$). WMF: white maize flour; WF: wheat flour; n: total parts of psyllium+water mix to make the paste; psyllium$^{P}$: amount of psyllium used to make the paste; water$^{P}$: amount of water used to make the paste.*

Cookies were made using 173.2 g of flour, 124.8 g of sugar, 77.6 g of margarine, 3.6 g of sodium bicarbonate and 25 g of tap water. With respect to the formulations with WMF, the amount of water in the formulation was modified (15 g) with the aim of producing doughs that could be correctly laminated. The margarine was melted for 1 min at 1000 W in a commercial microwave; later, the sugar was added over this melted fat. The batter and sugar were creamed using a professional mixer (Kitchen Aid, St. Joseph, Michigan, USA) at speed 4 (120 rpm) for 3 min, stopping each minute to remove the cream retained on the walls of the bowl using a flat beater. After that, the psyllium and water paste were added to the mixture, which was mixed for 1 min at speed 4 (120 rpm). Later, the water from the cookie formulation was added and mixed for 2 min at speed 4 (120 rpm). Finally, the flour and sodium bicarbonate were added and mixed for 2 min at speed 2 (90 rpm), stopping every 30 s to remove the dough retained at the borders. The dough was wrapped in a plastic film and rested for 30 min in a fridge (5 °C). The dough was laminated using a Salva L-500-J sheeter (Salva, Lezo, Spain) with a gap of 6 mm. The dough was cut using a cookie cutter with a diameter of 40 mm. Batches of at least 16 pieces of dough were baked in an electric oven (14 min at 185 °C). Baked cookies were packaged into plastic bags and stored at room temperature for 7 days before diameter, texture and colour measurements.

All the cookie formulations were made in duplicate.

### *2.2.2 Dough rheology*

The rheology test was carried out using a HAAKE RheoStress 1 rheometer from Thermo Scientific (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P water bath regulated to keep the analysis temperature at 25 °C. For this test, the cookie dough was laminated using a gap of 3 mm and a cookie cutter with a diameter of 60 mm, due to the 3 mm gap and the parallel-plate geometry (60 mm diameter titanium serrated plate – PP60 Ti) used in this analysis. Vaseline oil (Panreac, Panreac Química S.A., Castellar del Valles, Spain) was applied to the exposed borders of the sample to avoid drying during the test. The analysis started with resting the dough for 800 s before the strain sweep test, which was done using a stress range of 0.1–100 Pa at a constant frequency of 1 Hz, with the aim to identify a linear viscoelastic region. From the results, it was possible to identify a stress value included in this region, which was used in a frequency sweep test using a range of 10–0.1 Hz. The parameters obtained were the elastic modulus (G' [Pa]), viscous modulus (G" [Pa]), loss factor (tg delta = G"/G') as a function of frequency, and complex modulus (G*). The analysis was performed in duplicate.

### *2.2.3 Cookie properties*

Two diameters were obtained through perpendicular measurements of six different cookies of each formulation with the aim to obtain an average diameter. The cookies were weighed, and their thickness was obtained to calculate the spread factor (the relationship between the average diameter and thickness).

The cookies' moisture was measured according to AACC method 44-15.02 (AACC International, 2012) and it was done in duplicate.

Texture parameters were evaluated 7 days after baking with the aim of guaranteeing the stability of the cookies, as mentioned before. Cookies were analysed using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) and Texture Expert software. Cookies were analysed under the following experimental conditions: supports 30 mm apart, a trigger force of 49 mN and a test speed of 5.0

mm/s. The probe travel distance for all the cookies made with WMF and for the control with WF was 5 mm; for the cookies made with WF without fat, a distance of 35 mm was used, because the other distance did not allow correct breakage of the cookies, so it was not possible to have a real result. The maximum force (N) and the gradient of the curve (N/mm) were obtained. Six cookies were evaluated per formulation.

Cookie colour was analysed using a Minolta CN-508i spectrophotometer (Minolta, Co. Ltd, Tokyo, Japan) with D65 illuminant with the 2° standard observer. The centre of the surface of six different cookies for each formulation was measured with the aim to obtain values for crust colour in the CIE L*a*b* colour space.

### *2.2.4 Cookie sensory evaluation*

The sensorial analysis was carried out according to the protocol previously approved by the Committee of Tests and Research from the Hospital Rio Carrión (Palencia, Spain). Sensory evaluation of four different cookie formulations was performed using a hedonic scale. The cookies evaluated were controls (one made with WF and the other with WMF) and maize (n=5) and wheat (n=4) cookies without fat. They were chosen based on their similar rheologic and texture properties and to avoid having a huge number of cookies to be evaluated by the consumers. The evaluation was made by a total of 73 volunteers who were usual consumers of cookies, aged between 18 and 66 years old. The samples were presented on individual plastic plates, as whole pieces; each cookie was coded with a four-digit random number and plates were served in a random order. The cookies were evaluated 7 days after baking, according to their acceptability for appearance, odour, texture, taste and overall appreciation.

### *2.2.5 Statistical analysis*

Analysis of variance (ANOVA) was used to study the differences between batter and cookie characteristics. Tukey's HSD was used to describe means with 95% confidence. Analysis was performed using Statgraphics Plus V5.1 software (StatPoint Technologies, Warrenton, VA, USA).

## 3. Results and Discussion

### *3.1 Rheological properties*

Table 2 indicates the rheological values for the different cookie doughs. It must be considered that cookies made with WF had a greater amount of water in the control formulation. This addition was necessary to achieve correct lamination of the dough, without breaking (insufficient water) or sticking (excess of water) such as indicated by (Manley, 2000). All the doughs presented higher values of G' (storage modulus) than G" (loss modulus). Furthermore, the tg delta values were all smaller than 1, indicating that the doughs had more elastic than viscous properties (Lee and Inglett, 2006). This characteristic coincides with other studies of cookies made with both maize (Belorio, Sahagún and Gómez, 2019; Mancebo, Rodriguez and Gómez, 2016) and wheat (Lee and Inglett, 2006). The control doughs made with WF had lower values of G', G" and G*, but greater tg delta than the doughs made with maize, which could be related to the great amount of water presented in the formulation. Nevertheless, both doughs were properly laminated, which indicates that doughs made with wheat (lower G' and G") were less sticky than those made with WMF. This effect could be related to both the high amount of protein in WF and the water absorption capacity of wheat proteins (Belorio, Sahagún and Gómez, 2019; Manley, 2000; Pareyt and Delcour, 2008). With respect to fat substitution by psyllium paste, in all cases, the values of G’, G” and G* decreased and tg delta increased. In this case, three different effects must be considered; on one hand is the fat elimination and on the other is the incorporation of psyllium and water in amounts necessary for correct manipulation of the doughs. It is known that the addition of psyllium to cookie dough increases the values of G’ and G” (Raymundo, Fradinho and Nunes, 2014), and the decrease in fat has a similar effect when increasing the hardness of the dough (Pareyt et al., 2009; Pareyt et al., 2010). However, these studies scarcely increased the dough hydration, and the greater hardness values can be attributed to the increase in the amount of solids. In our study, the decrease in rheologic values is due to the increase of dough hydration. Although it is possible to observe a trend of small values of G’, G” and G* with an increasing amount of water in the dough, among the wheat cookies there was no significant difference with respect to the water content of psyllium paste. It must be considered that the doughs were submitted to refrigeration before lamination and rheological analysis, so the fat partially solidified, which contributed to these high rheologic values. Nevertheless, it was possible to achieve correct lamination of all doughs, but it was not viable to produce cookies made with a lower water content, because it was difficult to laminate those doughs once they broke due to low cohesiveness. This showed that fat contributes to cohesion of the rest of the ingredients and to producing doughs which are easy to manipulate (Pareyt and Delcour, 2008; Wade et al., 1988), and it is necessary to use more hydrated doughs when substituting fat by psyllium or other hydrocolloids (Sudha et al., 2007; Zbikowska, Kowalska and Pieniowska, 2018). In general, the decrease in the values of G’, G” and G* and the increase in tg delta were more pronounced in doughs from maize cookies, because of these cookies’ high initial values of G’ and G”. In fact, cookies without fat, made with wheat or maize, had similar values among them compared to the controls. So, it should be considered that fat covers the grains of flour, avoiding water absorption and the development of a gluten network (Colla, Costanzo and Gamlath, 2018).

*Table 2. Rheological properties of cookie doughs.*

| **Sample** | **G' (× $10^4$) (Pa)** | **G" (× $10^3$) (Pa)** | **tg delta** | **G* (× $10^4$)** |
|---|---|---|---|---|
| Control WMF | 187.00 ± 65.05e | 167.00 ± 43.84e | 0.094 ± 0.020d | 189.00 ± 65.76e |
| WMF n = 4 | 13.50 ± 2.62cd | 35.90 ± 8.20cd | 0.260 ± 0.010a | 14.00 ± 2.76cd |
| WMF n = 5 | 8.18 ± 0.69bc | 23.00 ± 1.77bc | 0.280 ± 0.001ab | 8.49 ± 0.71bc |
| WMF n = 6 | 7.42 ± 0.58abc | 21.00 ± 0.99abc | 0.280 ± 0.010ab | 7.72 ± 0.59abc |
| Control WF | 16.70 ± 7.15d | 49.90 ± 15.42d | 0.310 ± 0.040b | 17.40 ± 7.35d |
| WF n = 4 | 2.79 ± 0.57ab | 11.90 ± 2.05ab | 0.430 ± 0.010c | 3.04 ± 6.01ab |
| WF n = 5 | 1.57 ± 0.13ab | 6.94 ± 0.36a | 0.450 ± 0.010c | 1.72 ± 0.13ab |
| WF n = 6 | 1.16 ± 0.21a | 5.30 ± 1.06a | 0.460 ± 0.010c | 1.28 ± 0.24a |

*Values with the same letter in the same column are not significantly different ($p <$ 0.05). WMF: white maize flour; WF: wheat flour; n: total parts of psyllium+water mix to make the paste; G': elastic modulus; G": viscous modulus; tg delta: loss factor; G*: complex modulus.*

Thus, while eliminating fat and increasing the content of water, a gluten network can be developed (Pareyt, Brijs and Delcour, 2010), which can increase dough elasticity and compensate for the decrease in elasticity that can occur in the case of water elimination, but only in cookies made with WF.

### *3.2 Cookie properties*

Physical characteristics of cookies are indicated in Table 3. With respect to the cookie dimensions, the diameter and spread factor in both types of cookie decreased when fat was substituted by psyllium paste, regardless of the amount of water in the paste, and a trend for cookies with large diameters was even observed when increasing the water content. The decrease in diameter and spread factor was similar for both type of cookies, although wheat cookies had lower spread factor values than maize cookies. Nevertheless, fat substitution produced thicker cookies, as observed in Figure 1, but this effect was more evident in wheat cookies. Previous studies also found a decrease in cookie diameter when reducing the fat content (Blanco-Canalis et al., 2018; Pareyt, Brijs and Delcour, 2010; Rodríguez-Garcia et al., 2013; Sudha et al., 2007). This effect was attributed to the inhibitory action of fats on the gluten network, which generates harder doughs (Pareyt, Brijs and Delcour, 2010), since fat significantly affects neither cookie dough setting nor collapse (Pareyt et al., 2009). In the case of wheat cookies, an increase in water content can contribute to greater development of the gluten network (Pareyt, Brijs and Delcour, 2010) which enhances this explanation for the results obtained for cookies made with wheat. However, this explanation was not valid in the case of gluten-free cookies. This effect was attributed to the high values of G’ and G” for doughs with a reduced fat content, but in their case the amount of water in the formulation was not modified (Blanco-Canalis et al., 2018). In our study, doughs without fat had the smallest values of G’ and G”, since it was necessary to increase the amount of water in the formulation, so this explanation could not be accepted either. Other studies also found a decrease in cookie diameter when incorporating psyllium as a substitute for flour in the formulation (Fradinho, Nunes and Raymundo, 2014). Nevertheless, these studies are not comparable to ours, because they did not reduce the fat content or the amount of water in the formulations, which can explain the thickness of doughs, because of the thickening character of these hydrocolloids due to their high capacity to retain water. A feasible explanation for this effect is related to the fat melting during baking. It is known that fats generate doughs with higher values of G’ and G” than oils (Mancebo et al., 2017) and greater thickness in a farinograph (Jacob and Leelavathi, 2007). Thus, dough consistency is reduced when the fat melts, which favours both relaxation and expansion of dough (Pareyt and Delcour, 2008). This effect does not occur in doughs without fat. So, this effect would be common to cookies made with wheat and maize flour, but greater development of the gluten network would also be observed for wheat cookies, which could explain the increase in thickness of these cookies without fat. This result was previously found by Blanco-Canalis et al. (2018) and is related to the fact that the gluten network generates an extensible structure which is able to retain the gases produced during baking.

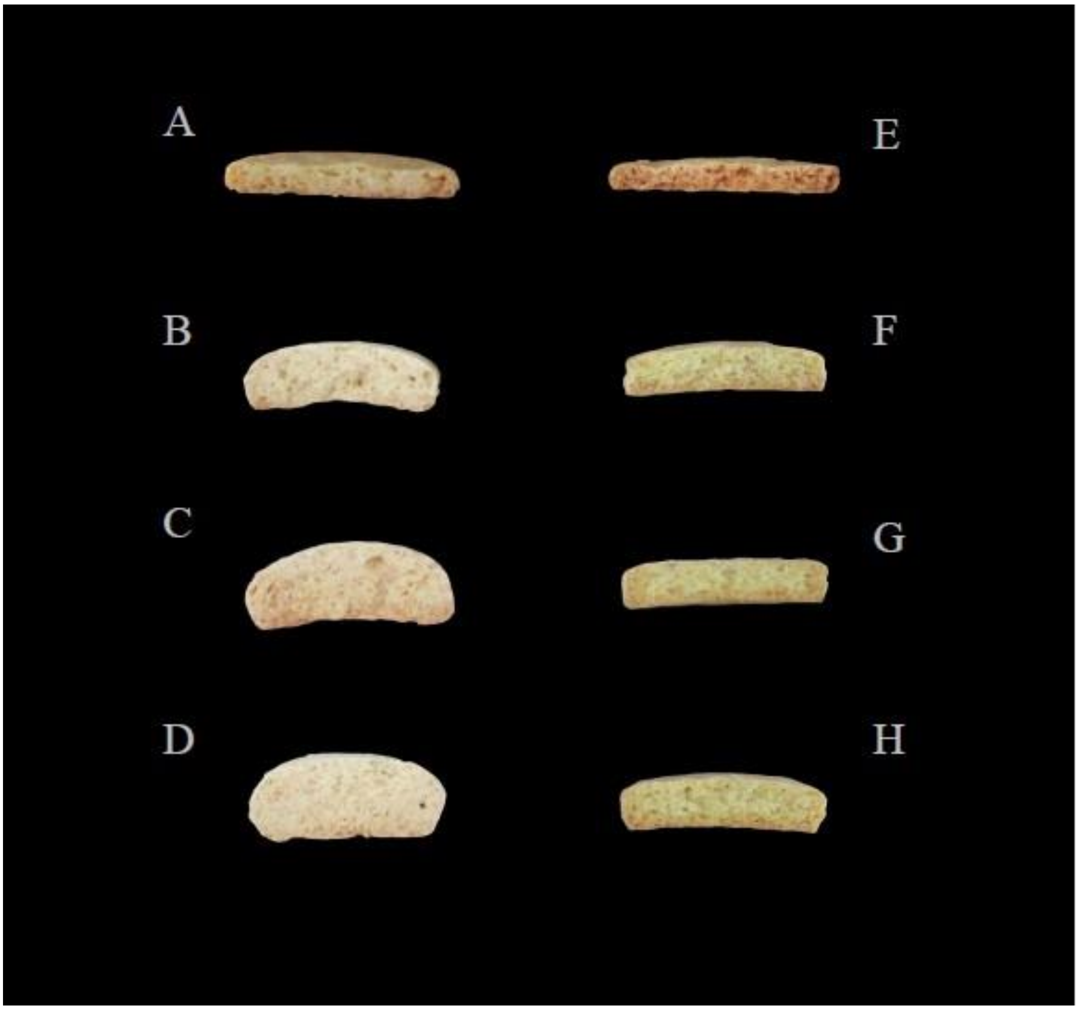

*Figure 1: Images of the cross section of final cookies (controls and cookies with fat substitution). n: total parts of psyllium+water mix to make the paste; A: Control WF; B: WF n=4; C: WF n=5; D: WF n=6; E: Control WMF; F: WMF n=4; G: WMF n=5; H: WMF n=6.*

*Table 3. Physical properties of cookies with fat substitution.*

| **Sample** | **Average diameter (mm)** | **Spread factor** | **Hardness (N)** | **Gradient (N/mm)** | **Moisture (%)** |
|---|---|---|---|---|---|
| Control WMF | 54.55 ± 3.80b | 8.17 ± 0.12e | 16.92 ± 0.31a | 25.52 ± 1.16ab | 2.34 ± 0.64a |
| WMF<br>n = 4 | 43.07 ± 0.43a | 4.19 ± 0.28c | 137.41 ± 9.56c | 122.96 ± 21.84c | 6.03 ± 0.01b |
| WMF<br>n = 5 | 44.60 ± 0.08a | 4.01 ± 0.13c | 137.83 ± 2.82c | 102.70 ± 7.11c | 6.71 ± 0.31b |
| WMF<br>n = 6 | 45.20 ± 0.50a | 4.15 ± 0.05c | 136.43 ± 9.03c | 109.59 ± 38.81c | 6.28 ± 0.30b |
| Control WF | 52.15 ± 2.16b | 7.17 ± 0.06d | 35.39 ± 5.64a | 58.15 ± 0.69b | 1.54 ± 0.64a |
| WF n = 4 | 43.80 ± 0.33a | 3.01 ± 0.13b | 127.54 ± 15.37c | 21.08 ± 14.64ab | 10.43 ± 0.62c |
| WF n = 5 | 44.29 ± 0.36a | 2.65 ± 0.16a | 76.00 ± 4.83b | 12.84 ± 1.46a | 11.94 ± 0.46d |
| WF n = 6 | 45.03 ± 1.13a | 2.41 ± 0.03a | 59.71 ± 17.11b | 5.38 ± 3.84a | 13.09 ± 0.87d |

*Values with the same letter in the same column are not significantly different ($p < 0.05$). WMF: white maize flour; WF: wheat flour; n: total parts of psyllium+water mix to make the paste.*

Considering the hardness of the cookies, an increase was observed when the fat content was reduced; this effect was previously found in other studies (Blanco-Canalis et al., 2018; Pareyt, Brijs and Delcour, 2010; Rodríguez-Garcia et al., 2013; Sudha et al., 2007; Zoulias, Oreopoulou and Tzia, 2002). Some of these studies justified this effect by the lubricating action of the fats. However, Pareyt, Brijs and

Delcour (2010) defatted the cookies and showed that this effect was maintained, so it seems that the lubricating action of fats does not influence the cookies texture. Another explanation is related to the cookies internal structure. Thus, previous studies have shown that cookies with less expansion during baking are harder (Belorio, Sahagún and Gómez, 2019; Mancebo et al., 2018; Rocha-Parra et al., 2019). Nevertheless, in our study differences were also observed between the behaviour of wheat and maize cookies. Cookies made with WMF had a great increase in both the hardness and the gradient of the curve for cookies with fat substitution, independent of the amount of water in the paste. For their part, cookies made with WF also showed an increase in hardness, but it was smaller and decreased on increasing the water content in the paste. In addition, the gradient of the curve reduced with fat substitution, and it was bigger with a greater amount of water in the paste. This could be related to the final moisture content of cookies, since it increased in cookies made with WMF and the addition of psyllium. This effect could be explained by both the high water content in the formulation and the high water retention capacity of psyllium (Dello-Staffolo, Sato and Cunha, 2017). However, in the formulations with a high water content, this excess was not retained, and the final moisture content was similar to that in all the cookies without fat. Despite this, the increase in moisture was much bigger for the wheat cookies, and it was greater in formulations made with pastes containing a high amount of water. This could be related to development of the gluten network in this type of cookie, because this network has a high capacity to retain water during the baking process (Boukid et al., 2019). For development of the gluten network, a minimum quantity of water and mechanical work is necessary. During the elaboration of cookie dough, there is mechanical work, but the amount of water, in addition to the presence of fat, can be a limiting condition for doughs with less water content, such as the controls. Obviously, in cookies made with WMF this network did not develop because of the absence of the specific proteins in this type of flour.

With respect to the colour of the cookies (Table 4), the substitution of fat by psyllium paste generated lighter cookies (Figure 2), independent of the water content in the pastes, and this effect was more intense in cookies made with WMF. In these cookies, fat substitution produced cookies with small values of a*, without affecting b*. Nevertheless, cookies made with WF had reduced values of b*, as previously observed by Conforti, Charles and Duncan (1997), but it was more significant for more diluted pastes, and no significant differences were observed for a*. An increase in lightness in bakery products with a reduced amount of lipids has been observed before in cakes (Belorio, Sahagún and Gómez, 2019). In the case of studies with cookies, Blanco-Canalis et al. (2018) observed a trend of increasing lightness when the fat was reduced, even if it was not significant. This could have been generated because the maximum reduction was 50%. Other studies found high lightness values for cookies with more than 50% fat substitution (Conforti, Charles and Duncan, 1997; Sudha et al., 2007). This effect is related to the influence of lipids in the Maillard reaction, since substances generated in their oxidation modify the final colour of the product (Hidalgo and Zamora, 2000).

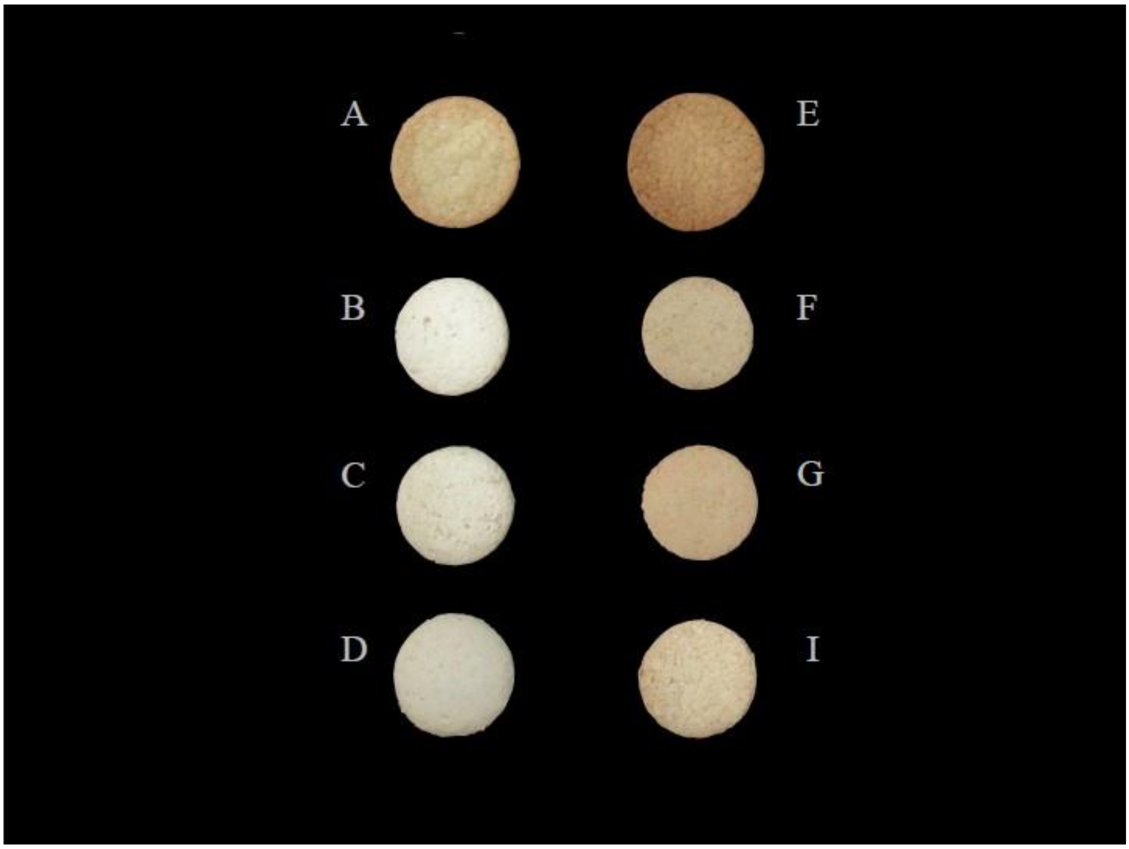

*Figure 2: Images of the final cookies obtained: controls and cookies with fat substitution. n: total parts of psyllium+water mix to make the paste; A: Control WF; B: WF n=4; C: WF n=5; D: WF n=6; E: Control WMF; F: WMF n=4; G: WMF n=5; H: WMF n=6.*

*Table 4. Colour parameters of cookies with fat substitution.*

| Sample | L* | a* | b* |
|---|---|---|---|
| Control WMF | 60.84 ± 3.22a | 7.56 ± 1.74d | 22.75 ± 2.82c |
| WMF n = 4 | 75.00 ± 2.08bcd | 3.85 ± 0.03c | 21.35 ± 0.31bc |
| WMF n = 5 | 73.47 ± 1.46bc | 3.81 ± 0.36c | 22.02 ± 0.66c |
| WMF n = 6 | 74.53 ± 0.66bcd | 3.72 ± 0.23bc | 22.37 ± 1.63c |
| Control WF | 71.00 ± 2.98b | 2.38 ± 2.38abc | 21.31 ± 1.39bc |
| WF n = 4 | 77.17 ± 0.14cd | 1.92 ± 0.46abc | 18.65 ± 0.05ab |
| WF n = 5 | 77.07 ± 0.70cd | 1.24 ± 0.52ab | 18.43 ± 0.45ab |
| WF n = 6 | 77.85 ± 1.47d | 0.79 ± 0.25a | 17.76 ± 0.86a |

*Values with the same letter in the same column are not significantly different ($p < 0.05$). WMF: white maize flour; WF: wheat flour; n: total parts of psyllium+water mix to make the paste.*

### *3.3 Sensory evaluation*

With respect to the sensorial evaluation of the cookies (Table 5), the controls were evaluated as better in all cases, and the cookies made with maize were evaluated as better than the wheat cookies, which was related to the high scores with respect to smell and taste. When fat was eliminated from cookie formulations, all the parameters had decreased evaluation scores, but for the wheat cookies it was more marked for the visual aspect than in maize cookies, which is logical since they were more different with respect to the control, because the cookies were thinner and more voluminous (Figure 1). On the other hand, cookies made with WMF and without fat had a bigger decrease in the scores related to chewing, flavour and texture, which could be related to the excessive hardness of these cookies. These parameters had a strong influence on the global score and meant that the reduction in the acceptance of wheat cookies without fat was greater than in WMF cookies.

*Table 5. Sensory acceptability of cookies with fat substitution.*

| **Sample** | **Appearance** | **Odour** | **Taste** | **Texture** | **Overall acceptability** |
|---|---|---|---|---|---|
| Control WMF | 7.03 ± 1.42c | 6.37 ± 1.55c | 7.01 ± 1.64c | 6.58 ± 1.72c | 7.04 ± 1.36c |
| WMF<br>n = 5 | 5.84 ± 1.70b | 5.37 ± 1.54ab | 4.49 ± 2.17a | 3.93 ± 2.00a | 4.52 ± 1.76a |
| Control WF | 6.67 ± 1.46c | 5.47 ± 1.72b | 6.00 ± 2.00b | 6.07 ± 1.81c | 6.22 ± 1.66b |
| WF<br>n = 4 | 4.99 ± 2.04a | 4.86 ± 1.46a | 4.93 ± 1.92a | 4.60 ± 2.11b | 4.81 ± 1.85a |

*Values with the same letter in the same column are not significantly different ($p < 0.05$). WMF: white maize flour; WF: wheat flour.*

## 4. Conclusion

The substitution of fat present in cookies by a paste made with psyllium and water generated a decrease in dough G' and G'', and an increase in tg delta. It also produced cookies with a smaller diameter, lower spread factor and harder texture. However, while the elimination of fat and the increase in water content of cookies made with WF can partially explain these changes, in gluten-free cookies they must be explained by the modifications in dough rheology and the influence of fats in its development during baking, such as the effect on its microstructure. The addition of a paste made with psyllium and water was not able to reverse the changes produced by fat reduction, and as it was necessary to add more water to the formulation, the cookies had a high moisture content. Nevertheless, the use of psyllium paste could be useful in partial reduction of fat content.

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## Conflict of Interest:

The authors declare that they have no conflict of interest.

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# CONCLUSIONES

# Conclusión

Tras los estudios realizados en la presente tesis doctoral, se puede afirmar que el psyllium, hidrocoloide de origen natural con importantes ventajas nutricionales, puede utilizarse como sustituto del gluten o de la grasa en la elaboración de productos de panadería y bollería.

- La presencia del psyllium aumenta las propiedades de hidratación del almidón de maíz y esos efectos son similares a los obtenidos con la goma xantana.

- El uso del psyllium junto al almidón de maíz no cambia significativamente la luminosidad de los geles producidos y la dureza de estos geles disminuye con el aumento de la concentración del psyllium.

- La hidratación óptima de formulaciones con psyllium para la elaboración de panes sin gluten es similar al de la goma xantana, generando panes con volúmenes específicos y pérdidas de peso similares.

- Panes con volúmenes específicos similares elaborados con HPMC presentan mayor dureza que los panes elaborados con psyllium o goma xantana.

- El uso de una mezcla de psyllium y agua como sustituto de la grasa en la elaboración de bizcochos permite una reducción de un 25% del aceite sin presentar diferencias significativas respecto al control.

- La producción de un bizcocho más sano se podría obtener reduciendo hasta un 75% el aceite de la fórmula, ya que estos bizcochos presentan una buena aceptabilidad.

- En la elaboración de galletas sin gluten es necesaria la presencia de un cierto porcentaje de partículas finas en la harina para conferir cohesividad a la masa y permitir su laminado. Pero un mayor tamaño de partícula incrementa la expansión y reduce la dureza de las galletas.

- El uso de una pasta de psyllium y agua como sustituto de la grasa genera galletas más duras y reduce la expansión.

- Las galletas con gluten, con psyllium y sin grasa presentan mayor humedad final que las sin gluten debido a la mayor hidratación de las masas y a la presencia del gluten.

- La sustitución del 100% de la grasa por pastas de psyllium y agua en galletas genera productos de baja calidad. Se recomiendan sustituciones parciales.

# ANEXO

# Anexo

Este anexo incluye otras publicaciones en las que el autor de la presente tesis también ha contribuido, aunque no forman parte de la tesis doctoral.

1. Jribi, S., Sahagún, M., Belorio, M., Debbabi, H., & Gómez, M. (2020). Effect of sprouting time on dough and cookies properties. *Journal of Food Measurement and Characterization, 14,* 1595-1600. https://doi.org/10.1007/s11694-020-00407-2

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