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TESIS DOCTORAL:

REFORMULACIÓN DE PANES Y GALLETAS DE MASA CORTA SIN GLUTEN: CAMBIOS EN LA REOLOGÍA DE LAS MASAS Y EN LA CALIDAD DE LOS PRODUCTOS

Presentada por Camino Martínez Mancebo para optar al grado de Doctora por la Universidad de Valladolid

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HACEN CONSTAR QUE:

La memoria titulada "Reformulación de panes y galletas de masa corta sin gluten: cambios en la reología de las masas y en la calidad de los productos" que presenta Dña. Camino Martínez Mancebo para optar al grado de Doctor por la Universidad de Valladolid, ha sido realizado bajo su dirección en La Escuela Técnica Superior de Ingenierías Agrarias de Palencia y que reúne las condiciones para ser defendida por su autora.

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Fdo: Manuel Gómez Pallarés

Fdo: Pedro A. Caballero Calvo

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Resumen

La falta de gluten en productos horneados, y especialmente en panificación, supone un reto para los científicos y tecnólogos de alimentos, ya que las harinas libres de gluten son incapaces de formar una masa viscoelástica con una funcionalidad adecuada para estos productos. En los últimos años se han logrado mejoras en los productos horneados sin gluten con el uso de ingredientes que intentan imitar las propiedades físico-químicas del gluten como gomas, hidrocoloides, proteínas y emulsionantes. Sin embargo, los productos horneados libres de gluten disponibles en el mercado continúan siendo de baja calidad.

El presente trabajo de tesis se ha centrado en la optimización de fórmulas de panes y galletas sin gluten para mejorar su calidad a través de la adecuada selección de los ingredientes y su cantidad. Para ello se han utilizado técnicas reológicas, texturales y técnicas sensoriales entre otras.

El primer objetivo de esta tesis consistió en optimizar mezclas de almidón de trigo, almidón de maíz y harina de arroz, a través de la metodología de superficie de respuesta (MSR). El estudio reveló que el uso de harina de arroz en panes sin gluten mejora el sabor y el color (corteza con menos brillo), con respecto a panes a base de almidón. Sin embargo, la harina de arroz redujo el volumen específico del pan y el número de alveolos por cm^2, pudiendo ser mejorados los panes con la adición de almidón de trigo, ya que éste proporcionó un mayor volumen específico y menor dureza que el almidón de maíz. Estos resultados confirman que la combinación de harina y almidones en la elaboración de panes sin gluten dan productos de mejor calidad que aquellos elaborados a partir de harina o almidones de forma separada.

La adición de grasas es una práctica común en panes comerciales sin gluten, puesto que facilitan el proceso mecánico y mejoran la estructura del pan a través de la estabilización de las burbujas. Sin embargo, no se conoce bien qué tipo y cantidad de grasa es la adecuada para este tipo de productos. Por este motivo, se continuó el estudio de panes sin gluten evaluando el efecto de la adición de diferentes cantidades de margarina y aceite de girasol sobre la calidad de los mismos. Los resultados mostraron que la incorporación de un 20 % o más de aceite de girasol mejoró el volumen específico y la textura del pan, el color de la corteza (tonos más marrones y oscuro) y aumentó el número de alveolos en la miga, mientras que la adición de margarina tuvo un efecto negativo sobre la calidad del pan reduciendo su volumen y aumentando la dureza.

Para finalizar el estudio de la mejora de la calidad de los panes sin gluten, se estudió el efecto de la combinación de dos hidrocoloides; HPMC y psyllium. Para ello, nuevamente se utilizó la MSR para optimizar la fórmula a base de harina de arroz con tres niveles de HPMC, psyllium y agua. Ambos hidrocoloides aumentaron los valores de G*, en el ensayo oscilatorio. Sin embargo, en el ensayo reológico de fluencia-recuperación el psyllium disminuyó la capacitancia, mientras que el HPMC apenas aumentó los valores de capacitancia. Esto coincide con los resultados de los parámetros de calidad del pan, ya que, el psyllium redujo el volumen específico y aumentó la dureza de los panes, mientras que el HPMC apenas ejerció un efecto en estos parámetros en los niveles de estudio. Esto implica que el ensayo de fluencia-recuperación puede ser un mejor predictor de la calidad de panes sin gluten que el ensayo oscilatorio. El estudio también demostró que, una mayor cantidad de agua añadida disminuye el efecto de los hidrocoloides, tanto en la reología de la masa como en el pan sin gluten, por lo que es necesario adaptar la cantidad de agua adicionada en función del tipo y cantidad de hidrocoloide añadido.

La presente tesis también se ha focalizado en la mejora de galletas sin gluten, en concreto "sugar-snap cookies" (SSC). Primeramente, se analizaron un amplio rango de harinas de cereales y pseudocereales sin gluten para determinar la harina más adecuada para este tipo de galletas, según sus características físico-químicas. En general, las galletas elaboradas con harinas sin gluten redujeron la expansión en el horneado y aumentaron la dureza de las galletas en comparación con la galleta control de trigo. Se encontraron correlaciones positivas entre el tamaño de partícula de la harina y el ratio de expansión en el horneado, ya que galletas elaboradas con harinas finas presentaron diámetros menores que las elaboradas con harinas de granulometría gruesa. También se encontraron correlaciones negativas entre el ratio de expansión y el contenido en almidón dañado y las propiedades de hidratación de las harinas.

Por último, se estudió el efecto de la adición de proteína de guisante y almidón de maíz en galletas de arroz. En general, la incorporación de proteína aumentó las propiedades de hidratación de la mezcla harina-almidón-proteína, así como la consistencia de la masa, produciendo galletas con una expansión limitada en el horneado, menor dureza y color más oscuro. De forma opuesta, la adición de almidón redujo las propiedades de hidratación de la mezcla, produciendo galletas con mayores dimensiones, sin afectar a la textura ni al color. Las galletas con mayor contenido en proteína presentaron mejor aceptación en la evaluación sensorial que aquellas con alto contenido en almidón y sin proteína. Se puede concluir, que la adición de almidón y proteína puede ser una estrategia adecuada para ajustar la fórmula de galletas sin gluten, con el fin de obtener la masa y las características del producto final deseadas en función de las necesidades de los fabricantes de galletas.

Finalmente, cabe destacar que, en todos los estudios las características reológicas de las masas sin gluten estuvieron relacionadas con los parámetros de calidad del producto final, tanto en panes como en galletas. Por lo tanto, el estudio de la reología de masas puede ofrecer información relevante a los tecnólogos de alimentos, permitiendo una selección adecuada de los ingredientes para la optimización del producto final.

Abstract

The lack of gluten in baked goods, especially in bread, is a challenge for food scientists and technologists, as gluten-free flours are unable to form a viscoelastic dough with proper functionality for this kind of products. In recent years, some progress has been made in gluten-free bakery products using ingredients that attempt to mimic the physicochemical characteristics of gluten such as gums, hydrocolloids, proteins and emulsifiers. However, commercial gluten-free baked goods continue to be of low quality.

This thesis work has focused on the optimization of gluten-free bread and cookie formulas choosing proper ingredients and their quantities in order to improve their quality. For this purpose, rheological, textural and sensory techniques among others were used.

The first objective of this doctoral thesis consisted on the optimization of a blend of wheat starch, maize starch and rice flour, employing a response surface methodology (RSM). The study revealed that using rice flour in gluten-free breads improves the taste and colour (less crust brightness) in comparison to starch-based breads. However, rice flour reduced the bread specific volume and the number of alveoli per cm^2, but rice-breads can be improved by adding wheat starch, since it provided higher specific volume and lower hardness than maize starch did. These results confirm that the combination of flour and starches in the preparation of gluten-free breads give products of better quality than those made from flour or starch separately.

Fat addition is a common practice in commercial gluten-free breads, since it makes the mechanical process easier and improves the bread structure through stabilization of bubbles. However, the type and amount of fat suitable in these products is not yet very well known. Therefore, the study was continued evaluating the effect of adding different amounts of sunflower oil or margarine in the quality of gluten-free bread. The results showed that the incorporation of 20% or even more of sunflower oil improved the bread specific volume and texture, the crust color (browner and darker) and increased the number of alveoli in the crumb. On the other hand, adding margarine had a negative effect on bread quality, reducing the specific volume and increasing the hardness.

In order to conclude the research about the improvement of gluten-free bread quality, the combination of two hydrocolloids (HPMC and psyllium) was studied. For this purpose, once again RSM was used to optimize the formula with rice flour and three levels of HPMC, psyllium and water. Both hydrocolloids increase the G* values in the dynamic oscillatory

test. Nevertheless, in creep-recovery test, psyllium decreased compliance values, meanwhile HPMC barely increased compliance. Therefore, the results of the creep-recovery test were in agreement with the effect on bread quality parameters, since the psyllium reduced the specific volume and increased hardness of bread, while the HPMC scarcely modified these parameters in the levels studied. As a consequence, our study showed that the creep-recovery test may be a better predictor of bread quality characteristics than the oscillatory test. Furthermore, the study showed that a higher amount of water in dough led to a lower effect of inclusion of hydrocolloids, both on dough rheology and on the characteristics of gluten-free breads, thereby it is necessary to adjust the amount of water added depending on the type and amount of hydrocolloid used.

This thesis also focused on improving gluten-free cookies, namely "sugar-snap cookies" (SSC). Firstly, a wide range of gluten-free cereal and pseudocereals flours were studied in order to determine the most suitable flour for such cookies, based on their physicochemical characteristics. In general, gluten-free flours produced cookies with lower spread during baking and greater hardness than wheat cookies. Interestingly, there was a positive correlation between particle size and spread factor, since cookies made from fine-grain flours showed smaller diameters than cookies made from coarse-grained flours. There were also negative correlations between spread factor and damaged starch and hydration properties.

Finally, the effect of the addition of pea protein and maize starch in cookies made from rice flour was also investigated. Generally, protein incorporation increased hydration properties of the flour-starch-protein mixture and dough consistency, producing cookies with limited spreading in the baking time, lower hardness values and darker colour. On the contrary, maize starch addition reduced hydration properties and gave rise to cookies with higher dimensions, but the texture and colour were not affected by the starch. Cookies with higher protein content showed higher acceptability than cookies with higher starch content and no protein addition in the sensorial evaluation. Therefore, protein and starch can be used to adjust the formula in gluten-free cookies in order to get the desired cookie dough and final product characteristics depending on the needs of cookie manufacturers.

Interestingly, for every study we noticed that the rheological characterisation of gluten-free doughs was related to quality indicators of the end-products, either breads or cookies. Accordingly, the study of dough rheology could give important information for food technologists, allowing the appropriate selection of ingredients to optimise the final product.

Índice

INTRODUCCIÓN

1. Introducción

1.1. ENFERMEDADES PATOLOGICAS ASOCIADAS AL TRIGO

Las intolerancias y sensibilidades que se encuentran relacionadas con los componentes del trigo incluyen la enfermedad celíaca (EC), la alergia al trigo (AT) y la sensibilidad al gluten no celiaca (SGNC).

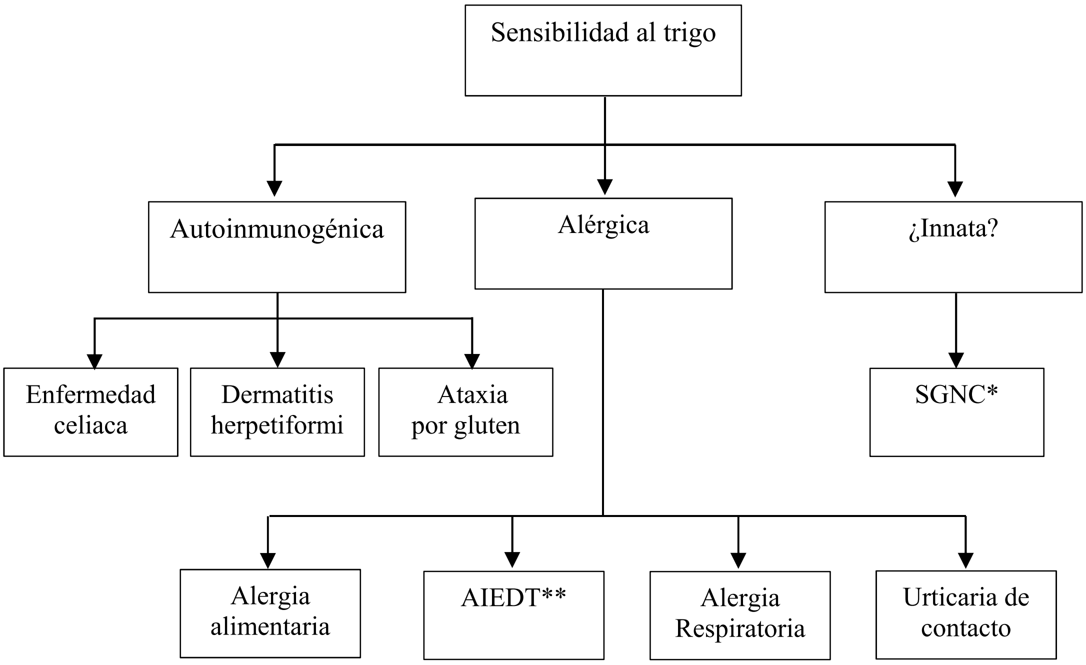

Figura 1- Clasificación de las patologías asociadas al trigo (Scherf y col., 2016); *Sensibilidad al gluten no celiaca; **Anafilaxia inducida por ejercicio dependiente del trigo

1.1.1. Enfermedad celiaca

La celiaquía puede ser definida como una enfermedad que se caracteriza por la inflamación del intestino delgado superior (duodeno y yeyuno) en individuos genéticamente susceptibles causada por la ingestión de gluten. La literatura científica percibe en parte la EC como un trastorno de sensibilidad a los alimentos donde el gluten es el antígeno responsable, y en parte, como una condición autoinmune (presencia de auto-anticuerpos séricos contra la transglutaminasa tisular (TG2), otras transglutaminasas (TG3, TG 6) y el endomisio) (Scherf y col., 2016).

La EC es provocada por el consumo de algunas proteínas presentes en los cereales, que por lo general se clasifican como prolaminas (Darewicz y col., 2008; Shan y col., 2002). Las prolaminas de trigo, llamadas gliadinas, junto con la proteína glutelina (gluteninas en el caso del trigo), forman el complejo llamado gluten, responsable de las propiedades reológicas y mecánicas de la masa y alimentos a base de trigo. En esta forma la proteína podría aislarse a partir de diversas especies del género *Triticum* (trigo blando, espelta, trigo duro, kamut), así como *Triticale*. Entre otras prolaminas, que también podrían provocar problemas en las personas con enfermedad celíaca, están las de centeno (secalinas) y las de cebada (hordeínas) (ambas de la tribu *Triticae*). Por otra parte, la avena (*Avena sativa*), a pesar de pertenecer a la misma subfamilia (*Pooideae*), pertenece a la tribu *Aveneae* por lo que posee algunas características diferentes. Aunque la avena se considera como una fuente segura de proteínas para la mayoría de los celíacos, ya que contiene bajas cantidades de prolaminas (Dickey y col., 2008; Fric y col., 2011; Kaukinen y col., 2013), se han observado reacciones inmunológicas en algunos pacientes (Arentz-Hansen y col., 2004, Richman, 2012) a algunas variedades de avena**.**

Son numerosos los síntomas asociados a la EC, los cuales varían entre las personas. Los síntomas en una manifestación típica son principalmente intra-intestinales, mientras que las manifestaciones atípicas tienen síntomas mayormente extra-intestinales (Slot y col., 2015). Concretamente, los síntomas intestinales clásicos que conlleva esta enfermedad son diarrea, vómitos, esteatorrea y dolor abdominal, pero pueden ocurrir otros síntomas como estreñimiento o reflujo gastroesofágico. Las manifestaciones extra-intestinales más comunes son generalmente causadas por la malabsorción de nutrientes, por ejemplo la deficiencia en vitaminas y minerales como anemia, disminución de la densidad mineral de huesos, dolor de huesos y fracturas, hipoplasia del esmalte dental y ceguera nocturna. En niños y adolescentes, son complicaciones asociadas a la EC ratios inferiores de crecimiento y retardo de la pubertad. En mujeres puede afectar a la salud reproductiva provocando infertilidad. Un menor número de pacientes muestra síntomas neurológicos y psiquiátricos como ansiedad y depresión, entre otros. La asociación del autismo, esquizofrenia o cáncer con la EC presenta aun cierta controversia. Además, la EC puede manifestarse de forma asintomática. En este caso, el paciente no muestra otros síntomas aparte de atrofia de las vellosidades o cambios serológicos (Scherf y col., 2016).

Antiguamente, la enfermedad celiaca era considerada como una patología infantil, sin embargo los avances en medicina, con una mejora en la detección serológica seguida de una pequeña biopsia intestinal, han puesto de manifiesto que el 1% de la población mundial padece esta enfermedad, y sólo del 10 al 15% de esta población ha sido diagnosticado y tratado (Guandalini y Assiri, 2014). Por lo tanto, la EC es una de las sensibilidades alimentarias más frecuentes en todo el mundo. Además la celiaquía puede aparecer a cualquier edad, siendo más común en mujeres que en hombres (Scherf y col., 2016).

Como enfermedad con un componente autoinmune (TG2), la EC comparte características importantes con otras enfermedades autoinmunes (Kaukinen y col., 2010). Por ejemplo, la diabetes *mellitus* tipo 1 es una de las enfermedades autoinmunes más asociadas con la enfermedad celiaca. Otras enfermedades asociadas a la celiaquía son la dermatitis herpetiforme y la ataxia del gluten. La dermatitis herpetiforme, también conocida como enfermedad de Duhring, es la contraparte cutánea de la EC, sin embargo su prevalencia es mucho menor que la de la EC. Los síntomas típicos son intensa picazón y ardor de la piel por grupos herpetiformes de pápulas pruriginosas urticantes y vesículas, especialmente en los codos, los antebrazos superiores, las nalgas y las rodillas. La ataxia por gluten es una manifestación neurológica también asociada a la EC (Scherf y col., 2016).

Actualmente, el único tratamiento disponible para los celiacos es una dieta estricta libre de gluten, por el resto de su vida. En los casos con EC clásica, los pacientes recuperan la función de la mucosa completamente y desaparecen los síntomas en pocos meses.

1.1.2. Alergia al trigo

La alergia al trigo se define como una reacción inmunológica adversa a las proteínas de trigo (Sapone y col., 2012). Dependiendo de la vía de exposición a los alérgenos y los mecanismos inmunológicos subyacentes la alergia al trigo se clasifica como: i) alergia alimentaria inmediata; ii) anafilaxia inducida por ejercicio dependiente del trigo (AIEDT); iii) alergia respiratoria, y iv) urticaria de contacto. Los anticuerpos específicos IgE juegan un papel crucial en las alergias alimentarias. Datos recientes indican que la alergia al trigo ocurre con más frecuencia de lo que se había pensado, al menos en ciertas regiones. Esto hace que sea un problema de salud pública de magnitud similar a la del EC. Varios estudios indican que la alergia al trigo representa entre el 11% y el 25% de toda la población clínica que presenta una alergia alimentaria (Hischenhuber y col., 2006).

Le alergia al trigo inmediata se produce dentro de unas pocas horas de la ingestión de alimentos. Las manifestaciones clínicas son similares a las de otras alergias a los alimentos con síntomas en la piel (por ejemplo, hinchazón, picazón, urticaria), y en el aparato respiratorio (como dificultad respiratoria, obstrucción bronquial) y gastrointestinal (por ejemplo, hinchazón, dolor abdominal dolor, diarrea). Incluso la anafilaxia se ha descrito en relación a la alergia al trigo. Uno o más de estos síntomas pueden ser sufridos tanto por niños como por adultos, pero posiblemente con diferentes patrones clínicos. Se estima una prevalencia del 0,2 al 4% (Scherf y col., 2016).

AIEDT es una forma peculiar de la alergia al trigo, ya que el consumo de trigo por sí solo no induce síntomas (Morita y col., 2007). Las reacciones alérgicas son provocadas sólo cuando se añade un cofactor de activación, como la actividad física, después de la ingesta de productos derivados del trigo. Otros cofactores son la ingesta de ácido acetilsalicílico, otros fármacos anti-inflamatorios no esteroideos, alcohol o el estado general del paciente. Los síntomas típicos de pacientes con AIEDT son prurito, urticaria, angioedema, disnea, hipotensión, síntomas gastrointestinales y obstrucción de las vías respiratorias. AIEDT es una enfermedad poco frecuente, con una prevalencia estimada por debajo del 0,1%.

El asma y la rinitis alérgica son respuestas alérgicas a la inhalación de harinas de trigo y otros cereales como el centeno y la cebada (Tatham y Shewry, 2008). Junto con otras proteínas de diferentes orígenes, las proteínas de trigo también están involucradas en la urticaria inmunológica de contacto. El sistema inmunológico del paciente se ha sensibilizado previamente al agente alergénico, que puede ocurrir a través de la exposición a la piel, membranas mucosas, tracto respiratorio o el tracto gastrointestinal. La mayoría de los casos de alergia respiratoria y urticaria de contacto están relacionados con el trabajo, siendo frecuente en molineros, panaderos y manipuladores de alimentos.

1.1.3. Sensibilidad al gluten no celiaca (NCGS)

NCGS, que frecuentemente se denomina sensibilidad al gluten o trigo, ha sido recientemente descrito como un trastorno mediado por el gluten (Catassi y col., 2013; Fasano y col., 2015; Mansueto y col., 2014). NCGS se puede definir como un trastorno dependiente del gluten (trigo) con síntomas similares a los de la EC, pero con la histología del intestino delgado mayormente normal y falta de anticuerpos anti-TG2. La prevalencia de NCGS en la población general es aún desconocida. El principal problema en el diagnóstico NCGS es la diferenciación de otros trastornos relacionados con el gluten, tales como EC y AT. En la actualidad, el diagnóstico se hace por exclusión.

1.2. IMPORTANCIA TECNOLÓGICA DEL GLUTEN EN PAN Y GALLETAS

Son numerosas las definiciones del término gluten, las cuales varían en gran medida de si se trata dentro de la disciplina de ciencia de los alimentos o medicina. En enero de 2009, se adoptó un Reglamento de la Comisión Europea sobre los alimentos sin gluten basado en la Norma del Codex 118-1979 (Codex Alimentarius, 2008). En el contexto de la intolerancia al gluten y para los efectos del presente Reglamento, "gluten" es una fracción proteica de trigo, centeno, cebada, avena o sus variedades cruzadas y derivados de los mismos, que algunas personas no toleran y que es insoluble en agua y solución al 0.5 mol/L de NaCl (Matos y Rosell, 2014).

El gluten frecuentemente se define como la proteína estructural esencial que contribuye enormemente a la apariencia y estructura de muchos productos fermentados y horneados tradicionales a base de trigo. Está compuesto principalmente por dos fracciones proteicas: la gliadina, que contribuye esencialmente a la viscosidad y extensibilidad de la masa, y la glutenina, responsable de la elasticidad de la masa (Lazaridou y col., 2007; Sluimer, 2005). En el caso del pan, esta masa viscoelástica tiene la habilidad de formar una fina película que retiene el gas procedente de la fermentación, permitiendo que la masa se expanda; además es responsable de la retención de agua y contribuye directamente a la formación de una estructura alveolar en la miga, que tras el horneado, confiere una textura suave y ligera y proporciona palatabilidad (Cauvain, 2007).

La red de gluten se forma cuando la harina de trigo se hidrata y se somete al trabajo mecánico del amasado, incluso a mano. La ausencia de gluten en panes provoca que el resultado del amasado de la harina, agua y otros ingredientes sea una masa sin cohesión y que, tras el horneado se obtenga un pan de textura inadecuada que tiende a desmigarse, con un color muy pobre y otros defectos sensoriales (Gallagher y col., 2004).

En la elaboración de galletas libres de gluten los problemas que provoca la ausencia de gluten en el pan, normalmente no se producen, ya que el desarrollo de la red de gluten en galletas es mínimo y muchas veces no deseable (Gallagher y col., 2004). Sin embargo, el gluten ejerce un efecto sobre la masa y las propiedades de la galleta que implica que su ausencia modifique la reología y características de la galleta final, como es en el caso de galletas de masa corta tipo "sugar-sap" que se estudiaran en el presente trabajo. De este modo, Pareyt y otros (2008a) observaron, en este tipo de galletas elaboradas a partir de almidón de trigo y gluten, como niveles altos de gluten disminuyeron el peso de la pieza de masa, su densidad, pegajosidad y dureza. Altos niveles de gluten también retrasaron el inicio de la expansión sin apenas afectar el fin de la expansión, lo que dio lugar a galletas de menor diámetro. La mayor expansión se obtuvo cuando no se añadió gluten pero estas galletas sin gluten presentaron una estructura inaceptable. Sin embargo, al no formarse la red de gluten, este efecto podría tratarse del efecto de cualquier proteína. Souza y col. (1994), llegaron a la conclusión de que el contenido total de proteínas es más importante para la calidad de la galleta que la composición de las proteínas. De este modo, galletas con bajo contenido en proteínas incrementan la expansión de la galleta. A pesar de ello, en la literatura no hay un consenso acerca de su papel y su influencia en la calidad de la galleta.

1.2.1. Reología en las masas panarias y de galletería sin gluten

La masa es el estado intermedio entre la harina y el producto terminado (Sai Manohar y Haridas Rao, 1999). La calidad de la masa queda determinada por la cantidad y calidad de los ingredientes empleados presentando cada masa unas cualidades particulares de consistencia, elasticidad, resistencia y moldeabilidad. Este comportamiento reológico de la masa es determinante en el proceso de formación y manipulación de la masa (amasado y laminado), la fermentación y el horneado, así como en la calidad de productos como el pan, galletas, pasta o noodles (Lazaridou y Biliaderis, 2009).

En la fabricación de galletas, el comportamiento reológico de la masa es de tal importancia que una de las clasificaciones de galletas se basa en el tipo de masa. Según Wade (1988) se distingue entre dos tipos fundamentales de galletas: “de masa dura” (*hard dough*) y “de masa corta” (*short dough*), siendo una de las diferencias fundamentales entre estos dos tipos de galletas la existencia o no de largas cadenas de gluten que confieren a la masa extensibilidad (Manley, 1991). Cuando el gluten está desarrollado la masa presenta un comportamiento viscoelástico, dando lugar a masas duras. Sin embargo, cuando la cantidad de grasa y azúcar es alta, el gluten no se puede desarrollar completamente y la masa “se queda corta” (falta de extensibilidad). Las galletas de masa corta aumentan su tamaño (*spread* o esparcimiento) durante los primeros estadios del proceso de horneado, mientras que las galletas de masa dura tienden a encoger longitudinalmente (Manley, 1991). En las galletas de masa corta el objetivo fundamental durante el amasado es que el gluten se desarrolle lo mínimo posible, aunque debe lograrse la dispersión adecuada de los ingredientes (Baltsavias y col., 1999).

En el caso del pan, la matriz de gluten es un factor decisivo en las características reológicas, la tolerancia al amasado y la capacidad de retención de gas (Schofield, 1986). El gluten proporciona a la masa extensibilidad, elasticidad y cohesividad, y contribuye a la capacidad de absorción de agua (Wieser, 2007).

En este sentido, parece importante el estudio de las propiedades reológicas de las masas para determinar los cambios en el procesado de masas de pan y galletas en las que se está sustituyendo el gluten, ya que las masas producidas a partir de formulaciones libres de gluten carecen de las características cohesivas y elásticas propias de las masas obtenidas a partir de la harina de trigo, debido a la ausencia de gluten. Sin embargo, sólo un pequeño número de estudios utilizan métodos reológicos fundamentales para medir las propiedades mecánicas de las masas sin gluten. Estas mediciones incluyen tanto pruebas oscilatorias como barridos de deformación y frecuencia, así como pruebas de fluencia-recuperación en formulaciones de masa sin gluten (Gujral y Rosell, 2004a, b; Lazaridou y col., 2007; Lazaridou y Biliaderis, 2009; Marco y Rosell, 2008c; Moreira y col., 2011; Sivaramakrishnan y col., 2004). Los ensayos de cizallamiento oscilante son ampliamente utilizados para evaluar simultáneamente las propiedades viscoelásticas de la masa, a través de la determinación del módulo elástico (G’) y el módulo viscoso (G’’). Sin embargo, en las masas panarias sin gluten, también se requieren pruebas reológicas utilizando grandes deformaciones para simular el procesado de estas masas (Lazaridou y col., 2007; Sivaramakrishnan y col., 2004). Los ensayos de fluencia-recuperación son útiles para establecer vínculos con los resultados de las técnicas empíricas. Esta técnica consiste en la aplicación de una tensión constante, y cuando se libera la tensión se observa una cierta recuperación, debida a los intentos del material para volver a su forma original.

Más escasos aún son los estudios que relacionan las características reológicas de la masa y la calidad del pan que podrían ser una guía para realizar una adecuada selección de ingredientes para optimizar los productos libres de gluten (Lazaridou y Biliaderis, 2009). Matos y Rosell (2014) recopilaron los datos de las características de la masa y la calidad del pan de recetas de pan sin gluten de diversos estudios para buscar una posible relación. Cuando se llevó a cabo el mismo análisis no se encontraron correlaciones significativas entre el módulo viscoelástico y los parámetros de volumen específico ni textura de miga. Lo mismo ocurrió al relacionar las propiedades de empastado obtenidas con el RVA al relacionarlo con los mismos parámetros de textura del pan. Probablemente esto fue debido al reducido número de datos disponibles de los manuscritos publicados. Sin embargo, la recopilación de los resultados de la literatura científica reveló que los parámetros de textura de miga de pan sin gluten obtenidos en ensayos de TPA están correlacionados con la consistencia de la masa y la retrogradación del almidón o gelificación; más concretamente, una baja consistencia de la masa y bajos valores de gelificación se relacionan con migas más suaves.

1.2.2. Ingredientes capaces de imitar las propiedades del gluten

La falta de gluten en productos horneados, y especialmente en panificación, supone un reto para los científicos y tecnólogos de alimentos, y para la industria alimentaria, ya que las harinas libres de gluten son incapaces de formar una masa viscoelástica con una funcionalidad acorde con las necesidades tecnológicas de sus procesos de fabricación (Arendt, y col., 2008). Este es uno de los problemas potenciales en el manejo de masas sin gluten, que en los últimos años se ha mejorado con el uso de ingredientes que intentan imitar las propiedades físico-químicas del gluten como gomas, hidrocoloides, ingredientes a base de proteínas y emulsionantes o el uso de nuevas tecnologías como el procesado enzimático, altas presiones hidrostáticas o la tecnología de extrusión (Deora y col., 2014; Matos y Rosell, 2014).

1.2.2.1. Hidrocoloides

Los hidrocoloides son polisacáridos solubles en agua con una estructura química diversa y que presentan un amplio rango de propiedades tecno-funcionales en función del tipo de hidrocoloide por las que son ampliamente utilizados en la industria alimentaria (Mir y col., 2016). Los hidrocoloides se utilizan habitualmente en panificación sin gluten como agentes estructurales en combinación con harinas y/o almidones libres de gluten tratando de sustituir al gluten por su función estabilizadora. Éstos imitan parcialmente el comportamiento viscoelástico y cohesivo del gluten (Toufeili y col., 1994), incrementando la capacidad de retención de gas y mejorando la textura del pan por su capacidad de formación de geles (Rosell y col., 2001). Los hidrocoloides aumentan la estabilidad de las burbujas en la masa mediante el aumento de la viscosidad, la floculación y la coalescencia.

El efecto del hidrocoloide tanto en las propiedades de la masa como en la calidad del pan sin gluten, depende principalmente de la naturaleza, el contenido y las propiedades de los hidrocoloides (Mil y col., 2016). Su efecto dependerá también de los parámetros del proceso y de las interacciones con otros ingredientes (Hager y Arendt, 2013; Houben y col., 2012). Se ha de tener especial cuidado con las interacciones hidrocoloide-almidón, ya que son altamente dependientes del tipo de hidrocoloide seleccionado (Gularte y Rosell, 2011). En panificación sin gluten se han investigado un gran número de hidrocoloides tales como hidroxipropilmetilcelulosa (HPMC), metilcelulosa (MC), carboximetilcelulosa (CMC), psyllium, goma de algarrobo, goma guar, goma xantana, carragenano o alginato (Lazaridou y col., 2007; Sabanis y col., 2009; Sabanis y Tzia, 2011; Sciarini y col., 2010a; Sciarini y col., 2012). Éstos se han utilizado solos o combinados, basándose en su funcionalidad tecnológica en las masas y el producto final (Xue y Ngadi, 2009; Torbica y col., 2010). Dentro de estos hidrocoloides, los más utilizados en panes sin gluten son el HPMC y la goma xantana por ser los que mejor imitan al gluten proporcionando los mejores resultados en el producto final (Anton y Artifield, 2008; Hager y Arendt, 2013). Sin embargo, son varios los estudios en los que se demuestra que el HPMC mejora en mayor medida el volumen y estructura del pan sin gluten en comparación con la goma xantana (Crockett y col., 2011b; Nishita y col., 1976; Schober, 2009).

El HPMC es un éter de celulosa obtenido por la unión química de grupos hidroxipropilo y metilo al esqueleto β-1,4-D-glucano celulósico (Hager y Arendt, 2013). Se caracteriza por su gran capacidad de retención de agua y por ser capaz de estabilizar las burbujas de gas por la acumulación de las mismas en un microgel elástico (Bell, 1990; Crockett y col., 2011b; Peressini y col., 2011). El HPMC se diferencia de la goma xantana en su superficie activa que le ayuda a dispersar las burbujas de aire favoreciendo la formación de pequeñas burbujas, previniendo la coalescencia de las mismas. Esto, añadido al aumento de la viscosidad hace que el HPMC estabilice las burbujas en la interfaz gas-líquido. Esto conlleva a que, durante el horneado, la capacidad de retener burbujas aumenta, y por lo tanto aumenta el volumen específico del pan sin gluten (Lazaridou y Biliaderis, 2009). Mientras tanto, la goma xantana forma una solución espesa en la que solo son atrapadas burbujas aisladas de mayor tamaño (Schober, 2009) que da lugar a panes de menor volumen en comparación al HPMC. Otros efectos del HPMC en el pan final son la disminución de la dureza de la miga y un aumento de la vida útil (Andersson y col., 2011; Peressini y col., 2011; Sciarini y col., 2010a).

Aunque con menos frecuencia, el uso del psyllium comienza a tener relevancia. El psyllium es una fibra que se añade por lo general con otros hidrocoloides como el HPMC (Cappa y col., 2013; Haque y Morris, 1994; Mariotti y col., 2009; Zandonadi y col., 2009). Este hidrocoloide mejora las propiedades físicas de las masas sin gluten debido a la formación de una red de gel débil capaz de atrapar CO_2 debido a la gelificación inducida por el calentamiento de HPMC y la capacidad de absorción de agua de psyllium (Haque y Morris, 1994; Mariotti y col., 2009). La estructura del gel débil que forma el psyllium es muy similar a la del gel formado por la goma xantana (Morris, 1990).

1.2.2.2. Proteínas

Debido a que las proteínas de harinas provenientes de cereales naturalmente libres de gluten no son capaces de formar una red similar a la creada por el gluten, numerosos estudios se han centrado en la suplementación con proteínas funcionales en la elaboración de panes libres de gluten (Gallagher y col., 2003; Marco y Rosell 2008a,b; Moore y col., 2004; Ziobro y col., 2013).

Algunos autores han comprobado cómo la adición de proteínas lácteas durante la formación de la masa aumenta la capacidad de retención de agua, disminuyen la pegajosidad de la masa y la hacen más plástica (Gallagher y col., 2003; 2004). También se ha demostrado un aumento en el volumen específico y la distribución de las burbujas (Van Riemsdijk y col., 2011) así como una mejora de las propiedades texturales, el sabor y el color de la corteza (Krupa-Kozac y col., 2013). Sin embargo, la adición de ingredientes derivados de la leche conlleva algunos efectos adversos ya que las personas con inflamación del intestino por EC, habitualmente sufren también intolerancia a la lactosa. Esto es debido a que la actividad de la lactasa se ve reducida o anulada por la atrofia de las vellosidades intestinales (Ortolani y Pastorello, 2006).

Las proteínas de huevo también han sido estudiadas como sustitutos del gluten. La coagulación de la yema de huevo durante el tratamiento térmico crea geles termo-irreversibles, por lo que se emplea en la industria de panadería como emulsionante. Moore y col. (2006) obtuvieron un aumento en el volumen del pan sin gluten así como un incremento del número de poros gracias a la adición de huevo entero en polvo. Por otro lado, Jonagh y col. (1968) y Eggleston y col. (1992) encontraron que la adición de albúmina de huevo y huevo ya espumado, podían incrementar la capacidad de retención de gas durante la preparación de la masa y estabilizar la estructura del pan. Otros autores han probado la adición de proteínas de soja, ricas en aminoácidos esenciales como la lisina, comprobando el aumento del volumen y la mejora de las propiedades texturales del pan (Crockett y col., 2011a; Sánchez y col., 2002).

1.2.2.3. Enzimas

En la última década, se ha incrementado el número de estudios enfocado al procesado enzimático de masas sin gluten, con el uso de enzimas que pueden mejorar la funcionalidad de las proteínas presentes en las harinas libres de gluten o las proteínas suplementadas en la formulación. Su aplicación se ha centrado en la modificación de las características reológicas y microestructurales de las masas sin gluten, buscando una mejora en la estabilización de gas en la masa y las propiedades texturales de los panes. Además, las enzimas generalmente están reconocidas como seguras (GRAS), lo que implica la ausencia de efectos negativos para la salud asociados a su excesivo consumo (Rosell, 2009).

Son numerosas las enzimas que pueden modificar las proteínas y se clasifican según su mecanismo de acción como de entre-cruzamiento directo, indirecto y proteólisis. Inicialmente, la formación de enlaces entre las proteínas presentes en las formulaciones sin gluten con el uso de enzimas, se consideró la mejor forma de imitar la funcionalidad del gluten en masas sin gluten (Rosell, 2009). Para este fin se utilizaron transglutaminasas (TGasa) y diferentes oxidasas. Sin embargo, no existe un consenso en la efectividad del uso de estas enzimas en la mejora de sistemas sin gluten.

Renzetti y Rosell, (2016) afirmaron que los entrecruzamientos de proteínas a través de TGasa puede ser una alternativa para crear redes internas en los sistemas sin gluten. Sin embargo, un aumento en los entrecruzamientos no está relacionado directamente con una mejora en la panificación de esta harina. Añadido a esto, la actividad de la TGasa depende de la accesibilidad de la glutamina y la lisina en las proteínas (Gerrard, 2002; Houben y col., 2012; Renzetti y col., 2008), por ello la fuente de harina tiene una gran influencia en la acción de la TGasa debido a su composición en aminoácidos. Además de las limitaciones que presenta el uso de la TGasa microbiana para la mejora de los sistemas sin gluten, cierta preocupación se ha planteado por su homología con la TGasa involucrada en la enfermedad celíaca, y su mayor reactividad de la IgA de los pacientes celíacos contra las prolaminas de panes tratados con TGasa (Cabrera-Chávez y col., 2008; Dekking y col., 2008). En la actualidad, no existen estudios que apoyen estas hipótesis.

Diferentes oxidasas (lipoxigenasa, sulfhidrilo oxidasa, glucosa oxidasa, polifenol oxidasa y peroxidasa) se han utilizado en aplicaciones de panadería tradicional (con harina de trigo), debido a su acción en el fortalecimiento de la masa y de estabilización (Van Oort, 1996), y como agentes de blanqueo de masa (Gelinas y col., 1998) o en la mejora de la calidad de pan fresco. En panificación sin gluten se ha estudiado el uso de glucosaoxidasas (GO) en masas de arroz (Gujral y Rosell, 2004b) y en masas de maíz, sorgo, arroz integral y teff (Renzetti y Arendt, 2009a) mostrando un efecto diferente en función del tipo de harina y la concentración de la enzima. Los estudios sobre la aplicación de lacasas en panes sin gluten son limitados. Renzetti y col. (2010) observaron un aumento del volumen específico y la suavidad de la miga en panificaciones con harina de avena que se había tratado con un preparado de lacasas y endo-β-glucanasa. Flander y col. (2011) también observaron un aumento en el volumen específico de panes de avena que combinaban la acción de una lacasa y una xilanasa.

Desde el punto de vista molecular, las diferencias en la estructura de las proteínas determina la eficacia del tratamiento enzimático. De este modo, los tratamientos enzimáticos dependen en gran medida del tipo de harina y los niveles de proteínas suministradas, así como del tipo y cantidad de enzima añadida. En consecuencia, cada tratamiento enzimático de masas sin gluten requiere una optimización específica del tipo y cantidad de enzimas (Renzetti y Rosell, 2016).

1.3. IMPORTANCIA DEL ALMIDÓN EN PRODUCTOS HORNEADOS SIN GLUTEN

La eliminación del gluten aumenta la importancia del papel del almidón para proporcionar estructura y textura en los productos de panadería, bollería y galletería sin gluten. Esto es debido a que los ingredientes principales en este tipo de elaboraciones son almidones y harinas libres de gluten, provenientes de diferentes orígenes botánicos, en las que el almidón es el componente principal.

Los cereales estimados como seguros para los enfermos celiacos son el arroz, maíz, sorgo, mijo y teff. También son seguros otros granos como los pseudocereales: trigo sarraceno, amaranto y quinoa. La inclusión de harinas provenientes de leguminosas también constituye una posibilidad para mejorar las propiedades nutricionales de este tipo de productos. Aunque estas fuentes de almidón mencionadas se pueden usar de forma separada, ya sea en forma de harina o de almidón, la mayoría de los estudios en los que se desarrollan productos sin gluten realizan mezclas de diferentes harinas (Brites y col., 2010; Moreira y col., 2013; Sciarini y col., 2010b; Torbica y col., 2012) o mezclas de harinas y almidones (López y col., 2004; Onyango y col., 2011; Sánchez y col., 2002).

1.3.1. Almidones libres de gluten

El almidón es uno de los polímeros de almacenamiento de muchas plantas. Se compone de dos tipos de moléculas, amilopectina y amilosa. En ambos casos, el bloque de construcción es un residuo de glucosa (α-D-glucopiranosa), formando una estructura lineal de enlaces α-1,4-glicosídicos en la amilosa y ramificaciones adicionales α-1,6-glicosídicos en las moléculas de amilopectina. Las diferencias en la estructura de ambos polímeros resultan en una variación significativa en sus propiedades. La amilosa es mucho más propensa al proceso de cristalización, llamado retrogradación, y puede formar geles duros y películas fuertes, mientras que la amilopectina puede ser dispersada en agua y retrograda mucho más lentamente, lo que se traduce en geles suaves y películas débiles (Fredriksson y col., 1998; Pérez y Bertoft, 2010).

El almidón nativo es organizado en gránulos presentes en las semillas, raíces y tubérculos, así como en los tallos, hojas, frutas e incluso en el polen. Se diferencian en el tamaño (0,1-200 mm) y forma (esferas, elipsoides, polígonos, plaquetas y túbulos irregulares) dependiendo principalmente de su origen botánico. El gránulo de almidón es parcialmente cristalino, lo que afecta de manera significativa a sus propiedades (Singh y col., 2003). El origen botánico también condiciona la presencia de componentes no almidonosos que afectan a las propiedades físico-químicas y funcionales del almidón (Perez y Bertoft, 2010).

Las fuentes de almidón más importantes son las de maíz, trigo, tapioca, arroz y patata. Mientras el uso de almidones de cereales naturalmente libres de gluten no presenta ninguna controversia, el uso de almidón de trigo (también los almidones de cebada, centeno o avena) en productos sin gluten ha sido cuestionada por su posible contaminación con gluten (Capriles y Areas, 2014). De hecho, el uso de almidón de trigo fue prohibido en algunos países y permitido en otros en función de la definición de la dieta sin gluten (Thompson, 2001). Sin embargo, hoy en día ya existe en el mercado almidón libre de gluten gracias a la mejora de los procesos de extracción del mismo. Peräaho y col. (2003) mostraron que los pacientes con enfermedad celiaca que ingirieron almidón de trigo sin gluten presentaron un histológico y una recuperación clínica similar a la de aquellos pacientes que siguieron una dieta libre de gluten sin consumo de almidón de trigo.

Las propiedades de los productos finales e intermedios a base de almidón son en gran parte determinadas por las propiedades del almidón utilizado en la receta. El origen y el tipo de almidón ejercen una influencia en la microestructura, la reología de la masa, la retención de agua y la estructura y calidad de los productos finales. De este modo, el tamaño de gránulo de almidón, su capacidad de absorción de agua y la solubilidad del mismo, juegan un papel importante en las propiedades finales de los productos a base de almidón. También son de gran importancia la capacidad de gelatinización y gelificación del almidón, las propiedades reológicas de la pasta y gel, y la capacidad de la amilosa para formar un complejo con las grasas y los emulsionantes de alimentos (Witczak y col., 2016). La microestructura del almidón también influye notablemente en el aspecto, la textura y la estabilidad del producto final (Kálab y col., 1995). La tecnología utilizada en la molienda de la harina, o en el aislamiento del almidón, ejerce un impacto en la granulometría, el peso específico y la cantidad de almidón dañado, determinando las propiedades de hidratación y empastado de las mismas (Witczak y col., 2016).

Son varios los estudios que demuestran la importancia de las características de los gránulos de almidón en el procesado de masas sin gluten y la calidad del producto final. Los almidones que forman un gel rígido se pueden utilizar para mejorar la consistencia de las masas sin gluten. Estos efectos están relacionados con las modificaciones en el comportamiento del almidón en el calentamiento en el horneado y las interacciones entre el almidón y otros compuestos presentes en el sistema, que depende de las características del almidón (Witczak y col., 2016).

El almidón de maíz dentado (normal o común) presenta una elevada funcionalidad tecnológica en productos de pan, galletas o pasta sin gluten. Al cocinar el almidón de maíz nativo da lugar a pastas densas, de elevada viscosidad y apariencia translúcida que cuando se enfrían forman un gel resiliente, de textura corta y apariencia opaca. Podemos distinguir, según el ratio amilosa:amilopectina entre el almidón de maíz normal, que contiene entre un 20 y un 30% de amilosa, y el almidón de maíz waxy que contiene <1% de amilosa. Al cocinar el almidón de maíz normal mantiene la integridad del granulo mucho mejor que el almidón waxy. Esto sugiere que las moléculas de amilosa del maíz normal están entrelazadas con las de amilopectina para mantener esta integridad del granulo durante el calentamiento en agua (Jane, 2009). Por otro lado, el almidón de maíz waxy debido a su alto contenido en amilopectina (>99%) forma pastas que mejoran la resistencia a la formación del gel y la sinéresis durante el almacenamiento en frío (Mason, 2009). El almidón de maíz nativo, además de en productos horneados sin gluten, se utiliza con frecuencia como molde de almidón, polvo secante, agente de relleno y de carga, y como agente estabilizante en ciertos alimentos enlatados como salsas o rellenos para tartas cuya vida útil es de unas horas o pocos días (Mason, 2009). Sin embargo, el envejecimiento del gel formado por el almidón de maíz nativo provoca su contracción hasta que se rompe desprendiendo un líquido de la matriz. Por ello, generalmente, se evita su uso en productos congelados y se sustituye o combina con almidones modificados.

Tradicionalmente, el almidón de arroz tiene asociadas ciertas ventajas frente a otros almidones. Estas características incluyen su hipoalergenidad, digestibilidad, aceptación por el consumidor, sabor neutro, granulos pequeños (2-10 μm), color blanco, mayor estabilidad frente a la congelación-descongelación de pastas, mayor resistencia al ácido, así como un amplio rango de ratio amilosa:amilopectina. Dependiendo del ratio de amilosa:amilopectina del almidón, podremos encontrar geles de diferente fuerza y texturas y diferencias en su resistencia a la acidez. Podemos distinguir de forma básica entre dos variedades de arroz según el contenido en amilosa: waxy (0-2% de amilosa) y común. Dentro de las variedades de arroz común, también se pueden clasificar por su contenido en amilosa en bajo (9-20%), medio (20-25%) y alto >25%). El contenido en amilosa también está asociado al tamaño del grano, de este modo, podemos distinguir entre granos largos (20-25% de amilosa), medianos (14-18%) y cortos (15-23%). Los almidones de arroz extraídos de arroz común tienden a tener altos picos de viscosidad al cocinarlo y una vez enfriado, así como dar lugar a una pasta de textura pálida y corta. La textura de una pasta de almidón de arroz waxy tiende a ser larga y fibrosa. Las aplicaciones del almidón de arroz dependerán de si proviene de arroz waxy o de arroz común. De este modo, para productos horneados sin gluten se utiliza el almidón de arroz común que también tiene otras aplicaciones (aglutinante en salsas, puddings, rellenos de pastelería, alimentación infantil,…). En cuanto al almidón de arroz waxy se utilizan en helados y aderezos para ensalada por su textura suave y cremosa. Además, el almidón de arroz waxy presenta una estabilidad superior en los ciclos de congelación-descongelación. Esta estabilidad permite su uso como espesante en rellenos de tartas, salsas y sopas congeladas (Mason, 2009).

La mayor aplicación de almidón de trigo continua siendo en productos horneados con gluten, ya que sus propiedades son muy similares a las del almidón presente propiamente en la harina de trigo, ingrediente mayoritario en este tipo de productos. No existe consenso en si existen diferencias en el volumen de pan obtenido cuando se utilizan almidones extraídos de distintos tipos de trigo (Mason, 2009). Sin embargo, se ha demostrado que los panes elaborados con almidón de trigo son de mayor calidad en comparación con los panes elaborados a partir de almidones de maíz, sorgo, avena, arroz y patata (Maningat y col., 2009). Las propiedades funcionales únicas del almidón de trigo también han sido demostradas en otros productos horneados con gluten como tartas, bizcochos, galletas o crackers, ya que equilibran o mejoran la calidad del producto final (Maningat y col., 2009). Resultados similares fueron obtenidos por Ronda y col. (2011) en bizcochos sin gluten cuando compararon el efecto de diferentes fuentes de almidón, donde el almidón de trigo dio lugar a mayores volúmenes. Esto es debido a un control de la expansión de la masa durante el horneado provocada por una mayor temperatura de gelatinización del almidón de trigo. Esto implica que el paso de masa, como emulsión aireada, a sólido, como estructura porosa, se produzca más tarde y puede aumentar de volumen durante mayor tiempo (Houben y col., 2012). El almidón de trigo ha sido menos estudiado en elaboraciones sin gluten debido a que históricamente no se garantizaba la ausencia de gluten. Éste almidón se caracteriza por poseer una distribución de tamaño bimodal debido a las diferentes estructuras moleculares de las cadenas de amilopectina, presentando forma de disco los gránulos grandes y forma esférica los gránulos pequeños (Jane, 2009). Esta distribución bimodal del almidón de trigo podría influir en el procesado de productos sin gluten. El almidón de trigo nativo así como el modificado se utilizan también en rebozados y empanados, cereales de desayuno, azúcar en polvo, toppings de helados, salsas,… Su función en estos productos es proporcionar adhesión, estructura, controlar la humedad y/o como espesante (Maningat y col., 2009).

Los almidones que provienen de raíces y tubérculos muestran un incremento en la viscosidad más pronunciado, además de mayores valores de viscosidad y de colapso (breakdown) que aquellos almidones que provienen de cereales. Los almidones de tapioca y patata también han sido ampliamente utilizados en panificación sin gluten. Se caracterizan por presentar contenidos en amilosa del 18% y 32 %, respectivamente, dando lugar a soluciones claras relativamente estables a pesar de su contenido en amilosa. Esto es debido a que la amilosa que contienen tiene un mayor peso molecular que la amilosa del maíz y quizá sea más ramificada (ambos factores interfieren en la retrogradación y la formación del gel). Los almidones de tapioca se han utilizado tradicionalmente como espesantes en puddings y rellenos de pastelería por su sabor neutro. Además se ha utilizado en alimentación infantil por su imagen natural. El almidón de tapioca nativo forma una pasta clara pero fibrosa y cohesiva, como el almidon de maíz waxy nativo, por lo que presenta ciertas limitaciones en su uso debiendo ser modificado para eliminar sus defectos texturales. El almidón de patata presenta gránulos largos que se hinchan y solubilizan de manera más rápida que los almidones de cereales. Produce pastas con alta viscosidad y cierta apariencia granulosa. Éste almidón, una vez cocinado, muestra una menor resistencia a las fuerzas de cizalla que los almidones de cereales. El almidón de patata nativo una vez cocinado y enfriado forma un gel claro, gomoso y flexible. Éste almidón se utiliza principalmente como espesante en sopas enlatadas para generar viscosidad aunque también como gelificante en golosinas, como espesante en rellenos de pastelería y en puddings instantáneos (Mason, 2009).

1.3.2. Harinas libres de gluten

En general, la funcionalidad de las harinas depende principalmente de su tamaño de partícula, de la distribución de las mismas (Houben y col., 2012), de su composición química así como de los posibles tratamientos sufridos (Witczak y col., 2016). Como componente principal, el almidón tiene un impacto directo sobre las propiedades de la harina, a parte de su apariencia física. Otros componentes de la harina son las proteínas de almacenamiento, carbohidratos no amiláceos, lípidos, minerales, vitaminas y enzimas. Estos componentes son altamente dependientes de su origen y procesamiento. El tipo de molienda y tamizado son decisivos en la composición química de la harina, porque el contenido de los componentes citados no es uniforme en varias partes de las semillas, lo que permite su separación física. El tipo de molienda también puede modificar las propiedades del almidón ya que una parte considerable de los gránulos de almidón son dañados en este proceso. El contenido en azúcares simples afecta significativamente a la fermentación, mientras que los polisacáridos no amiláceos participan en la absorción de agua y afectan a la reología de la masa. La proteína también afecta a la absorción del agua y al comportamiento reológico de la masa aunque su papel es mucho menos importante que en masas de trigo (Witczak y col., 2016).

La harina de arroz es una de las harinas más adecuadas para la elaboración de productos horneados sin gluten, debido a sus propiedades hipoalergénicas, su alta digestibilidad, su bajo contenido de sodio, su sabor suave y su apariencia pálida (Torbica y col., 2012). El arroz (*Oryza sativa*) es cosechado con la cáscara y posteriormente se descascarilla para obtener el denominado arroz integral. Éste es equivalente a los granos enteros de otros cereales sin cáscara, como el trigo. Eliminando el salvado y el germen del grano obtenemos el grano de arroz blanco. La harina de arroz blanco puede obtenerse de la molienda de los granos pulidos enteros o partidos, pero ya que los granos partidos son un subproducto de la industria arrocera y por lo tanto se venden a menor precio, la harina de arroz se obtiene normalmente de la molienda de éstos últimos. La molienda se realiza habitualmente sobre los granos blancos sin germen ya que la harina obtenida de la molienda del grano de arroz integral tiene una vida útil limitada debido a la actuación de las lipasas (Wilkinson y Champagne, 2004). El arroz presenta un bajo contenido proteico (7% aproximadamente) en comparación con otros cereales. Las limitadas propiedades funcionales de sus proteínas, ya que no forman una red proteica similar al gluten, hacen que en su aplicación en productos horneados requiera del uso de otros componentes que proporcionen textura y estructura en este tipo de productos (Capriles y Arêas, 2014). Por otro lado, estas proteínas parecen jugar un papel importante en las propiedades de hinchamiento de los gránulos del almidón, que supone un 80% del grano de arroz (Marshall y col., 1990). Como ya se ha comentado en el apartado 1.3.1. sobre almidones, la forma del grano se encuentra relacionada con el contenido en amilosa del almidón. De este modo, las variedades de grano largo tienen mayores temperaturas de gelatinización y contenidos de amilosa que las variedades de grano medio y corto, así como una mayor tendencia a la retrogradación (Moldenhauer y col., 2004).

Otra harina sin gluten muy utilizada es la harina de maíz (*Zea mays*). Esta harina presenta limitaciones en la elaboración de pan o galletas debido a su sabor y color amarillo característicos. Los panes de maíz se caracterizan por un color amarillento, escaso volumen específico y una miga densa y firme. Sin embargo, el color de los granos de maíz (proveniente del color del pericarpio) varía ampliamente dependiendo de la variedad desde blanco a amarillo incluso rojo y azul. El maíz blanco puede ser una alternativa interesante para productos sin gluten (Hager y col., 2012). En cuanto a su composición, el maíz destaca por su densidad energética debido al alto contenido en carbohidratos (73%) y grasas, aunque el contenido proteico es también importante (Walker y Li, 2008). Los granos de maíz contienen aproximadamente la misma cantidad de proteína que otros cereales (entre el 8 y el 10 %), pero gran parte está en forma de zeína. Como ocurre en el arroz, el contenido en amilosa del almidón de maíz también varía con la variedad (común o waxy).

El sorgo y el teff también son una alternativa para obtener una harina libre de gluten. El sorgo (*Sorghum bicolor*) se caracteriza por un grano que recuerda al de maíz en cuanto a que el endospermo está compuesto de una zona exterior rugosa y vítrea y una interior harinosa y opaca (Chandrashekar y Mazhar, 1999). El almidón presente en el sorgo se caracteriza por una alta temperatura de gelatinización respecto al almidón de patata y de trigo (Lineback, 1984) aunque hay diferencias respecto de los cultivares (Akingbala y Rooney, 1987). En lo que se refiere al teff (*Eragrostis tef*), es un cereal del que se obtiene harina integral, muy utilizado para realizar *injera* en Etiopía. También se utiliza como espesante por las características tecnológicas de su almidón, y para productos infantiles debido a su buen perfil de aminoácidos. Puede presentar diferentes colores que varían entre blanco, crema o marrón (Bultosa y Taylor, 2004).

En los últimos años ha aumentado el uso de harinas provenientes de algunos pseudocereales, tales como el amaranto, el trigo sarraceno o la quinoa. En términos botánicos, los pseudocereales no son cereales verdaderos, ya que son plantas dicotiledóneas a diferencia de los cereales, que son monocotiledóneas. Sin embargo, sus semillas se asemejan en función y composición a las de los verdaderos cereales (Alvarez-Jubete y col., 2010). Estos pseudocereales son elegidos en la elaboración de productos sin gluten, además de por ausencia de gluten, por su alto contenido en proteínas y minerales, así como por su valor nutricional (Schoenlechner y col., 2008). El amaranto (*Amaranthus sp*) es uno de los pseudocereales más comunes que, a pesar de no pertenecer a la familia de las gramineas, produce semillas pequeñas y secas que se asemejan en forma y usos a las de los cereales (Walker y Li, 2008). Su uso, tanto en forma de grano entero como en harina, se está incrementando como ingrediente minoritario en productos de panadería comercializados como "productos saludables". El trigo sarraceno (*Fagopyrum esculentum*) miembro de la familia de las poligonáceas, destaca por su contenido proteico, entre los más altos de las fuentes vegetales (Walker y Li, 2008). La harina de este pseudocereal es bastante oscura debido a que, a pesar de que el endospermo posee un color blanquecino, el salvado y partes de la cascarilla permanecen en el grano durante la molturación aportándole a la harina un color marrón. La quinoa (*Chenopodium quinoa*) presenta un alto contenido proteico junto con un contenido en aminoácidos bastante bien balanceado que le confiere una calidad nutricional superior a la mayoría de los cereales (Valencia-Chamorro, 2004). Las semillas deben ser limpiadas y pulidas antes de su utilización ya que poseen una cobertura amarga (Johnson y Ward, 1993).

1.4. PANIFICACIÓN SIN GLUTEN

La panificación es un proceso complejo que incluye diferentes pasos: amasado, fermentación, horneado y enfriamiento. El amasado es un paso clave en la panificación, ya que, el resultado de las fases posteriores de fermentación y horneado dependen de la homogeneización de los ingredientes, así como de la incorporación de aire en el amasado. Durante la fermentación, la estabilidad de las burbujas de gas, crucial para obtener un pan de volumen adecuado, se ve influenciada por las propiedades reológicas y viscoelásticas de la matriz. En la fermentación, se produce la expansión de la masa que resulta de la acción de las levaduras; responsable del aumento de la porosidad y los cambios en la estabilidad de la estructura alveolar. El CO_2, producido como resultado de la fermentación llevada a cabo por las levaduras, primero se disuelve en la fase líquida y posteriormente llena las celdas de gas formadas durante el amasado. Cuando la solución se satura, el dioxido de carbono se evapora en las celdas de gas, permitiendo que se expandan. La estabilidad de la masa refleja la capacidad de las celdas de gas de mantener su forma y volumen durante la expansión en la fermentación evitando el colapso de la estructura de la masa (Shehzad y col., 2010). En cuanto al horneado, engloba los fenómenos de evaporación del agua, expansión y aumento del volumen, inactivación enzimática y de la levadura, gelatinización del almidón, coagulación de la proteína y formación de la miga y la corteza (Capriles y Arêas, 2014; Sivam y col., 2010). Por último, en el enfriamiento del pan se produce una contracción de la miga y de la corteza, pérdida de humedad y peso.

En general, en panificación sin gluten las masas son más fluidas que las masas de trigo provocado por la falta de la red de gluten y, normalmente por un mayor contenido en agua. Por este motivo, frecuentemente son denominadas como batidos en lugar de masas (Houben y col., 2012) y se manejan de una manera similar a los batidos de pastelería en lugar de las típicas masas de pan (con harina de trigo).

Existen ciertas diferencias en el procesado para la elaboración de pan a base de harina de trigo y en panes sin gluten. En masas de pan a base de harina de trigo, un exceso de amasado provoca un colapso que da lugar a un menor volumen en el pan. Esto es debido a que, un exceso de amasado provoca daños en la red de gluten, que es la responsable de la retención del gas, escapándose el gas producido en la fermentación (Pyler y Gorton, 2009). Sin embargo, la retención del gas producido durante la fermentación en masas sin gluten, se basa en la consistencia de la masa, la cual no disminuye durante el amasado, y en la distribución de las burbujas del aire en la masa. El amasado, puede favorecer además de la incorporación de burbujas de aire, el acondicionado de la levadura para su crecimiento en las etapas iniciales lo que implica la producción de CO_2. Gómez y col., (2013) observaron que en masas sin gluten existe un tiempo mínimo de amasado necesario para la incorporación óptima de las burbujas de aire. Sin embargo, largos tiempos de amasado no dan lugar a masas que se colapsan, en lugar de esto, el volumen se puede incluso incrementar.

Los tiempos y temperaturas de fermentación también son importantes y se verán afectados por el tipo de formulación y el proceso de amasado. Un exceso de fermentación también puede provocar el colapso y ruptura de la estructura del pan escapándose el gas. Según Gómez y col. (2013) el tiempo óptimo de fermentación depende de la velocidad de desarrollo del volumen durante la fermentación, de modo que el óptimo se alcanza antes cuando el incremento en el volumen es más rápido. Estos autores observaron en masas sin gluten cómo los tiempos óptimos de fermentación establecidos estaban asociados al proceso de amasado. De este modo, tiempos largos de amasado produjeron más gas en las primeras etapas de fermentación, pudiéndose deber a dos motivos. El primero, debido a una mayor oxigenación durante el amasado que favoreció unas condiciones aerobias para el inicio de la fermentación llevada a cabo por las levaduras. Y el segundo, a un aumento en la temperatura al someter a la masa a un estrés mecánico más prolongado y/o más intenso, llegando de forma más temprana al rango óptimo de actuación de las levaduras (20-30ºC).

Sin embargo, a pesar del esfuerzo de científicos y panaderos en la adaptación de fórmulas y procesos para la obtención de panes libres de gluten de buena calidad, la elaboración de los mismos continua siendo un reto tecnológico. Tanto es así, que hoy en día los panes sin gluten comercializados continúan siendo en su mayoría de peor calidad que los panes de trigo, presentando menor volumen, menor intensidad de color y menor crujencia en la corteza (Matos y Rosell, 2012) además de su pobre composición nutricional, con bajo contenido en proteínas y elevada cantidad de grasas (Matos y Rosell, 2011).

1.4.1. Características necesarias en una harina para la elaboración de pan sin gluten

El tipo de cereal y la variedad, su composición química, el tamaño y distribución de las partículas de la harina, así como los tratamientos sufridos parecen ser importantes en la elaboración de pan sin gluten. Como ya se ha comentado, las harinas sin gluten más utilizadas son las de arroz, maíz y algunos pseudocereales, elegidos por su alto contenido en proteínas y minerales así como su valor nutricional (Schoenlechner y col., 2008). El uso de harina de arroz es preferido al uso de harina de maíz por su sabor neutro y color blanco encontrándose más extendido.

En lo que se refiere a la composición química de la harina, ya se ha hablado sobre la importancia del almidón en productos sin gluten en el punto 1.3. Sin embargo, a pesar de que se lleva investigando cerca de 25 años sobre la funcionalidad de las proteínas de almacenamiento en las masas libres de gluten, sigue sin haber muchos avances. Solamente se ha demostrado que la zeína (proteína del maíz) es capaz de formar una masa viscoelastica similar a la formada por el gluten. Esto solo se ha conseguido cuando la zeína ha sido extraída del maíz en su forma de α-zeína, la sub-clase más hidrofílica de la zeína (Taylor y col., 2016).

En cuanto al tamaño de partícula, de la Hera y col. (2013) proponen el uso de harinas de granulometría intermedia o gruesa frente a harinas finas ya que observaron cómo al elaborar panes a base de harina de arroz, aquellas harinas con un tamaño de partícula mayor produjeron panes con un volumen específico mayor y menor dureza.

Para mejorar la calidad sensorial y nutricional de los panes libres de gluten la mayoría de los estudios se han centrado en el uso de mezclas de harinas libres de gluten (Brites y col., 2010; Moreira y col., 2013; Sciarini y col., 2010b; Torbica y col., 2010) o el uso de mezclas de almidón y harina libre de gluten (López y col., 2004; Onyango y col., 2011; Sánchez y col., 2002). Estas mezclas pueden lograr mejores resultados que el almidón o la harina sola. Además, con el fin de formar una miga aceptable, las harinas libres de gluten requieren captar mayor contenido de agua que la harina de trigo (Capriles y Arêas, 2014). Teniendo esto en cuenta, Sánchez y col. (2002) aplicaron la metodología de superficie de respuesta (MSR) para optimizar una formulación de pan sin gluten a base de almidón de maíz, harina de arroz y almidón de tapioca, con y sin adición de soja. En la optimización el ingrediente predominante fue el almidón de maíz, seguido por la harina de arroz y en menor cantidad el almidón de tapioca. López y col. (2004) también estudiaron el uso de harina de arroz suplementado con almidón de tapioca y almidón de maíz en la producción de panes sin gluten. Primeramente estudiaron su efecto por separado, para posteriormente optimizar una mezcla donde la harina de arroz fue predominante por favorecer las características de textura y apariencia de la miga, así como por el color de la corteza. Sin embargo, los almidones también fueron incluidos, ya que el almidón de maíz proporcionó el mayor volumen y el almidón de tapioca favoreció la adhesividad, descendiendo el desmigado del pan, mejorando la compactación del pan. Las diferencias entre los resultados de estos dos estudios es debida a la adición de diferentes ingredientes a la fórmula y a las diferencias en el proceso (amasado, fermentación y horneado). Sciarini y col. (2010b) estudiaron el efecto de harina de soja en panes a base de harina de maíz y de harina de arroz. La adición de harina de soja mejoró todos los parámetros de calidad de ambos tipos de pan (de arroz y de maíz). Los panes con mejores resultados fueron aquellos con harina de arroz, maíz y soja: volumen específico elevado, buena apariencia de la miga, textura suave y menor envejecimiento. Onyango y col. (2011) cuando sustituyeron hasta un 50% de harina de soja por almidones de arroz, maíz, tapioca y patata en la elaboración de panes sin gluten observaron cómo los almidones que presentaron mayores temperaturas de gelatinización (almidón de arroz y de tapioca) dieron mayores volúmenes de pan.

En general, la inclusión de almidones aumenta el volumen del pan (Lopez y col., 2004; Mariotti y col. 2013; Onyango y col. 2011), sin embargo, los panes a base de almidón presentan una miga seca, color blanco y un sabor y sensación en boca de escasa calidad (Mariotti y col., 2013). La adición de harina de arroz da lugar a un mejor color de la corteza y mejor gusto, mientras que la harina de soja mejora la vida útil del pan. En cuanto a la estructura de las celdas, también se puede ajustar con los porcentajes de harina o almidón añadido. En los panes a base de trigo, comúnmente utilizado como modelo en panes sin gluten, una estructura alveolar más cerrada se corresponde con panes de molde, mientras que en panes de barra es preferible un alveolado más abierto. De este modo, un mayor porcentaje de almidón aumentará el tamaño del alveolo con respecto a mayores contenidos de harina de arroz (alveolado más cerrado). Se puede concluir que el uso de mezclas de harinas y almidones libres de gluten es una herramienta útil para optimizar fórmulas de pan sin gluten.

Las harinas libres de gluten se pueden someter al proceso de extrusión para pregelatinizar el almidón mejorando sus propiedades funcionales, como un aumento de la capacidad de absorción de agua, poder espesante, propiedades emulsionantes, propiedades gelificantes entre otras (Gómez y Martínez, 2016). La extrusión es un tratamiento físico que combina calor y esfuerzo mecánico sobre una mezcla harina-agua para modificar el almidón sin la necesidad de químicos. Dentro de las características de las harinas extrusionadas, su mayor capacidad de absorción de agua es relevante en la elaboración de pan sin gluten para aumentar la consistencia de la masa y reducir la típica sequedad de estos panes (Gómez y Martínez, 2016). La inclusión de almidones pregelatinizados disminuye también los efectos negativos del almacenamiento de productos almidonosos, como puede ser la retrogradación (Bojana y col., 2012). Además su capacidad emulsionante y propiedades gelificantes favorecen la retención de gas mejorando la estabilidad de la masa.

Son varios los autores que han observado las propiedades de las harinas pregelatinizadas y sus beneficios en panificación sin gluten (Clerici y El-Dash, 2006; Clerici y col., 2009; Defloor y col. 1991; Martínez y col. 2014b; Sánchez y col. 2008). En general, concluyeron que es necesario aumentar la cantidad de agua en la fórmula de panificación cuando se utilizan harinas extruidas con el fin de compensar la mayor consistencia de las masas. Martínez y col. (2014b) observaron como las harinas pregelatinizadas con un tratamiento de extrusión más intenso necesitan adicionar mayor cantidad de agua que aquellas con un tratamiento más suave. También observaron como la granulometría de la harina afecta a la calidad del pan pues, con la harina extrusionada de mayor tamaño de partícula se mitigaron los efectos negativos provocados por la harina extrusionada fina (menor volumen y mayor dureza del pan) y se mejoró la vida útil del pan.

1.4.2. Otros ingredientes importantes en panificación sin gluten. El agua y la grasa.

A parte de la importancia de los hidrocoloides en panificación sin gluten, ya destacada en el apartado 1.2.2.1. , existen otros ingredientes como el agua y la grasa relevantes por su funcionalidad en este tipo de elaboraciones. El efecto plastificante del agua es crucial a la hora de hacer pan sin gluten, ya que contribuye a las propiedades reológicas de la masa durante la mezcla (Marco y Rosell, 2008c). Se ha demostrado que contenidos de agua (en base harina y/o almidón) de 70-80% producen masas de textura suave pero faltas de elasticidad y extensibilidad (Schober, 2009). Por el contrario, las masas elaboradas con contenidos de agua por encima del 90% producen masas de consistencia similar a un batido. Estas últimas masas dan lugar a panes aceptables en contraposición a las masas poco hidratadas que apenas incrementan su volumen durante la fermentación, con lo que los panes resultantes son muy densos y de escaso desarrollo. De este modo, para obtener un pan de calidad parece clave la cantidad de agua añadida que ha de ser cuidadosamente ajustada para obtener una consistencia de masa o batido adecuada (Schober, 2009). De estos estudios se puede extraer que hay un límite inferior y superior de cantidad de agua a añadir en una formulación de pan sin gluten. Cuando se excede el límite superior la viscosidad de la masa es demasiado baja y la dilución de los ingredientes demasiado alta. Del mismo modo, cuando no se llega al límite inferior los ingredientes no pueden dispersarse ni hidratarse suficientemente para ofrecer sus aptitudes funcionales. Establecer el contenido óptimo de agua para una formulación de pan sin gluten es importante ya que el contenido de agua, junto con las características de las harinas y el hidrocoloide añadido, determinarán los parámetros reológicos de las masas resultantes (Ronda y col., 2013; Schober, 2009).

El uso de grasas y aceites es una práctica común en la elaboración de masas de trigo. Según Sluimer (2005), la adición de pequeñas cantidades de estos ingredientes conduce a una mayor flexibilidad y facilidad de trabajo de las masas, mayor volumen final, una estructura alveolar más fina y una textura más suave. La adición de grasas y aceites también retrasa la retrogradación del almidón debido a la formación de complejos entre los lípidos y los componentes del almidón prolongando la vida útil de los panes. Algunos de los efectos de las grasas y aceites se basan en las interacciones entre éstos y el almidón, pero la mayoría de sus efectos son debidos a interacciones con la red de gluten y la compleja estructura de la masa de trigo. Por ello, su comportamiento en la masa de panes sin gluten puede ser completamente diferente.

La adición de grasas y aceites es también una práctica común en panes comerciales sin gluten, como lo demuestra la amplia variación en el contenido de grasa de estos productos, que pueden alcanzar valores de hasta 26g / 100g (Matos y Rosell, 2011). En estas matrices, las grasas facilitan el proceso mecánico, ya que disminuyen la adhesión de las mismas en las superficies, y juegan un papel importante en la estabilización de las burbujas mejorando la estructura del pan (Litwinek y col., 2014). Este hecho puede explicar las observaciones realizadas por Eggleston y col. (1992) quienes constataron que la margarina aumentó el aire atrapado durante la mezcla de una masa con harina de tapioca. La adición de aceite y grasas también modifica la reología de las masas libres de gluten (Lorenzo y col., 2009; Moreira y col., 2012), lo cual puede afectar el comportamiento de la masa en el procesado, y por lo tanto la calidad del producto final. Son varios los autores que han observado mejoras en la calidad de panes sin gluten con la adición de grasas. Gujral y col. (2003), estudiando la adición de menos del 10 % de aceite a una masa de arroz, observaron que el aceite redujo la consistencia de la masa y dio lugar a panes con un mayor volumen y menor dureza. Hart y col. (1970) y Milde y col. (2012) observaron también que la adición de grasas vegetales mejoró la suavidad de panes de almidones de sorgo y tapioca.

1.5. GALLETAS DE MASA CORTA

Las galletas son productos alimenticios horneados elaborados con una mezcla de harina, azúcar, grasas comestibles y agua, adicionada o no, y de otros productos alimenticios o alimentarios (aditivos, aromas, condimentos, especias, etc.) sometidos a un proceso de amasado y al posterior tratamiento térmico, dando lugar a un producto de presentación muy variada.

Las galletas se diferencian de otros productos derivados de cereales en base a su contenido en agua. En general, se reconoce que las galletas poseen un contenido en agua inferior al 5%, a diferencia de otros productos horneados como el pan que posee un 35-40% de humedad o los bizcochos con un 15- 30% de humedad (Wade, 1988).

Según Manley (1991) las galletas se pueden clasificar en función de distintos criterios como la textura o dureza del producto final, el cambio de forma en el horno, la extensibilidad de la masa, o las diferentes formas de tratar la masa. De este modo, según Pareyt y Delcour (2008a), se pueden distinguir fundamentalmente dos tipos de amasado en galletas:

- Método simple (*single-method*), donde se mezclan todos los ingredientes en una sola etapa formando una masa no extensible.

- Método de punto pomada (*creaming-method*), donde primero se mezcla la mantequilla con el azúcar y los ingredientes minoritarios hasta alcanzar lo que se conoce en pastelería como "punto pomada" (*cream-up*) y, posteriormente, se añade la harina y el resto de ingredientes con un amasado mínimo, resultando una masa no elástica ni extensible y con un mínimo desarrollo de gluten.

Las galletas denominadas "sugar-snap cookies" (SSC) son un tipo de galletas que se caracterizan por presentar una elevada cantidad de grasa y azúcar que impide que el gluten se desarrolle completamente, lo que provoca que la masa se quede corta (poco extensible). Por este motivo, estas galletas se engloban dentro de las galletas de masa corta, cuyas características se han explicado en el punto 1.2.1. Para evitar este desarrollo del gluten el método de amasado utilizado en este tipo de galletas es el método de punto de pomada.

La calidad de la galleta se puede resumir en dos términos generales. La primera son las dimensiones de la galleta (tanto el diámetro como el espesor), considerándose el diámetro un parámetro importante de calidad (Hoseney, 1994). El diámetro de la galleta final está determinado por la velocidad de expansión de la masa y el tiempo en el que la masa deja de expandirse durante el horneado. La expansión de la masa parece estar controlada por la viscosidad de la misma (Miller y col., 1997), que a su vez, está determinada por una competencia por el agua disponible (Hoseney y Rogers, 1994). La segunda característica que determina la calidad de la galleta es la sensación de mordida de la galleta, que incluye la textura. Una galleta de buena calidad ha de quebraste con facilidad. Está forma de romperse al morder la galleta depende de la estructura de la misma y es la que da nombre a la galleta a estudio, ya que al término "snap" se refiere al sonido cuando la galleta rompe al ser sometida a una carga (Hoseney, 1994). Slade y col. (1993) describen la estructura de la galleta como una matriz vítrea continua de azúcar-agua embebida en gránulos de almidón no gelatinizados, gluten sin desarrollar y grasa.

1.5.1. Ingredientes relevantes en galletas de masa corta

Los constituyentes de la harina y sus propiedades, el azúcar, la grasa e incluso la cantidad de agua tienen un efecto importante sobre el procesado de la masa, el horneado de la galleta, y la calidad del producto final.

1.5.1.1. La harina

La harina es el mayor ingrediente en las fórmulas de galletas y se compone principalmente de almidón, agua y proteína. Dentro de los componentes de la harina, el mayor efecto sobre la galleta lo ejercen aquellos que absorben agua, como el almidón, la proteína y los arabinoxilanos, y por lo tanto limitan la expansión de la misma (Pareyt y Delcour, 2008b). Aunque estos tres componentes absorben agua, los mecanismos por los que modifican el diámetro de la galleta difieren considerablemente.

La mayoría de los autores están de acuerdo en el papel del almidón, componente mayoritario de la harina, en la elaboración de galletas. En general, un mayor contenido de amilosa da lugar a un diámetro mayor de la galleta (Kaldy y col., 1991). La cantidad de almidón dañado también juega un papel importante en la expansión de la galleta y en la calidad del producto final. El almidón dañado es probablemente el componente que controla en mayor medida la cantidad de agua requerida para producir una masa de la consistencia deseada (Wade, 1988). Donelson y Gaines (1998) observaron que niveles altos de almidón dañado dieron galletas de menor diámetro final. Estas afirmaciones coinciden con lo observado por numerosos investigadores (Gaines y col., 1988; Hoseney, 1994; Hoseney y Rogers, 1994; Miller y Hoseney, 1997; Barrera y col., 2007). Sin embargo, el almidón, como tal, no parece desempeñar un papel tan importante en estos sistemas como lo hace en el pan. Esto es debido a que la mayoría de los gránulos de almidón no gelatinizan por los altos niveles de azúcar (Chevallier y col., 2000) y agua insuficiente (Hoseney, 1994).

Por otro lado, las proteínas de la harina, que son cuantitativamente menores que el almidón, parecen tener una gran influencia en la calidad del horneado de la galleta y, en particular, sobre el diámetro de la galleta final. La cantidad y calidad de las proteínas presentes en la harina tienen un papel importante en el comportamiento reológico de la masa, particularmente cuando la harina es el principal constituyente de la fórmula (Maache-Rezzoug y col., 1998). En SSC a base de harina de trigo, es preferible que se elaboren a partir de harina floja de trigo blando, es decir, que el contenido de gluten sea bajo, para obtener galletas de calidad (Gaines, 1990; Hou y col., 1996; Kaldy y col., 1993). Souza y col. (1994), sin embargo, llegaron a la conclusión de que el contenido total de proteínas es más importante para la calidad de la galleta que la composición de las proteínas. De este modo, galletas con bajo contenido en proteínas incrementan la expansión de la galleta. A pesar de ello, en la literatura no hay un consenso acerca de su papel y su influencia en la calidad de la galleta. Al igual que el almidón dañado, la proteína tiene un gran efecto en las propiedades de absorción de agua de la harina al preparar la masa. Como regla general, el almidón en buen estado absorbe 33% de su propio peso en agua, mientras que el almidón dañado absorbe 100% de su propio peso. Debido a esto, y al hecho de que la proteína absorbe dos veces su peso en agua, tanto el contenido proteico como el de almidón dañado ejercen un gran efecto sobre la capacidad de absorción de agua de la harina (Manley, 2000).

El tamaño de partícula de la harina también es un factor decisivo en las propiedades de las galletas a base de harina de trigo (Gaines, 1985) y su efecto varía según el tipo de masa (Manley, 2000). Según Manley (2000) en galletas de masa corta un menor tamaño medio de partícula incrementa la expansión en el horneado.

1.5.1.2. El azúcar y la grasa

El azúcar, un importante ingrediente en la elaboración de galletas, ejerce un efecto sobre la viscosidad de la masa, la gelatinización del almidón, así como en el desarrollo del gluten.

La sacarosa es el azúcar más importante en la elaboración de galletas. Proporciona dulzor, influye en las propiedades estructurales y texturales de la galleta, y se cree que tiene una influencia en la incorporación de aire en la masa durante los procesos de mezclado y amasado. Además, en las galletas, la sacarosa disminuye la viscosidad de la masa (Maache-Rezzoug y col., 1998). Durante el horneado, el azúcar no disuelto previamente se disuelve progresivamente, y por lo tanto contribuye a la expansión de la galleta (Hoseney, 1994). Otros parámetros de la galleta que se ven afectados por el nivel de azúcar son la dureza, la crujencia, el color y el volumen. Finalmente, Hoseney (1994) cree que, durante el horneado, la recristalización de la sacarosa en la superficie de la galleta hace que aparezca el típico patrón de agrietamiento superficial. Sin embargo, en contraste, otros investigadores relacionan esta apariencia de agrietamiento superficial con el grado de colapso al final del horneado (Slade y col., 1993).

La grasa es el último de los tres ingredientes mayoritarios en SSC. Ésta afecta a la apariencia general del producto, mejorando la aireación y el volumen, la lubricación, y la textura final del producto, e interfiere en el desarrollo de la red de gluten (Pareyt y Delcour, 2008b). La grasa imparte suavidad y terneza al producto, mejora la sensación en boca, la intensidad de sabor y la percepción del mismo (Zoulias y col., 2002). Como ocurre con el azúcar, altos niveles de grasa aumentan el diámetro de la galleta y disminuyen su espesor. De este modo, la grasa también hace que las galletas se rompan más fácilmente (Maache-Rezzoug y col., 1998).

1.5.1.3. El agua

El agua, aunque es un componente minoritario en la fórmula de las galletas, juega un papel importante durante la preparación de la masa. Se la considera un catalizador en la elaboración de la galleta, que tiene que ser añadido para hacer la masa moldeable o para cambiar el carácter de los ingredientes, pero debe eliminarse casi completamente durante el horneado (Manley, 2000). A pesar de ello, existen gradientes de humedad en el producto al final tras el proceso de horneado que desaparecen por la migración de la humedad en el producto durante el enfriamiento y almacenamiento (Wade, 1988).

El agua en la elaboración de galletas es necesaria para la solubilización de otros ingredientes (Maache-Rezzoug y col., 1998), incluyendo azúcar, sal, o bicarbonato sódico, y ayudará a la dispersión de la grasa y otros ingredientes a través de la masa (Manley, 2000). El nivel de agua añadida para la fabricación de galletas afecta al desarrollo del gluten en la masa, la expansión de galletas durante el horneado, la retención de humedad, proporciona lubricación cuando la galleta se está comiendo y afecta a la calidad de los productos terminados (Lai y Lin, 2006). Estos efectos fueron observados por Miller y col. (1997) cuando variaron el contenido de agua en fórmulas de SSC de trigo. Observaron como un aumento del agua en la fórmula aumentó la velocidad de expansión, presumiblemente mediante la reducción de la viscosidad de la masa, al mismo tiempo que se vio reducido el tiempo de fin de expansión. Como resultado, el diámetro de la galleta final no sufrió cambios significativos.

1.5.2. Galletas de masa corta sin gluten. Estrategias

Aunque las SSC elaboradas con harina de trigo han sido ampliamente estudiadas, no existen apenas trabajos que estudien galletas sin gluten y en menor medida de este tipo. Sin embargo, la mayoría de los trabajos sobre galletas sin gluten se asemejan a los de galleta de masa corta por la estructura de estas masas y la falta de extensibilidad. No obstante los trabajos difieren en la proporción de harina/almidón, azúcar y grasa/aceite.

En la elaboración de galletas libres de gluten mayormente se han usado harina de amaranto (de la Barca y col., 2010; Gambus y col., 2009; Hozova y col., 1997; Tosi y col., 1996; Schoenlechner y col., 2006) y de trigo sarraceno (Gambus y col., 2009; Kaur y col., 2015: Schoenlechner y col., 2006) como sustitutos de la harina de trigo. Otros autores han estudiado el uso de harina de teff (Coleman y col. 2013), harina de arroz (Chung y col., 2014), harina de avena sin gluten (Duta y Culetu, 2015), harina de sorgo (Rao y col., 2016) y harina de castaña (Sarabhai y Prabhasankar, 2015). En la mayoría de estos estudios, el objetivo buscado fue una mejora nutricional de galletas sin gluten.

Por otro lado, otros estudios se han centrado en la combinación de diferentes harinas libres de gluten. Inglett y col. (2015) estudiaron el efecto de la sustitución de un 25 % harina de amaranto por harina integral de avena, salvado de avena o β-glucano de avena (Nutrim) en galletas sin gluten y las compararon con una galleta control de trigo. Las galletas más similares a la galleta control de trigo fueron aquellas que contenían harina integral de avena o concentrado de salvado de avena. Torbica y col. (2010) utilizaron mezclas de harina de arroz y de harina de trigo sarraceno en diferentes porcentajes (90/10; 80/20 y 70/30, respectivamente) para la elaboración de galletas sin gluten. Con estas proporciones buscaron unos contenidos en proteína, almidón y grasa semejante a los de la harina de trigo y unas galletas de calidad semejante a galletas de trigo. Más adelante, Hadnađev y col. (2013) estudiaron el efecto de la influencia de la sustitución parcial de harina de arroz por harina de trigo sarraceno y la adición de CMC en los parámetros reológicos y la calidad de galletas sin gluten utilizando la misma fórmula que Torbica y col. (2010). La adición de trigo sarraceno y CMC dio lugar a masas más similares a aquellas elaboradas con harina de trigo, en términos de resistencia a la deformación. Ambos estudios observaron cómo los mejores resultados sensoriales fueron obtenidos en las galletas con un 80% de harina de arroz y un 20 % de harina de trigo sarraceno. Altındağ y col. (2014) estudiaron el efecto de la incorporación de harina de maíz y de arroz en la elaboración de galletas semi-dulces sin gluten a base de trigo sarraceno. Para ello, realizaron diferentes mezclas de trigo sarraceno y maíz (50%-50%), trigo sarraceno y arroz (50%-50%) y trigo sarraceno-arroz-maíz (50%-25%-25%) además de una galleta a base de harina de trigo sarraceno al 100%. Los resultados de diámetro, espesor y expansión de la galleta fueron afectados por el tipo de harina. Observaron como la adición de harina de maíz y/o de arroz aumentaron la expansión de la galleta y el diámetro de la misma, parámetros valorados positivamente en las galletas. Esto puede ser debido a que el trigo sarraceno presenta mayor cantidad de proteína que aumenta la retención de agua. Esto resulta en masas de alta viscosidad y poca expansión en el horneado. De este modo, las galletas elaboradas solo con harina de trigo sarraceno obtuvieron los mayores valores de dureza que se vieron disminuidos con la adición de harina de maíz. Este efecto lo atribuyeron a que la harina de maíz presentó el menor contenido en proteína, pues Gaines y col. (1992)

demostraron que galletas con un mayor contenido en proteína resultaban en una estructura más dura al estudiar el efecto de diferentes tipos de harina de trigo en SSC. Rai y col. (2014) estudiaron el efecto de diferentes combinaciones de harinas de arroz, maíz, sorgo y mijo perlado. Observaron como las combinaciones con sorgo y mijo mejoraron significativamente las propiedades reológicas de la masa, así como las propiedades funcionales, sensoriales y nutricionales de las galletas. Las galletas con harina de arroz también presentaron buenos resultados en la evaluación sensorial. En general, estos estudios revelaron que harinas ricas en proteína como las harinas de trigo sarraceno, sorgo y mijo mejoraron las propiedades reológicas de la galleta (masas más manejables) pero aumentaron la dureza de las galletas, mientras que las harinas de arroz y maíz aumentaron la expansión y por lo tanto el diámetro de las mismas.

Pocos autores han estudiado el efecto de los almidones en galletas sin gluten. Arendt y col. (2002) estudiaron el efecto de diversos almidones que abarcaban desde almidón de maíz, soja, mijo, arroz y patata combinado con diferentes tipos de grasas en la elaboración de galletas sin gluten. Observaron como la combinación de almidón de arroz, patata, maíz y soja con alto contenido en grasas en polvo produjeron galletas de calidad muy similar a las galletas de trigo. Schober y col. (2003) estudiaron el efecto de la combinación de mezclas de harinas y almidones libres de gluten en galletas, las cuales comparó con una galleta control de harina de trigo y otra a base de un preparado sin gluten comercial que contenía principalmente almidón de trigo y leche en polvo. A pesar de que hicieron ensayos preliminares de las mezclas, en el estudio solo muestran los resultados de tres de ellas: 50 % de harina de arroz integral, 10 % de almidón de maíz, 10 % de almidón de patata y 10 % de harina de soja (RCPS); 50 % de harina de arroz integral, 30 % de almidón de maíz, 10 % de harina de trigo sarraceno y 10 % de copos de mijo (RPBM); 25 % de harina de arroz integral, 25 % de almidón de maíz, 25 % de almidón de patata y 25 % de copos de mijo (RCPM). Encontraron correlaciones entre la pegajosidad de la masa y algunos parámetros de calidad de la galleta. De modo que masas no pegajosas resultaron en galletas redondeadas, gruesas y más duras (aquellas a base de trigo y la mezcla RCPS). Esto lo atribuyeron a su mayor contenido en proteínas, que en el caso de la mezcla sin gluten era debido a la harina de soja. Mientras tanto, las mezclas RPBM y RCPM, así como el preparado comercial sin gluten dieron lugar a masas más blandas y pegajosas. Estas masas se caracterizaban por un aumento en el contenido en almidones y menor contenido en proteínas que puede contribuir a una estructura débil. Resultaron en galletas más ovaladas y gruesas. El mayor grosor fue probablemente debido a la mejor actuación del leudante químico en estas masas más blandas y las diferencias entre el diámetro mayor y menor más pronunciadas por el efecto de la pegajosidad de las mismas en el laminado.

Sin embargo, estos trabajos resultan confusos ya que realizan diferentes mezclas y no estudian estas harinas por separado. En el caso de los trabajos que estudian un único tipo de harina, en general no son comparables entre sí, ya que difieren en los ingredientes y cantidades de las fórmulas de galletas sin gluten e incluso en el procesado de las mismas lo que hace difícil comparar el efecto del origen de las harinas estudiadas. Añadido a esto, en su mayoría, estos estudios no tienen en cuenta características tan importantes de las harinas como el tamaño de partícula o contenido en almidón dañado que ejercen un efecto importante en la calidad final de la galleta como lo demuestran estudios sobre SSC a base de harina de trigo (Pareyt y Delcour, 2008b).

A pesar de los beneficios que presenta el uso de harina de arroz en la elaboración de productos horneados sin gluten, son pocos los estudios que desarrollan galletas a base de harina de arroz. Esto puede ser debido a que el color de galletas elaboradas con harina de arroz es excesivamente blanco en comparación con una galleta de trigo, lo cual se percibe como un defecto. La adición de proteínas es una práctica habitual para mejorar las características de los productos horneado sin gluten. La importancia de las proteínas de la harina en la elaboración de galletas, sugieren que la adición de proteínas o el uso de harinas naturalmente ricas en proteínas puede ser una buena alternativa para regular la expansión en el horneado y mejorar el color de galletas libres de gluten ya que éstas favorecen las reacciones de Maillard.

La falta de información sobre galletas sin gluten y especialmente en SSC sin gluten pone de relevancia la necesidad de estudiar la adecuación de las diferentes harinas sin gluten disponibles en el mercado para su elaboración, además del estudio de su combinación con almidones y proteínas, cuya eficacia está ampliamente demostrada en otros productos horneados sin gluten como es el pan.

OBJETIVOS

El objetivo general de esta investigación fue evaluar la influencia de diferentes ingredientes, comunmente utilizados en su mayoría en fórmulas de pan y galletas sin gluten, sobre la calidad de los productos finales para así optimizar las fórmulas de estos panes y galletas.

Para llevar a cabo este objetivo general, se han establecido los siguientes objetivos parciales:

1.-Optimizar una formulación de pan sin gluten con mezclas de harina de arroz, almidón de trigo y almidón de maíz.

2.- Estudiar el efecto de diferentes tipos y niveles de grasa en panes sin gluten.

3.- Estudiar la influencia de la combinación de psyllium y HPMC en pan sin gluten.

4.-Estudiar el efecto de las características físico-químicas de diferentes harinas provenientes de cereales y pseudocereales sin gluten sobre la calidad final de galletas de masa corta, con el fin de elegir la harina más adecuada en función del fin deseado.

5.- Optimizar una formulación de galletas de masa corta sin gluten con mezclas de harina de arroz, almidón de maíz y proteína de guisante.

6.- Buscar una relación entre las características de las harinas y la reología de las masas con la calidad del producto final.

ESTRUCTURA

La presente tesis doctoral se estructura en dos secciones diferenciadas según el tipo de producto sin gluten estudiado (pan o galletas) que se subdividen en tres y dos apartados, respectivamente, correspondientes a las publicaciones científicas a las que ha dado lugar la investigación.

Sección I: Estudio del efecto de la adición y/o combinación de diferentes ingredientes sobre la reología de masas y la calidad de pan sin gluten.

- Camino M. Mancebo, Cristina Merino, Mario M. Martínez y Manuel Gómez (2015) Mixture design of rice flour, maize starch and wheat starch for optimization of gluten free bread quality. Journal of Food Science and Technology, 52: 6323–6333, doi: 10.1007/s13197-015-1769-4.
- Camino M. Mancebo, Mario M. Martínez, Cristina Merino, Esther de la Hera, Manuel Gómez (2016) Relationship between dough rheology and quality characteristics of rice based breads with oil or shortening. Journal of Texture Studies, enviado 11/07/2016.
- Camino M. Mancebo, Miguel Ángel San Miguel, Mario M. Martínez y Manuel Gómez (2015) Optimisation of rheological properties of gluten-free doughs with HPMC, psyllium and different levels of water. Journal of Cereal Science 61: 8-15, doi:10.1016/j.jcs.2014.10.005.

Sección II: Efecto de las propiedades de diferentes harinas sin gluten, el almidón de maíz y la proteína de guisante sobre la calidad de galletas denominadas “sugar-snap cookies” sin gluten.

- Camino M. Mancebo, Javier Picón y Manuel Gómez (2015). Effect of flour properties on the quality characteristics of gluten free sugar-snap cookie. LWT - Food Science and Technology 64: 264-269, doi:10.1016/j.lwt.2015.05.057.
- Camino M. Mancebo, Patricia Rodríguez y Manuel Gómez (2016) Assessing the rice flour-starch-protein mixtures to produce gluten free sugar-snap cookies. LWT - Food Science and Technology 67: 127-132, doi:10.1016/j.lwt.2015.11.045.

SECCIÓN I: PANES SIN GLUTEN

MIXTURE DESIGN OF RICE FLOUR, MAIZE STARCH AND WHEAT STARCH FOR OPTIMIZATION OF GLUTEN FREE BREAD QUALITY

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Abstract

Gluten-free bread production requires gluten-free flours or starches. Rice flour and maize starch are two of the most commonly used raw materials. Over recent years, gluten-free wheat starch is available on the market. The aim of this research was to optimize mixtures of rice flour, maize starch and wheat starch using an experimental mixture design. For this purpose, dough rheology and its fermentation behaviour were studied. Quality bread parameters such as specific volume, texture, cell structure, colour and acceptability were also analysed. Generally, starch incorporation reduced G^* and increased the bread specific volume and cell density, but the breads obtained were paler than the rice flour breads. Comparing the starches, wheat starch breads had better overall acceptability and had a greater volume than maize-starch bread. The highest value for sensorial acceptability corresponded to the bread produced with a mixture of rice flour (59 g/100 g) and wheat starch (41 g/100 g).

Keywords: rheology; rice flour; wheat starch; maize starch; gluten-free bread; mixture design.

1. Introduction

The market for gluten-free products is increasing. Due to better diagnostic methods, more people are identified as having coeliac disease, which is a disorder of the small intestine that causes chronic malabsorption (Laurin et al. 2002). The production of gluten-free bakery products continues to be a major challenge for bakers and cereal scientists in the twenty-first century.

Bread is a staple food in which gluten has an important function, as it forms a three-dimensional protein network during dough preparation; in gluten-free baking, this function has to be taken over by other additives, such as hydrocolloids. Most research into the development of gluten-free products has focussed on the substitution of wheat flours by mixtures of gluten-free flours, starches, hydrocolloids and proteins (Schober 2009).

Rice flour is one of the most suitable flours for baking gluten-free products due to its hypoallergenic properties, low sodium content, mild flavour and pale appearance (Torbica et al. 2012). The particle size of rice flour is also known to be important in gluten-free breadmaking. Among the different types of flour, those with a larger particle size produce breads with a higher specific volume and lower hardness (De la Hera et al. 2012).

Several studies have focused on the use of mixtures of starch and gluten-free flour for making breads suitable for coeliac patients. These mixtures can achieve better results than starch or flour alone. Taking this into account, Sánchez et al. (2002) applied response surface methodology (RSM) to optimize a bread formulation from maize starch, rice flour and cassava starch, with and without soy addition. López et al. (2004) studied the use of rice flour supplemented with cassava and maize starch in the production of gluten-free breads. Sciarini et al. (2010) produced breads with mixtures of maize starch with rice and soy flour, and Onyango et al. (2011) used sorghum flour with the addition of different proportions of maize, cassava, potato and rice starch.

Historically there has been some controversy over the safety of gluten-free wheat starch-based products. In fact, the use of wheat starch was forbidden in some countries and allowed in others depending on the definition of the gluten-free diet (Thompson 2001). Nonetheless, Peräaho et al. (2003) showed that gluten-free wheat starch produce a similar histological and clinical recovery in patients with newly detected coeliac disease than a natural gluten-free diet do. The protein content of wheat starches that were used for food for normal consumption varied from 0.3 up to 5% (Deutsch et al. 2008). Under the new European Union regulations (2009) only foods that contain less than 20 mg gluten/kg can be labelled as gluten-free. Recently, guaranteed gluten-free wheat starch has appeared on the market. This starch has not been sufficiently studied for the production of quality gluten-free bread. However its flavour is closer to that of conventional products made with wheat flour, and it may be an alternative to the starches currently used in these products. In addition, wheat starch has a bimodal size distribution due to the different molecular structures of the amylopectin chains (Jane 2009). The large granules have a disk shape and the small granules have a spherical shape. This bimodal distribution of wheat starch could influence gluten-free breadmaking processes since Zeng et al. (2014) observed changes in starch gelatinization when the proportion of granules of different shape varied. In order to better explain the results, dough rheology and its behaviour during the fermentation process were also assessed.

RSM is a statistical technique that has been successfully applied in the development and optimization of gluten-free breads (Gallagher et al. 2003). For this purpose, a mixture design to optimize a combination of rice flour, maize starch and wheat starch for the baking quality of gluten-free breads (specific volume, texture, colour, porosity, and acceptability) was made. Dough rheology and its behaviour during the fermentation process were also studied.

2. Materials and methods

2.1. Materials

The white rice flour employed in this study was japonica rice flour provided by Harinera Castellana (Medina del Campo, Spain). The flour contained 8,01g/100g of protein, 74,35g/100g of starch (with 22,13g of amylose and 10,6g damaged starch per 100g of starch), and 12,5g/100g of humidity (data provided by the manufacturer). Flours were sifted 10 min in a Bühler MLI 300B sieve (Milan, Italy) with two screens, one of 106 microns and other of 180 microns. The flour fraction with particle size lower than 106 µm was removed as previous studies have shown that finer fractions gave poorer results in gluten-free breadmaking (de la Hera et al. 2013). Therefore, it was used flour with particle size of 106-180 µm. The maize starch, Merizet 100, was provided by Tate & Lyle (London, United Kingdom) and the gluten-free wheat starch with less than 20 mg/kg gluten content, according to the current European Union regulations (2009), provided by Roquette Laisa (Lestrem, France).

RVA curves from rice flour and maize and wheat starches are shown in Fig. 1.

Saf-Instant yeast (Lesaffre, Lille, France), dry refined salt (Esco European Salt Company, Niedersachsen, Germany), white sugar (AB Azucarera Iberia, Valladolid, Spain), refined sunflower oil (Langosta, Ciudad Real, Spain) and hydroxypropyl methylcellulose (HPMC) K4M (Rettenmaier & Sohne, Rosenberg, Germany) were also used in the bread formulation.

2.2. Methods

2.2.1. Experimental design

To evaluate the effect of the independent variables (proportion of rice flour, maize starch and wheat starch) on the dependent variables, an experimental design of mixtures in which the ingredients under investigation added up to 100% was established. Specifically, it was used a simplex centroid design in which the first trial groups were formulations of pure ingredients, the second group were mixtures of two ingredients at 50% and the third group consisted of mixtures of 33.3% of each ingredient. All the experiments were performed in duplicate. The response of each investigated parameter was analysed adjusting to cubic model (eq. 1) with least square regression in order to identify significant (P<0.05) effects of the variations in ingredient levels on the responses (Table 1).

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \tag{1}$$

Significance of the lack-of-fit error term, R^2 value and model significance were used to judge adequacy of model fit. For optimisation, a multiple response method was applied. The following responses were used: loaf specific volume (maximise), crust brightness (L*) (minimise), cell density (maximise); and loaf specific volume (maximise), crust L* (minimise), cell density (minimise).

2.2.2. Flour/Starch characterisation

The pasting properties of flours during the heating-cooling cycle was measured in duplicate with the Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia) in accordance with AACC method 61.02.01 (AACC 2012).

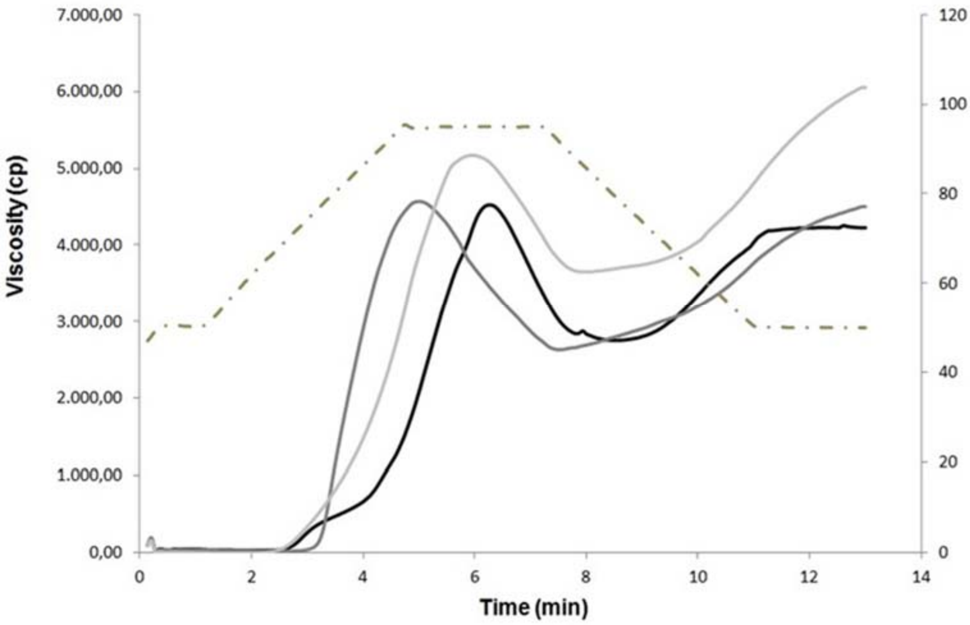

Fig. 1 Viscosity profiles of rice flour, maize starch and wheat starch determined by RVA. Rice flour (*black line*), maize starch (*dark grey line*), wheat starch (*light grey line*). Temperature (*dot-dash line*).

2.2.3. Dough rheology

The rheological behaviour of doughs was studied using a Thermo Scientific Haake RheoStress 1 controlled strain rheometer (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P water bath which controlled analysis temperature (set at 25ºC). The rheometer was equipped with parallel-plate geometry (60 mm diameter titanium serrated plate-PP60 Ti) with a 3-mm gap. After adjustment to the 3-mm gap, the excess dough was removed and Vaseline oil (Panreac, Panreac Química SA, Castellar del Vallés, Spain) was applied to cover the exposed sample surface. Dough samples for rheological test were prepared following the method explained in breadmaking section. In oscillatory tests, dough was rested for 300 s before measuring. Samples (without yeast) were analysed in duplicate. First, a strain sweep test was performed at 25 ºC with a strain range of 0.1 - 100 Pa and a constant frequency of 1Hz to identify the linear viscoelastic region. On the basis of the results obtained, a strain value included into the linear viscoelastic region was used in a frequency sweep test at 25 ºC with a frequency range of 100-0.1 Hz. Values of the complex modulus (G*[Pa]) were obtained for different frequency values (Hz) (Dobraszczyk and Morgenstern 2003).

In creep-recovery tests, the dough was rested for 600 s to allow relaxation before the measurement. Creep tests were performed by imposing a sudden step shear stress in the linear viscoelastic region for 60 s. In the recovery phase the stress was suddenly removed and the sample was allowed to rest for 180 s to recover the elastic (instantaneous and retarded) part of the deformation. Each test was performed in triplicate.

2.2.4. Dough development and gas production

Dough proofing behaviour was determined using a rheofermentometer (Chopin, Villeneuve-la-Garenne, France), obtaining information on dough development and gas production during fermentation. In contrast to the traditional method, the weight of dough was reduced to 200 g and the weights were removed from the piston due to the weakness of this kind of dough compared with those prepared with wheat flour. Analyses were run in duplicate.

2.2.5. Breadmaking

The following ingredients were used in breadmaking: water (90 g/100 g flour, starch or flour/starch mixture), Saf-Instant yeast (3 g/100 g), salt (1.8 g/100 g), oil (6 g/100 g), HPMC (2 g/100 g) and white sugar (5 g/100 g). In all tests, the water temperature was held between 20 ºC and 22 ºC. All the ingredients were mixed for 8 min in a Kitchen Aid 5KPM50 mixer (Kitchen Aid, Michigan, USA), after which 250 g of the bread dough were placed in aluminium pans of 232 x 108 x 43.5 mm (ALU-Schale, Wiklarn, Germany). Fermentation was performed at 30 ºC and 75 % RH for 90 min. After fermentation, doughs were baked in an electric modular oven for 40 min at 190 ºC. The loaves were removed from the moulds after a 60-min cooling period and were weighed. They were then introduced into polyethylene plastic bags and stored at 20 ºC until analysis. Measurements on the breads were performed 24 h after baking. All the elaborations were performed twice.

2.2.6. Bread characteristics

Bread volume was determined using a laser sensor with the Volscan Profiler (Stable Micro Systems, Godalming, UK). The volume measurements were performed on two loaves from each elaboration. The specific volume was calculated as the ratio of bread volume to its mass.

Crumb texture was measured with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) fitted with the *"Texture Expert"* software. A 25-mm diameter cylindrical aluminium probe was used in a *"Texture Profile Analysis"* (TPA) double-compression test to penetrate up to 50% of the sample depth at a test speed of 2 mm/s, with a 30 s delay between the two compressions. Firmness (N), cohesiveness and resilience were calculated from the TPA curve (Gómez et al., 2007). Texture analyses were performed on 30 mm thick slices. Analyses were performed on two slices from two loaves from each elaboration (each formulation). Each elaboration was performed in duplicate (2x2x2).

Bread colour was measured using a Minolta CN-508i spectrophotometer (Minolta, Co. LTD, Tokio, Japan) using the D65 illuminant with the 2º standard observer. Results are expressed in the CIE L*a*b* colour space. Crust colour was measured at two different points on two loaves from each type of elaboration (2x2).

Cell densities (number of cells/cm^2) of the breads were assessed using digital image analysis in black and white with an HP Scanjet G3110 scanner (HP, Palo Alto, EEUU). The analysis was performed on 15 x 15 mm squares taken from the centre of a slice. Images were processed using "WCIF ImageJ" software. Cell densities were measured on two slices from different loaves from each elaboration.

2.2.7. Consumer testing

Hedonic sensory evaluation of breads was conducted with 63 volunteers who used to consume bread as part of their diet on a regular basis. Samples were analysed 1 day after baking. Samples were presented as half pieces for appearance and 2 cm slices for sensory evaluation on white plastic dishes coded with three-digit random numbers and served in random order. Participants were provided with water for rinsing between each sensory evaluation. The elaboration of breads was evaluated on the basis of acceptance of their appearance, taste and overall liking on a nine-point hedonic scale. The scale of values ranged from "like extremely" to "dislike extremely", corresponding to the highest and lowest scores of "9" and "1" respectively.

2.2.8. Statistical analysis

The Statgraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, USA) was used both for the experimental design and to generate the corresponding response surfaces for evaluation of the independent variables.

3. Results and Discussion

3.1. Dough rheology

Figure 2a shows the *G** frequency sweep corresponding to the different doughs. It can be seen that dough made with rice flour had a higher complex modulus than all other doughs, and maize starch dough had a higher complex modulus than wheat starch. Generally, the mixtures presented intermediate *G** values that fell into the *G** range corresponding to its individual components. The differences in dough rheology can be attributed to the internal structure of the starchy base (flour or starch), as the geometry of the particles may lead to different degrees of compactness, as reported by de la Hera et al. (2012, 2013). Furthermore, the milling technique and kernel properties in the manufacture of flours (rice flour in our case), unlike the manufacture of starches, increase damage to starch, which enhances water binding (Schober 2009). Additionally, the higher protein content of doughs made with rice flour could also favour greater water absorption by the dough. This could be a key aspect in increasing complex modulus of rice flour doughs.

The results of the creep recovery test are shown in Fig. 2b. Van Bockstaele et al. (2011) have studied wheat doughs and shown that the creep recovery test can be an excellent measurement of overall dough consistency, due to the interaction between water absorption, protein content and other flour constituents. The creep recovery curves of gluten-free doughs exhibited a typical viscoelastic behaviour combining both viscous fluid and elastic responses, as described by Lazaridou et al. (2007). The doughs produced from rice flour showed a very different behaviour in comparison with starch-based doughs. They had lower compliance values both in the creep and recovery phases, indicating higher dough strength (Edwards et al. 2003). Maize starch-wheat starch dough showed the highest values for creep and recovery compliance.

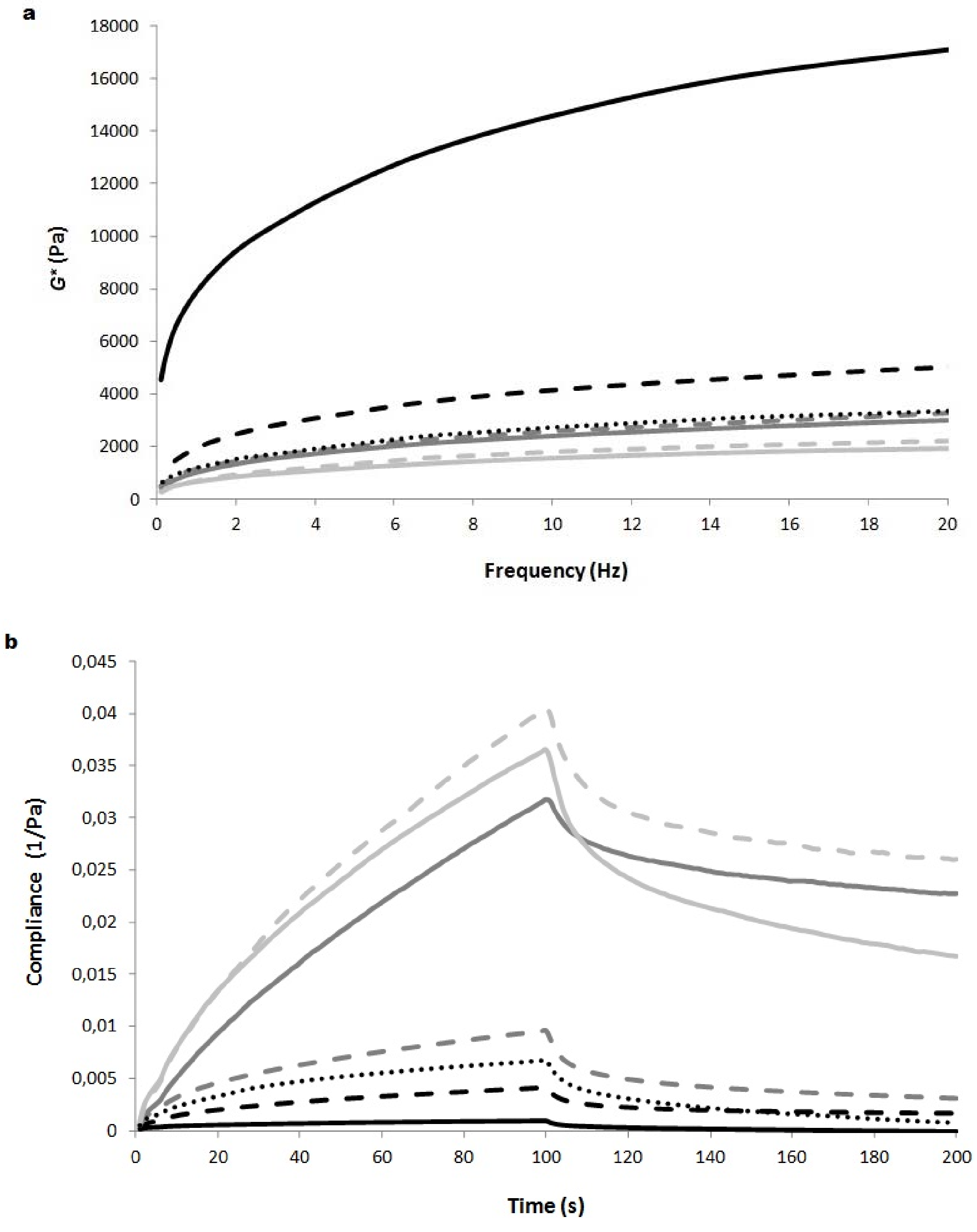

Fig. 2 Rheological behaviour of the different dough formulations: (a) *G** values and (b) creep recovery curves. Rice flour (*black line*), maize starch (*dark grey line*), wheat starch (*light grey line*), rice flour-maize starch (*discontinuous black line*), rice flour-wheat starch (*discontinuous dark grey line*), maize starch-wheat starch (*discontinuous light grey line*), rice flour-maize starch-wheat starch (*black dotted line*).

Flour doughs contain particles of larger size than doughs with starch granules and this leads to a more irregular structure (de la Hera et al. 2013). Additionally, the amount of damaged starch and the presence of proteins could favour greater water absorption by the dough, which would reduce the elastic component of maximum creep compliance (Lazaridou et al. 2007). The bimodal size distribution of wheat starch could promote greater continuity and density of the dough structure, as smaller granules would fit into the spaces between the larger granules, giving the dough a higher instant creep and recovery compliances. All formulations, except for the doughs made by starch-starch mixtures, which are more similar to the pure starch-based doughs, presented behaviour more similar to the rice-flour-based doughs, although with higher creep and recovery compliances.

3.2. Dough development and gas production

Figure 3 shows the curves corresponding to gas production during fermentation. Yeast first fermented the sucrose that had been added to the dough, followed by the maltose produced by the action of amylases on damaged starched granules. In all cases the curves show an abrupt decrease in gas production between 90 and 120 min, which indicates that all the sugars that can be fermented had been consumed by the yeast. Doughs containing rice flour showed greater gas production, particularly in the intermediate and final phases of fermentation; this could be related to greater starch damage (Schober 2009) increasing the amount of maltose available. The decrease in fermentation at 90-120 min would not have affected the fermentation process of the breads produced in this study as the fermentation time (90 min) was shorter than the time needed for consumption of all the sugar. Gas production in the initial phases of fermentation (until 30 min) was greatest with wheat-starch doughs than pure corn-starch and rice flour doughs, but it suffered a sharper decline before the others. Up to 60 min, the greatest gas production was in samples containing rice flour.

Regarding dough development (Fig. 3b), it may be observed that doughs made solely, by rice-flour had a greater initial development, but the dough structure became disassembled during the intermediate stage of fermentation, implying reduced gas retention, and dough height fell at 2 h after the initiation of fermentation. Including starch in rice-flour mixtures can resolve this problem and favour constant and greater dough development constant. Expansion in dough development could be related with the compliance results as samples with lower compliance values (rice flour doughs) had higher dough development.

Maize starch gave rise to good dough development, although slightly later than with rice flour. This could result in a lower bread volume if shorter fermentation times are used. However, wheat starch showed a very poor development that was not in accordance with the amount of gas produced. This analysis should be invalidated because the dough overflowed when the piston was assembled, and the recording of dough development was therefore innacurate; this may have been due to a lower *G** of wheat-starch doughs.

The differences between the different combinations of flour and starches at dough development and gas retention during fermentation could be related to the internal dough structure. Starch-based doughs are formed of smaller particles that are usually regular and round. However rice-flour doughs present a more irregular structure, containing some flour particles that are larger than starch granules, (de la Hera et al. 2013), while the rice starch granules themselves are smaller than maize or wheat starch granules.

The continuous phase composed of water, HPMC and other smaller components is able to join flour and starch particles forming a layer responsible for retaining the gas produced (de la Hera et al., 2013). Variations in this internal structure have a marked influence on dough development. It could be attributed to the observed variations of complex modulus. Previous studies of gluten-free breads have demonstrated a relationship between dough consistency and dough development during fermentation (de la Hera et al. 2012).

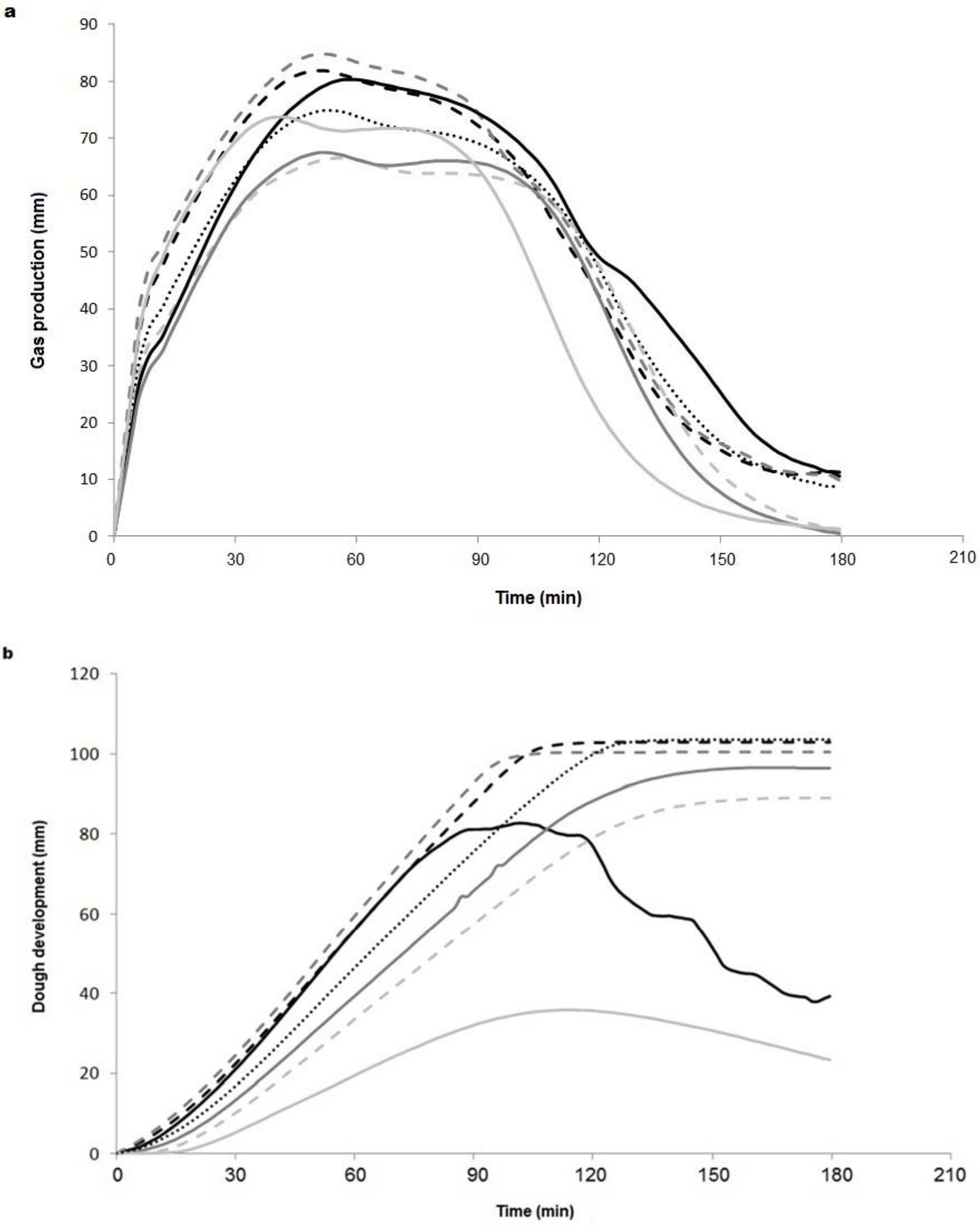

Fig. 3 Proofing behaviour of dough formulation determined by a rheofermentometer: (a) gas production and (b) dough development. Rice flour (black line), maize starch (dark grey line), wheat starch (light grey line), rice flour-maize starch (discontinuous black line), rice flour-wheat starch (discontinuous dark grey line), maize starch-wheat starch (discontinuous light grey line), rice flour-maize starch-wheat starch (black dotted line).

3.3. Bread properties

Figure 4a shows the changes in specific volume of breads prepared according to the experimental design. It can be seen that breads made with starches had a higher specific volume than rice flour breads. These results paralleled dough development, as doughs with starch showed a greater development (apart from wheat-starch dough which, as already mentioned, should not be taken into account). It was also observed that dough with a lower complex modulus produced bread with a greater specific volume; this correlation has been already reported in other studies (Matos and Rosell 2013) and has been attributed to the fact that a high consistency can restrict dough expansion during the proofing stage. Regarding the creep recovery test, lower J_{cmax} values were associated with lower specific volumes, indicating that less dough strength leads to greater development. Higher J_{steady} values, which are associated with greater dough elasticity (Moreira et al. 2012), also were in accordance with a greater bread specific volume. Our results coincide with those of other studies that have investigated the use of mixtures of starches and gluten-free flours and have found that the inclusion of starches generally increased bread volume (Lopez et al. 2004; Mariotti et al. 2013; Onyango et al. 2011). The difference in the specific volume of breads made with wheat starch and maize starch, and the differences in dough structure and G^*, may be due to variations in pasting behaviour. As can be seen from the RVA curves (Fig. 1), pure maize starch had an earlier pasting than pure wheat starch. Therefore expansion during baking could be lower with pure maize than pure wheat starch, as pasting signals the end of expansion.

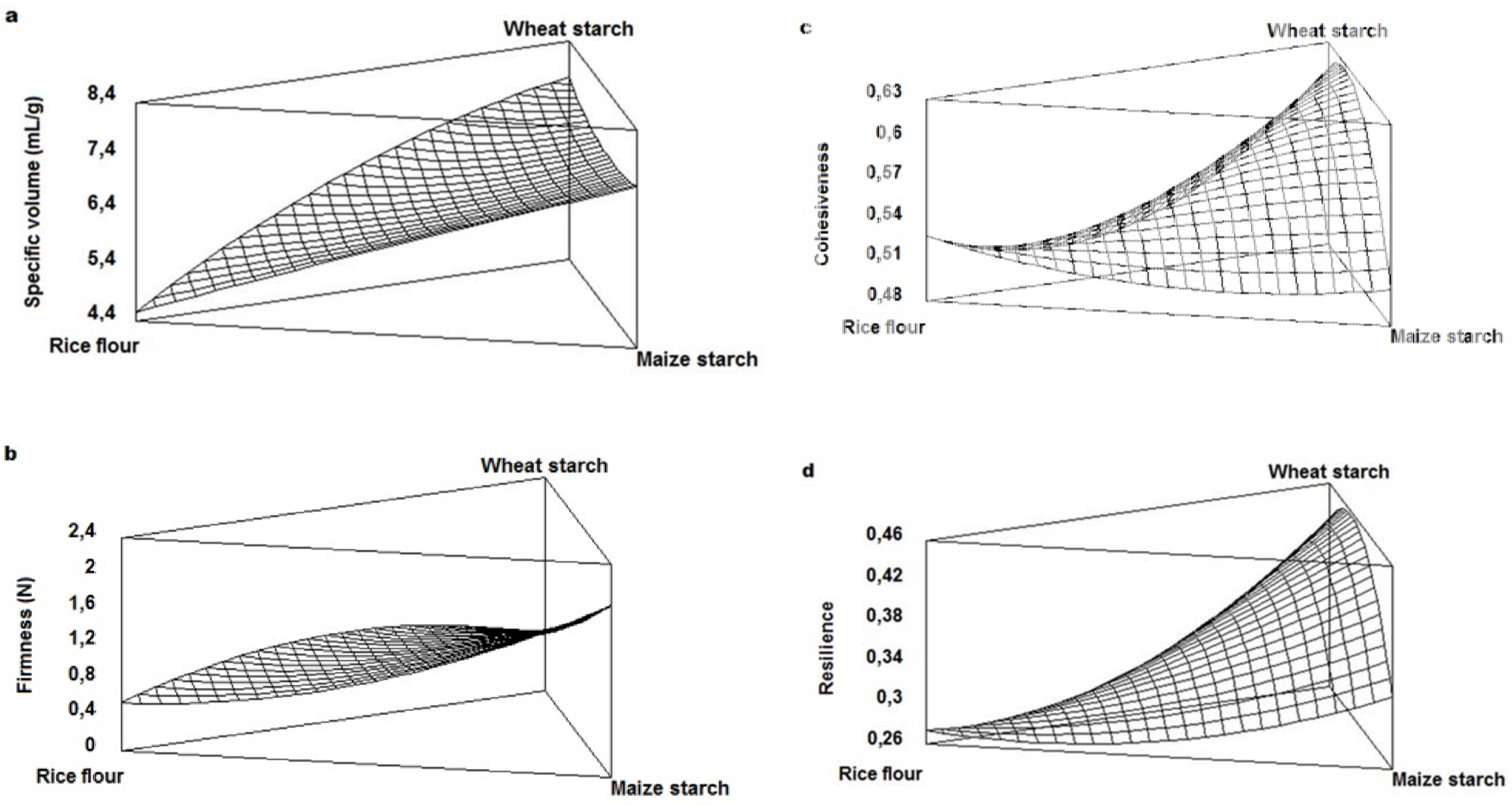

Fig. 4 Effect of rice flour, maize starch and wheat starch content on specific volume (a), Firmness (b), Cohesiveness (c) and Resilience (d) of the breads.

When the maize starch content was increased, the bread firmness also increased (Fig. 4b), but the differences between breads with rice flour or wheat starch were minimal. These results may seem surprising, since other studies have shown an inverse relationship between bread specific volume and firmness (Gallagher et al. 2003; Sabanis et al. 2009). However, these data may be related to the changes in internal bread and cell structure. Breads made with starch presented a more compact structure, with a larger number of cells and smaller mean cell area. Gueven and Hicsasmaz (2013) stated that the relationship between the texture and the cell structure of porous foods depends not only on the percentage of pores, but also on their distribution and shape. Breads made with wheat starch presented greater cohesiveness (Fig. 4c) and resilience (Fig. 4d) than rice-flour and maize-starch breads. The variations in these parameters may be related to the internal structure of the breads. One of the main problems with gluten-free breads is their lack of cohesiveness and resilience (Matos and Rosell 2012). Thus, these aspects can be improved by the incorporation of wheat starch.

Crust brightness (Fig. 5a) was affected by the type of mixture, and was lower in the case of breads with rice flour. However, there were no differences between the breads made with different starches. Our results agree with those reported by Miñarro et al. (2010) who observed that rice-flour breads had lower brightness values and that they were therefore less white than breads made with starches. These differences may be due to the higher protein content of doughs made with rice flour than doughs made with starches and therefore to a greater colour development through Maillard reactions. The excessively pale crust colour in gluten-free breads in comparison with standard wheat breads is a problem (Gallagher et al. 2002), but the inclusion of rice flours can minimize this defect. Figure 5b (cell density) displayed a lower cell density in breads made with rice flour whereas breads made with wheat or rice starch appeared with higher cell density. These trends coincide with the findings reported by Lopez et al. (2004), who studied mixtures of rice flours with maize and cassava starches. In wheat bread, which is commonly used as a model for gluten-free breads, the cell structure is preferred to be more closed for pan breads, but more open for other breads such as baguettes. The proportion of rice flour should therefore be varied according to the type of bread. As previously stated, this different crumb structure can affect the final bread texture (Mariotti et al. 2013), and the differences observed may be related to dough structure, how the air bubbles were integrated during the mixing stage, and how these bubbles grow during the fermentation and baking stages.

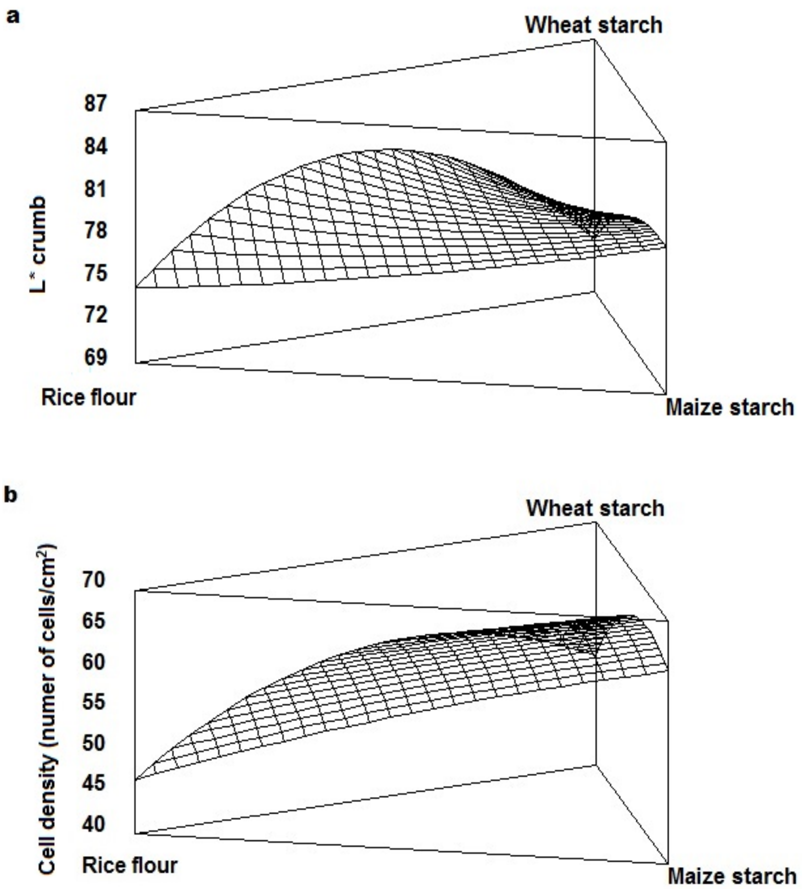

Fig. 5 Effect of rice flour, maize starch and wheat starch content on crumb L* (a) and cell density (b) of the breads.

3.4. Sensorial evaluation

Figure 6 shows the response surfaces for appearance (a), taste (b) and overall acceptability (c). The lowest scores for appearance were given to the starch breads, which were much paler than the other breads. Bread with rice-flour and wheat-starch achieved the highest score. Regarding taste, rice-flour breads and wheat-starch breads were rated similarly, whereas maize-starch bread was considered to have the poorest taste. This can probably be explained by the fact that the taste of wheat starch was much more similar to conventional bread. With regard to the overall acceptability, the bread with the lowest score was again the maize-starch bread and the highest score was for the bread with wheat starch and rice flour. Our results confirmed those reported by Lopez et al. (2004), which indicated that rice-flour bread is generally better rated with regard to appearance, taste and overall acceptability than maize-starch bread. Matos and Rosell (2012) studied the characteristics of gluten-free breads from different commercial doughs. Most of them were made from maize starch, except for one, containing a mixture of maize starch and rice flour. In the present study, the mixture formulation also exhibited the highest score for the three parameters measured.

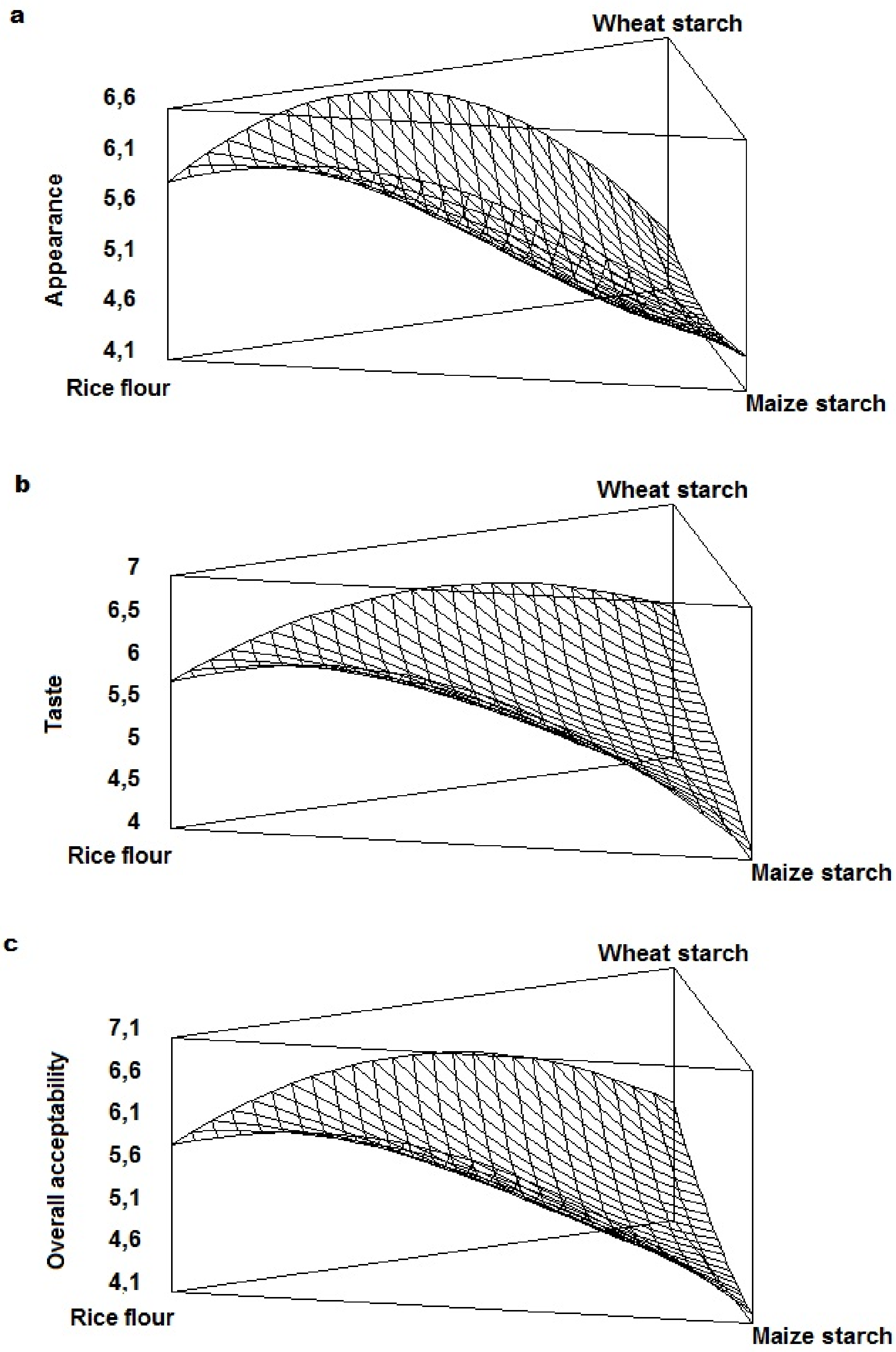

Fig. 6 Effect of rice flour, maize starch and wheat starch content on appearance (a), taste (b) and overall acceptability (c) of the breads.

3.5. Optimization

The highest value for sensorial acceptability was 6.50, corresponding to the bread produced with a mixture of rice flour (59 g/100 g) and wheat starch (41 g/100 g). Specific volume is one of the most commonly used parameters to evaluate bread quality and, when it was maximized the value obtained was wheat starch 100 g/100 g.

When only the instrumental values were took into account, using the results of the multiple response method, the optimal bread to achieve maximum specific volume and minimum crust luminosity contained 47 g/100 g rice flour and 53 g/100 g wheat starch when maximizing cell density; these proportions are relatively close to the optimal values obtained when maximizing for overall acceptability. However, when cell density was minimized, the optimal bread contained 98 g/100 g rice flour and 2 g/100 g wheat starch.

Table 1 Significant coefficients (95% confidence interval) of independent variables of the adjusted cubic model for bread characteristics (dependent analytical variables).

Variable	**a**	**b**	**c**	**ab**	**ac**	**bc**	**abc**	**R^2 (%)**
Specific Volume (mL/g)	7,75	4,72	7,20					92,34***
Firmness (N)	0,63	0,55	1,91	1,47		- 1,35		96,22***
Cohesiveness	0,61	0,52	0,50	- 0,21	0,23		0,42	95,90***
Resilience	0,42	0,26	0,32	- 0,17	0,31			95,94***
Crust brightness	81,39	68,91	84,46		19,65			94,40***
Cell density (number of cells/cm^2)	53,56	46,67	63,78	34,67	23,56	4,89	- 6,33	69,86*
Appearance	4,59	5,86	4,37	4,75		3,29	- 23,25	97,00***
Taste	5,78	5,73	4,10	2,64		3,7	- 16,92	94,82***
Overall acceptability	5,42	5,84	4,13	3,45		3,54	- 20,31	96,93***

Adjusted cubic model= a*Wheat starch + b*Rice flour + c*Corn starch + ab*Wheat starch*Rice flour + ac*Wheat starch*Corn starch + bc*Rice flour*Corn starch + abc*Wheat starch*Rice flour*Corn starch

Blanks correspond to non significant effects at level of significance of 5%. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: not significant.

These data show that bread specific volume cannot be considered individually as a quality indicator for gluten-free bread, as consumers also value other parameters, such as crust brightness and cell density. The addition of rice flour to this kind of product has the advantage of a better taste and less crust brightness. At the same time, it has the drawback of a lower specific volume and cell density, which can be improved by the addition of wheat starch to the mixtures, since in all cases it gave better results than maize starch for the manufacture of an optimal bread.

4. Conclusion

It was confirmed that the mixture design is an effective tool to optimize gluten-free flour and starch mixtures for making breads suitable for coeliac patients. It has also been shown that there is a relationship between dough rheology and bread specific volume. In addition, the incorporation of wheat starch into gluten free bread formulations was found to improve the specific volume and increased the cell density of breads made with rice flour resulting in higher scores for the taste and acceptability than those of maize starch breads. Wheat starch is therefore a very interesting alternative for the development of both industrial and traditional gluten-free breads.

Acknowledgements

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RELATIONSHIP BETWEEN DOUGH RHEOLOGY AND QUALITY CHARACTERISTICS OF RICE BASED BREADS WITH OIL OR SHORTENING

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Abstract

One of the main problems with gluten-free breads is their texture and their rapid staling. Fats are widely used for the improvement of texture and other quality parameters in gluten-free breads. The effect of oil and shortening in rice-breads quality and its correlation with dough rheology has been analysed. The inclusion of oil increased the specific volume of the breads and reduced their hardness, particularly with lower levels of hydration, whereas shortening did not modify specific volume or reduced it when hydration levels were higher. Oil, at levels of up to 30%, reduced the cohesiveness, springiness and resilience of breads, as well as the brightness of the crust, and increased the a* and b* values. Breads with oil also exhibited a greater number of pores per cm^2, especially in doughs with higher levels of hydration. An inverse correlation between G' and G" and bread specific volume has been observed, being the reciprocal-Y model a better predictor than the linear model to relate the bread specific volume.

Keywords: Gluten-free bread; dough rheology; fat; response surface methodology

1. Introduction

The relationship between dough rheology and quality characteristics of wheat based breads is widely proved. The rheological behaviour of wheat based doughs have been analysed by fundamental rheology techniques or specific empirical dough testing instruments such as alveograph or farinograph. In wheat breads, dough rheology is highly related with the gluten network. However, in gluten-free doughs, although several studies include rheological analyses, their information has been rarely used for predicting bread characteristics (Lazaridou and Biliaderis, 2009). In some of those studies a trend that relates a lower dough consistency with greater bread specific volume has been observed. Nevertheless, a deep study about those relationships has been never carried out. The use of fats and oils in the elaboration of wheat dough is common practice. According to Sluimer (2005), the addition of small amounts leads to greater flexibility and workability of the doughs, greater final volume, a finer alveolar structure and a softer final texture. The addition of fats and oils also delays starch retrogradation due to the formation of amylose-lipid complexes, prolonging the shelf-life of the breads. Some of the effects of fats and oils are based on the interactions between these products and starch, but most of their effects are due to interactions with the gluten network and the complex structure of wheat dough. Then the effects on the preparation of gluten-free breads may therefore be completely different.

Although the addition of fats and oils is also common practice in commercial gluten-free bread, as evidenced by the wide variation in the fat content of these products (with values of up to 26g / 100g [Matos and Rosell, 2011]), very few studies have been performed on the addition of fats and oils to gluten-free doughs, and most have only examined the addition of small amounts of these ingredients. Gluten-free doughs are more like batters than dough with gluten and Brooker (1993) showed that fats can play an important role in the stabilization of bubbles in batters. This fact may explain the observation by Eggleston *et al.* (1992) that margarine increased the air trapped during mixing in dough made with cassava flour. Similarly, Gujral *et al.* (2003), studying the addition of less than 10% oil to rice dough, noted that it reduced dough consistency and produced breads with a higher volume and lower hardness. Hart *et al.* (1970) and Milde *et al.* (2012) reported that the addition of vegetable fats improved softness in sorghum and tapioca starch breads. It has also been observed that rice bread is drier and crumblier than wheat bread and that it shows greater retrogradation during storage than wheat bread (Kadan *et al.* 2001); the use of oils or fats could therefore significantly improve rice bread quality. However, Schoenlechner *et al.* (2010) found no significant effect when incorporating fat into the formulas of gluten-free bread with amaranth. The addition of oil and fats also modifies the rheology of gluten-free dough (Lorenzo, *et al.* 2009; Moreira *et al.* 2012). This can impair dough control or its behaviour in the different phases of the baking process. The water content of the formulas can be changed in an attempt to compensate for these effects.

In the present study, it has been used response surface analysis to optimize the amount of oil or fat and the water content in a formulation of rice bread. Volume, weight loss, texture (initial and at 7 days), crumb and crust colour and alveolar structure have been analysed in the breads obtained. The rheological behaviour of the gluten-free batters has been studied and it has been correlated with bread quality parameters such as specific volume and hardness.

2. Materials and methods

2.1. Materials

Rice flours were supplied by Harinera Castellana S.A. (Medina del Campo, Valladolid, Spain). A particle size between 132 and 200 microns was chosen based on the results of previous studies (de la Hera *et al.* 2013). The protein content of the flour was 7.54g/100g, starch 73.6g/100g and amylose 22.56g/100g. Salt, sugar and sunflower oil were purchased from the local market. Fat (Argenta crema, Puratos, Barcelona, Spain), dry yeast (Saf-instant, Lesaffre, Lille, France) and hydroxypropyl methylcellulose (HPMC) (Vivapur K4M, J. Rettenmaier & Söhne, Rosenberg, Germany) were used.

2.2. Methods

2.2.1. Flour measurements

Flours were analysed according to AACC methods (2012). Protein content (AACC method 46-30) was determined using a Leco TruSpec®N nitrogen/protein analyser (St. Joseph, Michigan, USA). The total starch and amylose content were measured using the polarimetric method (AACC, 76-20).

2.2.2. Gluten-free breadmaking

A straight-dough process was employed, using a Kitchen-Aid Professional mixer (KPM5, KitchenAid, St. Joseph, Michigan, USA) with the K45DH dough hook. The following ingredients (as % on flour basis) were used in both formulas: sucrose (5%), salt (1.8%), instant yeast (3%) and HPMC (2%). Due to the influence of the addition of oil and fat on dough consistency, the oil and fat content was optimized with dough hydration by means of a response surface analysis. In the case of the oil, breads were prepared with 0%, 10%, 20% and 30% oil. In the case of fats, breads were prepared 0%, 5%, 10% and 15% fat, as breads with a higher content were of poor quality (lower specific volume and higher hardness). All breads were prepared with 70%, 85% and 100% water. The instant yeast was first rehydrated in half the volume of water. The doughs were kneaded for 8 minutes at speed 2 and 250 g of each dough was then moulded into aluminium pans of 232 x 108 x 43.5 mm. The pans were placed into a proofing chamber at 30ºC and 90% relative humidity for 90 minutes. After proofing, the breads were baked in an electric oven for 40 minutes at 190ºC. They were then demoulded, cooled for 50 minutes at room temperature and packed into sealed polyethylene bags to prevent dehydration.

2.2.3. Dough rheology

The rheological behaviour of doughs was studied using a Thermo Scientific Haake RheoStress 1 controlled strain rheometer (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P water bath that controlled analysis temperature (set at 25ºC). The rheometer was equipped with parallel-plate geometry (60-mm diameter titanium serrated plate-PP60 Ti) with a 3-mm gap. After adjustment to the 3-mm gap, the excess batter was removed and vaseline oil (Panreac, Panreac Química SA, Castellar del Vallés, Spain) was applied to cover the exposed sample surface. In oscillatory tests, dough was rested for 300 seconds before measuring. Samples (without yeast) were analysed in duplicate. First, a strain sweep test was performed at 25ºC with a stress range of 0.1–100 Pa at a constant frequency of 1 Hz to identify the linear viscoelastic region. On the basis of the results obtained, a stress value included in the linear viscoelastic region was used in a frequency sweep test at 25ºC with a frequency range of 100–0.1 Hz. Values of elastic modulus (G' [Pa]), viscous modulus (G'' [Pa]), complex modulus (G* [Pa]) and tg (δ) (G''/G') were obtained for different frequency values (ω [Hz]) (Dobraszczyk and Morgenstern, 2003).

2.2.4. Evaluation of bread quality

The evaluation of bread quality was done 24 hours after baking. Bread volume was determined using a laser sensor with the Volscan profiler 300 volume analyser (Stable Microsystems, Surrey, UK). The bread specific volume was calculated as the ratio between the volume of the bread and its weight. Weight loss was measured as the difference between the weight of the moulded dough and the weight of the bread after baking. Measurements were performed in duplicate.

Crumb texture was determined using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) with the "Texture Expert" software. A 25-mm diameter cylindrical aluminium probe was used in a 'Texture Profile Analysis' (TPA) double compression test to penetrate to 50% of the depth at a speed of 2 mm/s and with a 30-second delay between the first and second compressions. Hardness (N), cohesiveness, springiness and resilience were calculated from the TPA graph. Measurements were made on two central whole slices (30 mm thickness) from two breads from each elaboration. Texture was measured at 24 hours and 7 days.

Crumb grain characteristics of the breads were assessed using a digital image analysis (DIA) system. Images were acquired at 300 dots per inch (spatial resolution was 0.0843 mm^2/pixel) with a 1236USB Artec scanner (Ultima Electronics Corp., Taiwan). The analysis was performed on 34 x 34 mm squares taken from the centre of a slice. Images were processed using Leica QWin Pro V3.1 software (Leica Microsystems Imaging Solutions Ltd., UK). A cluster analysis method known as the "K-means algorithm" was used on each slice examined to obtain an optimum gray-level threshold to divide images into regions of cells and surrounded cell wall material. After cell detection, feature extraction was performed for each slice analysed. The following crumb grain characteristics were studied: mean cell area (mm^2), cell density (cells/cm^2; higher levels denote finer structure), cell to total area ratio (or void fraction, computed as the percentage of the total analysed square occupied by detected cells) and mean cell wall thickness (in mm; calculated as the averaged mean intercellular distance of neighbouring cells sampled). Crumb grain parameters were measured in triplicate.

Colour was measured using a Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Tokio, Japan). Results were expressed in the CIE L*a*b* colour space and were obtained using the D65 standard illuminant, and the 2º standard observer. Colour determinations on two breads from each formula were made 4x5 times on each slice of bread: crumb and crust colour was checked at four different points on each piece of bread and each point was measured five times.

Bread texture, colour and crumb grain characteristics were only analysed in oil based breads due to the poor results observed in bread made with shortening.

2.2.5. Statistical analysis

Response surface methodology was used to examine differences between the parameters. Two full factorial experimental designs were performed, one for oil and one for shortening, with one replicate and two factors. The factors analyzed were water content (three levels) for both designs and amount of oil (four levels) or fat (four levels), according to design. There were thus 24 elaborations for each design.

3. Results and discussion

The equations obtained after the analysis of response surface method for each parameter as well as the equation fit (r^2) are shown in table 1 (bread with shortening) and table 2 (bread with sunflower oil).

Table 1 Final equation in terms of coded factors for bread with shortening

	Cte	A (oil)	B (water)	AA	AB	BB	R^2	R^2 ajust
G'	1.05068E6		-20812.4		28.6067	103.665	99.24	99.02
G''	185590.		-3526.72		9.92533	16.9161	99.12	98.88
G*	1.06629E6		-21091.3		30.0337	104.929	99.24	99.02
tg (δ)	0.294882	-0.0021055	-0.00506244	0.000220833		0.0000560556	97.45	96.74
Specific volume	5.83	0.19	-0.18		-0.0027	0.0016	93.14	90.72
Weight loss	19.87	-1.55	-0.14		0.0139		91.52	88.53
Firmness	766.91		-15.57	-0.0693	0.0589	0.0787	98.04	97.35

Only significant coefficients (95 %) are shown

Table 2 Final equation in terms of coded factors for bread with sunflower oil

	Cte	A (oil)	B (water)	AA	AB	BB	R^2	R^2
G'	881466.00	-	-16839.4		60.7423	80.8772	97.62	96.95
G''	160670.00	-	-2944.42		13.508	13.5889	98.02	97.47
G*	895492.00	-	-17081.4		62.2065	81.9303	97.63	96.98
tg (δ)	0.123293		-				96.94	96.09
Specific	-32.37	0.29	0.75	-0.003	-002	-004	89.60	85.93
Weight loss	-43.74		1.37		0.003	-0.007	94	91.88
Firmness	439.65	-7.35	-8.37	0.051	0.061	0.040	79.48	72.24
Cohesiveness	0.5399	-0.0057	0.0007				91.51	88.51
Springiness	2.68	-0.03	-0.04	0.0005		0.0002	93.20	90.80
Resilience	0.4079	-0.0096	-0.0001				94.79	92.95
Firmness 7	913.31	-15.96	-17.31	0.11	0.13	0.08	80.31	73.36
Cohesiveness	0.3262	-0.0088					66.18	54.24
Springiness 7	3.25	-0.01	-0.06	0.0005		0.0003	83.09	77.12
Resilience 7	0.5488	-0.0094		0.0001			51.44	34.30
Crust L*	124.58	-2.97		0.0139	0.0240		89.97	86.44
Crust a*	-62.10	0.88		-0.0096	-0.0036	-0.0085	95.03	93.27
Crust b*	-79.82	0.97		-0.0197		-0.0130	90.12	86.64
Crumb L*	175.21	-0.55	-2.10		0.0136		76.75	68.54
Crumb a*	3.39						30.51	5.94
Crumb b*	83.71		-1.57	0.0043	0.0051	0.0081	83.75	78.02
Mean pore	-0.76	0.098	0.0194	0.0003	-0.0002		75.44	66.78
Pores/cm^2	-49.24		1.97				60.63	46.74

Only significant coefficients (95 %) are shown

3.1. Dough rheology

Figure 1 shows the results obtained through dynamic oscillatory tests. G' was higher than G'' in all gluten-free formulations, suggesting a solid-like behaviour of all gluten-free doughs. tg (δ) values were higher than 0,1 (soft gel) for all doughs (results no showed). As it could be expected, water content reduced G', G'' and G* values, since de la Hera *et al.* (2013); Mancebo *et al.* (2015b) and Ronda *et al.* (2015) observed similar results on rheological behaviour when the hydration content was modified in gluten-free doughs. This phenomenon can be attributed to the lower consistency of doughs made with more water than those made with a higher presence of other ingredients. However, the effect of fat on rheological behaviour depended in turn on the type of fat (oil or shortening). The inclusion of shortening hardly modified the values of G', G'' and G*, whereas the addition of sunflower oil had a similar effect to water (less marked), reducing the values of G', G'' and G*. This opposite trend as a function of the type of lipid incorporated or of its percent of solids at room temperature, was also observed by Lorenzo *et al.* (2009) when incorporating sunflower oil or margarine in pie-crust doughs. This effect could be due to the different consistency of the lipids. It was also noticed that the effect of oil addition was reduced when the hydration level increased.

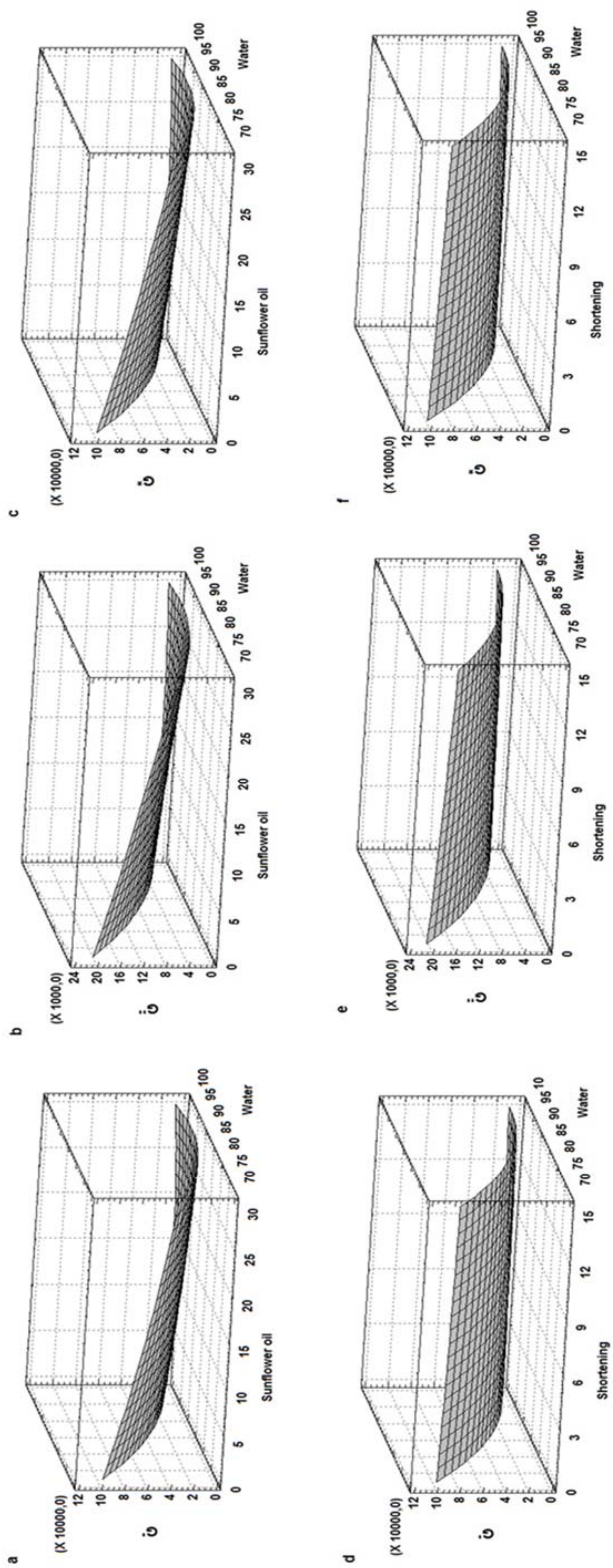

Fig. 1 Dynamic oscillatory test of the effect of sunflower oil or shortening and water content on G’ (a,d), G’’ (b,e) and G* (c,f): (a, b, c) sunflower oil vs. water; (d, e, f) shortening vs. water.

3.2. Bread properties

Figure 2 shows the spatial representation of how the values of specific volume and hardness of the loaves vary after modification of the water and oil or shortening content. It can be seen that while volume decreased slightly after the addition of shortening—an effect that became more noticeable with increasing amounts of water in the formulation—the addition of oil at levels of up to 30% increased bread volume, particularly in formulations with the lowest water content. It is in agreement with the observations of Gujral *et al.* (2013) and Milde *et al.* (2012) in breads with a lower oil content and low water content. In addition, the bread volume increase by oil incorporation coincided with reduced values of G', G'' and G*. in our study. The limited effect of the addition of shortening agrees with the findings reported by Schoenlechner *et al.* (2010). It can also be seen that the specific volume of breads with a low oil or shortening content increased with increasing water content of the formulation. In terms of hardness, the addition of shortening was found to have a minimal effect, and it even led to an increase in hardness when a greater water content. The addition of oil, on the other hand, reduced hardness, although this effect was minimized by increasing the amount of water in the formulation, and the combination of the highest percentages of oil and water content was associated with a slight increase in hardness. The first conclusion that may be drawn from these observations is that the effect of the addition of oil or shortening to rice bread is completely different from the effect of these same additions to wheat breads, in which an improvement in bread volume is only observed with low percentages of fat (less than 5%) and in which the addition of large amounts drastically reduces the volume (Sluimer, 2005). This finding confirms that the mechanism whereby gases formed during fermentation are retained, increasing the volume of the loaves, differs in the two types of bread-baking. In conventional baking (wheat breads), this gas retention correlates with the gluten network, and large amounts of oil or fat weaken this network; rice bread, on the other hand, is derived from a batter that is stabilized by the presence of hydrocolloids, and an excessive consistency is not suitable for that dough. The positive effect of increasing the water content of rice-bread formulations has already been reported in previous studies (Ylimaki *et al.* 1988; McCarthy *et al.* 2005; de la Hera *et al.* 2013). The incorporation of oil may help to reduce the consistency of the dough (Gujral *et al.* 2003; Moreira *et al.* 2012), as does the increase in the humidity of the dough (Lazaridou *et al.* 2007), thus improving their expansion. In Figure 2 it may be observed that in dough without oil, an increase in the water content of the formulation translates into an increase in the specific volume of breads; however, as oil content increases, the optimal percentage of water falls. This may be because a minimum consistency is necessary; de la Hera *et al.* (2013) have previously reported that an excess of water in the formulation could be detrimental to the volume of breads, especially in prolonged fermentation. The addition of shortening can increase the G* (increase the consistency of the dough) or, at least, not reduce it, so the effect on volume is not observed. These breads also presented a crude and irregular alveolar structure and an oral sensation of excessive shortening, so further analysis of other parameters was ruled out.

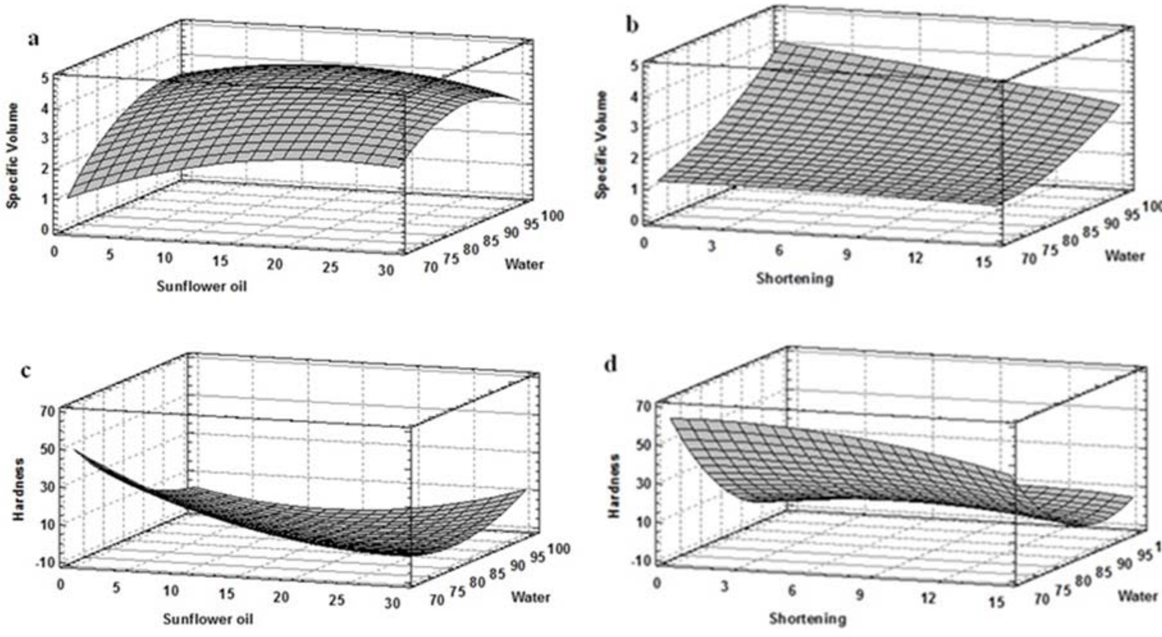

Fig. 2 Effect of sunflower oil or shortening and water content on specific volume (a, b) and hardness (c, d) of breads: (a, c) sunflower oil vs. water; (b, d) shortening vs. water.

Figure 3 shows the variation in weight loss and in the texture parameters of the breads with oil. It may be observed that weight loss during baking increased as the amount of water increased. This would appear logical, as the greater the water content of the dough, the easier it will be for evaporation to take place during baking. However, weight loss is also significantly influenced by the volume of the breads, as this will affect the surface area for evaporation. Indeed, a significant correlation of 99.9% (r = 0.72) was found between the specific volume and weight loss. For its part, the addition of oil barely affected weight loss during baking, and oil was not actually a significant factor in the weight loss equation.

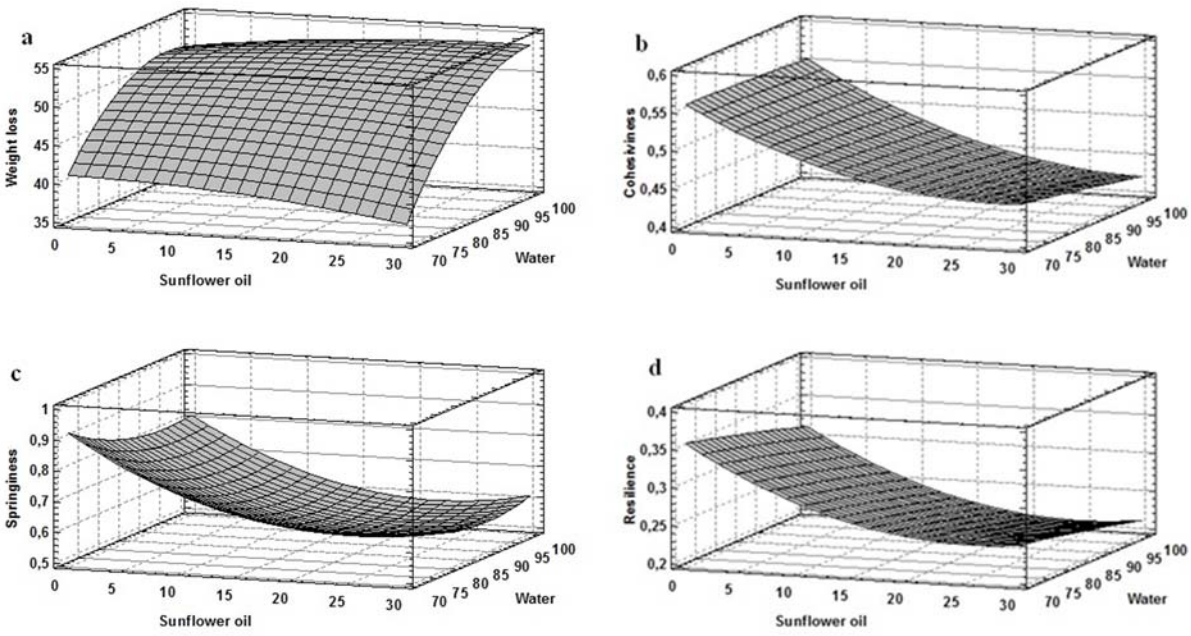

Fig. 3 Effect of sunflower oil and water content on weight loss, cohesiveness, springiness and resilience: (a) Weight loss; (b) Cohesiveness; (c) Springiness; (d) Resilience.

With regard to the texture parameters, it has already been commented that changes in the hardness of breads was closely related to changes in specific volume, as we detected a significant inverse correlation of 99.9% (r =-0.72) between the two parameters; so hardness is reduced by increasing the specific volume. This correlation has already been reported by Martínez *et al.* (2013) in a study on the texture of gluten-free bread. The other parameters show very similar changes, with significant correlations of 99.9% between them, and r values of 0.89 (springiness-cohesiveness), 0.92 (springiness-resilience) and 0.95 (cohesiveness-resilience). These correlations were also observed in the study by Martínez *et al.* (2013), although the values of the coefficients of correlation were somewhat lower. The incorporation of oil had a much greater effect on cohesiveness, springiness and resilience than was observed on increasing the percentage of water, which barely affects these texture parameters. In all cases, the addition of oil produced a fall in these parameters, although the reduction was more pronounced with the lower percentages of oil. From around 15% or 20%, the different texture parameters stabilize. We also studied the increase in hardness over time (data not shown). This parameter showed a very high correlation with initial hardness (r = 0.997), as already reported by Martinez *et al.* (2013), indicating that breads that are initially softer are also those that better maintain this condition; the incorporation of oil thus helps to increase the shelf-life of breads, but only when the water content of the dough was lower. The values of cohesiveness, resilience and springiness decreased over time, and while the amount of water had almost no effect, the incorporation of oil caused these values to be lower, although the differences were not as pronounced as with the initial texture.

Figure 4 shows the changes in the crust and crumb colour parameters. The colour of the bread is an important parameter for consumers and affects the acceptability of bread. Colour is one of the parameters that define a gluten-free bread (Pagliarini, *et al.* 2010; Matos and Rosell, 2012). The incorporation of oil led to a fall in crust brightness, and this was more evident with smaller amounts of water in the formulation; oil addition increased the a* and b* parameters, giving more intense brown tones. This is a positive effect because gluten-free breads have excessively pale colours if we compare them with the breads that contain gluten. This effect may occur because a reduction in the presence of water favours Maillard reactions, and hence the formation of brown pigment in the crust (Purlis, 2010). However, the influence of the oil on crumb colour was much less intense, with almost no change in the a* and b* parameters and only a slight increase in lightness. The crumb does not exceed 100°C and Maillard reactions would not therefore have occurred, so crumb colour is due mainly to the colour of the ingredients, and darker oils could therefore have had a greater influence on this colour. The amount of water in the formulation hardly affected crumb and crust colour, and an increase in water content was only found to increase the brightness of the crust in breads with a high oil content, thus confirming the influence of water on Maillard reaction activity.

Moisture content had a very clear effect on alveolar structure, increasing the number of alveoli per square centimetre; this effect was enhanced by a higher oil content and, therefore, a lower dough consistency. The average size of the alveoli also increased with increasing water content in the formulation, but this effect was minimized by the addition of oil, and was virtually undetectable with higher percentages of oil. The observed effect of the water content of the formulation on alveolar structure coincides with the findings of other authors investigating gluten-free breads (Schoenlechner *et al.* 2010; Tsatsaragkou *et al.* 2012).

According to our findings, lower G* values (related to lower dough consistency) can influence not only the creation of air bubbles in the initial batter (increasing them), but also their expansion, which is facilitated up to a certain limit of G* values.

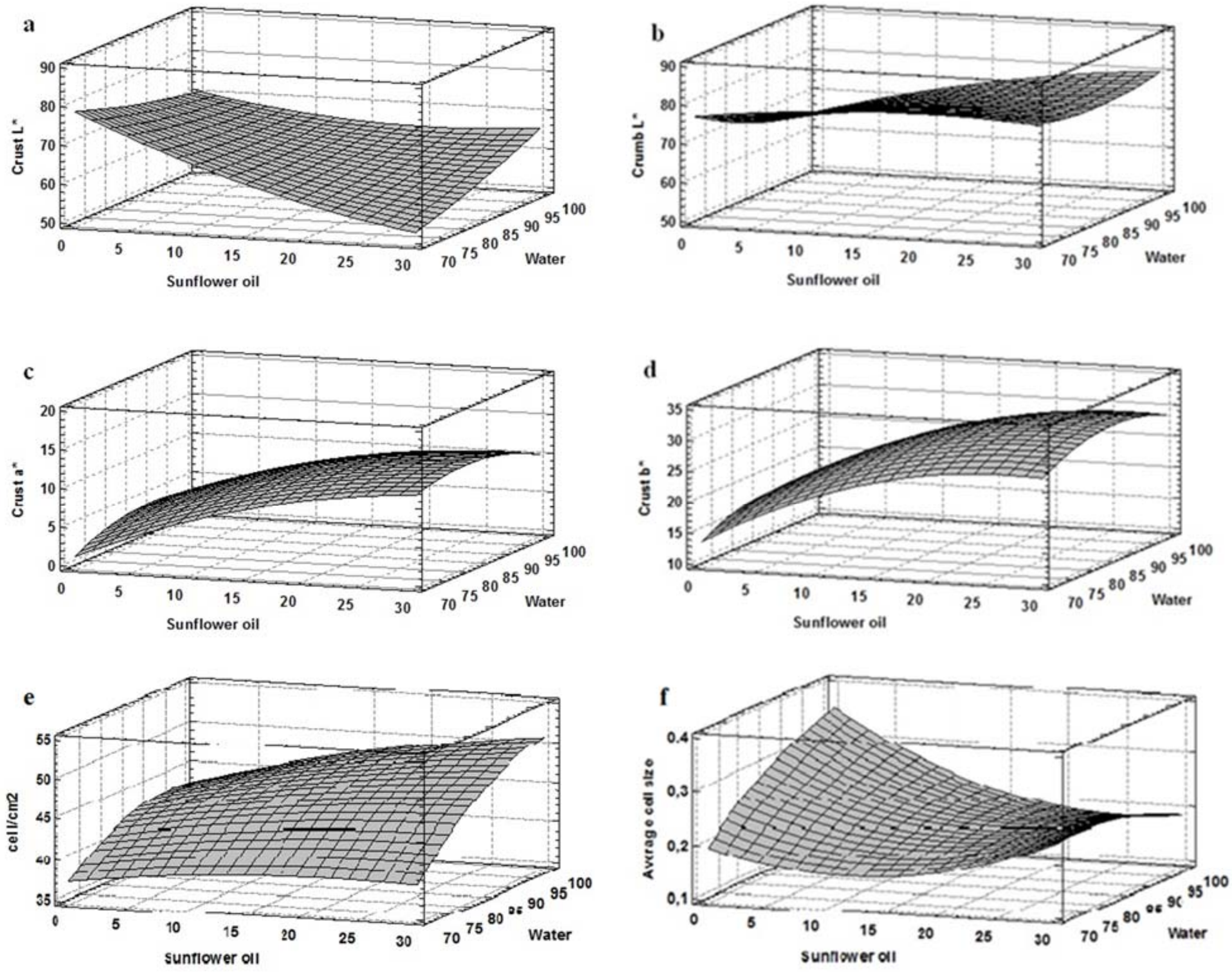

Fig. 4 Effect of sunflower oil and water content on colour parameters of crumb and crust: (a) Crust L*; (b) Crumb L*; (c) Crust a*; (d) Crust b* (e) Number of pores per cm^2; (f) Average cell size.

3.3. Correlation analysis between dough rheology and bread quality

After analysing the relationship between dough rheology and bread specific volume, it was found out that the lower the G’, G’’ and G* and the higher tg δ were, the greater bread specific volume and lower hardness (table 3). In general, a trend of an increase in the volume as dough consistency is reduced has been observed in different studies, even though this relationship has not been studied deeply. This interrelationship was observed in studies on the influence of dough hydration (Nishita *et al.* 1976), when starchy ingredients were changed (Mancebo *et al.* 2015a), when different hydrocolloids were added (Mancebo *et al.* 2015b) or when new ingredients were incorporated in the formula (Martínez *et al.* 2014; Rocha-Parra *et al.* 2015). In our study, a high correlation among all rheological parameters was observed, even displaying r values of 0.99 among G’, G’’ and G*, being these parameters better predictors than tg δ for bread quality. It would indicate that doughs with higher G', G'' and G* cannot expand properly in the fermentation and baking steps, and a reduction of G* to allow this expansion would be necessary. After a more exhaustive analysis, the linear model was observed not to be the most suitable for relating the rheological parameters with the bread specific volume, since although the latter increased as G', G'' and G* decreased, this increase is stronger as G' and G'' reach values close to 10000. This could be expected, since there must be a minimum bread volume corresponding to the doughs without expansion. Thus, values of r^2 of 74.19% between bread specific volume and G' can be obtained through a reciprocal-Y model (Y=1/(a+b*X), versus a r^2 value of 60.15% obtained through a linear model. If breads made from oil and breads made from shortening are analysed separately (Figure 5), r^2 values obtained by the reciprocal-Y model are 81.48% and 86.59% for breads made with oil and shortening respectively. It was also noted that, the increase of the volume as G' values are reduced is more intense in breads made with oil, thus the influence of oil or shortening addition on the specific volume of breads go beyond its influence on dough rheology. It is observed that G' values lower than 10000 from doughs made with oil gave rise to breads with lower specific volume than those from doughs with G' values around 20000, despite the observed trend of the increase of specific volume as G' values are reduced. In these breads, a slight volume reduction in the last fermentation or first baking stages was observed. Ronda *et al.* (2015), in a study on gluten-free breads enriched with beta-glucans, already observed that, after an increase of the bread specific volume as water content in the formulation was increased, reducing G', a decrease of the volume when water content was increases in excess was produced. This could indicate that there are minimum values for the rheological parameters from which doughs are too weak to keep the expansion, effect which can depend on the fermentation times and therefore on dough expansion.

Table 3 Correlation analysis between dough rheology and bread quality

	Firmness	G'	G"	G*	Tg δ
Specific volume	-0.788	-0.810	-0.818	-0.811	0.678
Firmness		0.872	0.877	0.872	-0.621
G'			0.997	1.000	-0.866
G"				0.997	-0.863
G*					-0.866

Correlations are significant at the $p \leq 0.001$

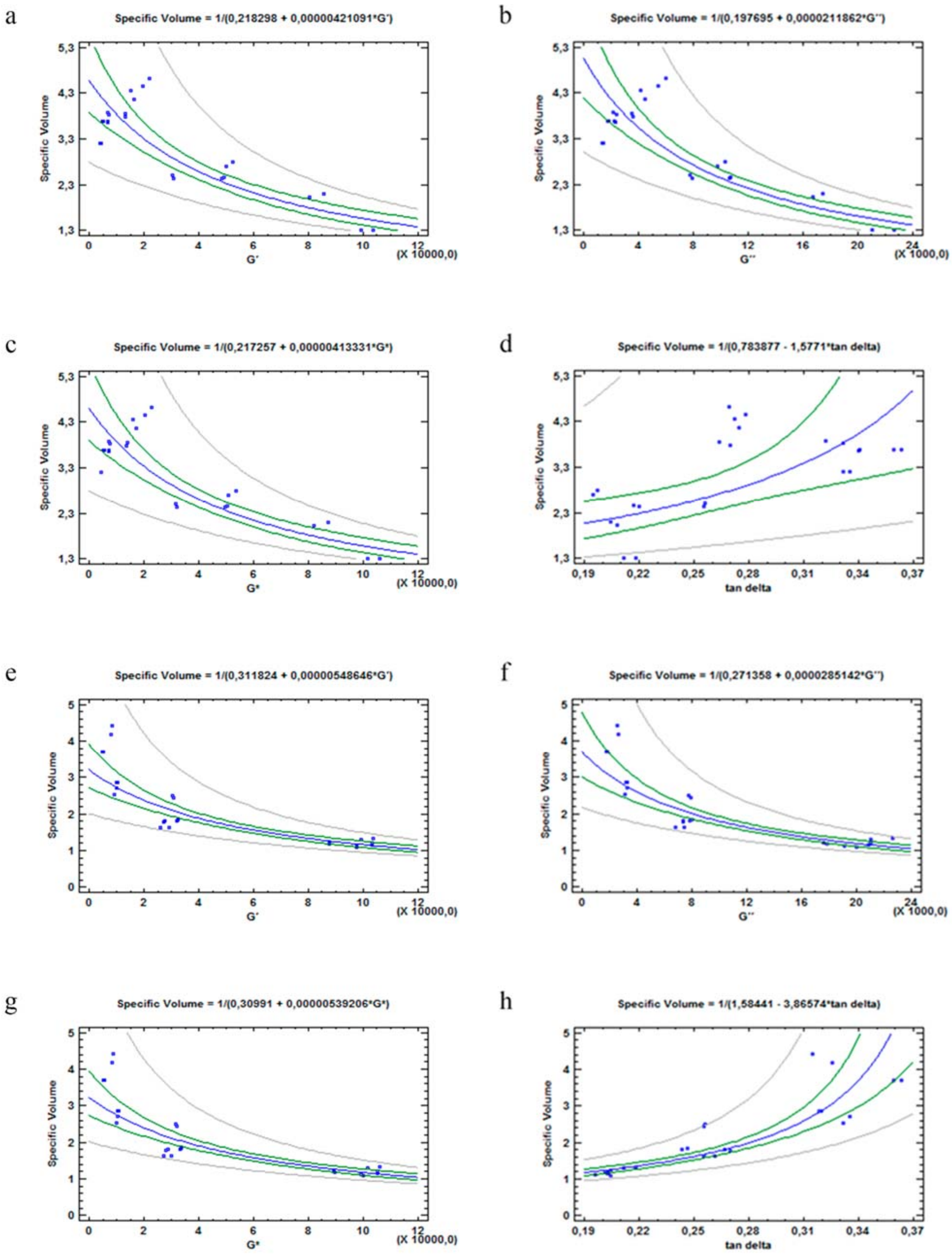

Figure 5 Reciprocal-Y-model analysis of the effect of sunflower oil (a, b, c, d) and shortening (e, f, g, h) on G' (a, e), G'' (b, f), G* (c, g) and tg (δ) (d, h).

4. Conclusions

A high correlation between the rheological parameters and bread specific volume and firmness was observed. Moreover the reciprocal-Y model was the best predictor to relate bread specific volume and G', G'' and G*. Nevertheless, this correlation should be studied for each formulation taking into account the processing variables. In general, it can be said that the incorporation of up to 20% oil increases the volume of the loaves, the a* and b* parameters of the crust and the cell density. It also decreases hardness, cohesiveness, springiness, resilience and the L* parameter. Converse to breads made with oil, the addition of shortening can negatively affect the quality of the breads.

Acknowledgements

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OPTIMISATION OF RHEOLOGICAL PROPERTIES OF GLUTEN-FREE DOUGHS WITH HPMC, PSYLLIUM AND DIFFERENT LEVELS OF WATER

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Abstract

Hydrocolloids have traditionally been investigated as an alternative to gluten for making good quality products for coeliac patients. This study investigated the interactions between hydroxypropylmethylcellulose (HPMC) (2–4 g/100 g of flour), psyllium (0–4 g/100 g of flour) and water level (90–110 g/100 g of flour) in gluten-free breadmaking. Psyllium incorporation reduced the pasting temperature and compliance values, and increased elastic (G') and viscous (G") moduli values. In contrast, HPMC addition had no important effects on pasting properties and compliance values, but also increased G' and G" values. Psyllium inclusion reduced bread specific volume and increased bread hardness, while there were hardly differences in the bread specific volume and hardness between the percentages of HPMC studied. In addition, when the dough hydration level was increased, there was a decrease in the influence of hydrocolloids on dough rheology and specific volume and hardness of breads.

Keywords: hydrocolloids; oscillatory test; creep recovery; rice flour.

Abbreviations

HPMC, hydroxypropylmethylcellulose; RVA, Rapid Visco Analyser; TPA, Texture Profile Analysis

1. Introduction

Three pathologies are associated with gluten intake, which appear to be increasing in importance: i) food allergy that affects 0.2–0.5% of the population, but has stronger clinical implications; ii) coeliac disease, which is an autoimmune disorder caused by the ingestion of gluten (from 0.1% to >1.6%); and iii) gluten sensitivity, a pathology of intolerance to gluten that has recently been rediscovered, which excludes coeliac disease and wheat allergy, with an estimated prevalence of 6% for the USA population (Rosell et al., 2014). Therefore, the demand for gluten-free products has increased, and for this reason, utilisation of new raw materials capable of simulating the viscoelastic behaviour of gluten is necessary. It has been reported by various authors that hydrocolloids improve dough development and gas retention through an increase in viscosity, producing gluten-free breads with higher specific volume (Gallagher et al., 2004; Haque and Morris, 1994; Lazaridou et al., 2007; Marco and Rosell, 2008a; Sciarini et al., 2012). Ylimaki et al. (1991) proposed the use of hydroxypropylmethylcellulose (HPMC) in gluten-free breadmaking, and nowadays it is widespread. HPMC is characterised by its great water-holding capacity in the solution state at lower temperature and it is believed to form stronger hydrophobic bonds with other HPMC chains, resulting in stronger gel networks at higher temperatures while simultaneously releasing water (Bell, 1990; Hager and Arendt, 2013; Lazaridou and Biliaderis, 2009). Psyllium fibre is less commonly used and it is usually added with other hydrocolloids (Cappa et al., 2013; Haque and Morris, 1994; Mariotti et al., 2009; Zandonadi et al., 2009). Psyllium generally enhances the physical properties of the gluten-free doughs due to the formation of a weak gel network capable of trapping CO_2 because of the gelling and water-absorbing abilities of psyllium and the heat-induced gelation of HPMC (Haque and Morris, 1994; Mariotti et al., 2009).

Several rheological techniques to monitor fundamental viscoelastic properties of gluten-free dough have been used. The rheological characterisation of gluten-free doughs is usually related to quality indicators of end-products and provides important information for food technologists, allowing the appropriate selection of ingredients to optimise the final product (Lazaridou and Biliaderis, 2009). However, only a small number of reports exist which deal with the mechanical properties of gluten-free doughs measured by fundamental rheological methods; these measurements include oscillatory tests, such as strain and frequency sweeps, as well as creep-recovery tests on gluten-free dough formulations (Gujral and Rosell, 2004a,b; Lazaridou et al., 2007; Lazaridou and Biliaderis, 2009; Marco and Rosell, 2008b; Moreira, et al., 2011; Sivaramakrishnan et al., 2004).

Oscillatory shear measurements are widely used to simultaneously evaluate the viscoelastic properties, *i.e.* elastic (G’) and viscous (G”) moduli. Nevertheless, small deformation tests hardly give clear relationships between the evaluated parameters and the dough characteristics during processing. In this way, rheological tests using large deformations are also required to adequately study these systems (Lazaridou et al., 2007; Sivaramakrishnan et al., 2004). Creep-recovery tests are useful to establish links with results from empirical techniques. This technique consists of the application of a constant stress and when the stress is released, some recovery is observed as the material attempts to return to its original shape. The deformation of the material is measured achieving strain values usually outside the linear domain.

Despite the importance of rheological characterisation of gluten-free doughs, there are no so much studies that analyse the rheological behaviour of these doughs with HPMC (Demirkesen et al., 2010; Gujral and Rosell, 2004b; Moreira, et al., 2011; Sivaramakrishnan et al., 2004) or psyllium (Haque and Morris, 1994; Marioti et al., 2009) and there are no studies about the synergistic or antagonistic effects of HPMC and psyllium, or about the hydration level effect on rheological characteristics of doughs that contain these hydrocolloids.

The objective of this work was to analyse the influence of HPMC (2–4 g/100 g) and psyllium (0–4 g/100 g) on rice-based gluten-free doughs with different hydration levels (90–110 g/100 g) by response surface methodology in order to study the influence of these ingredients on the rheological properties (dynamic oscillatory test and creep-recovery test). Further, the specific volume and hardness of breads was analysed, which are important bread quality parameters, as well as their relation with the dough rheology.

2. Materials and methods

2.1 Materials

The following ingredients were employed in this study: rice flour (8.01 g/100 g of protein, 22.13 g/100 g of amylose, 74.35 g/110 g starch and 12.5% of humidity) provided by Harinera Castellana S.L. (Medina del Campo, Spain), HPMC Vivapur® K4M Food-grade (JRS, Rosenberg, Germany) and Psyllium Vitacel® P95 Food-grade (JRS, Rosenberg, Germany). The rice flour employed in this study was coarse-grained rice flour (132–200 µm), as previous studies have shown that finer fractions give poorer results in gluten-free breadmaking (de la Hera et al., 2013).

Saf-Instant yeast (Lesaffre, Lille, France), dry refined salt (Esco European Salt Company, Niedersachsen, Germany), white sugar (Acor, Valladolid, Spain), refined sunflower oil (Langosta, Ciudad Real, Spain) and local tap water were also used in the breadmaking.

2.2 Methods

2.2.1 Experimental design

To evaluate the effect of the independent variables (level of HPMC, psyllium and water) on the dependent variables, we established a *Box-Behnken* experimental design using response surface methodology. It included three execution levels with a three-level full-factorial subset of trials (Table 1). It was also necessary to include centre points in which all factors are at their central values. Three-dimensional graphs of the models were used to visualise overall trends. Significance of the lack-of-fit error term, R^2 value, coefficient of variation and model significance were used to judge adequacy of model fit.

The Statgraphics Centurion XVI software package (StatPoint Technologies Inc., Warrenton, USA) was used both for the experimental design and to generate the corresponding response surfaces that allowed us to evaluate the independent variables.

Table 1 *Box-Behnken* experimental design with a three-level full-factorial subset of trials.

Trial	HPMC*	Psyllium*	Water*
1	4	0	100
2	4	2	110
3	3	2	100
4	3	0	110
5	2	2	110
6	3	0	90
7	2	0	100
8	3	4	110
9	2	2	90
10	2	4	100
11	3	2	100
12	4	2	90
13	3	4	90
14	4	4	100
15	3	2	100

* g/100 g of flour.

Water content (90, 100 and 110 g/100 g flour), level of HPMC (2, 3 and 4 g/100 g flour) and psyllium (0, 2 and 4 g/100 g flour).

2.2.2 Flour characterisation

The viscous behaviour of flours during the heating–cooling cycle was measured in duplicate with the Rapid Visco Analyser (RVA) (Newport Scientific, Warriewood, Australia) in accordance with AACC method 61.02.01 (AACC, 2012).

2.2.3. Breadmaking

The following ingredients were used in breadmaking (g/100 g rice flour): water (90, 100 and 110 g), Saf-Instant yeast (3 g), salt (1.8 g), oil (10 g), white sugar (5 g), HPMC (2, 3 and 4 g) and psyllium (0, 2 and 4 g).

Water temperature was held at 20ºC ± 0.5ºC. The instant yeast was first rehydrated in half the amount of water. All the ingredients, except the yeast, were mixed for 1 min at speed 1 in a Kitchen Aid 5KPM50 mixer (Kitchen Aid, Michigan, USA) with a dough hook (K45DH). After mixing the ingredients, the hydrated yeast was incorporated and the kneading process was continued for 8 minutes at speed 2. Gomez et al. (2013) observed that these kneading conditions were the most suitable in gluten-free breadmaking. The doughs were divided into 250-g pieces and moulded into aluminium pans of 232 x 108 x 43.5 mm. Pans were placed in a proofing chamber at 30ºC and 80% relative humidity for 60 minutes. After fermentation, doughs were baked in an electric modular oven for 40 minutes at 190ºC. The loaves were removed from the moulds after a 60-minute cooling period and were weighed. They were then placed into polyethylene plastic bags and stored at 20ºC until analysis. Bread volume and texture were performed 24 hours after baking. All the bread elaborations were performed twice.

2.2.4. Dough rheology

The rheological behaviour of doughs was studied using a Thermo Scientific Haake RheoStress 1 controlled strain rheometer (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P water bath that controlled analysis temperature (set at 25ºC). The rheometer was equipped with parallel-plate geometry (60-mm diameter titanium serrated plate-PP60 Ti) with a 3-mm gap. After adjustment to the 3-mm gap, the excess batter was removed and vaseline oil (Panreac, Panreac Química SA, Castellar del Vallés, Spain) was applied to cover the exposed sample surface. In oscillatory tests, dough was rested for 300 s before measuring. Samples (without yeast) were analysed in duplicate. First, a strain sweep test was performed at 25ºC with a stress range of 0.1–100 Pa at a constant frequency of 1 Hz to identify the linear viscoelastic region. On the basis of the results obtained, a stress value included in the linear viscoelastic region was used in a frequency sweep test at 25ºC with a frequency range of 100–0.1 Hz. Values of elastic modulus (G’ [Pa]), viscous modulus (G’’ [Pa]) and tangent δ (G’’/G’) were obtained for different frequency values (ω [Hz]) (Dobraszczyk and Morgenstern, 2003).

In creep-recovery tests, the dough was rested for 600 s to allow relaxation before the measurement. Creep tests were performed by imposing a sudden-step shear stress in the linear viscoelastic region for 60 s. In the recovery phase, the stress was suddenly removed and the sample was allowed to rest for 180 s to recover the elastic (instantaneous and retarded) part of the deformation. Each test was performed in triplicate. Creep data are described in terms of creep compliance, J, which is defined as the strain divided by the stress applied (maintained constant during the creep test). Parameters redily available from the creep-recovery curves are the maximum creep compliance J_{cmax} and the maximum recovery compliance J_{rmax}, measured at the end of the creep and recovery phase, respectively. The steady-state compliance J_{steady} is calculated by subtracting J_{rmax} from J_{cmax}.

2.2.5. Gluten-free bread quality

Bread volume was determined using a laser sensor with the Volscan Profiler volume analyser (Stable Microsystems, Surrey, UK). The volume measurements were performed on two loaves from each elaboration. The specific volume was calculated as the ratio of bread volume to its mass.

Crumb texture was measured with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) fitted with the “Texture Expert” software. A 25-mm diameter cylindrical aluminium probe was used in a compression test to penetrate to 50% of the sample depth at a test speed of 2 mm/s. Hardness (N) was calculated from the Texture Profile Analysis (TPA) graph. Firmness analyses were performed on 30-mm thick slices at 24 hours after baking. Analyses were performed on two slices from two loaves (2 x 2) from each type of elaboration.

3. Results and Discussion

3.1. Flour characterisation

As can be seen in Fig. 1 (a–d), there was hardly any difference between 2% and 4% HPMC inclusion in the flour pasting properties, apart from an increment in setback. However, the effect of the psyllium addition was clearer than the effect of HPMC. Pasting temperature (Fig. 1a) decreased with psyllium addition, mainly at low levels, since this parameter was not modified at levels higher than 2%. There was a weak peak viscosity increase (Fig. 1b) and higher setback increment (Fig. 1c) when psyllium content increased. The increment on setback with psyllium addition became stronger with higher HPMC doses. Meanwhile, the breakdown effect with either hydrocolloid was limited (Fig. 1d). Cappa et al. (2013) also observed how peak viscosity and setback values increased when psyllium was added (with sugar beet fibre) in gluten-free doughs, whereas HPMC addition had no significant effects ($P > 0.05$) in the same pasting properties. These trends underlined the strong ability of psyllium to bind water due to the hydrophilic nature of this biopolymer (Mariotti et al., 2009). Despite the fact that HPMC also possesses the ability to bind water, in this study there were no important differences in pasting properties. Differences could be due to the differences between psyllium water absorption and HPMC water absorption. Ronda et al. (2013), in contrast to our findings, found that the addition of HPMC linearly decreased peak viscosity. It could be explained because, in our study, the amount of HPMC was higher (2–4%) than their HPMC addition, which varied between 0.10% and 2.5%.

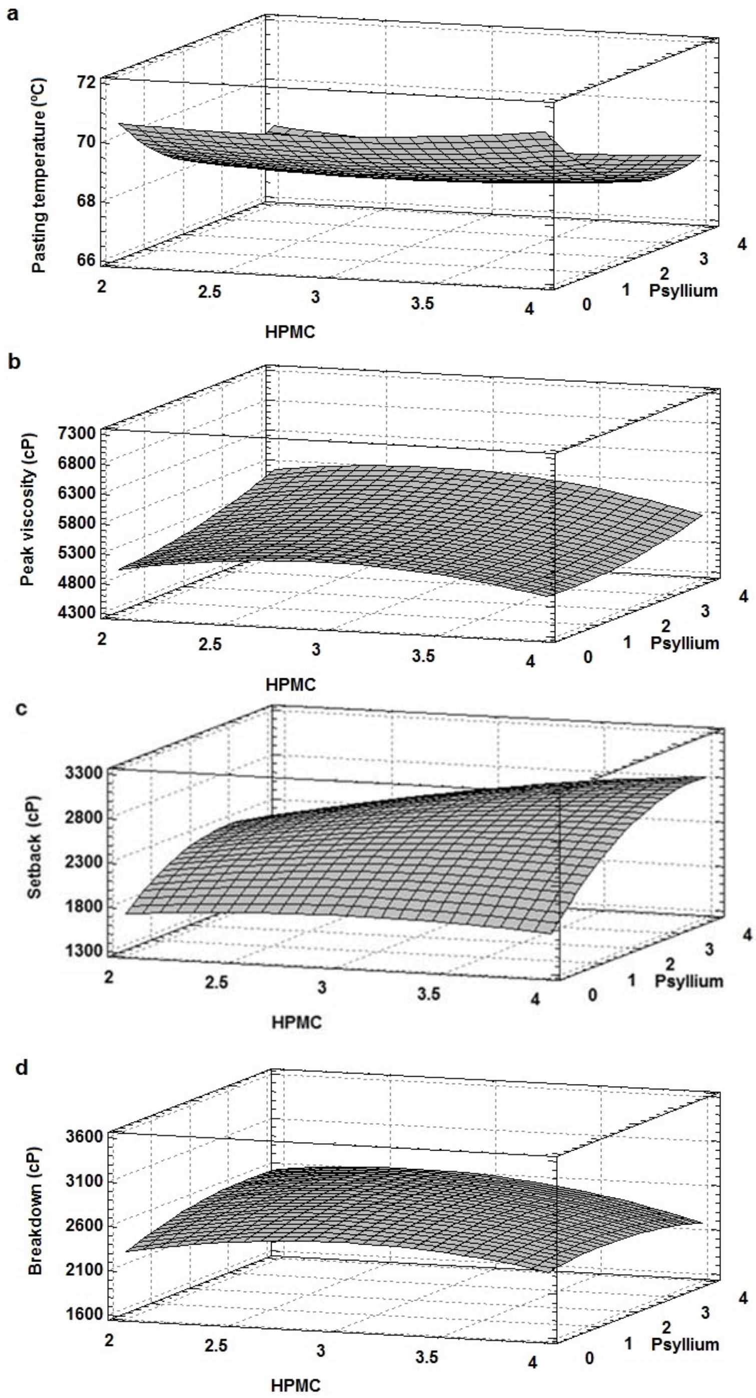

Fig. 1 Effect of HPMC and psyllium content on pasting properties at a level of water of 100 g/100 g rice flour. (a) Pasting temperature, (b) peak viscosity, (c) setback, and (d) breakdown.

3.2. Dough rheology

3.2.1. Dynamic oscillatory test

Fig. 2 shows the dynamic oscillatory test results. An increase in water content of dough decreased G' (Fig. 2b and c). This phenomenon is attributed simply to the dilution of constituents, and it has been well documented for wheat flour doughs (Autio et al., 2001) and for gluten-free doughs (Lazaridou et al., 2007; Ronda et al., 2013). It was also observed that psyllium and HPMC increased the elastic modulus (G') (Fig. 2a), particularly with reduced dough water levels (Fig. 2b and c). This could be expected because of the enhanced viscoelastic properties of polysaccharides in aqueous medium (Lazaridou et al., 2007). The effect of both hydrocolloids, psyllium and HPMC, was lower when the water percentage increased, becoming non-existent at 110 g/100 g hydration level when HPMC was added, due to the dilution effect being promoted by the large water content. Our results agree with those of other studies that have investigated the use of HPMC added to rice-flour dough where an increase of G' has also been observed (Crockett et al., 2011; Ronda et al., 2013; Sivaramakrishnan et al., 2004). In the case of psyllium, Haque and Morris (1994) confirmed the ability of this hydrocolloid to form a weak gel network that would increase the dough consistency. Matos and Rosell (2013) have already demonstrated that the enhancement of dough consistency is greatly dependent on the presence of ingredients with water-binding ability. However, in contrast to our findings, Mariotti et al. (2009) observed that the presence of psyllium in gluten-free doughs had no significant effect on G'. This could be due to the fact that these authors modified the water content in the formulation in order to achieve doughs with constant farinograph values.

In all gluten-free dough formulations the viscous modulus (G") showed similar tendency to G' (data not shown) and G' was greater than G", which suggests a solid elastic-like behaviour of all the gluten-free doughs studied. Therefore, tan (δ), ratio of viscous to elastic behaviour, was lower than 1 for all dough trials. A balance between elastic and viscous properties is required in gluten-free bread (Crockett et al., 2011; Lazaridou et al., 2007). In our research, the tan (δ) parameter slightly increased with increasing dough moisture content (Fig. 3b and c). It can be observed that psyllium decreased tan (δ) parameter and HPMC increased it (Fig. 3a), apart from a water content of 90g/100g where the HPMC had not effect (Fig. 3b). It could be said that HPMC required enough water to modify tan (δ). A tan (δ) increase was also observed by Crockett et al., (2011) when the HPMC content increased.

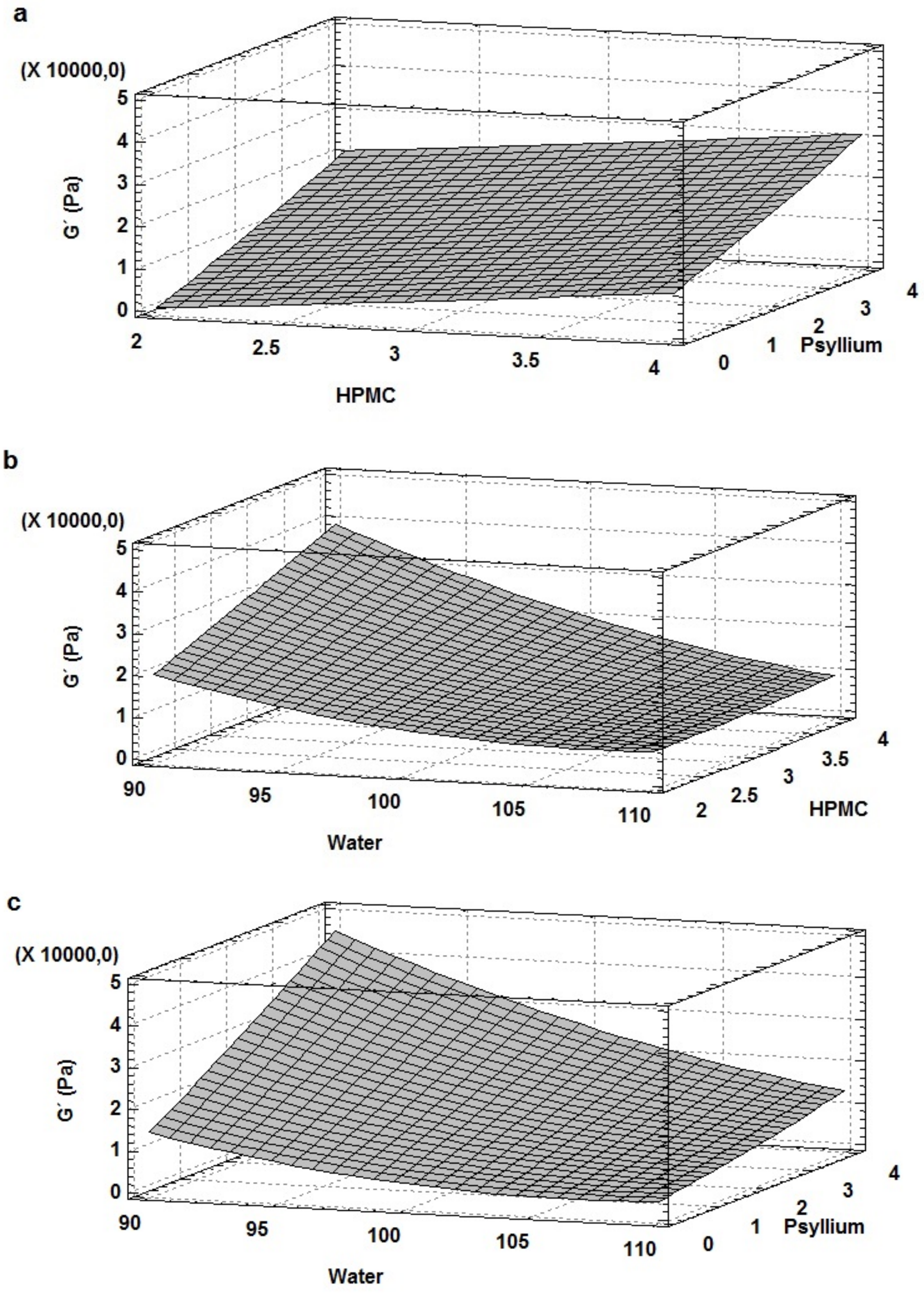

Fig. 2 Effect of HPMC, psyllium and water content on elastic modulus in oscillatory test. (a) HPMC vs psyllium at 100 g of water/100 g of flour, (b) water vs HPMC at 2 g of psyllium/100g of flour, and (c) water vs psyllium at 3 g of HPMC/100 g of flour.

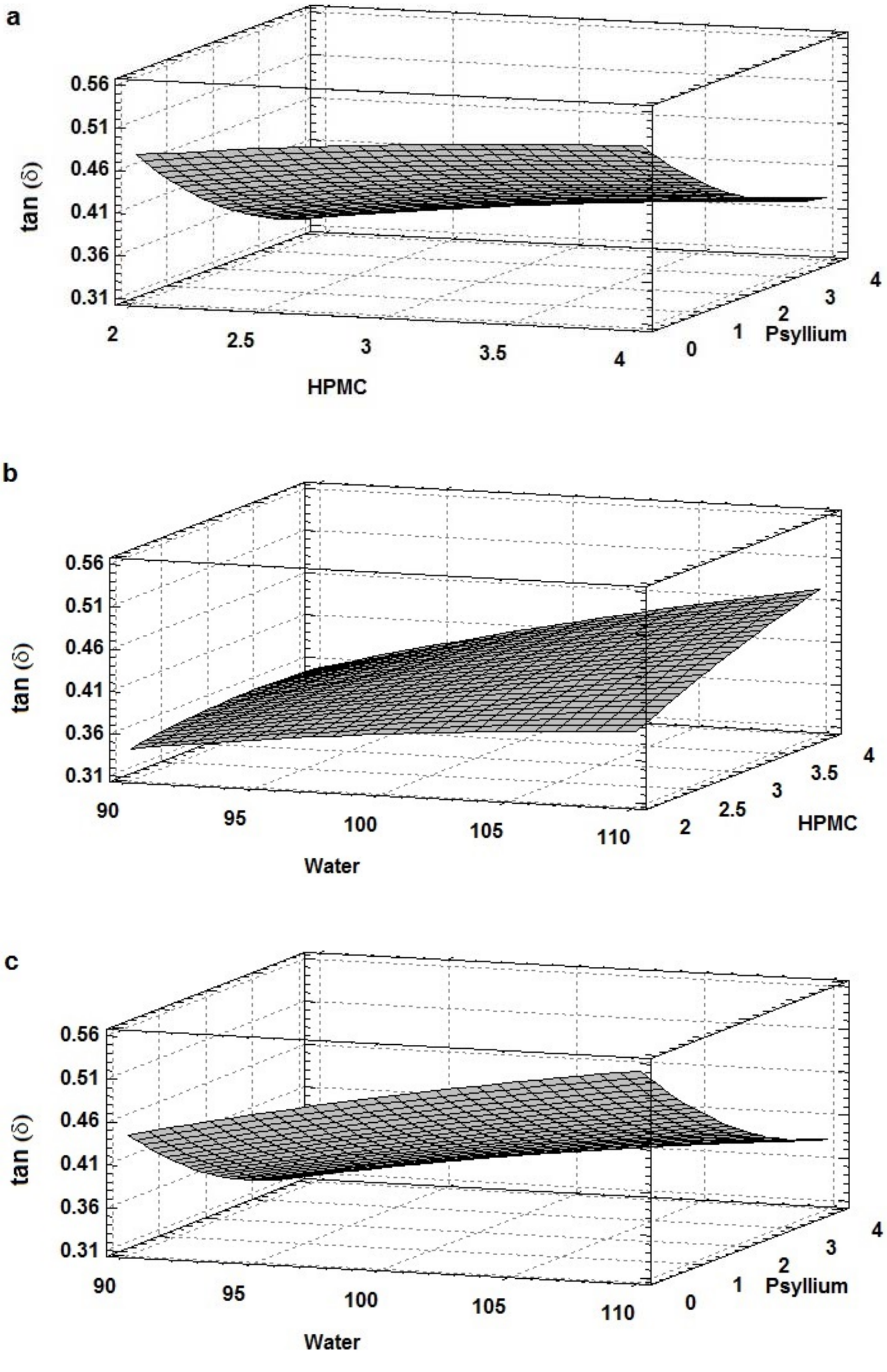

Fig. 3 Effect of HPMC, psyllium and water content on tan (δ) in oscillatory test. (a) HPMC vs psyllium at 100 g of water/100 g of flour, (b) water vs HPMC at 2 g of psyllium/100g of flour, and (c) water vs psyllium at 3 g of HPMC/100 g of flour.

3.2.2. Creep-recovery test

The creep-recovery curves of the gluten-free doughs obtained in this study (Fig. 4) exhibited a typical viscoelastic behaviour combining both viscous fluid and elastic components. They were similar to the corresponding curves obtained previously for rice-flour doughs (Sivaramakrishnan et al., 2004).

Fig. 4a shows compliance curves corresponding to the results for the mean HPMC value (3 g/100 g). It can be observed that the inclusion of psyllium reduced the compliance values (J), and its effect increased with greater amounts of water. When the water content was decreased in the formulation, the J values also decreased. This seemed to be in agreement with the higher G’ and G” values obtained for higher psyllium and lower water levels. Fig. 4b shows compliance curve corresponding to the results for the mean psyllium value (2 g/100 g), confirming the compliance reduction when the water content was decreased. Meanwhile, HPMC reduced the J value in more hydrated doughs (110 g/100 g), but the G’ values did not change. In contrast, with the lowest amount of water (90 g/100 g), HPMC addition increased compliance. Thereby, unlike in the case of psyllium and water (where an increase of compliance values was linked to a reduction in G’ and G’’ values), in the case of HPMC an increase of compliance values led to higher G’ and G’’ values. Ronda et al. (2013) investigated the rheological properties of two types of HMPC in gluten-free dough formulations and they also found that HPMC decreased J values. In addition, they found that one of those HPMC did not show any significant effect on J with the lowest hydrated doughs (70%), and therefore, the HPMC effect was minimised when the water hydration level was reduced, as in our results. Nevertheless, Sivaramakrishnan et al. (2004) found that the maximum creep compliance for short-grain rice flours increased with HPMC concentration. However, this study cannot be compared with ours because those authors corrected the amount of water based on farinograph tests. These results also reinforced that water is essential for optimising gluten-free formulations, and directly influences the dough rheological properties attributed to constituent concentration effects, and indirectly by interacting with hydrocolloids (Ronda et al., 2013).

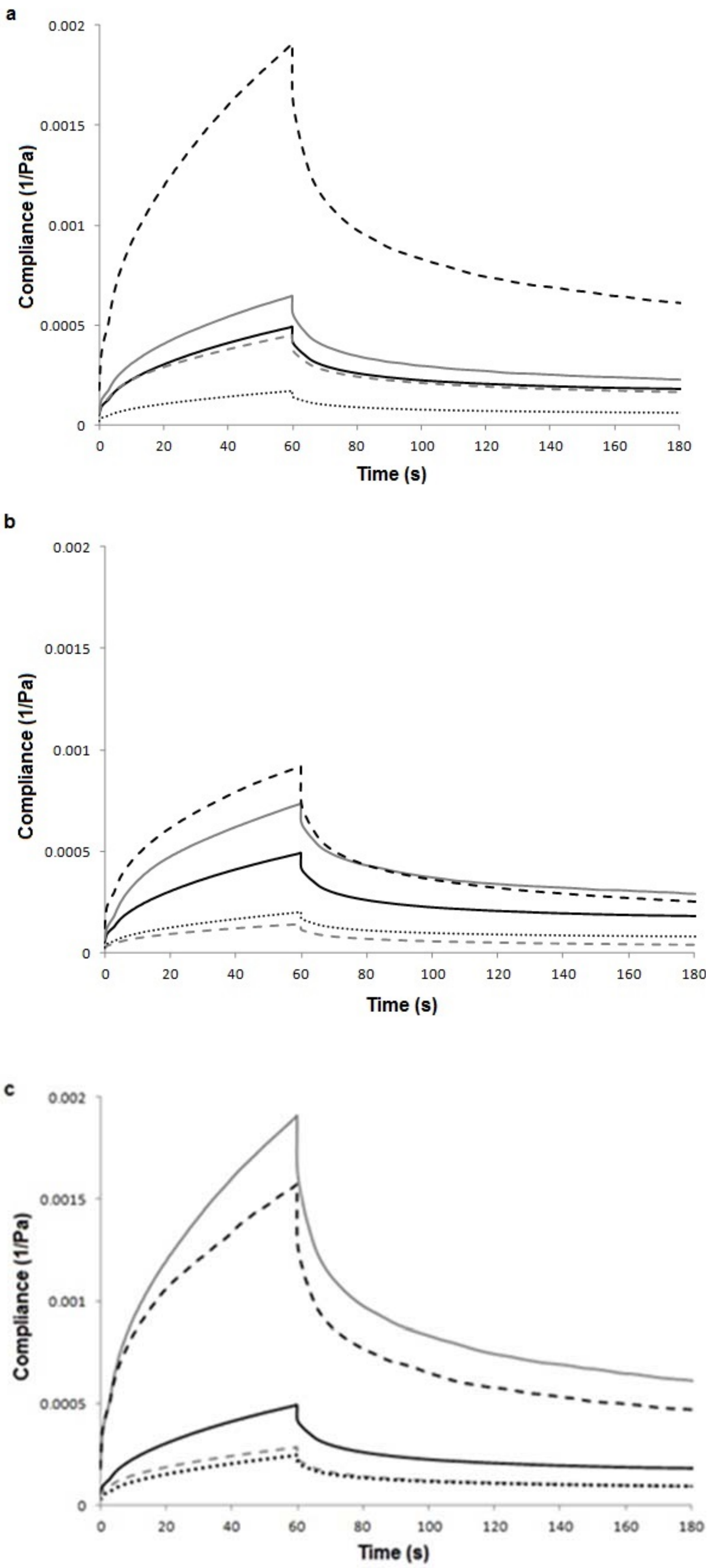

Fig. 4 Compliance results. (a) Constant HPMC value and different levels of psyllium and water. 2 % of psyllium and 100% of water flour basis (fb) (black line); 0 % of psyllium and 90 % of water fb (grey line); 0 % of psyllium and 110 % of water fb (discontinuous black line), 4 % of psyllium and 110 % of water fb (discontinuous grey line) and 4 % of psyllium and 90 % of water fb (dotted black line). (b) Constant psyllium value and different levels of HPMC and water. 3 % of HPMC and 100 % of water fb (black line); 4 % of HPMC and 110 % of water fb (grey line); 2 % of HPMC and 110 % of water fb (discontinuous black line); 2 % of HPMC and 90 % of water fb (discontinuous grey line) and 4 % of HPMC and 90 % of water fb (dotted black line). (c) Constant water value and different levels of HPMC and psyllium. 3 % of HPMC and 2 % of psyllium fb (black line); 4 % of HPMC and 0 % of psyllium fb (grey line); 2 % of HPMC and 0 % of psyllium fb (discontinuous black line); 2 % of HPMC and 4 % of psyllium fb (discontinuous grey line) and 4 % of HPMC and 4 % of psyllium fb (dotted black line).

Finally, compliance curves for the mean water value (100 g/100 g) are shown in Fig. 4c, and confirm that the increase of psyllium exhibited a great decrease in compliance values, indicating higher dough strength (Edwards et al., 2003). In contrast, HPMC barely increased compliance, with no effect in doughs with higher levels of psyllium. This shows that the rheological properties of psyllium were more dominant than those of the other dough components, such as HPMC. Therefore, the HPMC and psyllium effects were entirely different in their compliance values in spite of the similar G' and G" results. Lazaridou et al. (2007) claimed that, despite the concentration effect, the magnitude of the influence of hydrocolloids on rheological properties of gluten-free dough seems to be related to the molecular structure and chain conformation of the polysaccharide that determine the physical intermolecular associations (cross-links or entanglements) of the polymeric chains. It is obvious that psyllium and HPMC present different molecular structures. The weak gel structure of psyllium is similar to xanthan weak gel (Farahnaky et al., 2010; Haque et al, 1993a), which was formed by a tenuous association of rigid, ordered polysaccharide chains (Morris, 1990). In fact, in other studies about gluten-free doughs, xanthan gum addition also increased G' values (Crockett et al., 2011; Lazaridou et al., 2007) and decreased compliance values (Lazaridou et al., 2007). In a previous study, Guo et al. (2008) found that the alkaline extractable gel fraction of psyllium was an arabinoxylan with (1 → 4) linkages in the xylan backbone, which was heavily substituted by short arabinose branches. This structure could form a rigid, ordered polysaccharide chain conformation; however, the presence of branches could prevent molecules from associating with each other to form a stronger gel. Meanwhile, some modified cellulose derivatives such as HPMC have high water-retention properties due to their hydrophilic nature, but they also contain hydrophobic groups that induce additional properties, including increased interfacial activity within the dough system during proofing, and forming gel networks on heating during the breadmaking process, similar to those of thermosetting proteins (*e.g.* egg-white proteins) (Bell, 1990; Haque et al., 1993b). Such network structures serve to increase viscosity and to further strengthen the boundaries of the expanding cells in the dough, thus increasing gas retention through baking, and consequently leading to a better gluten-free loaf volume (Lazaridou and Biliaderis, 2009).

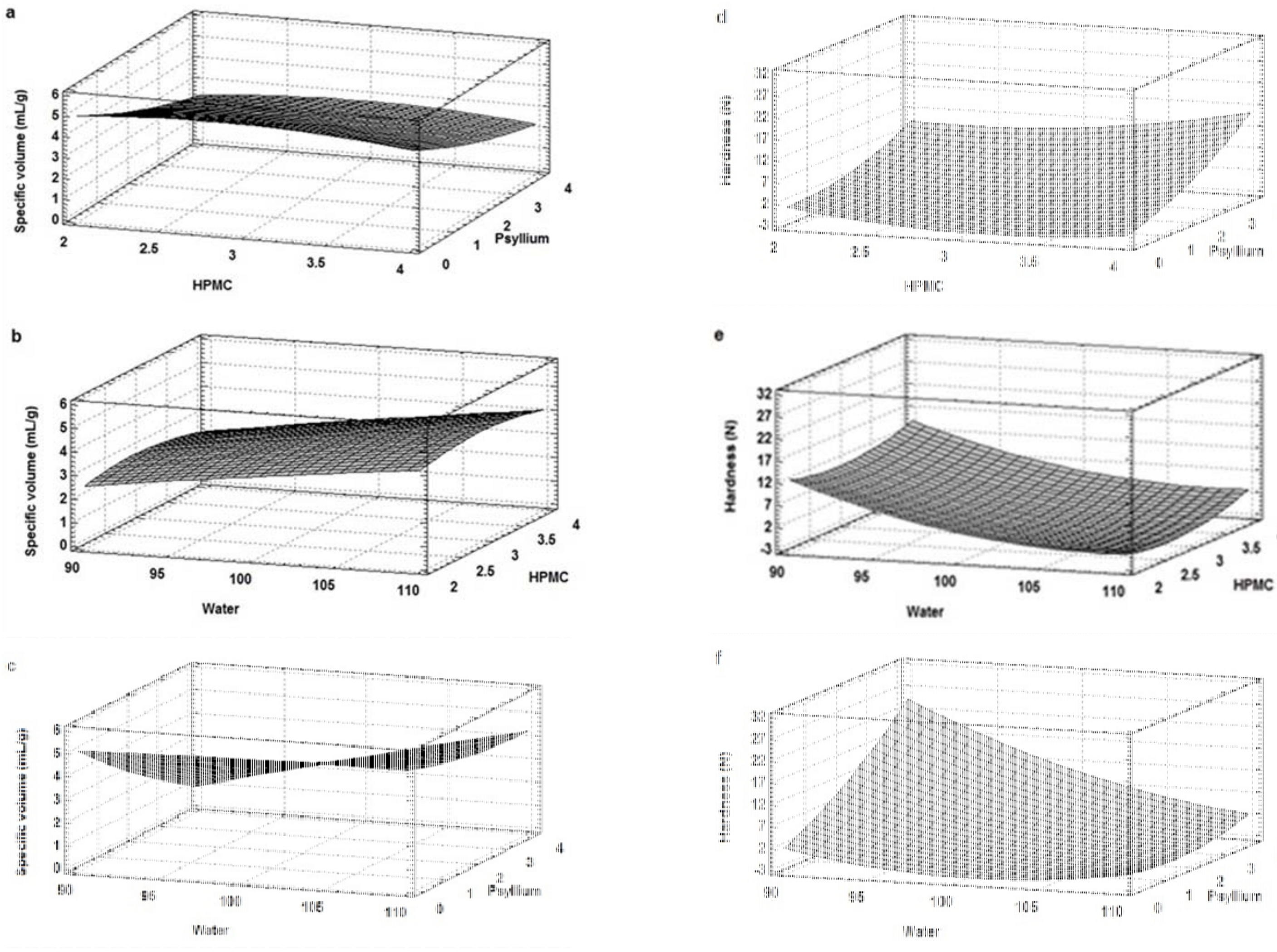

Fig. 5 Effect of HPMC, psyllium and water content on bread specific volume and hardness. Response surfaces of specific volume of breads, (a) HPMC vs psyllium at 100 g of water/100 g of flour, (b) water vs HPMC at 2 g of psyllium/100g of flour, and (c) water vs psyllium at 3 g of HPMC/100 g of flour. Responses surfaces of hardness of breads, (d) HPMC vs psyllium at 100 g of water/100 g of flour, (e) water vs HPMC at 2g of psyllium/100 g of flour, and (f) water vs psyllium at 3 g of HPMC/100 g of flour.

3.3. Gluten-free bread quality

Bread specific volume is one of the most important visual characteristics of breads and it is a key parameter observed when evaluating bread quality (Hager and Arendt, 2013). Fig. 5 shows the response surface of bread specific volume and hardness with different levels of HPMC, psyllium and water. Bread specific volume decreased when the content of psyllium increased (0–4%) (Fig. 5a and 5c), but this effect was reduced when the hydration level increased (Fig. 5c). However, important changes were not observed with the HPMC addition (2% – 4%) at any of the hydration levels studied (Fig. 5b). In contrast to our results, Haque and Morris (1994) observed a synergistic effect when HPMC and psyllium gum was added to rice-flour dough. However, they corrected the amount of water based on penetration tests, with higher water content when the hydrocolloid doses increased. It seems that bread volume can be increased up to a certain hydrocolloid concentration, but with further increases in the polymer level, the loaf volume decreased. Nonetheless, this effect depends on the dough hydration, which should be increased at the same time that the hydrocolloid quantity is increased. Other authors have also observed that a greater content of water in the formula, and therefore a lower dough consistency, increased gluten-free bread volume (de la Hera et al., 2013; McCarthy et al., 2005; Ylimaki et al., 1988). Matos and Rosell (2013) have already reported a negative correlation between initial dough consistency – analysed with the Mixolab device – and the specific volume of gluten-free breads.

Most studies have used a dynamic oscillatory test or a Mixolab device in order to find a connection between dough rheology and gluten-free bread quality. However, the low deformation conditions used for the oscillatory measurements are often inappropriate for processing situations, because they are carried out at rates and conditions very different from those experienced by the dough during processing or baking (Mariotti et al., 2009). Our study showed that the creep-recovery test was the best predictor of bread specific volume because when psyllium or water decreased G’ values in the oscillatory test it seemed to be correlated with higher bread specific volume. However, when HPMC content was increased, an increase in G' values was observed, especially with the minimum level of water, as happened with psyllium, but there was no decrease in the bread specific volume. Therefore, the creep-recovery test is a better predictor than oscillatory test, since when the psyllium content was reduced or the water content was increased resulted in a decrease in compliance values and bread specific volume but there is no a clear relation with the G' and tan (δ) values. In addition, when the HPMC content was modified, it hardly affected the compliance values and there were no changes observed in the bread specific volume. Other authors also found a good correlation between maximum recovery strain and bread volume for commercial wheat flour (Wang and Sun, 2002) and pure wheat cultivars (Van Bockstaele et al., 2008) that differed in quality.

Another important quality characteristic of bread is the texture, with consumers desiring soft and flexible crumbs, associated with low hardness values (Hager and Arendt, 2013). There was an inverse relationship between bread specific volume and initial firmness, with 99% significance, in agreement with other studies (Gallagher et al., 2004; Sabanis et al., 2009). An increase in hardness was observed when the content of psyllium increased (Fig. 5d and f), which could be explained by the lower bread specific volume due to the greater psyllium content. In addition, greater setback values – as observed when psyllium content was increased – could be associated with greater bread hardness. Other authors found similar results in crumb hardness when xanthan gum was added to wheat-flour breads (Guarda et al., 2004; Rosell et al., 2001) and in gluten-free breads (Lazaridou et al., 2007), and we already mentioned that xanthan gum has a similar structure to psyllium. In our study, psyllium effect in bread hardness decreased when the hydration level increased (Fig. 5f). Therefore, greater contents of water mitigated the negative psyllium effect in hardness and specific volume of breads. Important changes due to HPMC addition were not observed at any of the hydration levels, as in the case of specific volume (Fig. 5e).

4. Conclusion

We found that the addition of HPMC and psyllium modified the dough rheology in different ways and there were no synergistic effects between both hydrocolloids. Whereas HPMC and psyllium increased the elastic modulus similarly on the oscillatory test, only psyllium decreased compliance values. Thereby, psyllium showed opposite and more dominant rheological properties than HPMC on creep-recovery. In addition, there were hardly differences between 2 and 4 g/100 g of flour HPMC inclusion in bread specific volume and hardness, but psyllium addition had a greater and negative effect, the bread specific volume being reduced and bread hardness increased. Therefore, our study shows that the creep-recovery test may be a better predictor of bread quality characteristics than the oscillatory test. Furthermore, a higher amount of water in dough led to a lower effect of inclusion of hydrocolloids, both on dough rheology and on the characteristics of gluten-free breads.

Acknowledgements

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SECCIÓN II: GALLETAS DE MASA CORTA SIN GLUTEN

EFFECT OF FLOUR PROPERTIES ON THE QUALITY CHARACTERISTICS OF GLUTEN FREE SUGAR-SNAP COOKIES

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Abstract

The three main ingredients of cookies are wheat flour, fat and sugar. In gluten-free cookies the wheat flour must be replaced by other ingredients. The objective of the present study was to determine the effects of the flour properties of different gluten-free flours on cookie quality. A variety of different gluten-free flours, including yellow maize, precooked yellow maize, buckwheat, teff and short-grain and long-grain rice flour, were employed. The flour characteristics (protein, damaged starch content, particle size, flour hydration properties and oil absorption), dough properties (texture) and cookie parameters (final diameter, spread factor, texture, colour and acceptability) were evaluated. Coarse-grained rice flours produced cookies with a larger diameter and spread factor, darker colour and lower hardness. The rest of the gluten-free cookies had a lower spread ratio and greater hardness than wheat cookies, regardless of flour particle size. We have thus established that it is possible to obtain gluten-free cookies with organoleptic acceptability similar to that of cookies made from wheat flour. Nevertheless, cookie acceptability was hardly influenced by the cereal origin and their taste.

Keywords: sugar-snap cookie; gluten-free; particle size; damage starch; flour.

Abbreviations

Fine-grained short-grain rice flour (FSR), coarse-grained short-grain rice flour (CSR), fine-grained long-grain rice flour (FLR), coarse-grained long-grain rice flour (CLR), fine-grained maize flour (FM), coarse-grained maize flour (CM), fine-grained precooked maize flour (FPM), coarse-grained precooked maize flour (CPM), Water Binding Capacity (WBC), Water Holding Capacity (WHC), Swelling Volume (SV). Oil Absorption Capacity (OAC), dough peak force (DPF), dough elastic moduli (DEM).

1. Introduction

Three pathological conditions, which appear to be increasing in frequency, are related to gluten ingestion: i) food allergy, which affects 0.2%-0.5% of the population but has major clinical implications; ii) coeliac disease, which is an autoimmune disorder caused by the ingestion of gluten (between 0.1% and >1.6%); and iii) gluten sensitivity, a recently rediscovered condition due to gluten intolerance. Gluten sensitivity does not include coeliac disease and wheat allergy (Rosell, Barro, Sousa, & Mena, 2014). As a result, the market for gluten-free products is increasing. However, improving the quality of gluten-free products continues to be a major challenge for food scientists.

Cookies are a baked product characterized by a low final water content. Their three major ingredients are typically flour, sugar and fat; other ingredients which can be included in the cookie dough formula are chemical leavening agents, syrups, salt and emulsifiers, though these ingredients are usually only used at low levels (Pareyt & Delcour, 2008). The differences between the distinct types of cookie depend on cookie composition and cookie dough making and baking parameters. A particular type of cookie is the sugar-snap cookie. Due to the high levels of fat and sugar and the low water levels in these cookies, there is only limited development of the gluten network (Hadnadev, Torbica, & Hadnadev, 2013; Pareyt & Delcour, 2008). Flour is the main ingredient in cookie dough formulas and consists mainly of starch, water and protein. The most important components of the flour seem to be those that bind water, such as starch, protein and arabinoxylan, and thereby limit spreading of the cookie, (Pareyt & Delcour, 2008). Donelson & Gaines (1998) studied the starch-water relationship in wheat sugar-snap cookie dough systems and concluded that higher levels of damaged starch lead to smaller final cookie diameters. Those observations had already been reported by numerous investigators (Gaines, Donelson, & Finney, 1988; Hoseney & Roger, 1994; Hoseney, 1994; Miller & Hoseney, 1997) and confirmed by Barrera, Pérez, Ribotta, & León (2007). Flour particle size is also an important factor in wheat cookie properties (Gaines 1985) and its effects vary in different types of dough (Manley, 2011).

Most studies that have investigated gluten-free cookies have used amaranth (de la Barca, Rojas-Martínez, Islas-Rubio, & Cabrera-Chávez, 2010; Gambus et al., 2009; Hozova, Buchtová, Dodok, & Zemanovič, 1997; Tosi, Ciappini, & Masciarelli, 1996; Schoenlechner, Linsberger, Kaczyc, & Berghofer, 2006), buckwheat (Gambus et al., 2009; Hadnađev et al. 2013; Kaur, Sandhu, Arora, & Sharma, 2014: Schoenlechner, Linsberger, Kaczyc, & Berghofer, 2006) and/or rice flour (Chung, Cho, & Lim, 2014; Torbica, Hadnadez, & Hadnadev, 2012) as gluten-free substitutes for wheat flour. Other research have studied the use of teff flour (Coleman, Abaye, Barbeau, & Thomason, 2013), oat flour (Duta & Coletu, 2015) or starches (Demiete et al., 2000; Arendt et al., 2002) in gluten-free cookie making. Meanwhile, other studies have focussed on the combination of gluten-free flours with buckwheat, corn and rice flour (Altındağ, Certel, Erem, & Konak, 2014), with rice, maize, sorghum and pearl millet flours (Rai, Kaur, & Singh, 2014) or a mixture of gluten-free flours (brown rice flour, soya flour, buckwheat flour and millet flakes) and starches (corn starch, potato starch) (Schober et al. 2003). However none of those studies analysed the effect of gluten-free precooked flours or flours with different particle size. The aim of the present study was to compare the suitability of different gluten-free flours with a wide range of properties including precooked flours or flours with different particle size for the manufacture of sugar-snap cookies and to establish relationships between the different flour properties and the final cookie quality.

2. Materials and methods

2.1. Materials

The following ingredients were employed in this study: rice flour (8.01 g/100 g of protein, 22.13 g/100 g of amyloseThe gluten-free flours used in this study are commonly used in commercial gluten-free products and cover a wide range of different characteristics and origins. Wheat and yellow maize flours were supplied by Molendum Ingredients S.L. (Zamora, Spain), the pre-cooked yellow maize flour by PAN (Empresas Polar Caracas, Venezuela), buckwheat flour by El Granero Integral (BIOGRAN S.L., Seseña, Toledo, Spain) and wholegrain white teff flour by Salutef (Palencia, Spain). Rice flours were obtained from two rice grain types, short (japonica) and long (indica) (Hacendado, Arrocerías Pons S.A., Massanassa, Valencia, Spain), ground in a hammer mill (LM 3100) (Perten Instruments, Huddinge, Sweden). Other ingredients were white sugar (AB Azucarera Iberia, Valladolid, Spain), margarine 100 % vegetable (Argenta crema, Puratos, Barcelona, Spain), sodium bicarbonate (Manuel Riesgo S.A., Madrid, Spain) and local tap water.

The gluten-free flours were sifted for 10 min in a Bühler MLI 300B sieve (Milan, Italy) with a 106-micron screen, achieving two different particle-size fractions. It was not possible to obtain two different particle size fractions for teff flour because of the noticeable differences in composition between the two fractions, and for buckwheat flour because the fraction <106 was very small and it would have been extremely difficult to obtain a sufficient quantity of this fraction. Thus, 11 flours were studied: wheat flour as control, fine-grained short-grain rice flour (FSR), coarse-grained short-grain rice flour (CSR), fine-grained long-grain rice flour (FLR), coarse-grained long-grain rice flour (CLR), fine-grained maize flour (FM), coarse-grained maize flour (CM), fine-grained precooked maize flour (FPM), coarse-grained precooked maize flour (CPM), buckwheat flour and teff flour.

2.2. Methods

2.2.1. Flour characterisation

2.2.1.1. Particle size and protein content

Flours were analyzed in accordance with AACC method 46-30.01 for protein content (AACC, 2012) using a Leco TruSpecN analyser (St. Joseph, Michigan, USA). Particle size distribution was determined using a Mastersizer 3000 particle size analyzer (Malvern Instruments, Malvern, United Kingdom). Measurements were carried out in duplicate.

2.2.1.2. Damaged starch content

The damaged starch content of the flour samples was determined in accordance with AACC method 76-31.01 (AACC, 2012), using the Megazyme starch damage test kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Starch damage was determined as a percentage of flour weight on dry basis. Three replicates were performed for each sample.

2.2.1.3. Flour hydration properties and oil absorption

Swelling volume (SV) was evaluated by adding 100 mL of distilled water to 5 g (±0.1 g) of flour sample in a test tube and allowing it to hydrate for 24 h. Water holding capacity (WHC) was determined on the same suspension used to evaluate swelling; the hydrated solid was weighed after removing the excess of water and values were expressed as grams of water per gram of solid. Water binding capacity (WBC) was measured as described in AACC method 56-30.01 (AACC, 2012). Hydration properties were analysed in duplicate.

The method described by Lin et al. (1974) was used to determine oil absorption capacity (OAC). Flour (100.0 ± 0.2 mg) was mixed with 1.0 ml of refined sunflower oil (Langosta, Ciudad Real, Spain). The mixture was stirred for 1 min with a wire rod to disperse the sample in the oil. After a period of 30 min in the vortex mixer, tubes were centrifuged at 3000 × g and 4◦C for 10 min. The supernatant was carefully removed with a pipette and tubes were inverted for 25 min to drain the oil and the residue was weighed. The oil absorption capacity was expressed as grams of oil bound per gram of sample on dry basis. Three replicates were performed for each sample. OAC was calculated by Eq. (1):

$$\text{OAC (g/g)} = \text{Wr} / \text{Wi} \quad (1)$$

where Wr is the residue weight and Wi is the sample weight (g, db)

2.2.2. Cookie preparation

All formulations were prepared using the same quantities of ingredients except for water, which was added to adjust dough moisture content to 15.0% and the flour type. The following ingredients (as g/100 g on dough basis) were used: flour (43.3 g/100 g), sugar (31.2 g/100g), margarine (19.4 g/100g), water (5.2 g/100g) and sodium bicarbonate (0.9 g/100 g). Eleven different cookie elaborations were made according to Pareyt & Delcour (2008). Cookie dough was cut with a circular cookie cutter (internal diameter, 40 mm). Each different cookie elaborations were performed twice.

2.2.3. Dough and cookie characteristics

The texture of the dough and the cookies was measured using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) fitted with the *"Texture Expert"* software. Dough texture was measured using a modification of the method described by Laguna, Salvador, Sanz, & Fiszman (2011). A 25-mm diameter cylindrical aluminium probe (P/0.25S) was employed in a *"Texture Profile Analysis"* (TPA) compression test to penetrate to 50% of the dough sample depth at a test speed of 1 mm/s, with a 30 second delay between the two compressions. The maximum force (N) and the adhesiveness were measured. Dough texture analyses were performed on four 40-mm diameter dough discs from each elaboration.

Cookie texture was measured 60 minutes after baking, using parameters from a modification of the puncture test described by Laguna, Salvador, Sanz, & Fiszman (2011), in which cookies were penetrated to a depth of 3 mm with a 0.25 mm diameter spherical probe (P/ 0.25S). The maximum force at penetration (N) and the elastic moduli (N/mm) attained during penetration were measured on six cookies from each elaboration.

Sixty minutes after baking, four cookies were weighed and their widths (diameter) and thicknesses were measured to calculate the spread factor. The diameter of each cookie was measured twice, perpendicularly, in order to calculate an average diameter. The spread factor of the cookies was calculated by dividing the average width (W) by the thickness (T) of the cookies.

Measurements at the centre of the upper surface (crust) colour of four sugar-snap cookies from each elaboration were carried out with a Minolta CN-508i spectrophotometer (Minolta, Co. LTD, Tokyo, Japan) using the D65 illuminant with the 2º standard observer. Results are expressed in the CIE L*a*b* colour space.

2.2.4. Consumer testing

Hedonic sensory evaluation of the cookies was conducted with 63 volunteers who were habitual cookie consumers. Samples were analysed one day after baking. For sensory evaluation, samples were presented as whole pieces on white plastic dishes coded with four-digit random numbers and served in random order. The cookies were evaluated on the basis of acceptability of their appearance, odour, texture, taste and overall appreciation on a nine-point hedonic scale. The scale of values ranged from “like extremely” to “dislike extremely”, corresponding to the highest and lowest scores of “9” and “1” respectively.

2.2.5. Statistical analysis

Differences between the parameters of the different formulations were studied by analysis of variance (ANOVA). Fisher’s least significant difference (LSD) was used to describe means with 95% confidence intervals. The statistical analysis was performed with the Statgraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, USA).

3. Results and Discussion

3.1. Flour characteristics

Particle size, water content, protein content, damaged starch content, hydration properties and oil absorption of flours are shown in Table 1. The lowest protein content was detected in the two maize flours (native and precooked), whereas teff flour had the highest protein content, followed by buckwheat and wheat flour. Altındağ, Certel, Erem, & Konak (2014) also observed a lower protein content in maize flour and higher content in buckwheat flour when they studied gluten-free cookies made with rice, buckwheat and maize flour. Fine-grained flours had a lower protein content than coarse-grained flours of the same type, except for precooked maize flour. Our results coincide with those of de la Hera, Gómez, & Rosell, (2013c) who studied rice flours. Protein content showed negative correlations with WHC (r=-0.4400; 95 %) and WBC (r=-0.5121; 95 %) hydration properties. Similar results were reported by de la Hera et al. (2013a, 2013b), who also observed a negative correlation between protein content and WBC in rice flours. Water-holding and water-binding properties are more dependent on starch content and this finding may therefore be due to a lower starch content of flours with high protein content. Particle size of wheat and buckwheat flours was larger but similar to fine-grained flours, with the exception of FPM. On the other hand, teff flour showed a particle size intermediate to coarse-grained maize flour and coarse-grained rice flours. Coarse-grained flours had the largest particle size.

With regard to damaged starch content, the precooked flours, as expected, had a significantly higher damaged starch content than the other flours, because precooking pregelatinizes starch (Martínez, Calviño, Rosell, & Gómez, 2014a; Martínez, Oliete, Román, & Gómez, 2014b ; Mason, 2009). Maize flours and the fine fractions of rice flours showed more damaged starch than wheat flour. Buckwheat flour showed the lowest damage starch content, followed by teff and both coarse-grained rice flours. Torbica, Hadnadev, & Hadnadev (2012) also obtained the lowest amount of damaged starch with buckwheat flour on comparison with rice and wheat flour. The higher content of damaged starch observed in rice, maize and wheat flours compared with buckwheat and teff flours may have been due to the highly compact nature of the rice, maize and wheat kernels, which could have produced greater starch damage content during the milling process (Torbica, Hadnadev, & Hadnadev, 2012). Fine-grained rice-flour fractions showed a higher content of damaged starch than coarse-grained fractions of the same flour. These results agree with those reported by Hasjim, Li, & Dhital (2012).

It was observed that the two precooked maize flours had the highest hydration capacity. This may have occurred because precooking creates pregelatinized starch and affects these properties (Martínez, Calviño, Rosell, & Gómez, 2014a; Martínez, Oliete, Román, & Gómez, 2014b). In general, fine-grained flours showed higher WBC values than coarse-grained flours, except for maize flour. The highest WHC values were for CPM and FM following by FPM, teff and FSR. The other flours had lower WHC values, with no differences between the different flours. We also observed that the two precooked maize flours had the highest SV and no clear trend was observed in SV among the remaining flours. Correlation analysis indicated positive correlations between damaged starch and hydration properties, including WHC (r=0.5501; 99 %) and WBC (r=0.8014; 99%) and SV (r= 0.4881; 95%). Many authors have also reported an increased hydration capacity with increasing damaged starch content (Martínez, Calviño, Rosell, & Gómez, 2014a; Martínez, Oliete, Román, & Gómez, 2014b).

Unlike hydration properties, the OAC of precooked maize flours did not differ significantly from that of other flours and particle size had no clear effect on the OAC. Only buckwheat, CPM and both fine fractions of rice flours had lower OAC values than wheat. The other flours showed no differences with wheat. In general, no clear tendency in OAC results and nor correlations with other flour parameters or cookie quality parameters were found.

Table 1 Flour properties

Flour	Damaged Starch (%)	Protein (%)	Average particle size (μm)	WBC	WHC	SV	OAC
W	6.07d	8.92i	81.3d	83.44a	7.35a	150.00ab	1.82def
FSR	10.25f	7.46e	65.2a	130.75e	10.41b	165.28abcd	1.63ab
CSR	4.01bc	8.71h	228.0j	115.14d	8.06a	230.00e	1.79de
FLR	10.62f	7.74f	72.6c	131.55e	8.20a	150.00ab	1.65abc
CLR	4.41c	8.47g	250.0k	99.00b	7.17a	183.34bcd	1.69abcd
FM	7.95e	5.63a	68.0b	157.04f	20.38e	190.91cd	1.94f
CM	8.75e	6.19b	150.0g	168.89g	8.57a	138.89a	1.78cde
FPM	13.86g	7.28d	97.4f	282.11i	18.52d	266.67f	1.83ef
CPM	13.52g	6.99c	172.0h	264.10h	20.83e	371.43g	1.65abc
B	1.24a	9.96j	83.6e	106.96c	8.70a	160.72abc	1.57a
T	3.53b	10.475k	174.0i	126.69e	14.09c	200.00de	1.73bcde

Values followed by the same letter in the same column are not significantly different ($p < 0.05$).
Flours: W: wheat; FSR: fine-grained short-grain rice; CSR: coarse-grained short-grain rice; FLR: fine-grained long-grain rice; CLR: coarse-grained long-grain rice; FM: fine-grained maize; CM: coarse-grained maize; FPM: fine-grained precooked maize; CPM: coarse-grained precooked maize; B: buckwheat; T: teff; WBC: Water Binding Capacity; WHC: Water Holding Capacity; SV: Swelling volume; OAC: Oil absorption capacity

3.3. Dough and cookie properties

Table 2 shows the results of dough and cookie texture and of cookie shape and colour. In general, flour particle size did not affect dough peak force (DPF), but it did influence stickiness and dough elastic moduli (DEM), with higher absolute values being observed in both parameters with fine-grain flours. The average particle size of flours showed a positive correlation with stickiness (r=0.74) and a negative correlation with DEM (r=-0.55) with a confidence of 99%, which could have been due to the greater surface area of flours with a smaller particle size. Flour particle size could also affect the internal dough structure, which would be more compact with fine flour fractions. Native maize flours showed the highest DPF values but otherwise we detected no clear tendency in DPF.

The coarse-grained rice flours (CLR and CSR) produced the greatest spread and were the widest cookies. Chung, Cho, & Lim, (2014) also observed an increase in spread factor when wheat flour was substituted by white rice flour, though they did not analyse flour particle size. In contrast, there were no significant differences in spread factor between the other gluten-free cookies though all had a lower spread and diameter than wheat cookies. This would agree with the findings published by Kaur, Sandhu, Arora, & Sharma (2014), who observed a reduced spread ratio of gluten-free biscuits made from wheat flour with substituted buckwheat flour. Among the gluten-free cookies with no significant differences in spread, cookies made from buckwheat and teff flours, with a higher proportion of protein in their composition, were among the widest in this group. However, there are studies on the use of wheat flour to make cookies that found reasonable negative correlations between wheat flour protein content and cookie diameter (Gaines, 1985; Gaines, Donelson, & Finney, 1988; Miller & Hoseney, 1997). It might be considered that this is due to gluten content, as Chung, Cho, & Lim, (2014) reported that the spread factor of cookies increased as non-wheat protein content increased. Cookies made from fine-grain flours showed smaller diameters than cookies made from coarse-grained flours, which agrees with the observations reported by Gaines (1985) and Gaines, Donelson, & Finney, (1988). However, this difference was much more pronounced with rice than with maize. In addition, there were no significant differences in spread factor between the maize flours; this may be due to the lower flour particle size of the coarse maize flours compared with the coarse rice flours used in this study. Interestingly, there was a positive correlation between particle size and spread factor (r=0.75; 99%), although this correlation was strongly influenced by the different results with the coarse and fine rice flours. Altındağ, Certel, Erem & Konak (2014) also observed an increase in the spread factor when maize and rice flours were incorporated into gluten-free cookie formulae instead of buckwheat; the rice and maize flours used by those authors had a larger particle size than the buckwheat. In addition, cookie diameter was affected by damaged starch content and WHC showing negative correlations of r=-0.62 and r=-0.55, respectively and a confidence of 99 %. There were also significant negative correlations between spread factor and damaged starch and WHC (r=-0.47 and r=-0.44, respectively) with a confidence of 95%. This relationship between the spread factor and damaged starch, which increased WHC, coincided with the findings of other studies based on cookies made from wheat or triticale flours (Barak, Mudgil, & Khatkar, 2014; Barrera, Pérez, Ribotta, & León, 2007; Donelson & Gaines, 1998; Gaines, Donelson, & Finney, 1988; Hoseney, 1994; Hoseney & Rogers, 1994; Miller & Hoseney, 1997). In general, cookie spread factor appears to be dependent on dough viscosity (Hoseney, Wade, & Finley, 1988; Hoseney & Rogers, 1994; Miller & Hoseney, 1997; Yamazaki, 1959). Flour components that absorb large quantities of water reduce the amount of water that is available to dissolve the sugar in the formula; this will make the initial dough viscosity higher and the cookie will thus spread less during baking (Yamazaki, 1955, Hoseney & Rogers, 1994). Flours with low hydration properties will therefore produce cookies with greater spread (Yamazaki, 1962).

Table 2 Dough and cookie properties (n=2)

Flour	*Dough peak force (N)*	*Stickiness (N)*	*Dough elastic moduli (N/mm)*	*Cookie width (mm)*	*Spread factor*	*L**	*a**	*b**	*Cookie peak force (N)*	*Cookie elastic moduli (N/mm)*
W	13.37abc	-2.832a	8.56cde	54.47g	8.17b	72.36bcd	3.94b	24.55d	40.21ab	83.26a
FSR	12.40abc	-1.965bc	8.46bcde	46.77f	5.86a	75.64cde	0.62a	22.51cd	50.26bc	109.39cd
CSR	7.73a	-0.985d	3.88a	63.07h	14.83c	52.40a	8.54e	17.49ab	29.46a	87.97ab
FLR	17.58cd	-2.144abc	12.47e	43.49cd	5.47a	76.99de	1.07a	22.14bcd	62.02cd	113.32d
CLR	12.77abc	-1.067d	5.50abc	62.78h	14.88c	49.86a	9.645ef	15.31a	30.30a	89.01abc
FM	27.57e	-2.944a	19.00f	41.39ab	5.06a	80.08e	4.195bc	35.42e	95.07f	116.55d
CM	23.34de	-1.799bcd	12.13e	44.26cde	5.00a	76.23cde	5.495cd	35.72e	74.77e	102.95abcd
FPM	16.48bcd	-2.600ab	10.33de	39.96a	5.47a	70.31bc	9.51ef	39.50e	66.00de	112.88d
CPM	7.38a	-1.307cd	4.19ab	42.94bc	5.53a	68.24b	10.26f	37.69e	43.71b	101.62abcd
B	8.70ab	-1.935bc	5.14abc	45.68ef	5.48a	67.02b	5.96d	18.37abc	87.63f	111.61d
T	16.21bcd	-2.185ab	7.26abcd	45.09def	5.93a	55.27a	8.59e	22.63cd	71.90de	107.01bcd

Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

Flours: W: wheat; FSR: fine-grained short-grain rice; CSR: coarse-grained short-grain rice; FLR: fine-grained long-grain rice; CLR: coarse-grained long-grain rice; FM: fine-grained maize; CM: coarse-grained maize; FPM: fine-grained precooked maize; CPM: coarse-grained precooked maize; B: buckwheat; T: teff

Regarding the colour of the cookies, this is related not only to the colour of the flour used but also to Maillard and caramelisation reactions, which take place during baking (Ameur et al. 2007). Cookies made from maize flours (more yellowish due their higher carotenoid content) thus presented the highest b* values. Altındağ, Certel, Erem, & Konak (2014) also observed the highest b* values with maize flour. Cookies made from coarse-grained rice flours showed lower brightness (darker) and b* values and higher a* values (more brown) than cookies made from fine-grained rice flours. This effect could have been caused by the greater spread of these cookies and the oil released during the baking processes, which could produce a higher concentration of sugars, leading to a more intense caramelization phenomenon, with the production of brown polymers, which contribute to the surface coloration of the cookies (Manley, 2011; Wade, 1988). When coarse-grained rice flours were excluded, cookies made from buckwheat and teff were the darkest (lowest L*). This effect may be due to their higher protein content, which would enhance the Maillard reactions between reducing sugars and amino acids.

Textural properties are one of the major factors contributing to the eating quality of cookies. Hardness, which is one of the most important textural characteristics for cookies, is measured as the peak force to snap the cookie. In our study, hardness was significantly affected by flour particle size: cookies made from fine-grained flour required a significantly higher peak force than cookies made with course-grained flour of the same flour type. This may be related to a more compact structure of cookies made with fine-grained flours. The statistical analysis revealed that the highest hardness values were obtained with cookies prepared using buckwheat, teff and maize flours (with the exception of CPM). The high hardness of teff and buckwheat cookies could be due to the high protein content of these flours since Maache-Rezzoug et al. (1998) observed how the effective force increased with protein content when studying the mechanical parameters of cookies made from wheat flour. Conversely, Hadnadev, Torbica, & Hadnadev (2013) observed that the replacement of rice with buckwheat flour led to a decrease in cookie hardness. This may be because Hadnadev, Torbica, & Hadnadev (2013) used rice flour that was finer than buckwheat flour (they did not specify particle size). The elastic moduli of gluten-free cookies was lowest in those made with coarse-grained rice flours; this may have been due to their greater spread. There were no major differences between the other flours. However, cookies made from wheat flour had similar hardness values to those made from coarse-grained rice flour, despite their greater spread factor values; this would suggest a certain functionality of wheat proteins, compared with other proteins, which would affect the texture of the cookies.

3.4. Consumer test

The cookies made from wheat flour were selected as the control cookies for the consumer test, the cookies made from buckwheat as an example of flour with a high protein content, and the cookies made from FSR and FPM (rice and maize flours), as examples which produced cookies with similar instrumental characteristics to the control cookie. The results of the cookie sensory evaluation are shown in Table 3. Consumers rated cookies prepared from FPM with the highest quality. These cookies had the best sensorial scores for appearance, taste and overall acceptability, although there were no significant differences in the last parameter between FPM cookies and the cookies made from wheat. The scores obtained would appear to indicate that consumers found the slightly more yellowish colour (Fig. 1) of those cookies appealing. Cookies made from FSR did not show significant differences with the control cookies in appearance, taste and overall acceptability, but they achieved a slightly lower score in odour and texture. Conversely, cookies made from buckwheat had the lowest sensorial scores, except for the odour parameter, which can be strongly influenced by their particular herbaceous taste and greater hardness. Similarly, Baljeet, Ritika, & Roshan (2010) observed that an increase in the level of buckwheat flour in wheat-flour based cookies produced a fall in sensory scores. Cookies made from wheat alone achieved higher scores than the other cookies in texture because consumers perceived them to be less hard and more brittle, which agreed with the cookie elastic moduli and peak force.

Table 3 Consumer test results

Sugar-snap cookie	*Appearance*	*Odour*	*Taste*	*Texture*	*Overall acceptability*
W	5.9b	6.2b	5.7b	5.9c	5.9bc
FSR	5.9b	5.6a	5.5b	5.1b	5.7b
FPM	6.8c	6.3b	6.4c	5.0b	6.3c
B	5.4a	5.4a	4.5a	4.3a	4.8a

Values followed by the same letter in the same column are not significantly different ($p < 0.05$).
Flours: W: wheat; FSR: fine-grained short-grain rice; FPM: fine-grained precooked maize; B: buckwheat.

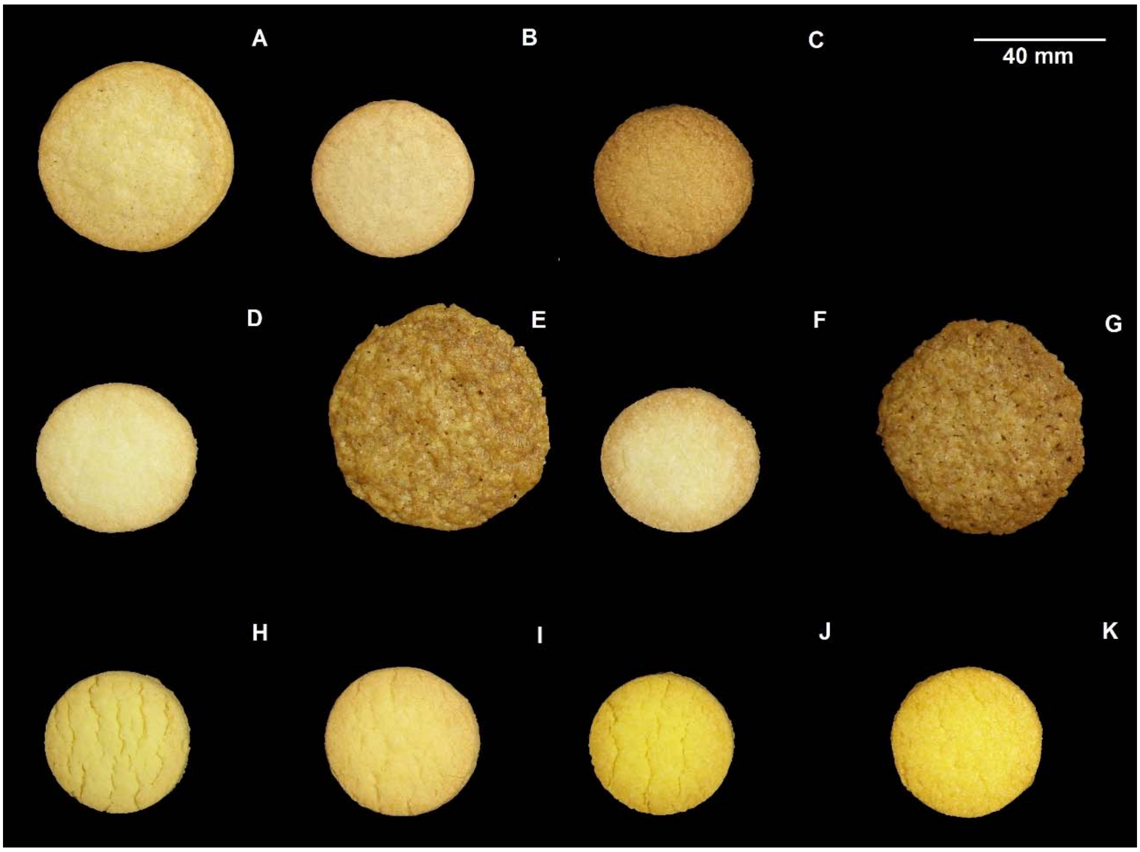

Fig. 1 Appearance of sugar-snap cookies containing different flours: A, wheat; B, buckwheat; C, teff; D, fine-grained short-grain rice; E, coarse-grained short-grain rice; F, fine-grained long-grain rice; G, coarse-grained long-grain rice; H, fine-grained maize; I, coarse-grained maize; J, fine-grained precooked maize; K, coarse-grained precooked maize.

4. Conclusion

The results of the study indicate that it is possible to obtain gluten-free cookies with a similar quality to cookies made with wheat flour, without any additives. Nevertheless, flour parameters such as particle size, damaged starch or protein content had a significant influence on cookie spread and texture. In general, gluten-free flours produced cookies with lower spread and greater hardness than wheat cookies, but flours with a very large particle size showed the opposite tendency. The origin of the gluten-free flour, and its consequent effect on cookie taste, significantly affected the overall acceptability of the cookies.

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ASSESSING THE RICE FLOUR-STARCH-PROTEIN MIXTURES TO PRODUCE GLUTEN FREE SUGAR-SNAP COOKIES

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Abstract

The mixture of rice flours, starches and proteins is common in gluten-free bakery products such as bread or cake. The aim of this study was to determine the effects of starch and/or protein addition in rice flour gluten-free cookie quality. For this purpose, the hydration and oil absorption properties of flour-starch-protein mixtures, dough rheology and quality cookie parameters (thickness, final diameter, spread factor, texture, colour and acceptability) were analysed. Generally, protein incorporation increased hydration properties of the mixture and dough consistency, producing cookies with limited spreading in the baking time, lower hardness values and darker colour. However, maize starch addition reduced hydration properties and gave rise to cookies with higher thickness and width, but the texture and colour were not affected by the starch. Cookies with higher protein content showed higher acceptability than cookies with higher starch content and no protein addition. Therefore, protein and starch can be used in order to adjust the desired cookie characteristics depending on the cookie formulation and the needs of manufacturers.

Keywords: maize starch; pea protein; dough rheology; cookie texture; sensory analysis.

1. Introduction

Cookies are a baked product that typically has three major ingredients; flour, sugar and fat. There are distinct types of cookies depending on cookie composition, the making of cookie dough and baking parameters. Sugar-snap cookie is a particular type of cookie with high levels of fat and sugar and low water levels characterised by a limited development of the gluten network (Hadnadev, Torbica, & Hadnadev, 2013; Pareyt & Delcour, 2008). In addition, because of the insufficient water content of the cookie dough, most of the starch granules do not gelatinize during the cookie baking process (Pareyt & Delcour, 2008). Due to the minimal gluten development of sugar-snap cookies, there is the possibility to produce gluten-free cookies made from gluten-free flours without any gluten substitute (Donelson, 1988). However, gluten-free flours produce cookies with different physico-chemical characteristics in comparison with cookies made from wheat flour, depending on the cereal origin and the milling process (Mancebo, Picón, & Gómez, 2015).

Most studies that have investigated gluten-free cookies have used different gluten-free flours such as amaranth (de la Barca, Rojas-Martínez, Islas-Rubio, & Cabrera-Chávez, 2010;

Gambus et al., 2009; Hozova, Buchtová, Dodok, & Zemanovič, 1997; Tosi, Ciappini, & Masciarelli, 1996; Schoenlechner, Linsberger, Kaczyc, & Berghofer, 2006), buckwheat (Gambus et al., 2009; Hadnađev et al., 2013; Kaur, Sandhu, Arora, & Sharma, 2015: Schoenlechner, Linsberger, Kaczyc, & Berghofer, 2006) and/or rice flour (Chung, Cho, & Lim, 2014; Torbica, Hadnadez, & Hadnadev, 2012) or a mixture of these flours with other cereal flours (maize, sorghum or millet) or legume flours (Altındağ, Certel, Erem, & Konak, 2014; Rai, Kaur, & Singh, 2014). However, many commercial bakery products are mainly made from maize starch mixed, greater or lesser extent, with gluten free flours, starches from tubers and / or proteins. It has been proven that the protein and starch proportion in cookies made from wheat flour play an important role in cookie quality, because of their water absorption capacity, their effect in dough rheology and their spread in the baking process (Pareyt & Delcour, 2008). In general, soft wheat flour, which is characterised by a low protein content and weak gluten strength, is preferred in sugar-snap cookie elaboration (Souza, Kruk, & Sunderman, 1994) since they give rise to cookies with higher spread and cookie set time in the baking process (Kaldy, Kereliuk, & Kozuk, 1993; Miller & Hoseney, 1997). Thereby, starch and protein addition could adjust the expansion in the baking process and gluten-free cookie diameter. It has also been shown that protein content affected dough rheology and texture of cookies, at least in the case of wheat cookies (Gaines, 1990). There are few studies about starch and protein addition in gluten-free cookies. Schober et al. (2003) added starches in gluten-free cookies formulations but they were mixed with three gluten-free flours and only three mixtures were analysed, therefore the effect of starches could not be clearly compared. Sarabhai et al. (2015) studied the effect of protein concentrate (soya and whey protein), however they were added with emulsifiers.

The aim of this study was to determine the effect of the addition of starch and/or protein to rice flour on dough rheology and gluten-free sugar-snap cookies quality.

2. Materials and methods

2.1. Materials

The following ingredients were employed in this study: rice flour (8.01 g/100 g of protein and 74.35 g/100 g starch) provided by Harinera Castellana S.L. (Medina del Campo, Valladolid, SpainEspaña), maize starch (DAESANG, Korea), Nutralys F85M pea protein (80 % protein content) (Roquette, Leutrem, France), white sugar (AB Azucarera Iberia, Valladolid, Spain), margarine 100 % vegetable (Argenta crema, Puratos, Barcelona, Spain), sodium bicarbonate (Manuel Riesgo S.A., Madrid, Spain) and local tap water.

2.2. Methods

2.2.1. Mixture hydration and oil absorption properties

The different flour-starch-protein mixtures were characterised by their hydration and oil absorption properties.

Swelling volume (SV), or the volume occupied by a known weight sample, was evaluated by adding 100 mL of distilled water to 5 g (±0.1 g) of flour sample in a test tube and allowing it to hydrate for 24 h. Water holding capacity (WHC), defined as the amount of water retained by the sample without being subjected to any stress, was determined on the same suspension used to evaluate swelling; the hydrated solid was weighed after removing the excess water and values were expressed as grams of water per gram of solid (AACC method 88-04, 2012). Water binding capacity (WBC), or the amount of water retained by the sample after it has been centrifuged, was measured as described in AACC method 56-30.01 (AACC, 2012). Hydration properties were analysed in duplicate.

The method described by Lin, Humbert, & Sosulski (1974) was used to determine oil absorption capacity (OAC). Flour (100.0 ± 0.2 mg) was mixed with 1.0 mL of vegetable oil. The mixture was stirred for 1 min with a wire rod to disperse the sample in the oil. After a period of 30 min in the vortex mixer, tubes were centrifuged at 3000 × g and 4◦C for 10 min. The supernatant was carefully removed with a pipette and the tubes were inverted for 25 min to drain the oil and the residue was then weighed. The oil absorption capacity was expressed as grams of oil bound per gram of sample on dry basis. Three replicates were performed for each sample. OAC was calculated by Eq. (1):

$$\text{OAC (g/g)} = \text{Wr} / \text{Wi} \qquad (1)$$

Where Wr is the residue weight and Wi is the sample weight (g, db)

2.2.2. Cookie preparation

All formulations were prepared using the same quantities of ingredients except for water, which was added to adjust dough moisture content to 15.0 %, and the proportions of flour, starch and protein added (Table 1). The flour-starch-protein mixture moisture was determined by the AACC 44-15.02 method (AACC, 2012). The following ingredients (as g/100 g on dough basis) were used: flour-starch-protein mixture (43.3 g), sugar (31.2 g), margarine (19.4 g), water (5.2 g) and sodium bicarbonate (0.9 g). The margarine and sugar were then creamed at speed 4 for 180 s in a Kitchen Aid 5KPM50 mixer (Kitchen Aid, Benton Harbor, Michigan, USA) with a flat beater, scraping down every 60 s. The water was then added and mixing was continued at speed 4 for 120 s with intermediate scraping. After mixing, the cream was scraped down. Finally, flour and sodium bicarbonate were added, followed by mixing at speed 2 for 120 s, whilst scraping down every 30 s. After mixing, the dough was allowed to stand for a predefined period of 30 min. The dough pieces were then laminated with a salva L-500-J sheeter (Salva, Lezo, Spain) (gap width 6.00 mm). Cookie dough was cut with a circular cookie cutter (internal diameter, 40 mm) and weighed. Batches of at least 15 dough pieces were baked in an electric modular oven for 14 min at 185 ºC. All the cookie elaborations were performed twice.

Table 1 Experimental design of flour-starch-protein mixtures

Trials	Mix (F-S-P)	Rice flour[a]	Maize starch[a]	Pea protein[a]
1	100-0-0	100	0	0
2	90-0-10	90	0	10
3	80-0-20	80	0	20
4	70-30-0	70	30	0
5	65-25-10	65	25	10
6	60-20-20	60	20	20
7	40-60-0	40	60	0
8	35-55-10	35	55	10
9	30-50-20	30	50	20

Mix (F-S-P): Mixture of rice flour, maize starch and pea protein (g/100 g of flour)
Each mixture was performed in duplicate (n = 2).
[a] g/100 g of flour.

2.2.3. Dough rheology properties

The rheological behaviour of doughs was studied using a Thermo Scientific HaakeRheoStress 1 controlled strain rheometer (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P water bath that controlled analysis temperature (set at 25ºC). The rheometer was equipped with parallel-plate geometry (60-mm diameter titanium serrated plate-PP60 Ti) with a 3-mm gap. After adjusting the 3-mm gap, vaseline oil (Panreac, Panreac Química SA, Castellar del Vallés, Spain) was applied to the exposed surfaces of the samples to prevent them drying during testing. In oscillatory tests, dough was rested for 800 s before measuring. First, a strain sweep test was performed at 25ºC with a stress range of 0.1–100 Pa at a constant frequency of 1 Hz to identify the linear viscoelastic region. On the basis of the results obtained, a stress value included in the linear viscoelastic region was used in a frequency sweep test at 25ºC with a frequency range of 10–0.1 Hz. Values of elastic modulus (G' [Pa]), viscous modulus (G'' [Pa]), complex modulus and tangent δ (G''/G') were obtained for different frequency values (ω [Hz]). Samples were analysed in duplicate.

2.2.4. Cookie properties

The texture of the cookies was measured sixty minutes after baking on eight cookies from each elaboration, using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) fitted with the *"Texture Expert"* software. The cookies were broken using the three point bending rig probe (HDP/3PB). The experimental conditions were: supports 30 mm apart, a 20 mm probe travel distance, a trigger force of 5 g and a test speed of 2.0 mm/s.The maximum force (N) and the displacement at rupture (mm) were measured.

Four cookies were weighed and their widths (diameter) and thicknesses were measured with caliper to calculate the spread factor. The diameter of each cookie was measured twice, perpendicularly, in order to calculate an average diameter. The spread factor of the cookies was calculated by dividing the average width by the thickness of the cookies.

Measurements at the centre of the upper surface (crust) colour of six sugar-snap cookies from each elaboration were carried out with a Minolta CN-508i spectrophotometer (Minolta, Co. LTD, Tokyo, Japan) using the D65 illuminant with the 2º standard observer. Results are expressed in the CIE L*a*b* colour space.

2.2.5. Consumer test

Hedonic sensory evaluation of the cookies was conducted with 66 volunteers, staff and students from the Agricultural Engineering College in Palencia (Spain), between the ages of 18–66 and of various socioeconomic backgrounds, who were habitual cookie consumers. Samples were analysed one day after baking. For sensory evaluation, samples were presented as whole pieces on white plastic dishes coded with four-digit random numbers and served in random order. The cookies were evaluated on the basis of acceptability of their appearance, odour, texture, taste and overall appreciation on a nine-point hedonic scale. The scale of values ranged from "like extremely" to "dislike extremely", corresponding to the highest and lowest scores of "9" and "1" respectively.

2.2.6. Statistical analysis

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

3. Results and Discussion

3.1. Mixture characteristics

As can be seen in table 2, protein addition increased every hydration property significantly (WBC, WHC and swelling volume). These results agree with those reported by Traynham, Myers, Carriquiry, & Johnson (2007) when evaluated the WHC for flour blends. It is well-known that protein has a profound effect on the water absorption properties of the flour when preparing dough, absorbing twice its weight in water, and meanwhile undamaged starch absorbs 33 % of its own weight in water (Manley, 2011). An increase in starch content, reduced WBC, WHC and swelling volume. However, the effect of the starch in WHC and SV was lower than in WBC and the starch effect in WHC and SV was greater as the protein content was increased. The starch effect could be due to high levels of starch in rice flour, and therefore the insignificant differences in total protein content when flour is replaced by starch. In contrast, there were no significant differences in OAC between the different formulas based on the starch or protein addition.

Table 2 Flour hydration properties and oil absorption capacity

Mix (F-S-P)	WBC (g water /g solid)	WHC (g water /g flour)	SV (ml/g)	OAC (g oil/g solid)
100-0-0	1.380d	8.8a	1.255b	1.89ab
90-0-10	1.735g	13.4cd	1.815d	1.87ab
80-0-20	2.014i	17.4f	2.315f	1.89ab
70-30-0	1.145b	9.1a	1.235b	1.82a
65-25-10	1.465e	12.0bc	1.670cd	1.84ab
60-20-20	1.790h	14.9e	2.070e	1.91ab
40-60-0	0.930a	8.9a	1.000a	1.92ab
35-55-10	1.300c	11.0b	1.250b	1.88ab
30-50-20	1.700f	14.4de	1.630c	1.96b
Standard error	*0.007*	*0.5*	*0.058*	*0.05*

Mix (F-S-P): Mixture of rice flour, maize starch and pea protein.
WBC: Water binding capacity (n = 2); WHC: Water Holding Capacity (n =2); SV: Swelling volume (n = 2); OAC: Oil Absorption Capacity (n = 3).
Mean values followed by the same letter in the same column are not significantly different ($p < 0.05$).

3.2. Dough properties

Dough properties depend on the different ingredients added, such as starch, protein or the water present, and their quantity which in turn influence the handling properties. If the dough is too soft or too firm, it is not easy to handle; the dough must be sufficiently cohesive to hold together during the different processing steps and viscoelastic enough to separate cleanly when cut by the mould (Gujral, Mehta, Samra, & Goyal, 2003). Dough rheological results are shown in Table 3. It was observed that elastic moduli (G') was greater than viscous moduli (G'') throughout the frequency range for all samples, which suggests a solid elastic-like behaviour of all the cookie doughs studied. Protein addition definitely increased G', G" and G* values, and decreased tg δ, which agrees with the observations reported by Inglett, Shen & Liu, (2015) when wheat flour was substituted with flours with a higher protein content than wheat flour in cookie. In general, no clear tendency of starch addition was found in dough rheological properties. A positive correlation between dough rheology and hydration properties with a confidence of 99.9 % was found (data not shown), which suggest that the water absorption of the mixture affects the dough rheology.

Table 3 Dynamic oscillatory test results of the dough for gluten-free cookies prepared from mixtures of rice flour, maize starch and pea protein.

Mix (F-S-P)	*G' (Pa)*	*G''(Pa)*	*G**	tan δ
100-0-0	148750a	36810ab	153250a	0.25e
90-0-10	278450b	57540cd	284400b	0.21d
80-0-20	672500e	94035ef	679350e	0.14a
70-30-0	105465a	28960a	109750a	0.28f
65-25-10	218400b	47730bc	223750b	0.22d
60-20-20	509400d	84410e	516500d	0.17b
40-60-0	107450a	28010a	111150a	0.26e
35-55-10	355500c	66595d	362250c	0.19c
30-50-20	712200e	101195f	720000e	0.14a
Standard error	*19974*	*3701*	*20192*	*0.01*

Mix (F-S-P): Mixture of rice flour, maize starch and pea protein (g/100g of flour).
G': elastic moduli; G": viscous moduli; G*: complex moduli; tan δ: tangent delta.
Mean values (n = 4) followed by the same letter in the same column are not significantly different ($p < 0.05$).

3.3. Cookie properties

Cookie properties are shown in table 4. No differences were found in cookie moisture content between the mixtures studied, which means that starch and protein did not have any clear effect in this parameter. However, cookie dimensions were affected by the different proportions of flour, starch and protein. On the one hand, thickness and width (diameter) decreased when protein content increased in the formula. In this way, there were no differences observed in spread factor when protein content was modified, since width results were compensated for thickness results. On the other hand, the addition of starch increased cookie thickness and width. Despite this, the resultant dimension for cookies with the higher level of starch studied (60 %) were not representative, since the cookie dough for this formula was excessively sticky and some difficulties were found in the process and it was necessary to add flour in the dough lamination. Just like the incorporation of protein, spread factor was not affected by starch addition, with the exception of the cookie with the highest starch content. The lower dough expansion during the baking process promoted by the protein addition, was also observed by Kaldy et al. (1993) and Miller and Hoseney (1997) in cookies made from wheat flour. It could be related to the protein effect on apparent glass transition temperature which determines the cookie set time (Payret & Delcour, 2008). Another explanation of protein effect on dough expansion could be the higher dough viscosity confirmed by other authors (Hoseney & Rogers, 1994; Miller & Hoseney, 1997). In fact, our study revealed a high correlation between G” values and cookie diameter with 99.9 % confidence. In addition, a high correlation (99.9 %) between hydration properties of mixtures (WHC, SV and WBC) and cookie diameter was observed. It is in agreement with the results of other authors such as Barrera, Pérez, Ribotta, & León (2007) and Barak, Mudgil, & Khatkar (2014), and it could be related to the dough hydration effect on dough rheology.

Regarding the texture of the cookies, it was found that protein incorporation decreased hardness (maximum breaking strength), which is consistent with the observations of Sarabhai et al. (2015) when incorporated protein concentrates and mixtures of emulsifiers, and the results of Hadnadev et al. (2013) who substituted rice flour with buckwheat flour, which has higher protein content, both in gluten-free cookies. Conversely, Singh and Mohamed (2007) found no differences in the texture of cookies fortified with gluten or soy protein, which may be due to the wire cut cookie formula used, and especially to the modifications in the water content of the formula based on the farinograph absorption. No clear trend of starch addition was showed in cookie texture. It should be highlighted that texture data of the cookies with the maximum quantity of starch was not representative because of the processing problems already explained. In fact, it was the only cookie that has a significantly larger displacement at rupture and there were no significant differences among the other cookies. No significant correlations between the values of texture and hydration of mixtures or texture and shape cookie (thickness or width) were found, therefore, differences in texture may have been caused by the internal structure.

Table 4 Quality parameters of cookies based on rice flour, maize starch and pea protein.

Mix (F-S-P)	Moisture (%)	Thickness (mm)	Width (mm)	Spread	F max (N)	Distance (mm)	L*	a*	b*
100-0-0	2.76ab	8.87c	44.27c	4.99a	28.30d	0.34a	78.63e	0.67a	22.73bc
90-0-10	3.29bc	8.01b	42.57b	5.32a	27.26cd	0.37a	76.63cd	4.55b	23.17c
80-0-20	2.77abc	7.32a	40.52a	5.53a	25.30bc	0.34a	73.94b	6.77d	26.68d
70-30-0	2.93abc	9.59d	48.56d	5.06a	28.25d	0.41a	77.95de	0.22a	20.21ab
65-25-10	2.47ab	8.72c	44.29c	5.08a	22.82a	0.34a	77.10cde	3.89b	23.49c
60-20-20	3.88c	8.05b	42.11b	5.23a	25.43bc	0.37a	75.18bc	5.61c	26.23d
40-60-0	2.51ab	7.16a	57.98e	8.15b	24.03ab	0.57b	70.95a	0.82a	19.61a
35-55-10	1.86a	9.76d	47.86d	4.90a	27.01cd	0.36a	76.70cd	3.97b	22.92c
30-50-20	3.12bc	8.57bc	44.44c	5.19a	22.37a	0.35a	77.01cde	5.69c	23.40c
Standard error	*0.35*	*0.20*	*0.49*	*0.25*	*1.52*	*0.04*	*0.60*	*0.22*	*0.81*

Mix (F-S-P): Mixture of rice flour, maize starch and pea protein (g/100g of flour).
Spread (width/thickness). F max (N): The maximum force (N); Distance: displacement at rupture (mm).
Mean values (n = 2) followed by the same letter in the same column are not significantly different ($p < 0.05$).

It was observed that the addition of protein increased a* and b* values of cookie colour. Thereby protein incorporation produced more red-looking and yellow-looking cookies. Protein also reduced L* values on cookies without starch, although this effect was smaller as the amount of starch was increased in the formula. However, including starch hardly influenced the colour of the cookies. The results of the cookies with the maximum quantity of starch (60 % of starch) should not be taken into account, since there were difficulties at lamination and formation and thereby had greater spread ratio, which probably influenced the colour development and give cookies with lower L* values than the others. The higher protein level, and therefore the greatest amount of amino acids can increase the Maillard reactions and therefore the generation of brown compounds, which contribute to the surface colouration of the cookies (Manley, 2011). Other authors found similar effects when they incorporated isolated or concentrated protein in the formulation of cookies (Singh & Mohamed, 2007; Rababah, Al-Mhasneh, & Ereifej, 2006) and when they compared different protein content flours (Mancebo et al., 2015). In contrast, starch had no effect on the colour, it hardly modified the overall proportions of amino acids and / or reducing sugars. The darkening of the cookies can be a positive effect as cookies made from rice flour often have a clearer colour than cookies made from wheat flour (Mancebo et al., 2015). Thereby, the incorporation of protein could minimize these differences.

3.4. Consumer test

After the instrumental analysis of the different cookies, four types of them were selected for a consumer test (Table 5). The cookies made from 100 % rice flour were selected as the control cookies (100-0-0), a cookie with the highest dose of protein but without starch (80-0-20), another with the highest dose of protein and high starch content (30-50-20) and the last cookie without protein but with high starch content (70-30-0). The results of the cookie sensory evaluation are shown in Table 5. Cookies with protein had the best scores for texture and for odour, in this case, when no starch was added. Meanwhile, cookies with starch and without protein got the lowest appearance and texture values. However, this cookie did not show differences in texture with the control cookie. No significant differences in taste between the different cookies were observed. Consumers rated cookies prepared from protein with the highest overall acceptability, although it was not significantly different from the control cookie. However, the cookie with high starch content and no protein got the worst overall acceptability. The higher scores of cookies with high protein content than the scores of cookies made with starch but without protein may be motivated by the darker colour (similar to cookies made from wheat flour) and the lower hardness of these cookies.

Table 5 Consumer test results of cookies based on rice flour, maize starch and pea protein.

Mix (F-S-P)	Appearance	Odour	Texture	Taste	Overall acceptability
100-0-0	6.06b	5.68a	5.26ab	5.68a	5.73ab
80-0-20	5.91b	6.22b	5.77bc	5.80a	5.98b
70-30-0	5.29a	5.42a	4.86a	5.32a	5.23a
30-50-20	6.44b	5.59a	5.97c	5.68a	5.92b
Standard error	*0.20*	*0.18*	*0.20*	*0.21*	*0.19*

Mix (F-S-P): Mixture of rice flour, maize starch and pea protein (g/100g of flour).
Mean values (n = 66) followed by the same letter in the same column are not significantly different (p < 0.05).

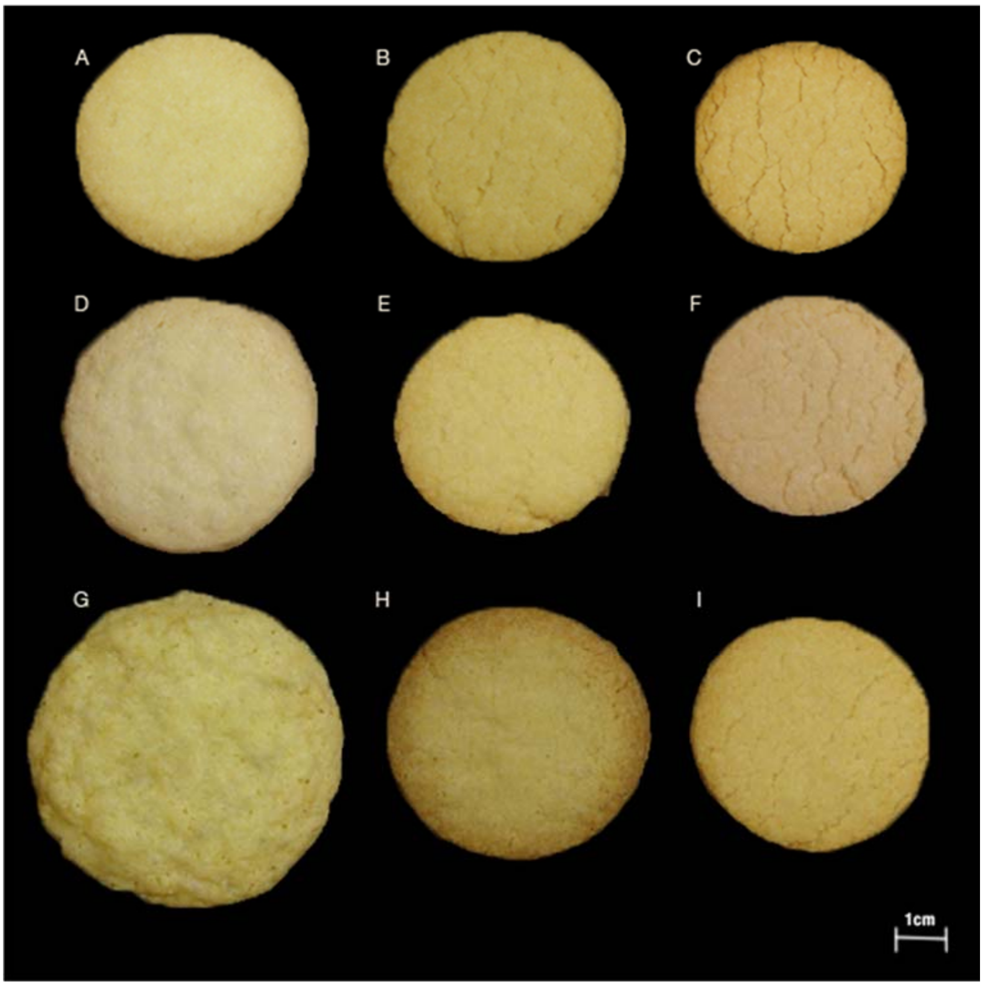

Fig. 1 Images of gluten-free cookies made from rice flour (F) substituted by maize starch (S) and pea protein (P) with different substitution levels (g/100 g of flour): A) 100 g flour, 0 g starch and 0 g protein (100F-0S-0P); B) 90F-0S-10P; C) 80F-0S-20P; D) 70F-30S-0P; E) 65F-25S-10P; F) 60F-20S-20P; G) 40F-60S-0P; H) 35F-55S-10P; I) 30F-50S-20P.

4. Conclusion

The substitution of rice flour with protein or starch can help to modify the characteristics of gluten-free cookies. Thus, the incorporation of protein in the formula reduced the size of the cookies (thickness and width), giving rise to less hard and darker cookies. In contrast, starch addition increased the cookie size (thickness and width) without affecting the texture or colour. Starch or protein incorporation did not show a negative effect in sensory evaluation if they are compared with the control cookie. However, it should be taken into account that protein addition modified dough rheology of the cookies, producing more consistent doughs, which could solve problems in cookie lamination and formation if the dough is too soft.

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RESUMEN Y DISCUSIÓN DE LOS RESULTADOS

Sección I: Panes sin gluten

El proyecto de la presente tesis doctoral se basa en la mejora de la calidad de productos libres de gluten llevándose a cabo sobre dos matrices alimentarias: pan y galletas. En el caso del pan, el estudio se centró en panes de arroz, a los cuales se les añadieron ingredientes utilizados comúnmente en elaboraciones sin gluten como son almidones, grasas o hidrocoloides, estudiando el efecto de los mismos en la calidad del producto final.

En la actualidad, el almidón de trigo se puede encontrar libre de gluten en el mercado gracias a la mejora de los procesos de extracción del mismo y su uso presenta una buena alternativa en panificación sin gluten, pudiendo imitar en mayor medida las características organolépticas de un pan de trigo. Por ello, el primer paso fue optimizar mezclas de almidón de trigo, almidón de maíz y harina de arroz, en base a los parámetros de calidad del pan a través de la metodología de superficie de respuesta (MSR). Este tipo de mezclas pueden conseguir mejores resultados que el almidón o la harina por separado, como lo demuestran Sánchez y col. (2002), quienes también aplicaron el método MSR a la optimización de una formulación de pan de almidones de maíz, arroz y tapioca, con y sin la adición de soja. Nuestro estudio reveló que el uso de harina de arroz en panes sin gluten mejora el sabor y el color (corteza con menos brillo), con respecto a panes a base de almidón. Sin embargo, la harina de arroz redujo el volumen específico del pan y la densidad alveolar (número de alveolos/cm^2), pudiendo ser mejorados estos panes con la adición de almidón de trigo, ya que éste proporcionó un mayor volumen específico y menor dureza que el almidón de maíz. Estos resultados confirman que la combinación de harina y almidones en la elaboración de panes sin gluten dan productos de mejor calidad que aquellos elaborados a partir de harina o almidones de forma separada.

Primeramente se estudió la reología de masas. Las masas que contenían harina de arroz mostraron los mayores módulos complejos (G*) y menores valores de capacitancia, indicando una mayor consistencia y mayor fuerza de masa respecto al resto de mezclas, mientras que las masas con almidón de trigo presentaron los menores valores de G*. Las diferencias en reología pueden ser atribuidas a las diferencias estructurales de la base almidonosa que da lugar a diferentes grados de compactación (Hera y col. 2012, 2013) así como a la mayor capacidad de absorción de agua de la harina con respecto a los almidones, provocada por su mayor contenido en proteína y mayor cantidad de almidón dañado, producido en función de las técnicas de molienda y propiedades del grano (Schober, 2009).

También se estudió la producción de gas y desarrollo de la masa durante la fermentación. Las masas que contenían harina de arroz presentaron una mayor producción de gas, posiblemente relacionado con su mayor contenido en almidón dañado (Schober, 2009), que incrementa la cantidad de maltosa disponible. Además, presentaron un desarrollo inicial mucho mayor pero se desmoronaron en las fases intermedias de la fermentación implicando una menor retención de gas. La masa con almidón de maíz presentó un gran desarrollo de la fermentación, aunque más tardío que la masa con harina de arroz. Sin embargo, el análisis de la masa con almidón de trigo hubo que obviarlo ya que la masa desbordó en el ensayo por su escasa consistencia y los resultados grabados no fueron precisos.

Los panes elaborados a partir de almidones mostraron los mayores volúmenes específicos, siendo el mayor volumen el del pan elaborado a partir de almidón de trigo. Estas diferencias pueden ser debidas a sus propiedades de empastado, puesto que el almidón de maíz mostró una temperatura de gelatinización menor al almidón de trigo, indicando posiblemente una menor expansión durante el horneado.

Con respecto a la optimización de los panes, el pan óptimo con el mayor volumen específico y menor valor de luminosidad contenía 45 g/100 g de harina de arroz y 53 g/ 100 g de almidón de trigo cuando se maximizó la densidad alveolar. Debido a que el tipo de pan condiciona la densidades alveolar deseada (en pan de molde se busca una densidad alveolar mayor y en panes de barra menor), se realizó esta optimización minimizando la densidad alveolar obteniéndose un pan óptimo con 98 g/100 g de harina de arroz y 2 g/100 g de almidón de trigo. La mayor aceptabilidad sensorial correspondió al pan elaborado con una mezcla de harina de arroz (59 g/100 g) y almidón de trigo (41 g/100 g).

Teniendo esto en cuenta, y a pesar de que las mezclas de harina y almidón mejoran la calidad del pan final (cuyas proporciones óptimas dependen del tipo de pan), para simplificar la fórmula en los siguientes estudios se continuó utilizando únicamente harina de arroz para un mejor estudio de los ingredientes añadidos, evitando interacciones con las variables a estudio. De este modo, en un segundo estudio se evaluó el efecto de dos grasas diferentes (aceite de girasol y margarina) en panes a base de harina de arroz. Para ello, el contenido de grasa y la hidratación de la masa se optimizaron mediante el MSR.

La reología de masas reveló como al aumentar el contenido en agua se redujo G' y G''. Por otro lado, la margarina apenas afectó a los parámetros reológicos estudiados, mientras que el aceite de girasol tuvo un efecto similar al agua aunque menos intenso, reduciendo también

G’ y G’’. Estas diferencias pueden ser debidas a las diferencias en la consistencia de los lípidos a la temperatura ambiente (temperatura del ensayo). También se observó como el efecto del aceite se vio disminuido a medida que se incrementó el contenido en agua.

En cuanto al producto final, el aceite aumentó el volumen específico del pan y redujo su dureza, particularmente en las fórmulas con menor contenido de agua. La incorporación de aceite también aumentó la vida útil del pan, aunque solo en los panes con bajo contenido en agua. A su vez, se observó como el volumen específico del pan aumentó a medida que se aumentaba el contenido en agua en aquellos panes con bajo contenido en grasa. Sin embargo, a medida que el contenido en aceite aumenta, el porcentaje óptimo de agua cae. Esto puede significar que las masas necesitan una mínima consistencia, como previamente de la Hera y col. (2013) observaron al comprobar que un exceso de agua contribuyó negativamente al volumen del pan. Por el contrario, la adición de margarina redujo ligeramente el volumen específico y aumentó también ligeramente la dureza del pan, efecto que se hace más intenso con mayor contenido de agua en la fórmula. Además, los panes con margarina presentaron una estructura alveolar irregular por lo que no se analizaron más parámetros de estos panes.

La incorporación de aceite también mejoró el color del pan, reduciendo la luminosidad y favoreciendo un tono más marrón en la corteza, especialmente al reducir el contenido en agua, lo cual es deseable en panes sin gluten que tienden a ser pálidos. La densidad alveolar aumentó con el contenido en agua, viéndose incrementado este efecto con mayores contenidos de aceite, es decir, con una consistencia menor.

Correlación entre los parámetros de reología de masas y los parámetros de calidad del pan fueron encontradas, de modo que a mayores valores de G’, G’’ y G* y menores de tg δ, mayor volumen específico y menor dureza del pan. Se observó como el modelo recíproco Y=1/(a+b*X) relacionaba mejor los parámetros de reología de masas con el volumen específico del pan que el modelo lineal.

Para finalizar el estudio de la mejora de la calidad de los panes sin gluten, se estudió el efecto de la combinación de dos hidrocoloides; HPMC y psyllium. Para ello, nuevamente se utilizó el MSR para optimizar la fórmula a base de harina de arroz con tres niveles de HPMC, psyllium y agua. Debido a las correlaciones observadas anteriormente entre la reología de masas y los parámetros de calidad del pan sin gluten, en este estudio se pretendió definir qué análisis reológico predice mejor los parámetros de calidad del pan sin gluten. Para ello se realizaron ensayos oscilatorios de frecuencia y esfuerzos así como ensayos de frecuencia-recuperación.

Tanto el HPMC como el psyllium aumentaron los valores de G*, es decir, aumentaron la consistencia de las masas en el ensayo oscilatorio. Esto era esperado por las propiedades viscoelásticas realzadas de los polisacáridos en medio acuoso (Lazaridou y col., 2007). El efecto de ambos hidrocoloides fue menor cuando se aumentó el contenido en agua debido al efecto de dilución provocado por la misma. En el ensayo reológico de fluencia-recuperación el psyllium disminuyó la facilidad de deformación de las masas (menor capacitancia), mostrando un efecto más dominante que el HPMC, que no presentó un efecto claro.

En relación al efecto de los hidrocoloides sobre el producto final, el psyllium aumentó la dureza de los panes, pudiedo estar asociado a los mayores valores de retrogradación encontrados cuando el contenido de este hidrocoloide fue en aumento. Además, el psyllium redujo el volumen específico, mientras que el HPMC apenas ejerció un efecto sobre estos parámetros en los niveles de estudio. Estos resultados van en concondancia con los resultados del análisis de fluencia-recuperación, lo que indica que éste análisis reológico puede ser un mejor predictor de la calidad de panes sin gluten que el ensayo oscilatorio. En el estudio también se observó como, una mayor cantidad de agua añadida disminuye el efecto de los hidrocoloides, tanto en la reología de la masa como en el pan sin gluten, por lo que es necesario adaptar la cantidad de agua adicionada en función del tipo y cantidad de hidrocoloide añadido.

Sección II: Galletas de masa corta sin gluten

Como se ha comentado con anterioridad, la presente tesis también se ha centrado en la mejora de galletas de masa corta sin gluten, en concreto en “sugar-snap cookies” (SSC). Aunque este tipo de galletas han sido profundamente estudiadas en elaboraciones a base de harina de trigo, no existen apenas estudios en galletas de masa corta sin gluten. Por ello, con el fin de elegir la harina más adecuada para este tipo de galletas se realizó un estudio donde se analizaron harinas provenientes de diferentes cereales y pseudocereales sin gluten que se pueden encontrar de forma habitual en el mercado. Considerando la importancia del tamaño de partícula de la harina, además de la procedencia del cereal, las harinas estudiadas se tamizaron obteniendo dos fracciones, una superior a 106 micras y otra inferior a 106 micras. No fue posible obtener dos fracciones diferentes en la harina de teff y de trigo sarraceno. De este modo once harinas fueron estudiaras: harina de trigo como control, harina de arroz de grano corto (fina y gruesa), harina de arroz de grano largo (fina y gruesa), harina de maíz (fina y gruesa), harina de maíz precocido (fina y gruesa), harina de trigo sarraceno y harina de teff.

De estas harinas se analizaron el contenido proteico, almidón dañado, tamaño de partícula y sus propiedades de hidratación así como la capacidad de absorción de aceite estudiando su efecto sobre las propiedades texturales de las masas y los parámetros de calidad de la galleta (expansión en el horneado, diámetro, textura y color).

La harina con más proteina fue la de teff, seguida por el trigo sarraceno y la harina control de trigo. El contenido proteico no solo se vio afectado por el origen del cereal, si no también por el tamaño de partícula, siendo mayor en la fracción fina del mismo tipo de harina.

Como era de esperar, las harinas de maíz precocido presentaron más almidón dañado, debido a la pregelatinización del almidón en el proceso de cocción, lo que también le confirió las mayores propiedades de hidratación. Se observó un mayor contenido en almidón dañado en las harinas de arroz, maíz y trigo, quizá por la naturaleza más compacta de éstos granos con respecto al trigo sarraceno o el teff que promueve la formación de mayor cantidad de almidón dañado en la molienda (Torbica y col., 2012).

La expansión en el horneado y el diámetro de la galleta, se vieron reducidos por la presencia de proteína y almidón dañado, lo que concuerda con las correlaciones negativas encontradas entre el ratio de expansión y el contenido en almidón dañado. El análisis de correlaciones indicó también correlaciones positivas entre el contenido en almidón dañado y las propiedades de hidratación. Muchos autores han reportado también un aumento en la capacidad de hidratación con mayores contenidos en almidón dañado (Martínez, y col. 2014a; Martínez, y col. 2014b).

En general, las galletas elaboradas con harinas sin gluten redujeron la expansión en el horneado y aumentaron la dureza de las galletas en comparación con la galleta control de trigo, a excepción de las galletas elaboradas a partir de las fracciones gruesas de las harinas de arroz. De este modo, correlaciones positivas entre el tamaño de partícula y el ratio de expansión fueron halladas. Asimismo, el tamaño de partícula afectó a la dureza de la masa y de la galleta, presentando mayor dureza la masa y las galletas elaboradas a partir de la fracción fina del mismo tipo de harina, por ser más compactas.

Por último, se evaluaron sensorialmente la galleta control de trigo y otras tres galletas representativas de la diversidad de harinas estudiadas. La galleta peor valorada fue la de trigo sarraceno, mientras que, en general, las galletas de maíz precocido fino y arroz corto fino presentaron resultados similares a la galleta control de trigo. A pesar de ello, la textura de la galleta de trigo fue la mejor valorada ya que la percibieron como menos dura y más crujiente, lo que concuerda con el análisis instrumental de textura.

En base a estos resultados, se consideró la harina de arroz corto como la harina más adecuada para SSC sin gluten. En cuanto al tamaño de partícula, es conveniente que la granulometría sea intermedia para evitar un exceso de compactación en las galletas, proporcionado por las fracciones finas, y el exceso de expansión con granulometrías muy gruesas.

Para finalizar, se estudió el efecto de la adición de proteína de guisante y almidón de maíz en SSC sin gluten a base de harina de arroz corto. En general, la incorporación de proteína aumentó las propiedades de hidratación de la mezcla harina-almidón-proteína, así como la consistencia de la masa, como lo demuestran los mayores valores reológicos de G', G'' y G*.

Las diferentes proporciones de harina-almidón-proteína afectaron las dimensiones de la galleta. Tanto el diámetro como el espesor se vieron reducidos con el contenido en proteína, mientras que el almidón ejerció el efecto contrario. En cuanto a la dureza de la galleta, ésta se vio reducida con la incorporación de proteína, lo que es consistente con las observaciones de Sarabhai y col. (2015) cuando incorporaron concentrados de proteína y mezclas de emulgentes y los resultados de Hadnađev y col. (2013) al incorporar harina de trigo sarraceno, más rico en proteína, a galletas de harina de arroz. La proteína también modificó el color de la galleta, favoreciendo la formación de un color más rojizo y amarillento y disminuyendo la luminosidad. En lo que respecta al almidón, no afectó ni a la textura ni al color de la galleta.

Se evaluaron sensorialmente la galleta producida con un 100% de harina de arroz, una galleta con alto contenido en proteína y nada de almidón, una galleta con alto contenido en almidón y nada de proteína, y una mezcla con alto contenido en almidón y proteína. Las galletas que contenían proteína fueron mejor valoradas en textura, olor, apariencia y aceptación global. Sin embargo, la galleta con almidón y sin proteína fue la peor valorada en todos los parámetros sensoriales. La mejor aceptación de las galletas con proteína puede deberse a su color más oscuro, semejante a las galletas de trigo y a su menor dureza. Se puede concluir que la inclusión de proteína puede mejorar la manejabilidad de la masa y la calidad de galletas de harina de arroz.

CONCLUSIONES

Las conclusiones principales que se extraen del presente estudio son:

- Los panes a base de harina de arroz presentan mejor sabor y color en la corteza. Sin embargo, su volumen específico y densidad alveolar son reducidos. Estos parámetros pueden ser mejorados con la adición de almidón de trigo, ya que proporciona mejores resultados que el almidón de maíz en volumen, textura y aceptación global.

- Mezclas de harina de arroz y almidón de trigo dan lugar a panes óptimos de mayor volumen y menor luminosidad. La mayor aceptación sensorial también se consigue con mezclas de harina de arroz y almidón de trigo. Esto demuestra que, la combinación de harina y almidón en la elaboración de panes sin gluten da productos de mejor calidad que aquellos elaborados a partir de harina o almidones de forma separada.

- La adición de aceite en panes sin gluten favorece el incremento del volumen específico, reduce la dureza del pan, mejora el color de la corteza y el alveolado de la miga. Contrariamente, la adición de margarina no produce un efecto positivo sobre la calidad del pan.

- El psyllium ejerce un efecto dominante y opuesto al HPMC sobre la reología de masas en el ensayo de fluencia-recuperación. Este ensayo reológico puede ser un mejor predictor de la calidad de panes sin gluten que el ensayo oscilatorio ya que, la capacitancia muestra mejor relación con los parámetros de calidad del pan.

- El incremento de la cantidad de agua añadida a fórmulas de pan con HPMC y psyllium disminuye el efecto de los hidrocoloides, tanto en la reología de la masa como en el pan sin gluten. Esto revela que la cantidad de agua añadida a la fórmula de pan debe ajustarse en función del tipo y cantidad de hidrocoloide.

- Características físico-químicas de la harina como el tamaño de partícula, el almidón dañado o el contenido en proteína ejercen una influencia importante en los parámetros de calidad de galletas de masa corta. Un mayor contenido en almidón dañado aumenta las propiedades de hidratación y disminuye la expansión en el horneado, mientras que un incremento en el tamaño de partícula aumenta el ratio de expansión.

- La adición de proteína en la elaboración de galletas de arroz aumenta las propiedades de hidratación, así como la consistencia de la masa. La proteína también afecta a los parámetros de calidad de la galleta reduciendo sus dimensiones y su dureza y favoreciendo la formación de un color semejante al color de las galletas de trigo mejorando la aceptación de las galletas si gluten.

- La inclusión de almidón en galletas a base de harina de arroz, aumenta las dimensiones, sin afectar a la textura ni al color de la galleta.

- La adición de almidón y proteína puede ser una estrategia adecuada para optimizar la fórmula de galletas sin gluten, con el fin de obtener una masa manejable y galletas de características similares a galletas de trigo.

- Las propiedades reológicas de la masa se encuentran relacionadas con los parámetros de calidad del producto final, tanto de panes como de galletas. Por lo tanto, el estudio de la reología de masas confiere información estructural de utilidad para los tecnólogos de alimentos, permitiendo una selección adecuada de los ingredientes para la optimización del proceso y del producto final.

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