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FORMULACIÓN Y DESARROLLO DE PRODUCTOS HORNEADOS LIBRES DE GLUTEN A BASE DE HARINA DE ARROZ ENRIQUECIDOS CON PROTEÍNAS

TESIS DOCTORAL

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Dedicatoria

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Resumen

La creciente demanda de productos libres de gluten ha favorecido el desarrollo de numerosos productos de panadería buscando imitar las características de calidad de sus homólogos elaborados con trigo. Sin embargo, en dichos desarrollos ha primado la calidad tecnológica y se han obviado otros aspectos como el nutricional. El objetivo de este estudio ha sido el diseño científico de productos horneados sin gluten (panes y magdalenas) elaborados utilizando harina de arroz, integrando aspectos tecnológicos, sensoriales y nutricionales. El estudio incluyó la evaluación de panes libres de gluten de origen comercial y el diseño de nuevas formulaciones sobre las cuales establecer correlaciones entre las propiedades de las masas y los parámetros tecnológicos de los productos horneados. En el diseño de magdalenas se puso especial énfasis a la evaluación del rol de las proteínas sobre las propiedades reológicas de las masas formuladas y las características tecnológicas del producto final. Los panes sin gluten comerciales mostraron un perfil nutricional muy variable, en general con un bajo contenido en proteína y alto en grasa. Los productos formulados, tanto panes como magdalenas sin gluten presentaron un buen contenido de proteínas y gran variabilidad en las características tecnológicas. El análisis reológico de las masas libres de gluten y los parámetros tecnológicos y sensoriales de los productos horneados resultantes permitieron establecer correlaciones positivas entre las propiedades de hidratación de la miga y algunos parámetros de textura, y entre la dureza-TPA y los parámetros reológicos de las masas caracterizadas mediante el Mixolab, los cuales podrían utilizarse como

predictores de la calidad para los panes libres de gluten. El estudio reológico de las mezclas formuladas para elaborar magdalenas a base de harina de arroz demostró que las propiedades reológicas de las masas-batidas estuvieron gobernadas por el tipo de proteína utilizada en cada formulación. En general, la presencia de clara de huevo confiere propiedades viscoelásticas a la masa-batida basada en harina arroz que permitieron obtener magdalenas con mejores características tecnológicas.

Resum

La creixent demanda de productes lliures de gluten ha afavorit el desenvolupament de nombrosos productes de forn buscant imitar les característiques de qualitat dels seus homòlegs elaborats amb blat. No obstant això, en estos desenvolupament ha primat la qualitat tecnològica i s'han obviat altres aspectes com el nutricional. L'objectiu d'este estudi ha sigut el disseny científic de productes enfornats sense gluten (pans i magdalenes) elaborats utilitzant farina d'arròs, integrant aspectes tecnològics, sensorials i nutricionals. L'estudi va incloure l'avaluació de pans lliures de gluten d'origen comercial i el disseny de noves formulacions sobre les quals establir correlacions entre les propietats de les masses i els paràmetres tecnològics dels productes enfornats. En el disseny de magdalenes es va posar especial èmfasi a l'avaluació del rol de les proteïnes sobre les propietats reològiques de les masses formulades i les característiques tecnològiques del producte final. Els pans sense gluten comercials van mostrar un perfil nutricional molt variable, en general amb un davall contingut en proteïna i alt en greix. Els productes formulats, tant pans com magdalenes sense gluten van presentar un bon contingut de proteïnes i gran variabilitat en les característiques tecnològiques. L'anàlisi reológic de les masses lliures de gluten i els paràmetres tecnològics i sensorials dels productes enfornats resultants van permetre establir correlacions positives entre les propietats d'hidratació de la molla i alguns paràmetres de textura, i entre la dureza-TPA i els paràmetres reològiques de les masses caracteritzades per mitjà del Mixolab, els quals podrien utilitzar-se com a predictors de la qualitat per als pans lliures de gluten.

L'estudi reològic de les mescles formulades per a elaborar magdalenes a base de farina d'arròs va demostrar que les propietats reológicas de les masses- batudes van estar governades pel tipus de proteïna utilitzada en cada formulació. En general, la presència de clara d'ou conferix propietats viscoelastiques a la massa-batuda basada en farina arròs que van permetre obtindre magdalenes amb millors característiques tecnològiques.

Abstract

The increasing demand of gluten free products has prompted the launching of numerous bakery gluten free products with similar quality to their wheat containing counterparts. Nevertheless, those products are mainly design focused on the technological quality and without considering the nutritional quality. The objective of this research was the scientific design of baked gluten free products (breads and muffins) based on rice flour, from technological, sensorial and nutritional point of view. The study included the evaluation of commercial gluten free breads and the design of new formulations to establish the correlations between the dough properties and the technological parameters of the baked products. In the muffins design, special emphasis was put on determining the role of proteins on the rheological properties of the formulated doughs and the product characteristics. Commercial gluten free breads showed great variation in the nutritional profile; in general they had low protein content and high content in fats. The formulated products, gluten free both breads and muffins, had adequate protein content and great variability in the technological characteristics. The rheological analysis of the gluten free doughs and the technological and sensorial parameters of the baked goods, allowed establishing positive correlations between the hydration properties of the crumb and some textural parameters and also between the TPA-hardness and the dough rheological parameters obtained with the Mixolab, which can be used as quality predictors for gluten free breads. The rheology of the formulated doughs for making muffins based on rice flour confirmed that the rheological properties of the batters are governed by the type of protein

added. In general, the egg white protein conferred to the batter the necessary viscoelastic properties for obtaining the best quality muffins.

Índice General

INTRODUCCIÓN

La presente introducción describe algunos aspectos fundamentales que contribuirán a un mayor entendimiento del tema y a una mejor ubicación en el entorno actual de lo concerniente a lo “libre de gluten”. Se define gluten y se refiere su importancia como componente fundamental en la elaboración de panes y productos de panadería, su composición química y su funcionalidad, lo cual le confiere la calidad única de formar una masa viscoelástica capaz de ser horneada para producir el pan. Se definen los productos libres de gluten (de acuerdo al Reglamento (CE) No 41/2009 de la Comisión de las Comunidades Europeas y a la Norma Codex relativa a los alimentos para regímenes especiales destinados a personas intolerantes al gluten, adoptada por la Comisión del Codex Alimentarius en su 31ª sesión de julio de 2008), señalando los límites permitidos en la cantidad de gluten, y los cereales que lo contienen. Se define la enfermedad celiaca (EC), el colectivo especial que la sufre, su incidencia a nivel mundial y en España. Por otro lado se presenta información relacionada con las tendencias de consumidores y de mercado en relación a los productos libres de gluten, lo cual refleja la realidad de la creciente demanda de este tipo de productos y la necesidad de mejorar su calidad sensorial, nutricional y su abastecimiento; tomando en cuenta que la percepción del consumidor que compra este tipo de productos es que los mismos son “más saludables que su contraparte”, a pesar de que esto no es necesariamente cierto. Seguidamente se presenta una breve y actualizada revisión de los aspectos que limitan la elaboración de productos horneados libres de gluten, a partir de harinas y otros ingredientes que no lo contienen, pero que han sido diseñados para satisfacer las necesidades específicas de personas con intolerancia al gluten. Finalmente se presenta algunos antecedentes derivados de recientes estudios científicos en relación al diseño de productos horneados libres de gluten (panes y magdalenas) utilizando harina de arroz, almidones de diferentes fuentes, hidrocoloides y proteínas, entre otros, como sustitutos funcionales del gluten. Es importante destacar que en todos estos estudios se ha puesto énfasis en evaluar el efecto de los ingredientes sobre las propiedades de las masas o sobre las características de los productos finales, pero no se han establecido claras relaciones entre ambas; dejando así un vacío en el entendimientos del comportamiento de estos complejos sistemas panarios. Aunado a ello, y a pesar de la importancia que tienen estos productos como sustitutos de un producto básico en la alimentación, como lo es el pan, es poca la información científica publicada en relación al estudio y mejora de la calidad nutricional de los productos libres de gluten que se desarrolla y diseñan. En tal sentido, y con miras a contribuir al avance del conocimiento científico, tecnológico y nutricional de los productos libres de gluten y tomando en cuenta la real y creciente demanda por parte de los consumidores, de productos con mejor sabor y textura que lo ya existentes, y dada la importancia de mejorar la pobre calidad nutricional de la mayoría de los productos que existen en el mercado, se han establecido y realizado los objetivos que justifican el desarrollo de esta Tesis Doctoral.

1. Gluten

De acuerdo al Reglamento No 41/2009 de la Comisión de las Comunidades Europeas sobre la composición y etiquetado de productos alimenticios apropiados para personas con intolerancia al gluten (con aplicación a partir del 1 de enero de 2012). En su artículo 2:

Se entenderá por «**gluten**»: una fracción proteínica del trigo, el centeno, la cebada, la avena o sus variedades híbridas y derivados de los mismos, que algunas personas no toleran y que es insoluble en agua y en solución de cloruro sódico de 0,5 M.

Y se entenderá por «**trigo**»: cualquier especie de *Triticum*.

En términos generales, el gluten es una mezcla compleja de proteínas de almacenamiento presentes en el trigo (Hoseney, 1986; Catassi y Fassano, 2008) y en otros granos de cereales, tales como el triticale, la cebada, el centeno. Actualmente, la avena está considerada como un cereal que contiene gluten, debido a la posible contaminación que puede presentar con trigo, cebada o centeno.

Las proteínas de almacenamiento del trigo son capaces de formar el gluten. La formación del gluten es un artefacto del procesamiento de la harina, se forma como resultado de la interacción de las dos principales clases de proteínas las gliadinas y las gluteninas, las cuales interactúan cuando la harina es mezclada con agua para formar la masa viscoelástica. Aunque las proteínas de almacenamiento están presentes en otros cereales (triticale, cebada y centeno y avena), el comportamiento viscoelástico de gluten de trigo y su funcionalidad lo distingue de otros granos o proteínas vegetales (Hoseney, 1986).

El gluten está definido como un gel formado por las proteínas de almacenamiento del grano de trigo cuando se trabaja mecánicamente una mezcla de harina y agua. Está formado por un 80% de proteína y un 8% de lípidos, base sustancia seca, con un resto de hidratos de carbono y cenizas (Hoseney, 1986).

En general, las proteínas que constituyen el gluten son: las gliadinas, que contribuyen esencialmente a la viscosidad y a la extensibilidad de la masa (Don y col., 2003) y las gluteninas, que son responsables de la fuerza y elasticidad de la masa (Xu y col., 2007). Esta estructura distintiva es crucial para las características de la textura y de la miga del pan del trigo (Hüttner y Arendt, 2010).

1.1. Importancia tecnológica del gluten

Debido a la naturaleza única de su propiedad viscoelástica, el gluten ofrece un sin número de propiedades funcionales para ser utilizado en sistemas alimenticios. Las propiedades funcionales del trigo son amplias, entre ellas se encuentran: la capacidad de desarrollar viscoelásticidad, la capacidad de formar películas, sus propiedades termoestables y su capacidad de absorción de agua (IWGA, 2012).

La capacidad del gluten de trigo para formar una masa viscoelástica cuando está totalmente hidratado lo distingue de las demás proteínas vegetales disponibles en el mercado. La propiedad formadora de película del gluten es una consecuencia de su viscoelásticidad.

La formación de película es una de las propiedades que proporciona la capacidad para que la masa retenga partículas sólidas en suspensión. La formación de película también es importante para atrapar las burbujas de gas producidas durante la fermentación del pan, lo cual resulta en una miga de textura deseable (uniforme) y la expansión del volumen. La estabilidad de las burbujas de gas depende de la elasticidad y de la fuerza de la película de gluten que forma las paredes de las burbujas. El colapso individual de las burbujas de gas puede conducir a la formación de grandes agujeros (cavidades) en la masa o resultar en panes con poco volumen. Por otra parte, la retención de humedad en masas y en la corteza durante el horneado es importante para permitir la expansión en el volumen de la masa y del pan. La capacidad de retener humedad del gluten también es clave en la obtención de la textura húmeda en la miga de productos horneados (Khan y Nygard, 2006).

Ningún área de procesamiento de alimentos goza de mayores beneficios de la funcionalidad del gluten que la industria de la panadería. Las propiedades de viscoelásticas exclusivas del gluten de trigo mejoran la fuerza de la masa, la tolerancia al mezclado y a la manipulación. Su capacidad de formar película proporciona retención de gas y expansión controlada lo cual permiten mejorar el volumen, la uniformidad y la textura, sus propiedades termoestables contribuyen a la rigidez de la estructura necesaria y a las características de la mordida; su capacidad de absorción de agua mejora el rendimiento del producto horneado, permitiendo obtener productos más suaves, y con mayor vida útil (IWGA, 2012). Las proteínas del gluten juegan un papel primordial en la determinación de las características únicas del trigo durante el horneado, debido a que le confieren capacidad reabsorción de agua, cohesividad, viscosidad, extensibilidad, elasticidad, resistencia al estiramiento, tolerancia al mezclado, y capacidad de retener gas (Lazaridou y col., 2007).

2. Alimentos libres de gluten

2.1. Definición de alimentos libres de gluten

A efectos del Reglamento (CE) No 41/2009 de la Comisión de las Comunidades Europeas (vigente a partir del 2012) sobre la composición y etiquetado de productos alimenticios apropiados para personas con intolerancia al gluten (artículos 2, 3 y 4), se entenderá por:

- «Productos alimenticios para personas intolerantes al gluten»: los productos alimenticios destinados a una alimentación particular elaborados, tratados o preparados especialmente para responder a las necesidades nutricionales particulares de las personas intolerantes al gluten.

- Los productos antes definidos que se comercializan como tales, deben llevar la indicación «**contenido muy reducido de gluten»** o «**exentos de gluten**» de conformidad con las disposiciones establecidas en el referido reglamento. Estas disposiciones pueden ser logradas mediante el uso de productos alimenticios tratados especialmente para reducir el contenido de gluten de uno o varios ingredientes que contienen gluten o productos alimenticios cuyos ingredientes con gluten han sido sustituidos por otros ingredientes exentos de forma natural.

Productos alimenticios para personas con intolerancia al gluten.

Los productos alimenticios para personas con intolerancia al gluten, constituidos por uno o más ingredientes procedentes del trigo, el centeno, la cebada, la avena o sus variedades híbridas, que hayan sido tratados de forma especial para eliminar el gluten, no contendrán un nivel de gluten que supere los 100 mg/kg en los alimentos tal como se venden al consumidor final.

El etiquetado, la publicidad y la presentación de los productos con un nivel de gluten que no supere los 100 mg/kg, llevarán la mención «contenido muy reducido de gluten».

Pueden llevar el término «exento de gluten» si el contenido de gluten no sobrepasa los 20 mg/kg en total, medido en los alimentos tal como se venden al consumidor final.

La avena contenida en alimentos para personas con intolerancia al gluten debe ser producida, preparada o tratada de forma especial para evitar la contaminación por el trigo, el centeno, la cebada, o sus variedades híbridas y su contenido de gluten no debe sobrepasar los 20 mg/kg.

Los productos alimenticios para personas con intolerancia al gluten constituidos por uno o más ingredientes que sustituyan el trigo, el centeno, la cebada, la avena o sus variedades híbridas, no contendrán un nivel de gluten que supere los 20 mg/kg en los alimentos tal como se venden al consumidor final. El etiquetado, la presentación y la publicidad de esos productos deberá llevar la mención «exento de gluten».

Los términos «contenido muy reducido de gluten» o «exento de gluten» deberán aparecer muy cerca del nombre comercial del producto.

Otros productos alimenticios adecuados para las personas con intolerancia al gluten.

El etiquetado, la publicidad y la presentación de los siguientes productos alimenticios pueden llevar el término «exento de gluten» si el contenido de gluten no sobrepasa los 20 mg/kg, medido en los alimentos tal como se venden al consumidor final:

a) productos alimenticios para el consumo normal;

b) productos alimenticios destinados a una alimentación particular elaborados, tratados o preparados especialmente para responder a las necesidades nutricionales particulares distintas de las de las personas con intolerancia al gluten pero que son sin embargo adecuados, en virtud de su composición, para cubrir las necesidades dietéticas especiales de las personas con intolerancia al gluten.

El etiquetado, la publicidad y la presentación de estos alimentos no llevarán la mención «contenido muy reducido de gluten».

De acuerdo al CODEX ALIMENTARIO (CODEX STAN 118 – 1979):

La norma Codex relativa a los alimentos para regímenes especiales destinados a personas intolerantes al gluten, adoptada por la Comisión del Codex Alimentarius en su 31ª sesión de julio de 2008, indica que:

- Los alimentos libres de gluten son alimentos dietéticos:

a) consiste en uno o más ingredientes que no contengan trigo (es decir, todos las especies de *Triticum*, como el trigo, espelta y kamut), centeno, cebada, avena o sus variedades, y el nivel de gluten no bebe exceder de 20 mg/kg en total, basado en el alimento tal y como es vendido o distribuido al consumidor,

b) consisten de uno o más ingredientes provenientes de trigo (es decir, todos las especies *Triticum*, como el trigo, espelta y kamut), centeno, cebada, avena o sus variedades, que hayan sido especialmente procesados para eliminar el gluten, y el nivel de gluten no debe superar los 20 mg/kg en total, basado en el alimento tal y como es vendido o distribuido al consumidor.

- Alimentos especialmente procesados para reducir el contenido de gluten a un nivel por encima de 20 y hasta 100 mg/kg.

Estos alimentos consisten en uno o más ingredientes de trigo (es decir, todos las especies de *Triticum*, como el trigo, espelta y kamut), centeno, cebada, avena o sus variedades mestizas, que se han procesado especialmente para reducir el contenido de gluten a un nivel por encima de 20 y hasta 100 mg/kg en total, basados en el alimento tal y como es vendido o distribuido al consumidor.

Tanto el REGLAMENTO (CE) No 41/2009 como en la norma adoptada por el Codex Alimentario establecen los límites de presencia de gluten entre 20 mg/kg y 100 mg/kg. Sin embargo, a diferencia del Codex Alimentario, en el REGLAMENTO (CE) No 41/2009, se establece que el etiquetado, la publicidad y la presentación de estos productos llevará la mención «exento de gluten» si el contenido de gluten no sobrepasa los 20 mg/kg y la mención «contenido muy reducido de gluten» si el contenido de gluten no sobrepasa los 100 mg/kg en total, medido en los alimentos tal como se venden al consumidor final.

A nivel mundial, se utilizan símbolos que identifican a los productos libres de gluten (figura 1), los cuales representan básicamente la prohibición del trigo, sin embargo no existe ningún símbolo con carácter universal para tal fin.

Figura 1. Algunos símbolos utilizados a nivel mundial en el etiquetado de productos libres de gluten (Fuente: Imágenes Google.com)

2.2. Productos libres de gluten como dieta terapéutica

La existencia de un colectivo especial que requiere de productos que no contengan gluten es una realidad a nivel mundial. El 1% de la población mundial sufre de la enfermedad celiaca o de algún otro tipo de intolerancia al gluten (Catassi y Yachha 2009). Ciertos individuos experimentan alguno de los muchos tipos de reacciones alérgicas al gluten, estas pueden incluir asma, erupciones de la piel, dermatitis, y el desorden intestinal grave llamado enfermedad celíaca. Por otro lado, un grupo más reducido de personas tiene una alergia específica al trigo y la exposición a éste puede producir erupciones, asma y choque anafiláctico.

Los términos *enfermedad celiaca* (EC), *celiac sprue disease*, *enteropatía por sensibilidad al gluten* (ESG) e *intolerancia al gluten* se utilizan para describir la enfermedad celiaca. La EC es una enteropatía inmune sufrida por la ingestión de gluten en individuos genéticamente susceptibles (Brussone y Asp 1999; Wrigley y Bietz 1988). Quienes padecen la EC pueden sufrir una reacción autoinmune al ingerir incluso pequeñas cantidades de gluten. La enfermedad celiaca es una enfermedad digestiva autoinmune que daña la vellosidad del intestino delgado e interfiere en la absorción de nutrientes del alimento. Esta lesión disminuye la superficie disponible para la absorción de nutrientes incluyendo hierro, ácido fólico, calcio y vitaminas solubles en agua (Catassi y Fasano, 2008), trayendo como consecuencia carencias nutricionales

Específicamente, los granos que provocan las reacciones celiacas incluyen trigos hexaploides y trigos durum, triticale, centeno, cebada, espelta, Kamut, escaña (“einkorn”), avena y las variedades híbridas de los referidos cereales. En particular, los pacientes celiacos son intolerantes a algunas prolaminas presentes en algunos cereales, dichas prolaminas contienen una secuencia tóxica específica de oligopéptidos. La fracción gliadina en el trigo, secalina en el centeno, hordeína en la cebada; y la avenina en algunas variedades de avena (Comino y col., 2011), son las prolaminas que están involucradas en el mecanismo de la enfermedad celiaca (Wrigley y Bietz, 1988; Brussone y Asp, 1999; Comino y col. 2011).

En el presente, el único tratamiento efectivo para el tratamiento de la EC es mantener una estricta dieta libre de gluten. Sin embargo, el seguimiento de esta pauta dietética no es sencillo, considerando que los granos que contienen gluten, especialmente el trigo, son el principal ingrediente en la elaboración de alimentos populares de consumo masivo de muchas culturas, tales como panes, pasta y pasteles. Por otra parte, estos granos y sus derivados son ampliamente utilizados como aditivos, conservantes, ligantes y espesantes en una vasta mayoría de alimentos procesados (caldos, salsas, carnes procesadas, alimentos enlatados, gelatinas y medicamentos).

Afortunadamente, tanto el conocimiento médico, como la calidad de la dieta libre de gluten continúan mejorando en la medida que aumenta la conciencia del incremento de la EC a lo largo del mundo (Cureton y Fasano, 2009).

2.3. Prevalencia de la Enfermedad Celiaca

La distribución geográfica de la EC estuvo principalmente restringida a Europa y otros los países desarrollados, tales como Estados Unidos, Canadá y Australia. Sin embargo, nuevos estudios epidemiológicos han revelado que este desorden es común en muchos países en desarrollo; además han demostrado que la “aldea global de la enfermedad celiaca” tiene de hecho una distribución mundial. En consecuencia la EC es uno de los desórdenes crónicos más comunes, y afecta a cerca del 1% de la población mundial (Catassi y Yachha, 2009). Se ha estimado que en Europa, dependiendo del país que se trate, una de cada 85-500 personas sufre la EC (Farrell y Kelly, 2001). Recientemente, se ha conducido un estudio en una amplia muestra de la población europea (Finlandia, Alemania, Italia y Reino Unido), incluyendo niños y adultos, para investigar si la prevalencia de EC varía significativamente entre las diferentes zonas del continente europeo (Mustalahti y col., 2010). Los resultados indican que la prevalencia global de EC (previamente diagnosticada) es de 1.0%. Por otra parte, en sujetos de 30 a 64 años la prevalencia fue del 2,4% en Finlandia, de 0,3% en Alemania y de 0,7% en Italia. Adicionalmente, el 68% de las personas estudiadas mostraron cambios en la mucosa del intestino típicos para la EC. Los investigadores concluyen que la EC es común en Europa y que la prevalencia de esta enfermedad en la edad adulta muestra grandes e inexplicables diferencias entre las poblaciones de los diferentes países europeos. En la población del Reino Unido, la prevalencia de la enfermedad celíaca está estimada en 0,8 a 1,9 %, estudios internacionales reportan hallazgos similares (NICE, 2009).

Recientemente Packaged Facts (2011), ha publicado algunas cifras que ponen de manifiesto la incidencia de la enfermedad celiaca en los Estados Unidos de América:

- Uno de cada 133 americanos tiene la enfermedad celiaca.
- 3 millones de americanos de todas las razas, edades y géneros sufren la EC.
- Se estima que el 85% de los americanos que tienen enfermedad celiaca no ha sido diagnosticado o ha sido diagnosticado con otras afecciones.
- Del 5 al 22% de pacientes celiacos tienen un miembro inmediato de la familia (pariente del primer grado) que también tiene la EC.

Particularmente en España, la prevalencia de la EC oscila entre 1 de cada 80 adultos y 1 de cada 300 menores de 15 años, con gran variabilidad regional, atribuible a diversos sesgos en los procedimientos de diagnóstico, y un predominio de las formas silentes frente a las formas clásicas con síntomas. Así, señaló la Dra. Enriqueta Román (Hospital Puerta de Hierro, Majadahonda, Madrid) al presentar los datos más novedosos recogidos en el Registro Español de Pacientes Celíacos (REPAC) (SEEC, 2010).

El incremento en la incidencia de la EC, y el resultante incremento en la demanda de productos libres de gluten, ha originado un creciente interés por parte de muchas compañías en investigar y desarrollar una amplia gama de productos sustitutos, los cuales puedan presentar una alta aceptabilidad sensorial. Hasta hace algunas décadas, incluso encontrar algunos productos alimenticios convenientes para los celiacos era una preocupación importante. Una vez superado este problema, la investigación se ha centrado en identificar las materias primas que sean tecnológicamente similares a la harina de trigo, el cual es el ingrediente principal en la dieta occidental (Pagliarini y col., 2010).

3. Productos libres de gluten: Tendencia de consumidores y de mercado

Los productos libres de gluten originalmente fueron destinados a personal con intolerancia al gluten, no obstante son aptos para toda la población. Son productos con características propias y distintas a las de sus homólogos hechos con harina de trigo.

Una gama de consumidores siguen dietas sin gluten, incluyendo aquellos con enfermedad celíaca, sensibilidad de gluten, autismo, condiciones neurológicas, síndrome de intestino irritable, esclerosis múltiple, cáncer y aquellos que lo ven como una dieta "saludable" (Nachay, 2010).

En los últimos años la expresión “¿Es usted libre de gluten?”, describe, más que al sector industrial de los productos alimenticios y de las bebidas, a la sensación de un colectivo especial que ha transformado en tendencia esta dieta en apenas algunos años. Mucha gente es “libre de gluten” por necesidad, debido a que sufre de enfermedad celiaca o de una alergia del alimentos. Pero un número creciente es “libre de gluten” por elección propia, ya que emerge la evidencia de que esta dieta puede tratar las condiciones médicas que se extienden desde el autismo en niños a la artritis reumatoide en adultos. Otros encuentran que “una vida libre de gluten” simplemente les hace sentir mejor. Recientemente, un informe del mercado de los alimentos y las bebidas libres de gluten realizado en los Estados Unidos por Packaged Facts (2011), una división de MarketResearch.com, reveló que la motivación número uno para comprar productos alimenticios libres de gluten es que estos productos están considerados más sanos que sus homólogos convencionales. Adicionalmente, el informe señala que las ventas de productos libres de gluten alcanzaron más de 2,6 mil millones de dólares a finales de 2010. (http://www.marketwire.com). Packaged Facts, adicionalmente proyecta que en Estados Unidos las ventas de alimentos y de bebidas libres de gluten excederán los 5 billones de dólares antes de 2015 y los 6,6 billones de dólares en 2017.

El incremento en el diagnóstico de la enfermedad celiaca y de las alergias por consumo de alimentos, el aumento en la conciencia de estas dolencias entre pacientes, el personal médico y el público en general, la disponibilidad de más y mejores productos, y la tendencia que tienen los familiares y amigos de los pacientes celiacos -para dar apoyo- de comer productos libres de gluten, están entre los factores que estimulan la continua expansión del mercado de productos libre de gluten. No obstante, la convicción de que los productos sin gluten son generalmente más saludables es la principal motivación de compra para los consumidores de estos productos.

El mercado de alimentos "libres de" ha crecido en los Estados Unidos y Europa Occidental, según una nueva investigación de Leatherhead Food Research. De acuerdo a este informe, en términos de declaraciones de propiedades saludables, “sin gluten” es la de más rápido crecimiento, lo que indica que este mercado tiene gran potencial de crecimiento en los próximos años. El mercado de los alimentos sin gluten en los Estados Unidos y Europa occidental tuvo un valor estimado de 3.500 millones de dólares en 2010. Gran parte del crecimiento previsto en el sector “libre de gluten” está vinculado a las percepciones saludables de los alimentos sin gluten, especialmente entre los grupos de consumidores que cada vez son más conscientes de su dieta, salud y bienestar, que por lo tanto buscan activamente productos “adecuados para"/"libres de” sin tener una alergia diagnosticada (Nachay, 2010).

Es una realidad que los productos libres de gluten están en auge. La cantidad de productos comercializados como libres de gluten continúa aumentando en el mercado internacional. Más del 5% de los lanzamientos de alimentos y bebidas identificados por Innova Market Insights en el 2009 se comercializaron como libres de gluten, porcentaje que supera el 10% en Australia y Nueva Zelanda y cae a menos del 1% en Asia. En el Reino Unido, el interés por estos productos también ha sido incentivado por los minoristas líderes, que en su mayoría ofrecen sus propios productos “libres de”, incluyendo la categoría libre de gluten. Estados Unidos posiblemente sea el mercado para alimentos libres de gluten más grande del mundo, con ventas estimadas en más de 1.500 millones de dólares anuales. La mayoría de los mercados europeos son mucho más pequeños, lo que refleja la existencia no solo de poblaciones más reducidas en general sino además de mercados de alimentos procesados mucho menos desarrollados (www.innovadatabase.com).

4. **Limitaciones en el diseño de los productos libres de gluten**

Aunque la industria de alimentos ha asumido los desafíos asociados a la eliminación del gluten en las formulaciones y un considerable número de productos está disponible en el mercado, muchas de las formulaciones libres de gluten todavía poseen baja calidad tecnológica y nutricional (Mariotti y col., 2013). A menudo, los productos de panadería sin gluten tienen inferior textura, poco color y corta vida útil (Gallager y col., 2004). Sin embargo, la demanda de los consumidores de estos productos está presionando a los fabricantes a mejorar la calidad del producto (O'Neill, 2010).

Como ha sido discutido previamente, las propiedades únicas que presenta la harina de trigo para formar una masa cohesiva que puede ser horneada para producir pan o para formar pastas, son derivadas de las proteínas presentes en el gluten (Gómez y col., 2007). La ausencia del gluten a menudo resulta en una mezcla líquida, más parecida a las masas batidas, de consistencia semi-líquida, producidas para elaborar tartas y pasteles que a una masa panaria. Las masas libres de gluten son mucho menos cohesivas y elásticas que las masas provenientes del trigo, presentan textura lisa, son más pegajosas y pastosas y difíciles de manipular. En la literatura, con frecuencia estas masas libres de gluten son llamadas “batter ó batidos” en lugar de masas. Estas masas-batidas no son realmente amasadas, sino mezcladas mecánicamente (Houben y col., 2012). La masa de pan sin gluten solamente puede retener gas si otro gel reemplaza al gluten. Es por ello que, los productos libres de gluten, y en especial los tipo pan, requieren de sustancias poliméricas que mimeticen las características viscoelásticas del gluten. El uso de almidones, gomas e hidrocoloides es la estrategia más antigua y la más ampliamente utilizada para mimetizar las propiedades del gluten en la elaboración de productos horneados libres de gluten, debido a las propiedades que tienen estos ingredientes para actuar como agentes estructurantes y enlazadores de agua, previniendo el envejecimiento del pan y la retención de las burbujas de aire producidas durante la fermentación (Gallagher y col., 2004; Schober, 2009). De acuerdo con la evolución en el desarrollo de panes libres de gluten descrita en la literatura, los primeros panes se hicieron a partir de formulaciones simples basadas principalmente en la combinación de almidones puros con algún tipo de hidrocoloide, posteriormente se desarrollaron fórmulas incorporando primeramente harinas de cereales libres de gluten (sorgo y arroz) y posteriormente otras harinas de cereales, pseudocereales y sus mezclas (maíz, amaranto) siempre en combinación con hidrocoloides. En los desarrollos más avanzados y novedosos se utilizan mezclas complejas de ingredientes resultantes de la combinación de harinas, almidones e hidrocoloides con proteínas de diferentes fuentes, fibras y enzimas a fin de obtener panes de mejor calidad tecnológica, sensorial y nutricional (Marco y Rosell, 2008a;b).

Recientemente se han publicado extensas revisiones que incluyen numerosos estudios científicos dirigidos a evaluar la optimización de formulaciones, el uso de ingredientes y técnicas que permitan desarrollar diferentes tipos de productos de panadería libres de gluten (panes, pizzas, pastas, galletas, pasteles, etc.) con mejor calidad tecnológica y sensorial (Schober, 2009; Hüttner y Arendt, 2010; Houben y col., 2012).

El arroz es uno de los cereales más adecuado para preparar alimentos libres de gluten (Rosell y Gómez, 2006; Rosell y Marco, 2008a), ya que su harina se caracteriza por tener un sabor suave y color blanco, es fácil de digerir y adicionalmente es hipoalergénica, debido al tipo de proteínas que contiene. Además presenta bajo contenido de sodio y alto contenido de carbohidratos fácilmente digeribles (Rosell y Gómez, 2006). Sin embargo, la calidad de productos horneados a base de arroz es inferior a la de productos preparados con trigo debido a la carencia de gluten. Por ello, el uso de sustancias poliméricas tales como hidrocoloides, es requerido a menudo para mejorar la calidad de los productos a base de arroz (Rosell y Gómez, 2006). La cantidad relativamente pequeña de prolaminas presentes en el arroz, conlleva a la necesidad de utilizar algún tipo de aditivo o ingrediente tales como, hidrocoloides, emulsificante, enzimas o productos lácteos, que junto con la harina del arroz, permitan obtener apropiadas características viscoelásticas (Rosell y Gómez, 2006; Marco y Rosell, 2008b).

5. Diseño con base científica de panes y magdalenas libres de gluten.

Panes

En la última década ha sido notable el incremento en el número de estudios publicados dirigidos al desarrollo de panes libres de gluten (Gujral y col., 2003a;b; Gujral y Rosell, 2004 a,b,c; Lazaridou y col., 2007; Pruska-Kedzior y col., 2008; Sciarini y col., 2008; Marco y Rosell, 2008a; Korus y col., 2009; Rosell y col., 2009; Rosell, 2009; Demirkesen y col., 2010; Brites y col., 2010; Krupa y col., 2010; Sciarini y col., 2010; 2012a,b; Onyango 2011; Sabanis y Tzia, 2011; Smith y col., 2012; Mariotti y col., 2009; 2013). El objetivo de estos estudios ha sido la incorporación de almidones (trigo, maíz, patata, tapioca), gomas e hidrocoloides - goma xantana, carboximetilcelulosa (CMC), hidroxipropilmetilcelulosa (HPMC), goma guar, β-glucanos, entre otras -, proteínas (soja, guisante, huevo, leche) y otras harinas de cereales (arroz, maíz, sorgo) o pseudocereales (amaranto, trigo sarraceno) en una mezcla libre de gluten con la intención de mimetizar las características viscoelásticas del gluten y obtener panes de buena calidad.

Las formulaciones diseñadas para elaborar panes libres de gluten, contienen principalmente harinas de arroz o maíz combinadas con almidones de trigo, patata o maíz (Gallagher y col., 2004; Gujral y Rosell, 2004a; Sabanis y col., 2009; Demirkesen y col., 2010; Pagliarini y col., 2010; Torbica y col., 2010; Brites y col., 2010; Sciarini y col., 2010; 2012a,b; Crockett y col., 2011). Para elaborar panes libres de gluten de buena calidad se ha descrito es uso de la harina del arroz en combinación con hidrocoloides (Kadan y col., 2001; Gujral y col., 2003a,b; McCarthy y col., 2005; Ahlborn y col., 2005; Moore y col., 2006; Lazaridou y col., 2007; Marco y Rosell, 2008 a,b; Pruska-Kędzior y col., 2008; Demirkesen y col., 2010; Sciarini y col., 2010; 2012). Entre todos los hidrocoloides estudiados el HPMC es el más adecuado ya que rinde panes con óptima expansión de volumen y masas con propiedades similares a las obtenidos con trigo (Gujral y Rosell, 2004a,b; Marco y Rosell, 2008a; Sabanis y Tzia, 2011; Smith y col., 2012; Mariotti y col., 2013). El uso de HPMC, permite el incremento de la viscosidad de la masa durante el mezclado; y promueve la dispersión de las burbujas de gas durante la fermentación, y en el horneado incrementa la capacidad de retener el gas; en consecuencia se puede incrementar en el volumen del pan (Rosell y Gómez, 2006).

Para obtener una red similar a la desarrollada por el gluten en la producción de pan, además de los hidrocoloides, las proteínas pueden ser añadidas en las formulaciones (Marco y Rosell, 2008a), obteniéndose adicionalmente el beneficio de mejorar el valor nutricional de los productos. Los panes producidos a partir de la mezcla de almidones y gomas con alguna proporción de harinas de cereales libres de gluten tienen muy bajo contenido de proteínas y son deficientes en lisina. Las proteínas de diversas fuente pueden ser añadidas con el objetivo de introducir un agente estructurante, incrementar el valor nutricional de los productos libres de gluten y mejorar la apariencia, el volumen y en algunos casos los aspectos sensoriales del pan (Gallagher y col., 2003; Moore y col., 2006; Crockett y col., 2011). Para incrementar el papel estructurante de las proteínas se ha propuesto la combinación de proteínas (albúmina de huevo, suero de leche) o aislados proteicos (soja y guisantes) con enzimas entrecruzantes (transglutaminasa, glucosa oxidasa) (Gujral y Rosell, 2004 a,b; Marco y col., 2007; Marco y Rosell, 2008 a,b) con miras a obtener panes con mejor calidad tecnológica y nutricionalmente enriquecidos (Marco y Rosell, 2008c).

Recientemente, Crockett y col (2011) formularon panes libres de gluten a partir de harina de arroz, almidón de tapioca, levadura instantánea, azúcar y HPMC (methocel E15), con adición de aislado de proteína de soja (APS) y clara de huevo en polvo (CHP). El estudio tuvo como objetivo evaluar el efecto de la adición de APS y CHP sobre la distribución del agua y las propiedades reológicas de las masas; así como caracterizar los atributos de calidad de los panes resultantes. Los resultados obtenidos en el estudio permitieron concluir que la adición de APS a la mezcla harina-almidón-HPMC redujo la estabilidad de la masa por supresión de la funcionalidad del HPMC, alterando la distribución del agua dentro de la masa, debilitando la interacción del HPMC con la matriz de almidón y reduciendo la estabilidad de la espuma. Por otra parte, encontraron que la adición de CHP produjo un efecto antagónico similar con el HPMC en la masa. Sin embargo, a concentraciones de 15% de adición, la CHP se convirtió en el principal estructurante de la masa y superó las interacciones negativas con la HPMC. Se logró mediante la formación de una matriz interconectada mejorar la regularidad en la miga y el volumen del pan. Sin embargo, esta formulación requiere de optimización en el sabor y la humedad percibida en el producto final.

Magdalenas

Contrariamente al pan, en la literatura hay un número limitado de estudios en otros productos horneados libres de gluten, tales como pizzas, pastas, galletas, pasteles, tartas y magdalenas (Turabi y col., 2008 a,b; Gularte y col., 2011; Park y col., 2012; de la Hera y col., 2012). En todos estos productos horneados también se utilizan mezclas de ingredientes (almidones de trigo, maíz o patata, harinas de arroz o maíz y sus mezclas, harina de soja, harinas de pseudocereales, gomas como guar o xantana, proteínas de soja o huevo, y otros ingredientes propios de la formulación de cada producto similares a los utilizados en la elaboración de panes libres de gluten, pero en las proporciones que establecen las formulaciones y los procesos tecnológicos específicos (Gallagher, 2008; Schober, 2009; Turabi y col., 2008).

Al igual que el pan libre de gluten, las magdalenas, tartas y otros productos horneados libres de gluten son fabricados comercialmente tratando de parecerse a aquellos hechos con harina de trigo. Sin embargo, los productos de bollería comerciales sin gluten suelen presentan defectos en la calidad sensorial (sabor, color, textura) y bajo valor nutritivo. Las recetas de magdalenas, bizcochos y tartas sin gluten contienen harina de arroz como ingrediente principal o almidones de diferentes fuentes (arroz, maíz, patata y trigo); así como otros ingredientes (azúcar, polvo de huevo o huevo líquido, leche, levadura, aceite vegetal, sal, hidrocoloides y emulgentes) que contribuyen a mejorar la calidad final del producto. En los últimos años se ha enfatizado la investigación en el desarrollo de productos dulces sin gluten (tartas, pasteles y bizcochos), con miras a mejorar su textura, sensación en la boca, aceptabilidad, vida útil y calidad nutricional (Gularte y col., 2011; Park y col., 2012; de la Hera y col., 2012). Otras investigaciones se han direccionado hacia la evaluación del efecto de la adición de gomas e hidrocoloides sobre las propiedades reológicas de las masas y la calidad tecnológica del producto final (Turabi y col., 2008b; Ronda y col., 2011) y en la optimización de procesos alternativos de horneado (Turabi y col., 2008a). Estudios recientes han evaluado la incorporación de proteínas como aislados proteico de soja (Ronda y col., 2011), harinas de soja y otras legumbres (Gularte y col., 2011), huevo y remplazadores del huevo (Geera y col.,

2011) en las formulación de este tipo de productos de repostería, con el objetivo de mejorar su calidad nutricional. Los resultados reportados en todos estos trabajos dejan ver que en la mayoría de los casos se han obtenido productos finales de calidad aceptable, sin embargo aún prevalecen algunos defectos tecnológicos y de calidad sensorial (sabor, color, textura).

Las magdalenas son un tipo de producto horneado que se consumen popularmente en el desayuno o merienda. Son productos de bollería dulces, y con alto nivel calórico, muy apreciados por los consumidores debido a su sabor y textura suave. La receta tradicional española de magdalenas se basa principalmente en harina de trigo, azúcar, aceite vegetal, huevo y leche (Sanz y col., 2009). En muchos productos de repostería, tales como bizcochos, pasteles, “muffins” o magdalenas no es deseable el completo desarrollo del gluten, en lugar de ello son los ingredientes como el huevo, la grasa y el azúcar quienes juegan un papel primordial en el logro de la estructura física del producto final. Esta es una ventaja cuando se trata de diseñar productos de bollería libres de gluten. *A priori*, es esperable que la formulación y elaboración de magdalenas a partir de harinas libres de gluten presente menos problemas, en todo caso las limitaciones tecnológicas pueden derivarse más de las formulaciones que de la necesidad del desarrollo del gluten. En estos productos, otros aspectos, tales como el color y el sabor deseados, son los que constituyen el principal reto (Schober, 2009).

Existe escasa información relacionada con la producción de magdalenas libres de gluten. Schamne y col. (2010) formularon magdalenas utilizando harina de arroz y almidones de maíz y tapioca y derivados de soja para obtener aceptación sensorial y buen valor nutricional del producto. La formulación óptima para la aceptación sensorial fue 20% de harina de arroz, 30% de almidón de tapioca y 50% de almidón de maíz. La adición de concentrado de soja incremento el contenido de proteínas sin alterar la calidad sensorial de las magdalenas. Geera y col. (2011) evaluaron el efecto del reemplazo del huevo en la formulación de magdalenas, utilizaron huevo líquido, huevo en polvo y tres tipos diferentes de reemplazadores de huevo comerciales. Las características físicas (color, textura. volumen y altura) y las propiedades sensoriales de las magdalenas fueron evaluadas. Los resultados indicaron que no fue posible el reemplazo del 100% del huevo en las formulaciones, debido a que no se obtiene las características deseadas para la aceptabilidad de las magdalenas. Sin embargo, se desconoce el papel de las proteínas en este tipo de sistemas libres de gluten.

De la revisión y análisis de la información científica disponible en la literatura consultada se desprende que aunque se ha estudiado el comportamiento de las masas sin gluten y las características de los productos finales, en ninguna de las investigaciones previamente citadas se han obtenido conclusiones, ni se han establecido relaciones que permitan predecir las características del producto final, partiendo de los resultados obtenidos en la optimización de las formulaciones y los procesos.

Únicamente, Pagliarini y col. (2010) describieron la existencia de una buena correlación entre las mediciones sensoriales y las instrumentales utilizadas para identificar los descriptores sensoriales que mejor pudieran caracterizar panes libres de gluten. Las variables más significativas que permitieron discriminar entre las muestras fueron los descriptores sensoriales porosidad, color de la corteza y de la miga, suavidad al tacto y en la boca, olor a queso, olor a maíz y olor a fermentado, dulce, salado, adhesivo y gomoso; mientras que los parámetros instrumentales fueron los asociados al color de la corteza y de la miga y la textura.

Por otra parte existe un considerable número de investigaciones dirigidas al diseño de formulaciones con diversos sustitutos del trigo que evalúan las propiedades tecnológicas y en algunos casos los atributos sensoriales que determinan la calidad del producto final (Ahlborn y col.,2005; Brites y col., 2010; Torbica y col., 2010; Sabanis y Tzia, 2011; Laureati y col., 2012), sin embargo no se han encontrado estudios que separada o simultáneamente evalúan la calidad nutricional de los productos finales provenientes de las formulaciones diseñadas.

En general se puede decir, que es evidente la escasa atención que se ha dado al estudio nutricional de los panes libres de gluten provenientes de los numerosos desarrollos encontrados en la literatura consultada, a pesar de que este aspecto es uno de los que presenta más necesidad de evaluación considerando que en general los productos libres de gluten presentan un desequilibrio en el contenido de nutrientes (Thompson y col, 2005) y en consecuencia considerables deficiencias nutricionales (Catassi y Fasano, 2008; Thompson y col., 2005).

Por todo lo antes expuesto, en el presente estudio se evaluaron nutricionalmente tanto muestras de panes comerciales como aquellas desarrolladas a nivel de laboratorio. Adicionalmente se puso especial énfasis primeramente en establecer relaciones entre los parámetros instrumentales de calidad y las características sensoriales de muestras comerciales de panes libres de gluten. Y en segundo lugar en establecer posibles indicadores de calidad a través de la relación entre las características reológicas de la masa y las propiedades tecnológicas y sensoriales del producto tipo pan obtenido a partir de formulaciones complejas basadas en harina de arroz y diferentes tipos de proteínas, las cuales fueron diseñadas para tal fin. Finalmente se diseñaron formulaciones dulces para obtener productos horneados no fermentados del tipo magdalenas, a partir de mezclas complejas basadas en harina de arroz y diferentes tipos de proteínas, con miras a ampliar el entendimiento de la función de las proteínas sobre la reología de las masas-batidas y las propiedades de calidad del producto obtenido, considerando el doble papel, tanto nutricional como funcional que tienen las proteínas en estos sistemas.

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OBJETIVOS

Objetivo General

Formular, desarrollar y caracterizar productos horneados libres de gluten elaborados a base de harina de arroz y enriquecidos con proteínas.

Objetivos Específicos

• Identificar el patrón de calidad instrumental, nutricional y sensorial de los productos libres de gluten comerciales tipo pan. Este objetivo incluye evaluar la calidad nutricional (composición química, contenido de fibra dietética y digestibilidad del almidón) de muestras comerciales de panes libres de gluten consumidos en España; y establecer posibles relaciones entre parámetros instrumentales de calidad y características sensoriales de panes libres de gluten.

• Definir posibles predictores de calidad de los productos libres de gluten tipo pan a través de la relación entre las características reológicas de la masa y las propiedades tecnológicas y sensoriales del producto horneado obtenido. Asimismo, diseñar formulaciones complejas basadas en harina de arroz y diferentes tipos de proteínas para obtener productos horneados libres de gluten tipo pan, con miras a evaluar el efecto de la incorporando de las proteínas sobre las propiedades reológicas de las masas formuladas.

• Investigar la funcionalidad de las proteínas en productos dulces libres de gluten, especialmente sobre la reología de las masas-batidas y las propiedades de calidad de los productos tipo magdalenas obtenidos. Este objetivo incluyó el diseño de formulaciones complejas basadas en harina de arroz y diferentes tipos de proteínas para obtener productos libres de gluten dulces no fermentados tipo magdalenas.

CAPÍTULO 1

CHEMICAL COMPOSITION AND STARCH DIGESTIBILITY OF DIFFERENT GLUTEN-FREE BREADS

María E. Matos and Cristina M. Rosell

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Abstract

The increasing demand for gluten free products has favoured the design of numerous gluten free bakery products which intended to mimic the quality characteristics of wheat bakery products. The objective of this study was to evaluate the nutritional pattern of gluten free breads representative of the Spanish market for this type of products. The protein, fat and mineral content of the gluten free breads showed great variation, ranging from 0.91g/100g to 15.05g/100g, 2.00g/100g-26.10g/100g and 1.10g/100g to 5.43g/100g, respectively. Gluten free breads had very low contribution to the recommended daily protein intake, with a high contribution to the carbohydrate dietary reference intake. Dietary fiber content also showed great variation varying from 1.30g/100g to 7.20g/100g. *In vitro* enzymatic hydrolysis of starch showed that the most predominant fraction was the rapidly digestible starch that varied from 75.6 g/100g to 92.5g/100g. Overall, gluten free breads show great variation in the nutrient composition, being starchy based foods low in proteins and high in fat content, with high glycaemic index.

Key words Gluten free bread, Nutrient composition, Fibers, Starch digestibility.

1. Introduction

Bread has been regarded for centuries as one of the most popular and appealing food product both because of its relative high nutritional value and its unique sensory characteristics (texture, taste, and flavor). However, an increasing number of individuals are suffering from celiac disease (CD), the life-long intolerance to the gluten fraction of wheat, rye and barley. In particular, celiac patients are intolerant to some cereal prolamins containing specific toxic oligopeptide sequences. The gliadin fraction of wheat, secalins of rye, hordeins of barley, and possibly avenins of oats are involved in the CD mechanism.

In CD patients, ingestion of gluten leads to inflammation and mucosal damage of the small intestine. The typical lesion in the small intestinal epithelium is villous atrophy with crypt hyperplasia, leading to malabsorption of most nutrients including iron, folic acid, calcium, and fat-soluble vitamins [1]. This can lead to associated diseases such as osteoporosis, anaemia and type I diabetes and skin disorders [2]. An acceptable treatment is strict adherence to a 100g/100g gluten-free diet for life, which results in clinical and mucosal recovery. Nevertheless, the manufacture of bread without gluten results in major problems for bakers, and currently, many gluten free products available on the market are of low quality.

In recent years there has been increasing interest on gluten-free breads. A large number of flour and starches as well as many ingredients such as gums, enzymes, soybean proteins, and have been used to mimic the viscoelastic properties of gluten and contribute to improved structure mouthfeel, acceptability, and shelf life of gluten free breads [3-6]. In such studies various technological parameters and formulations have been extensively investigated for making good quality gluten free bread. However, the nutritional concept of the gluten free baked goods has been scarcely addressed. Some approaches have considered the use of mixed amaranth flours for making gluten free breads and cookies [7] or even blends of plantain and legume flours [8], obtaining gluten free products with high nutritional value and acceptable quality , and also protein enrichment of gluten free breads has been carried out by incorporating soy protein isolates [9].

Historically, nutrition counseling for celiac disease has focused on the foods to avoid in a gluten free diet but they should be advised on the nutritional quality of gluten- free. There are growing concerns over the nutritional adequacy of the GF dietary pattern because it is often characterized by an excessive consumption of proteins, and fats, and a reduced intake of complex carbohydrates, dietary fibre, vitamins and minerals [1,10] . As a consequence, the long life adherence to gluten free products has been associated to undernourished and also minerals deficiencies that could conduct to anemia, osteopenia or osteoporosis [10].

The aim of this work was to evaluate the nutritional pattern of gluten free breads regarding their chemical composition in order to determine their contribution to the daily intake of nutrients. Special emphasis has been addressed to the fiber content of those breads and also to the *in vitro* starch digestibility due to their always high content in starch.

2. Materials and Methods

2.1. Materials

Gluten-free breads (GFB) from the major brands of these specialties were acquired in the Spanish market. Those breads were representative of the most consumed products in Spain. Eleven kinds of gluten-free breads were selected and purchased in general and specialized supermarkets. Duplicates of each sample from different batch were used for the characterization. Information on the ingredient composition, according to the labeling, is given in Table 1. α-Amylase from porcine pancreas (Pancreatin, Cat. No. P-1625, activity 3_USP/g) was purchased from Sigma Chemical Company (St. Louis, MO, USA). Amyloglucosidase (EC 3.2.1.3., 3300 U/mL) and glucose oxidase–peroxidase assay kit GOPOD (Cat. No. K-GLUC) were purchased from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland).

2.2. Analytical methods

The chemical composition of GFB samples was determined according to ICC corresponding standard methods [11]. Total carbohydrates were determinate by difference subtracting 100 g minus the sum of protein, ash and fat expressed in grams/100 grams [12]. For the estimation of dietary fiber, samples were finally powdered to pass through a sieve of 250 µm. Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) contents were determined following the AACC method [13] AACC International (2000) . Determinations were done in triplicate for obtaining mean values.

2.3. *In vitro* starch digestibility and estimated glycaemic index

Gluten free breads were frozen, freeze-dried and ground in a blender. Starch digestibility of gluten free bread was determined in the powders using AACC methods [13], with the modification reported by Gularte and Rosell [14]. According to the hydrolysis rate of starch, three different fractions were quantified as suggested Englyst et al. [15]. Rapidly digestible starch (RDS) was referred to the percentage of total starch that was hydrolyzed within 30 min of incubation, slowly digestible starch (SDS) was the percentage of total starch hydrolyzed within 30 and 120 min, and resistant starch (RS) was the starch remaining unhydrolyzed after 16 h of incubation. The percentage of total starch hydrolyzed at 90 minutes (H90) was also calculated.

The *in vitro* digestion kinetics was calculated in accordance with the procedure established by Goñi et al. [16]. A non-linear model following the equation [$C = C\infty(1 - e^{-kt})$] was applied to describe the kinetics of enzymatic hydrolysis, where C was the concentration at t time, $C\infty$ was the equilibrium concentration or maximum hydrolysis extent, k was the kinetic constant and t was the time chosen. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (0–180 min) of the sample by the area of a standard material (white bread) over the same period of time. The expected glycaemic index (eGI) was calculated using the equation described by Granfeldt et al. [17]: $eGI = 8.198 + 0.862HI$.

2.4. Statistical analysis

The results were expressed as mean values ± standard deviation. Datawere analyzed using one-way analysis of variance (ANOVA) to determine whether there was significant difference between gluten-free breads types by using Statgraphics Plus V 7.1 program (Statistical Graphics Corporation, UK). Fisher's least significant differences (LSD) test was used to differentiate means with 95% confidence.

3. Results and discussion

3.1. Chemical Composition

Commercial gluten free breads, according to suppliers' information (Table 1), were based on corn starch, potato starch, or rice flour, either enriched with milk solids, soy protein, eggs or lupine proteins. All of them contained corn starch as main ingredient, with the exception of GFB4 that was based on potato starch and GFB6 also contained rice flour. Other differences among breads were encountered in the protein

source. Eggs were the most common source of proteins, but also caseinate (GFB4), soy (GFB4) or lupine proteins (GFB6) were present.

Some types of bread (GFB8, GFB9, GFB10 and GFB11) did not contain any source of proteins among the ingredients. Vegetable oil or margarine was present in the formulations, with exception of GFB8 and GFB9 that did not contain any fat source. Yeast and raising agents were used in combination as leaving agents, with the exception of GFB2, GFB3 and GFB4 that only contained yeast. In addition, salt, emulsifiers,

Table 1. Ingredients in gluten free breads (GFBs) according to the producer labelling.

Product code	Ingredients
GFB1	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, yeast, thickener, emulsifier, salt, preservative, raising agents, antioxidants. May contain traces of soy.
GFB2	Corn starch, water, vegetal margarine, emulsifiers, salt, acidifier, preservative, antioxidants, aromas and colouring (betacarotene), egg, sugar, yeast, dextrose, humidifier, stabilizers, salt.
GFB3	Corn starch, water, vegetal margarine, emulsifiers, salt, acidifier, preservative, antioxidants, aromas and colorant, egg, sugar, yeast, dextrose, humidifier, stabilizers, salt.
GFB4	Potato starch, water, corn starch, caseinate (milk protein), sugar, vegetal oil, corn flour, yeast, soy protein, stabilizers, salt, preservative.
GFB5	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, stabilizers, yeast, emulsifiers, salt, raising agents, anise, cinnamon, and antioxidant.
GFB6	Corn starch, water, rice flour, vegetal oil, sugar, stabilizer, lupine protein, yeast, salt, vegetal fibre, aroma, emulsifiers.
GFB7	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, yeast, thickener, emulsifier, salt, raising agents, antioxidants. May contain traces of soy.
GFB8	Corn starch, water, sugar, yeast, thickeners, salt, raising agent, preservative.
GFB9	Corn starch, water, sugar, thickeners, emulsifier, salt, yeast, preservative, raising agents, antioxidants. May contain traces of egg.
GFB10	Corn starch, vegetal margarine, salt, sugar, emulsifier, raising agents, antioxidant, thickener, preservative, and yeast.
GFB11	Corn starch, vegetal margarine, salt, sugar, emulsifier, raising agents, antioxidant, thickener, preservative, and yeast.

preservatives and a variety of other food grade additives were present in the formulations. There was important significant differences ($p<0.05$)
among the proximate composition of all the GFB samples (Table 2). The protein content of GBF, which ranged from 0.91g/100g to 15.05g/100g, was found to be the highest in GFB4 while GFB9 closely followed by GFB8 showed the lowest values. This increase in the protein content must be associated to the presence of milk and soy proteins in the formulation, since those ingredients are used as protein sources in gluten free breads [9, 18]. GFB8 and GFB9 presented the lowest values of fat content (2.00g/100g), which agrees with the absence of fat ingredient in the formulation. Conversely, GFB10 showed the highest fat value (26.10g/100g), followed by GFB11, GFB2 and GFB3 due to the contribution of the vegetal oil or margarine in these gluten free bread formulations. Large variations were observed in ash contents that ranged from 1.10g/100g to 5.43g/100g. GFB9 had the highest ash content, mainly derived from the level of salt. The total carbohydrate content varied from 68.42g/100g to 92.96g/100g. The different proximal composition of GFB commercial samples studies could be affected by many factors such as the wide range of complex ingredients added and their combinations, besides the additives used to improve the structure, mouthfeel, acceptability and shelf-life of these products [4, 9].

Recently, Yazynima et al. [19] reported the nutritional composition of two kinds of gluten free crispy breads, which contained 3.5-6.0g/100g of proteins, 3.0-6.5g/100g of fats and 80-71g/100g of carbohydrates. The present study shows that marketed gluten free breads are carbohydrate

Table 2. Chemical composition, expressed as gram/100 gram on dry matter, of eleven types of commercial gluten free breads (GFBs).

Product	Protein (g/100g d.m.)	Fat (g/100g d.m.)	Ash (g/100g dm)	Total Carbohydrate* (g/100g dm)
GFB1	3.16 ± 0.09 e	8.51 ± 0.00 d	2.12 ± 0.03 c	86.21 ± 0.07
GFB2	6.94 ± 0.07 i	16.91 ± 0.20 g	1.10 ± 0.07 a	75.05 ± 0.22
GFB3	7.31 ± 0.15 j	16.56 ± 0.07 g	1.66 ± 0.15 b	74.47 ± 0.22
GFB4	15.05 ± 0.09 k	7.33 ± 0.08 c	1.85 ± 0.06 bc	75.76 ± 0.06
GFB5	5.13 ± 0.03 h	10.64 ± 0.06 e	2.01 ± 0.15 c	82.22 ± 0.19
GFB6	4.92 ± 0.07 g	4.86 ± 0.03 b	2.03 ± 0.02 b	88.18 ± 0.12
GFB7	3.96 ± 0.00 f	8.28 ± 0.05 c	4.53 ± 0.00 e	83.22 ± 0.03
GFB8	1.01 ± 0.02 b	2.00 ± 0.10 a	4.03 ± 0.01 e	92.96 ± 0.11
GFB9	0.91 ± 0.02 a	2.03 ± 0.37 a	5.43 ± 0.33 f	91.63 ± 0.04
GFB10	1.91 ± 0.00 c	26.10 ± 0.05 h	3.57 ± 0.04 d	68.42 ± 0.14
GFB11	2.80 ± 0.02 d	18.32 ± 0.00 f	3.98 ± 0.02 d	74.91 ± 0.03

(*)Total Carbohydrate (d.m) by difference: 100 – (weight in grams [protein + fat + ash] in 100 g of food) (FAO, 2003).Values are means ± standard deviation (n=3). Different letters within a column mean significant differences ($p<0.05$).

based products. They have great variation in their protein, fat and mineral content, in contrast to the very narrow variation in the proximate composition observed in wheat based bread products [20].

3.2. Contribution to dietary reference intakes (DRIs)

Table 3 shows the contribution of macronutrients, protein and carbohydrates intakes (%), to the relevant DRIs consuming an average portion (200g) of gluten free breads. Considering the Dietary Reference Intakes (DRIs) (NRC, 2001) [21] of an adult male and female, an average daily portion of bread (200g) would meet 2.2-39.2% and 2.7- 47.7% of DRIs for proteins, respectively (Table 3). GFB4 showed the highest value of DRIs for proteins on both male (39.2% and female (47.7%). Only that sample gives a similar protein contribution to that reported for white wheat bread (35.7% and 43.5% of DRI for male and female when consuming a 200g portion, respectively) [22]. Very low contribution to the recommended daily protein intake could be obtained with the consumption of the other evaluated breads. Regarding the intakes for carbohydrates, the contribution to DRIs ranged from 53.7% to 109.2%, obtaining the highest value with GFB8. Considering that white wheat bread provide an average of 43% of carbohydrate [20] and thus the contribution of a 200 g portion to the carbohydrate DRI will be around 66%, studied gluten free breads are richer in carbohydrates, with the exception of GFB10 and GFB11. Therefore, 200-gram portion of gluten free breads has higher contribution to the carbohydrate dietary reference intake than their wheat containing counterparts.

Table 3. Contribution of macronutrient intakes (%) to the relevant DRIs* consuming an average portion of 200g of gluten free breads (GFB)

Macronutrient	Gender	DRIs(*) (g/day)	Contribution to DRIs (%) of GFB										
			1	2	3	4	5	6	7	8	9	10	11
Proteins	Male	56	7.9	17.0	18.4	39.2	13.5	10.3	9.4	2.9	2.2	4.3	5.8
	Female	46	9.7	20.6	22.4	47.7	16.4	12.5	11.4	3.5	2.7	5.3	7.1
Carbohydrates	Adults	130	78.4	71.2	77.9	74.6	85.4	71.0	70.6	109.2	86.1	54.6	53.7

(*) Sourse: NRC (National Research Council) Dietary Reference Intakes (DRIs) for protein and carbohydrate (2002/2005). This report may be accessed via http://www.nap.edu

3.3. Soluble, insoluble and total dietary fibre

TDF ranged from 3.60g/100g to 7.20g/100g, except for GFB3 (1.30g/100g) and GFB8 (2.00g/100g) samples, showing that all gluten- free breads contained good amount of dietary fiber (>3g/100g) (Figure 1). High values of TDF and SDF were obtained in GFB7, GFB10 and GFB11 samples. In general, gluten free bread samples showed higher amount of soluble dietary fiber than insoluble dietary fraction. The clear exception to the last statement was GFB4 and GFB9, in which 83% and 71% of the total dietary fiber were insoluble, respectively. Values obtained for these gluten free breads slightly differ from those reported by Korus et al. [23], when studied the addition of resistant starch to gluten free formulations as fiber source. Those authors found values of IDF, SDF and TDF in gluten free breads that ranged 2.77- 4.99g/100g, 1.23-1.45g/100g and 3.61-6.30g/100g, respectively. Formulations of GFB usually contain gums or hydrocolloids used as thickeners or stabilizers. Hydrocolloids like xanthan gum, guar gum, carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC), pectin, or varied combinations of those hydrocolloids contained in the formulations might improve the content of TDF, contributing to increase the level of soluble dietary fibers.

Thompson [24] reported values of dietary fiber in commercial gluten free bread samples from 1.2 to 5.6 g/100g, whereas in fiber enriched bread those values varied from 6.1 to 9.6 g/100. Only for comparative purposes, it is worthy to note that white bread contains 0.81g/100g, 3.13g/100g and 3.84g/100g of IDF, SDF and TDF, respectively [25].

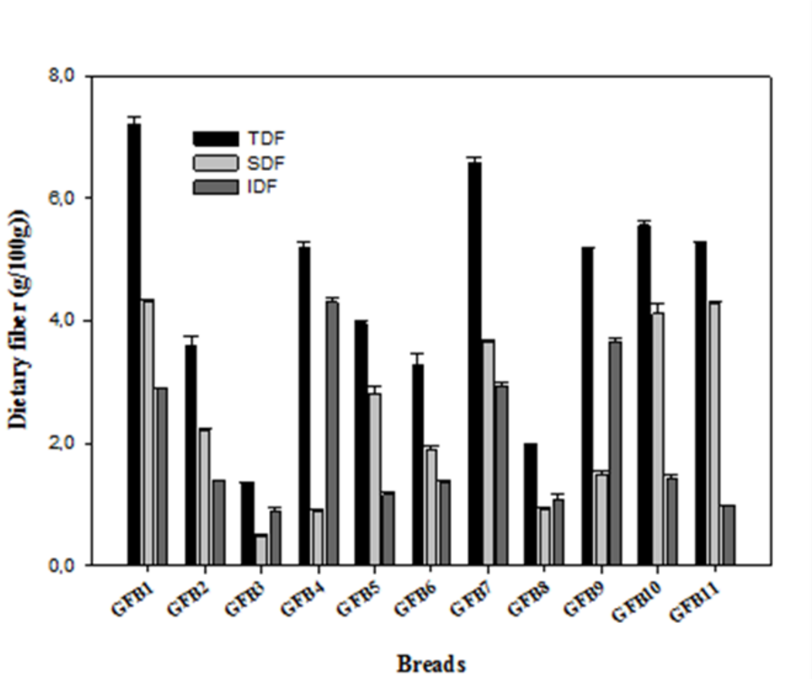

Figure 1. Total, soluble and insoluble dietary fiber (TDF, SDF and IDF) content, expressed as gram/100 grams (as is basis), in different gluten free breads (GFBs).

3.4. Starch digestibility in gluten free breads

The most predominant starch fraction was the RDS that varied from 75.6 g/100g to 92.5g/100g of the total starch (Figure 2). This pattern agrees with the one reported for starchy foods, where starch is highly gelatinised and product structure is very porous, resulting in rapid degradation of starch in small intestine and very rapid rise of blood glucose level (high GI) [26]. SDS and RS of GFB samples ranged between from 2.4g/100g - 21.1g/100g, and 1.0g/100g -2.9g/100g, respectively. GFB9 showed the highest value of SDS content (21.1g/100g), which is more desirable than

RDS. SDS is slowly digested in the small intestine and induces gradual increase of postprandial plasma glucose and insulin levels [27], although Englyst et al. [15] reported that the breakdown of solid starchy foods could predict the postprandial response in vivo but SDS has limited effect on the glycaemic response although it is available as sugar.

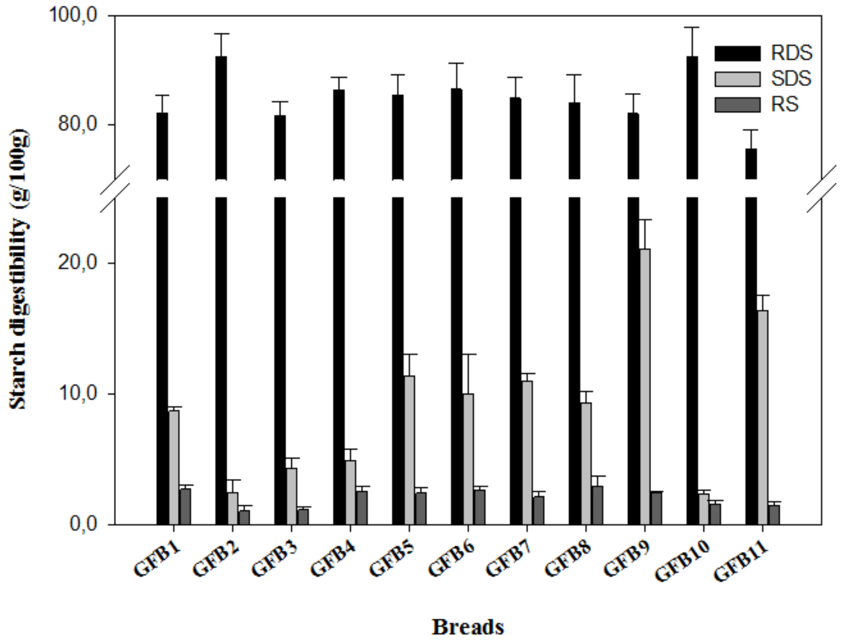

Figure 2. Starch digestibility in different gluten free breads (GFBs) determined by in vitro enzymatic hydrolysis. RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch, expressed as gram/100 grams (as is basis).

3.5. Kinetic of the *in vitro* starch hydrolysis and expected glycaemic index.

Primary and secondary parameters derived from the *in vitro* digestion of the gluten free breads evaluated are listed in Table 4. The maximum hydrolysis, $C_{\infty,}$ or hydrolysis degree when the enzymatic reaction reaches a plateau, of gluten free breads was very high, which was associated with the high levels of rapidly hydrolyzed starch. The kinetic constant (*k*), indicative of the hydrolysis rate in the early stage, showed significant differences among the GFBs. The lowest values were observed in GFB9 and GFB11, which were the samples with higher fractions of slowly digested starch. Gelencsér et al [28] reported values of rate constant comprised between 0.015 and 0.025 (min-1) in pasta products and the addition of resistant starch did not significantly modify that constant. Therefore, higher kinetic constant is obtained for gluten free breads than those determined for pasta, showing the high susceptibility of these starchy products to enzymatic hydrolysis. The hydrolysis index (HI) of GFBs ranged from 87 to 100 and estimated glycaemic index (eGI) values were between 83.3 and 96.1. All samples showed very high *in vitro* starch digestibility index, being practically hydrolyzed between 60 to 90 min of assay, as indicated the H90.

Differences among breads should be attributed to variations in composition (Table 1 and 2). Bernal et al. [29] also observed slightly higher digested starch in gluten free infant cereals. That result was due to the higher starch digestibility of rice and corn (103.98g/100g for rice and 107.05g/100g for corn) compared to white bread (100g/100g) [28]. Therefore, although GFBs are mainly starchy foodstuff, the very complex formulation of those breads might be responsible of the reduction observed in those values. In fact, the

Table 4. Kinetic parameters of the in vitro starch hydrolysis and estimated glycaemic index

Samples	C∞ (g/100g)		*k* (min^{-1})		AUC 180		H90		HI		eGI	
GFB1	90.7	b	0.0782	c	22345	b	91	b	91	b	87	b
GFB2	94.9	c	0.1218	e	23764	d	95	cd	95	d	90	d
GFB3	86.9	a	0.1458	f	21664	a	86	a	87	a	83	a
GFB4	91.2	b	0.0973	d	22587	b	91	b	91	b	87	b
GFB5	97.8	d	0.0713	b	23740	d	97	e	97	e	91	e
GFB6	96.4	cd	0.0756	c	23653	d	96	e	96	e	91	e
GFB7	95.8	c	0.0723	b	23608	d	96	d	96	d	91	e
GFB8	93.2	b	0.0768	c	23100	c	93	d	93	c	89	c
GFB9	100.1	e	0.0527	a	24732	e	100	f	102	f	96	f
GFB10	94.8	c	0.1232	e	23797	d	95	cd	94	c	89	cd
GFB11	92.0	b	0.0574	a	22127	b	91	b	92	b	87	b

a Mean of four replicates. Values followed by different letters in each column and each starch indicate significant differences ($p \leq 0.05$).
b C∞, equilibrium concentration; k, kinetic constant; HI, hydrolysis index; AUC 180, area under curve; eGI, estimated glycaemic index.

glycaemic response to bread varies widely according to the type of bread studied [30]. Low to moderate GI (<70) are considered favorable to health. The glycaemic index could vary from 27 (barley bread with 75g/100g substitution) to 95 (extremely porous French baguette). This extreme variability reflects very different rates of starch digestion. The starch from a French baguette is rapidly digested, leading to glycaemic response close to that of glucose (GI=100), whereas starch from bread containing intact cereal grains is digested more slowly [30].The results obtained in the present study showed that all samples could be considered as food with rapidly digested starch and high glycaemic index. The number and variety of ingredients of gluten free bread can be considered important factors that will determine the starch digestibility.

4. Conclusions

The nutritional evaluation of different commercial gluten free breads revealed that they are mainly starchy foods with great divergences in fat and protein composition, due to the occasional protein enrichment. In consequence, these products have very low contribution to the recommended daily protein intake, but higher contribution to the carbohydrate dietary reference intake than their gluten containing counterpart. The majority of gluten free breads evaluated contained good amount of dietary fiber (>3g/100g), and in most cases the amount of soluble dietary fiber was higher than the insoluble dietary fraction. The presence of hydrocolloids needed in the formulation of these products could be partially responsible of that pattern. The *in vitro* hydrolysis of the starch of the gluten free breads showed that RDS was the major starch fraction distantly followed by SDS and RS, indicating the high starch digestibility. The estimated glycaemic index of the gluten free breads varied between 83.3 and 96.1, thus all samples could be considered as food with high glycaemic index. Overall, gluten free breads shows great variation in the nutrient composition, being starchy based foods low in proteins and high in fat content.

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CAPÍTULO 2

RELATIONSHIP BETWEEN INSTRUMENTAL PARAMETERS AND SENSORY CHARACTERISTICS IN GLUTEN-FREE BREADS

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Abstract

Numerous bread-like gluten free products have been lately developed due to the rising demand on wheat free foods. A range of parameters has been used to describe these products, but there is no general agreement about the most suitable assessment to characterize them. The objective of this research was to characterize diverse gluten free like breads (GFB) in order to discriminate them and to establish possible correlations among descriptive parameters of GFB features determined by instrumental methods and sensory analysis. Statistical analysis showed that all physical, physicochemical characteristics (specific volume, moisture content, water activity, L^*, a^*, b^*, hue and chroma), hydration properties (swelling, water holding capacity and water binding capacity), texture profile analysis (TPA) parameters (hardness, springiness, chewiness, cohesiveness and resilience) and structural analysis of the crumbs (number of cells and total area) significantly ($p<0.05$) discriminated between the GFB types tested. Sensory analysis revealed great divergences in crumb appearance, odour, springiness, crumbliness and colour of samples, but not significant differences ($p<0.05$) in flavour, aftertaste and hardness of them. Certain significant correlations were established within the parameters determined by instrumental methods. Hydration properties of the crumb showed to be positively correlated with cohesiveness and resilience. Significant correlations, but scientifically meaningless, were observed among the instrumental and sensory parameters, because correlation coefficients were rather low, which represent very weak or low linear correlations ($r\leq0.35$). The principal component analysis showed that sensory parameters described in this study and also hydration properties besides texture parameters would be suitable for characterizing bread like gluten free products.

Key words: gluten-free, bread, quality, crumb, sensory characteristics.

1. Introduction

Celiac disease (CD), also known as gluten-sensitive enteropathy, is a chronic disorder of the small intestine caused by exposure to gluten in the genetically predisposed individuals [1,2]. It is characterized by a strong immune response to certain amino acid sequences found in the prolamin fractions of wheat, barley, rye, and certain varieties of oats, resulting in inflammation and damage of the small-intestine mucosa and leading to malabsorption of nutrients [1,3]. Nowadays, the general prevalence of CD was estimated to be 1 in 300, although population-based screening studies carried out in 2008 suggest that the prevalence may be 1 in 100 [4]. Persons with CD are unable to consume some of the most common products in the market, including breads, baked goods, and other food products made with wheat flour. Until now, the only effective treatment for CD is strict adherence to gluten-free (GF) diet throughout the patient's lifetime [4].

The apparent or real increase in celiac disease or other allergic reactions and intolerances to gluten consumption has prompted the rising demand for gluten-free products. A range of bread-like gluten-free products has been designed trying to resemble wheat bread. The gluten-free bread recipes contain mainly rice or maize flours combined with potato, maize or wheat starches [5-7]. In recent years there has been extensive research for the development of gluten-free bread, involving diverse approaches, like the use of different starches (maize, potato, cassava or rice), dairy products, gums and hydrocolloids, emulsifiers, other non-gluten proteins, prebiotics or combinations thereof, as alternatives to gluten, to improve the structure, mouthfeel, acceptability and shelf-life of gluten-free bakery products [5-6, 8-16]. The development of such bread is frequently difficult having in mind that gluten is the main structure-forming protein in wheat flour, responsible for the elastic and extensible properties to produce good quality bread [17].

In those researches, different features of the gluten free breads have been evaluated to assess their quality. Despite the different characteristics of the gluten free bread compared to its wheat counterparts, the same evaluation methods have been usually applied. Instrumentals analysis, including loaf weight and volume, specific volume, colour parameters, and textural parameters have been frequently used to characterize gluten-free breads [12, 14, 16, 18-22]. Sensory analysis has been also considered in some of the studies when developing gluten-free breads [7, 10, 13-15, 20, 23, 24]. Other researchers have also characterized the crumb microstructure by using image analysis [19, 23] or scanning electron microscopy [12].

Therefore, instrumental measurements and sensory analysis have been applied to characterize gluten free breads. However, no correlation between instrumental parameters and sensory analysis has been previously established in this type of products, which would be very helpful for defining the best quality attributes of gluten-free breads. Additionally, principal components analysis (PCA) could be used to identify the best parameters or descriptors of the quality of gluten-free breads that allow the discrimination among bread features.

The aim of this research was to characterize a range of gluten free breads in order to establish possible correlations among descriptive parameters of gluten free bread like features determined by instrumental methods and sensory analysis. For that purpose, eleven gluten-free breads like products, which represent a large range of commercial gluten-free breads, were evaluated regarding physicochemical analysis, hydration properties, crumb microstructure, crumb texture and sensory analysis.

2. Materials and methods

2.1. Materials

Eleven specialties of gluten-free breads (GFB) with either loaf or sliced presentations were selected and purchased in general and specialized supermarkets. Gluten-free breads are marketed in polyethylene pouches and packaged under modified atmosphere for keeping their characteristics during at least four months. All breads were purchased within the first month after its production. Breads were kept at 20ºC till analysis. Information on the ingredients of each bread type, according to the labeling is given in Table 1. Due to commercial sensitivity the branded bread (n=11) varieties were labeled as GFB. Abbreviations of the samples are listed in Table 1. Samples from two different batches were used for the characterization.

2.2. Physicochemical analysis

Bread moisture content was determined following the ICC Standard Methods (110/1) [25]. Volume was determined by rapeseed displacement

Table1. Ingredients in gluten free breads (GFBs) according to the producer labeling.

Product code	Ingredients
GFB1	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, yeast, thickener, emulsifier, salt, preservative, raising agents, antioxidants. May contain traces of soy.
GFB2	Corn starch, water, vegetal margarine, emulsifiers, salt, acidifier, preservative, antioxidants, aromas and colouring (betacarotene), egg, sugar, yeast, dextrose, humidifier, stabilizers, salt.
GFB3	Corn starch, water, vegetal margarine, emulsifiers, salt, acidifier, preservative, antioxidants, aromas and colorant, egg, sugar, yeast, dextrose, humidifier, stabilizers, salt.
GFB4	Potato starch, water, corn starch, caseinate (milk protein), sugar, vegetal oil, corn flour, yeast, soy protein, stabilizers, salt, preservative.
GFB5	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, stabilizers, yeast, emulsifiers, salt, raising agents, anise, cinnamon, and antioxidant.
GFB6	Corn starch, water, rice flour, vegetal oil, sugar, stabilizer, lupine protein, yeast, salt, vegetal fibre, aroma, emulsifiers.
GFB7	Corn starch, water, sugar, egg, vegetal margarine, acidifier, preservative, aromas and colorant, yeast, thickener, emulsifier, salt, raising agents, antioxidants. May contain traces of soy.
GFB8	Corn starch, water, sugar, yeast, thickeners, salt, raising agent, preservative.
GFB9	Corn starch, water, sugar, thickeners, emulsifier, salt, yeast, preservative, raising agents, antioxidants. May contain traces of egg.
GFB10	Corn starch, vegetal margarine, salt, sugar, emulsifier, raising agents, antioxidant, thickener, preservative, and yeast.
GFB11	Corn starch, vegetal margarine, salt, sugar, emulsifier, raising agents, antioxidant, thickener, preservative, and yeast.

method and specific volume (cm^3 /g) of the individual loaf was calculated by dividing volume by weight. Water activity (aw) of bread samples was measured using an Aqua Lab Series 3 (Decagon devices Pullman, USA) at 22ºC. The colour of the bread crumbs was measured at three different locations by using a Minolta colorimeter (Chromameter CR-400/410. Konica Minolta. Japan) after standardization with a white calibration plate (L*= 96.9, a*= -0.04, b*=1.84). The colour was recorded using CIE-L*a*b* uniform colour space (CIE-Lab) where L* indicates lightness, a* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis. Data from three slices per bread were averaged. Additionally the cylindrical coordinates: hue or hue angle (hab) and Chroma (C*ab) were defined by the following equations:

$C^*ab = \sqrt{((a^*)^2 + (b^*)^2)}$

$hab = \arctan (b^*/a^*)$

Hue angle is the angle for a point calculated from a* and b* coordinates in the colour space. Chroma is the quantitative component of the colour [26], which reflected the purity of colour in the CIELAB space.

2.3. Hydration properties

Swelling or the volume occupied by a known weight of sample was evaluated by mixing 5g (±0.1 mg) of dried gluten-free bread with 100 mL distilled water and allowing it to hydrate during 16h. Water holding capacity (WHC) defined as the amount of water retained by the sample without being subjected to any stress was determined by suspending 5g (±0.1 mg) of commercial gluten-free bread sample with 100mL distilled water and allowing them to hydrate overnight. After removing the excess of water, the hydrated solid was weighed and expressed per one gram of solid. Water binding capacity (WBC) or the amount of water retained by the bread after being subjected to centrifugation was measured as described the AACC International method (56-30.01) [27].

2.4. Crumb cell analysis

Images of the gluten-free bread slice (10-mm thick) were captured using a flatbed scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP scanjet 4400C, Hewlett–Packard, USA). The default settings for brightness (midtones 2.2) and contrast (highlights 240, midtones 2.2, and shadows 5) of the scanner software were used for acquiring the images. The images were scanned full scale at 1200 pixels per inch and analysed in levels of grey (8 bits, readout 0–255) and captured in jpeg format for each measurement. A 30x30-mm square field of view (FOV) was evaluated for each image. This FOV captured the majority of the crumb area of each slice. Images were analysed by Image J software (National Institutes of Health, Bethesda, MD, USA) using the Otsu's algorithm for assessing the threshold according to Gonzales-Barron and Butler [28]. Data derived from the crumb structure analysis included: number of cells or alveoli, average cells area and cell circularity, and were used for comparing purposes among different samples. Circularity was calculated using the following equation:

Circularity = $4 \times \pi \times \text{area} / (\text{perimeter})^2$. A value of 1.0 indicates a perfect circle.

2.5. Crumb texture analysis

Crumb texture analysis was measured on uniform slices of 10mm thickness. Three slices from the center of each loaf were taken for evaluation [29]. Texture profile analysis (TPA) was performed using a universal testing machine TA-XT2i (Stable Micro Systems, Surrey, UK) equipped with a 30 Kg load cell and 25 mm aluminium cylindrical probe. The settings used were test speed of 2.0 mm/s with a trigger force of 5 g to compress the middle of the bread crumb to 50% of its original height at a crosshead speed of 1mm/s. Values were the mean of three replicates.

2.6. Sensory evaluation

A descriptive sensory analysis was performed for evaluating the sensory characteristics of commercial gluten-free breads. Bread slices, including crust and crumb, were presented (1cm thick) on plastic dishes coded and served in randomised order. A quantitative descriptive sensory analysis was carried out with twelve trained panellists under normal lightening conditions and at room temperature. The range of time that test panellist had participated in descriptive analysis and scale rating of a wide range of bread products varied from 3 to 20 years. Preliminary training test was performed, in which they were sat in a round table and after evaluating the sample, an open discussion was initiated for defining and describe the best descriptors for characterizing the product. Evaluation included perception at first glance of the bread slice (crust and crumb included) and mastication with the molar teeth up to swallowing. The attributes assessors finally agree were appearance (by observing the product slice), flavour, colour, taste, aftertaste (taste remaining in the mouth after swallowing), texture attributes during chewing and springiness (ability to regain original shape after pressing down the crumb with the middle finger). The descriptors for each attributes were appearance (visually liking or disliking), flavour (scale goes from high when typical of bread or bakery products to low, uncharacteristic of bakery products), colour (scales goes from high yellow/beige to low when brown or grey), taste (scale goes from high when typical taste of bread or bakery products to low, uncharacteristic of bakery products), aftertaste (scale goes from high when agreeable taste to low when distaste after swallowing), texture attributes during chewing (scales goes from hard-soft, crumbly-cohesive). Attribute intensity was scored on a scale varying from 1 (disliked extremely) to 5 (like extremely). Two samples were evaluated during one session. Breads were considered acceptable if their means score for overall acceptance were above 2.5.

2.7. Statistical analysis

The results were expressed as mean values. For each quality parameter, a one way analysis of variance (ANOVA) was applied using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). Fisher's least (LSD) test was used to assess significant differences ($p<0.05$) among samples that might allow discrimination among them. Simple correlations were performed using Statgraphics V.7.1 software. Principal component analysis (PCA) was also performed to determine the number of principal components that significantly ($p< 0.05$) discriminated samples.

3. Results and discussion

3.1. Technological and sensory characteristics of gluten free bread

The characterization of diverse gluten-free breads was carried out to identify the most discriminating parameters. With that purpose, an in-depth analysis of the gluten free breads was carried out (Table 2, 3). The analysis included physical, physicochemical properties, crumb structure analysis, also hydration properties of the crumb and sensory analysis. Mean values from two different batches for each sample are showed in table 2. Analysis of data collated using ANOVA showed that all physicochemical characteristics significantly (p<0.05) discriminated between the breads tested. GFB samples presented specific volume values that ranged from 1.54 to 4.79 mL/g. Those agree with the ones reported by Sabanis, Lebesi and Tzia [13] when they evaluated enrichment of gluten-free baked products with different cereal fibres (2.7 to 3.9 mL/g), or with Marco and Rosell [12] findings (1.57 to 2.71 mL/g). Moisture content values ranged from 21.10 g/100g (GFB8) to 42.03 g/100g (GFB11). The present study included a range of marketed GFB specialties, thus probably differences might be attributed to the different bread formulations. In general, the moisture content values reported for gluten-free breads obtained from different formulations are rather high, for instance rice based bread enriched with proteins showed values of 41.66- 46.13 g/100g [12] and the enrichment of gluten-free breads with fibres even enhances those values (49-53 g/100g) [13]. Water activity values of crumb were also high (Table 2). Those values agree with the findings of Lazaridou, Duta, Papageorgiou, Belc and Biliaderis

Table 2. Different quality characteristics of different gluten-free breads.

Sample codes	Specific volume ml/g		Moisture g/100 g		a_w		Swelling ml/g		WHC g water/g solid		WBC g water/g solid		*L**		*a**		*b**		Chroma		Hue angle º	
GFB1	3.37	cde	29.63	d	0.91	b	1.49	a	2.55	ab	2.31	a	64.71	a	-2.01	cd	11.85	a	12.02	a	-80.36	def
GFB2	3.47	de	31.63	f	0.95	e	1.58	bc	2.63	ab	2.47	ab	72.93	f	0.50	d	21.78	g	21.78	f	88.67	h
GFB3	1.54	a	29.50	d	0.94	d	1.49	a	2.41	a	2.39	a	71.86	ef	0.97	d	19.86	f	19.88	e	87.20	g
GFB4	4.79	f	27.17	c	0.94	d	1.38	a	2.50	ab	2.60	bc	65.77	a	-1.63	abc	10.72	a	10.84	a	-81.37	cd
GFB5	3.88	e	26.27	b	0.89	a	1.99	de	3.23	c	2.90	d	67.95	b	-0.25	bcd	15.97	de	15.97	c	-89.10	a
GFB6	2.89	c	41.66	i	0.97	g	1.59	ab	2.84	b	2.70	c	72.77	f	-2.74	a	17.17	e	17.39	d	-80.93	cde
GFB7	3.14	cd	33.60	g	0.94	d	1.79	bc	2.72	ab	2.41	ab	69.21	bc	-2.44	a	13.97	b	14.18	b	-80.09	ef
GFB8	4.77	f	21.10	a	0.92	c	2.58	e	3.49	c	3.19	e	83.83	h	-2.21	a	11.92	a	12.13	a	-79.44	f
GFB9	2.31	b	31.33	e	0.96	f	3.48	f	3.86	d	3.35	e	80.20	g	-2.28	a	15.86	de	16.02	cd	-81.82	c
GFB10	3.70	e	36.13	h	0.97	g	2.09	d	3.25	c	2.78	cd	71.13	de	-1.99	a	14.09	bc	14.23	b	-81.99	bc
GFB11	3.47	de	42.03	j	0.97	g	1.90	cd	3.24	c	2.72	cd	70.37	cd	-1.90	ab	15.44	cd	15.55	bc	-83.00	b
p-value	0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.000		0.000	

For each parameter values followed by the same letter are not significantly different at $p \leq 0.05$.
WHC: water holding capacity (ml/g); WBC: water binding capacity (g water/g solid).

Table 3. Analysis of crumb microstructure and texture.

Sample codes	Number of alveoli/cm^2	Total area alveoli mm2/cm2	Hardness N	Springiness	Chewiness N	Cohesiveness	Resilience
GFB1	4 ab	9.07 a	20.50 e	0.95 de	5.77 d	0.29 b	0.11 abc
GFB2	6 ab	7.53 a	80.20 g	0.95 de	32.90 g	0.43 c	0.17 d
GFB3	6 ab	36.70 b	14.53 c	0.85 bc	3.53 abc	0.29 b	0.09 ab
GFB4	6 ab	24.26 ab	14.60 cd	0.90 cd	4.83 cd	0.37 c	0.13 bcd
GFB5	2 a	2.50 a	11.27 abc	0.76 a	2.33 ab	0.24 ab	0.08 ab
GFB6	16 c	130.03 c	11.47 abc	0.88 c	4.04 bcd	0.37 c	0.15 cd
GFB7	2 a	8.80 a	10.83 ab	0.79 ab	1.69 a	0.20 a	0.06 a
GFB8	5 ab	18.70 ab	18.23 de	1.00 f	14.94 e	0.82 d	0.39 e
GFB9	4 ab	23.50 ab	32.77 f	0.96 de	24.07 f	0.77 d	0.40 e
GFB10	7 b	21.33 ab	12.57 bc	0.95 de	3.74 abcd	0.38 c	0.15 cd
GFB11	6 ab	3.17 a	8.47 a	0.87 c	3.60 abc	0.44 c	0.18 d
p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

For each parameter values followed by the same are not significantly different at $p \leq 0.05$.

[10], that reported water activity values of GFB crumb in the range of 0.97-0.99. Likely, the high water activity as well as the moisture retention might be ascribed to the high water holding capacity of the incorporated hydrocolloids [30] that are usually added to GFB formulations as thickeners for improving volume (see Table 1). It has been reported 0.95 as typical aw value for breads [31]. Therefore, GFB samples tested, according to the above results, covered a good range of characteristics previously reported for this type of breads.

The colour of the crumb has been also an important parameter for characterising GFB. Lower L^* value indicates darker crumb, a^* positive value is associated with crumb redness, whereas b^* positive value indicates yellow colour. To obtain a good characterisation of the colour, it is necessary to bear in mind the psychophysical parameters, which correspond with the cylindrical coordinates: hue (h_{ab}) and chroma (C^*_{ab}). Great variability was observed in lightness. GFB8 and GFB9 showed the highest values (83.83 and 80.20, respectively), indicating more reflectance of light when compared with the rest of the breads. Additionally, darker crumb was observed for GFB1, GFB4, GFB5 and GFB7. The darkening of the crumb colour is desirable as gluten-free breads usually tend to have lighter colour than wheat breads [23], and darker bread are usually associated with whole grains and wholesomeness [15]. Regarding a^*, only GFB2 and GFB3 showed low positive value indicating hue on red axis, whereas the other breads presented negative a^* value (hue on green axis). In addition, all samples presented positive b^* value (indicating hue on yellow axis), showing significant differences among them (p<0.05). In relation to hue (h_{ab}) and chroma (C^*_{ab}) colour attributes, great variation was observed (Table 3). The majority of the GFB samples presented negative hue values that reflected yellow-greenish hue, with the exception of GFB2 and GFB3 samples that presented hue positive values, which reflected yellow-orange hue. Chroma is the quantitative component of the colour associated to the colour purity in the CIELAB space. Both GFB2 and GFB3 showed chroma values higher than the other samples, which revealed its higher purity of colour related to major intensity of the yellow component (Figure 1).

Gluten-free breads have low ability to retain moisture during storage [11], thus hydration properties of the bread crumbs might be interesting properties to characterize this type of products. Hydration parameters are generally used for assessing the water uptake ability of different ingredients like hydrocolloids or fibers. GFB9 exhibited the highest values for swelling, WHC and WBC indicating that it can retain significantly more water than the other breads (Table 2). In addition, GFB4 showed the lowest value for swelling while GFB3 presented lowest values to WHC and WBC. In GFB, dietary fibre (mainly hydrocolloids incorporated as ingredient into gluten-free bread formulations) might be a major determinant of the water retention capacity of these products. Significant differences were found among the samples, which could be useful for discriminating GFB and maybe those properties could be related to sensory attributes. Presumably, water retention capacity of the crumb could affect the perception of textural properties when these samples are eaten.

Parameters from the image analysis of the gluten-free bread crumbs (Figure 1) showed a large variability among crumb bread structures (Table 3). GFB6 exhibited significantly high cells or alveoli number value and total area value, whereas lower values were seen for GFB5 and GFB7. The unique reported values of this parameter in gluten-free breads ranged from 15 to 20 cells/cm2 [32]. No significant differences were

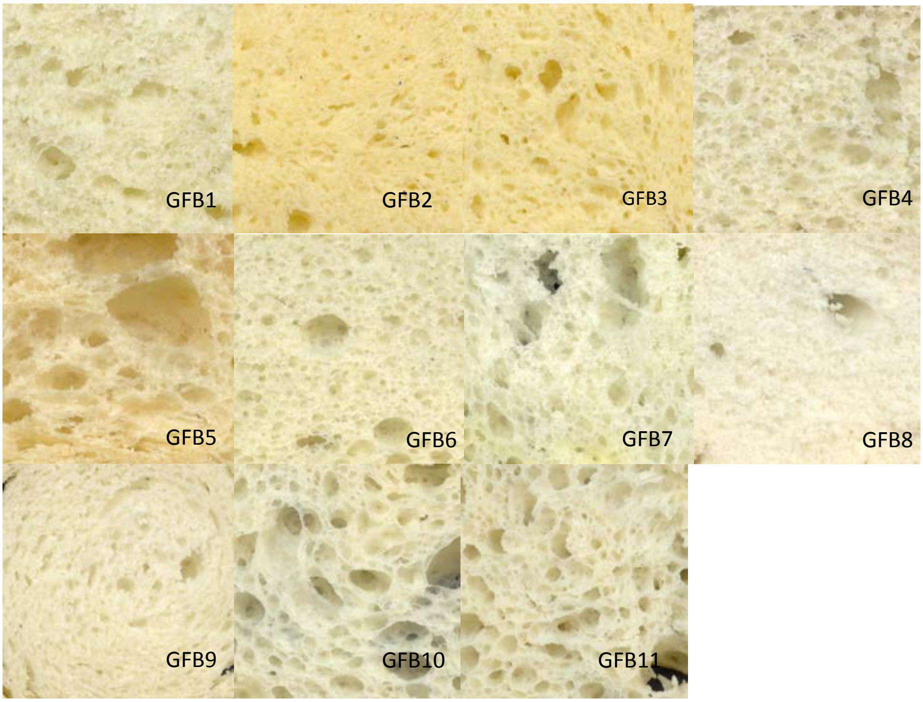

Figure 1. Digital images of commercial gluten-free bread crumb samples (30x30 mm field of view of GFB).

observed for average cell area (mm2). Nevertheless, significant differences were found for circularity values ($p<0.05$). It has been described that up to certain limit, the number of cells/cm^2 increases as HPMC and water increase [24]. Nonetheless, the combination of high levels of both decreases the cell/cm^2, likely due to the coalescence of many gas cells into one large cell. Carboxymethyl cellulose and xanthan gum has been associated with higher cell average size, while breads with carrageenan and alginate had smaller cell sizes [22]. Gluten free crumbs had circularity values ranging from 0.60 to 0.81, indicating less uniform shape (Figure 1). Beside, cell (air) total area of bread crumbs showed significant differences among gluten-free breads.

In addition, significant differences were observed in the crumb texture properties of the different gluten free breads (Table 3). Gluten free bread like products due to their complex formulation, mainly based in carbohydrates [33], present high crumb hardness, which agree with the results of crumb image analysis. The majority of GFBs presented hardness values ranging from 10.33N to 14.60N; however GFB2 and GFB11 had the highest and lowest values, respectively. With respect to springiness, GFB8 showed the highest value, while GFB5 presented the lowest. Springiness is associated to a fresh and elastic product; therefore high quality bread will be related to high springiness values. Marco and Rosell [12] found springiness values that ranged from 0.77 to 0.94 when study the protein enrichment of rice based gluten-free breads. Low springiness value is indicative of brittleness and this reflects the tendency of the bread to crumble when is sliced [24]. Cohesiveness characterises the extent to which a material can be deformed before it ruptures, reflecting the internal cohesion of the material. Bread with high cohesiveness is desirable because it forms a bolus rather than disintegrates during mastication, whereas low cohesiveness indicates increased susceptibility of the bread to fracture or crumble [16]. With the exception of the GFB8 and GFB9, low cohesiveness values (0.20-0.44) were observed, which implies that lower compression energy was required and consequently those breads more easily crumbled. Chewiness varied from 1.69 to 32.90 N, but the majority of breads presented values comprised between 2.33 to 5.77 N and only GFB2 showed higher value. Therefore, the time required masticating a bread piece prior to swallow showed great variation. Low chewing value means easy break of the bread in the mouth like a biscuit. It was also observed that hardness and chewiness showed similar traits for all breads. Resilience values showed that GFB7 had the lowest elasticity, whereas GFB8 and GFB9 presented the highest values. It has been reported that the reduction in resilience or springiness characterizes loss of elasticity [16].

A quantitative descriptive analysis was performed for the sensory evaluation of the breads. Although 50 panellists are recommended for this analysis, in this study 12 long trained judges participate in the sensory evaluation, which agree with method of Heenan et al [34]. According to ANOVA results, the gluten-free breads differed significantly ($p<0.05$) in crumb appearance, odour, springiness and crumbliness, also significant differences ($p<0.1$) were found in colour (Table 4). Conversely, no significant differences were observed in taste,

Table 4. Sensory analysis of different gluten-free bread like products.

Sample codes	Crumb appearance		Taste	Odour		Color	Aftertaste	Springiness		Hardness	Crumblines	
GFB1	2.57	bc	2.71	2.28	bc	3.43	3.14	1.86	a	3.14	2.29	ab
GFB2	2.83	abc	2.33	2.67	c	3.00	2.67	3.33	bc	2.67	3.00	bc
GFB3	2.33	ab	2.66	3.00	c	3.00	3.33	2.50	ab	3.33	2.50	ab
GFB4	2.83	bcd	1.33	2.00	abc	3.50	2.50	4.83	d	4.17	1.33	a
GFB5	3.33	bcd	3.00	2.16	abc	3.83	2.67	2.67	ab	3.67	3.83	c
GFB6	4.00	d	2.66	2.83	c	3.83	3.83	4.17	cd	3.67	2.50	ab
GFB7	3.50	bcd	2.83	3.00	c	3.50	3.33	3.33	bc	3.16	2.33	ab
GFB8	3.16	bcd	2.66	2.66	c	3.17	3.50	4.33	cd	3.33	2.00	ab
GFB9	1.16	a	2.16	1.16	a	1.83	2.67	2.17	ab	2.16	2.83	bc
GFB10	3.50	bcd	1.83	1.50	ab	2.83	1.83	1.33	a	2.17	2.33	ab
GFB11	3.67	cd	2.50	2.66	c	3.67	3.83	4.17	cd	3.83	2.33	ab
p-value	0.01		0.24	0.030		0.078	0.101	0.000		0.130	0.033	

For each parameter values followed by the same are not significantly different at $p \leq 0.05$.

aftertaste and hardness. GFB6 showed the highest appearance score. The less intense odour was perceived in GFB9. GFB4 received the highest score for springiness. In general, GFB6 was scored higher for majority of the sensorial attributes evaluated. Conversely, GFB9 and GFB10 were scored lower for most of the sensory attributes. These results clearly revealed great variability on sensory quality.

3.2. Relationship among technological and sensory parameters of gluten free bread like products

The assessment of technological or instrumental quality is the most preferred analysis for characterizing gluten-free breads because they are not subjected to consumer perceptions, which are greatly dependent on individual backgrounds, locations and so on. Therefore, the establishment of possible relationship between sensory and quality parameters or within the technological parameters would be very useful. With that purpose multivariate data handling was applied by using Pearson correlation analysis.

Significant correlations were observed within the parameters used for characterizing gluten free bread like products, but they were mainly obtained within the instrumental parameters (Table 5). Strong linear relationships were observed within the colour parameters, but also a strong positive linear relationship was obtained between $L*$ and cohesiveness ($p<0.001$) and resilience ($p<0.001$). Presumably, crumb structure has great influence on the texture properties and the luminosity of the crumb. The initial observation about the hardness and chewiness

Table 5. Correlation matrix (correlation coefficients and *p*-value) between characterizing parameters of gluten-free bread like products.

	Specific volume	*L**	*a**	*b**	Chroma	Tono º	Hardness	Springiness	Cohesiveness	Chewiness	Resilience	Moisture content	Swelling	WHC	WBC
Instrumental parameters															
*b**	-0.6049***		0.6375***												
Chroma	-0.6049***		0.6232***	0.9998***											
Tono º	-0.6049***		0.8082***	0.7737***	0.7688***										
Hardness			0.4333***	0.5434***	0.5413***	0.6235***									
Springiness		0.4659***	-0.2515*				0.3569**								
Cohesiveness		0.8650***	-0.2829*					0.6643***							
Chewiness				0.4103***	0.4111***	0.4364***	0.9043***	0.5273***	0.6002***						
Resilience		0.858***	-0.3076*					0.6197***	0.9895***	0.6034***					
Moisture content	-0.3628**		-0.296*	0.2846*	0.2934*				-0.2707*		-0.2579*				
Aw	-0.2781*		-0.2823*	0.2417*	0.2511*			0.2859*				0.7431***			
Total area			-0.3173**									0.4118***			
Swelling		0.5210***	-0.4993***	-0.3849**	-0.3801**	-0.5864***	-0.4517***		0.5613***		0.6195***				
WHC		0.6186***	-03422**			-04446***			0.6604***	0.2442*	0.7020***		0.8146***		
WBC		0.7083***	-0.2905*			-03943***			0.7633***	0.3017*	0.7901***		0.8014***	0.9323***	
Sensory parameters:															
Appearance										-0.3184**					
Odour													-0.3086*	-0.3321**	-0.3098*
Colour		-0.2662*							-0.2860*	-0.2909*				-0.2493*	
Springiness	0.2829*	0.4659***													
Crumbliness				0.3047*	0.3034*										

p≤0.05 *; p≤0.01**. p≤0.001***

trend was confirmed with the high relationship ($r<0.9043$) detected between those parameters. Additionally, cohesiveness was strongly linear related to resilience ($r<0.9895$), showing the importance of the internal cohesion of the crumb on the ability to recover after compressing. In this type of products, water activity showed a significant positive relationship with the moisture content. It must be highlighted the relationships observed among the crumb hydration properties and some other parameters, since those properties have not been previously determined in bread crumbs. Water hydration properties (swelling, WHC and WBC) were significant positively related within them. Moreover, strong positive relationships were observed between the WHC with resilience ($r<0.7020$) and between WBC with cohesiveness ($r<0.7633$) and resilience ($r<0.7901$).

Some relationships between sensorial parameters and instrumental parameters were statistically significant, although the correlation coefficients were rather low, which represent very weak or low linear correlations ($r\leq0.35$). With these types of products no linear relationships were detected between the instrumental and sensory parameters likely due to their complex formulations.

In order to propose a small number of parameters that allow gluten free bread characterization, a principal component analysis (PCA) with the significant quality parameters was carried out. Significant quality parameters analysed by PCA indicated that six principal components significantly ($p< 0.05$) discriminated between breads, which accounted for 91% of the variability in the original data (data not showed). This analysis described 35% and 18% of variation on principal components 1 (PC1) and 2 (PC2), respectively (Figures 3 and 4). Component 1 was defined by hydration properties, instrumental cohesiveness, resilience and springiness, and luminosity (*L**) along the positive axis, which were present in GFB8 and GFB10. Along the negative axis, PC1 was described by sensory parameters, moisture content and area and number of alveoli that were present in the majority of the gluten free breads tested. Conversely, the component 2 was mainly defined by specific volume, colour parameters (*a**, *b**, chroma and hue) and hardness, along the positive and negative axis, respectively. GFB8 and GF10 were positively located along PC1 and PC2 (Figure 4). On the other hand, the breads located along the negative axis of PC1 and PC2 were GFB2 and GFB3. Therefore, PCA allowed discriminating among gluten free breads and it showed that crumb hydration properties, besides texture parameters like cohesiveness, resilience and springiness could be of great importance for characterizing gluten free breads. In addition, most of the gluten free breads tested (GFB1, GFB4, GFB5, GFB6, GFB7, GFB11) were mainly grouped by the sensory parameters. Descriptive sensory attributes have been reported for discriminating among different wheat bread types [34]. In that study, porous appearance and odour attributes were the most important descriptors.

Simultaneously, quality parameters obtained from instrumental analysis have been selected for defining the consumers' acceptability of wheat breads, which have been useful for identifying the main discrepancies of wheat breads produced by different breadmaking processes [35].

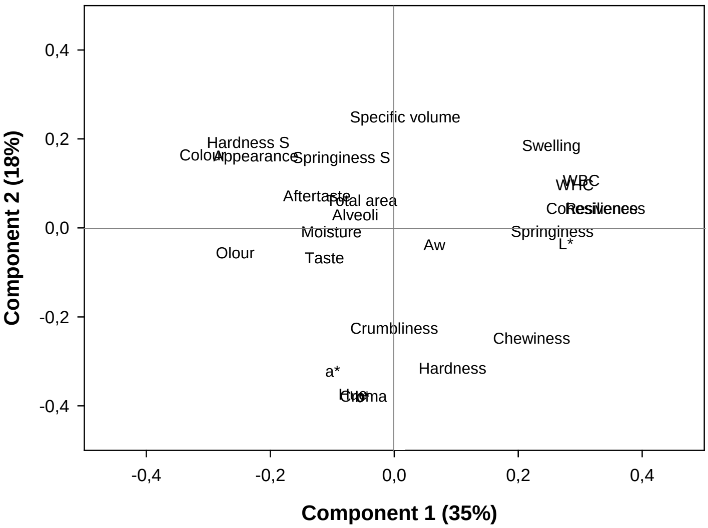

Figure 3. Correlation loadings plot from principal component analysis showing the quality parameters of the eleven gluten free breads evaluated.

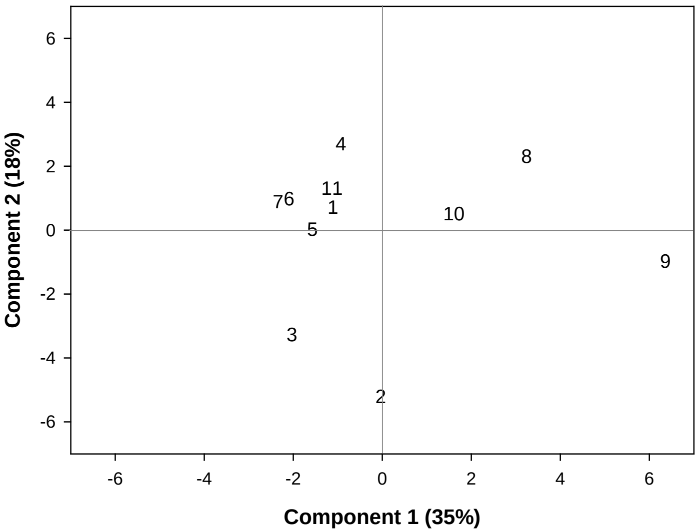

Figure 4. Scores plot from principal component analysis of the eleven gluten free breads evaluated.

Conclusions

The assessment of the physicochemical, hydration properties, crumb texture and microstructure of a range of gluten free breads showed great divergence among their properties and the same observation was perceived in the sensory analysis. Sensory analysis revealed also great divergences in crumb appearance, odour, springiness, crumbliness and colour. Among all the assessed parameters, from the correlation matrix it was observed that colour, texture and hydration parameters were highly correlated within them. In addition, hydration properties were significantly positive correlated with cohesiveness and resilience. Significant but scientifically meaningless correlations were found between sensory and instrumental parameters. According to the principal component analysis, gluten free breads could be classified along the first component on the basis of sensory properties (negative side) and hydration properties, instrumental cohesiveness, resilience and springiness (positive side). Therefore, sensory parameters described in this study and also hydration properties besides texture parameters would be suitable for characterizing bread-like gluten free products.

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CAPÍTULO 3

QUALITY INDICATORS OF RICE BASED GLUTEN FREE BREAD-LIKE PRODUCTS: RELATIONSHIPS BETWEEN DOUGH RHEOLOGY AND QUALITY CHARACTERISTICS

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Abstract

The design of gluten-free bread-like products involves the study of gluten-free dough rheology and the resulting baked product characteristics, but little information has been obtained connecting dough and baked product properties. The aim of this study was to determine quality predictors of gluten-free bread like products at dough level by defining possible correlations between dough rheological properties and both instrumental parameters and sensory characteristics of the those products. Diverse rice based gluten-free doughs were defined and rheologically characterized at dough level and the technological and sensorial quality of the resulting baked products was investigated. Dough Mixolab® parameters, bread-like quality parameters (moisture content, specific volume, water activity, colour, and crumb texture), and chemical composition significantly ($P<0.05$) discriminated between the samples tested. In general, the highest correlation coefficients ($r>0.70$) were found when quality instrumental parameters of the baked products were correlated with the dough Mixolab® parameters, and lower correlation coefficients ($r<0.70$) were found when sensory characteristics were correlated with dough rheology or instrumental parameters. Dough consistency during mixing (C1), amplitude and dough consistency after cooling (C5) would be useful predictors of crumb hardness; and C5 would be also predictor of perceived hardness of gluten-free bread-like products.

Key words: Rice flour; Gluten-free; Wheat free; Dough behaviour; Bread quality

1. Introduction

Gluten-free breads are products initially designed for people who have intolerance to some specific peptides comprised in the gluten proteins (Catassi & Fasano, 2008). Nevertheless, there is an increasing number of people interested in wheat free foods motivated by health concern or because they want to avoid wheat in the diet. Particularly, gluten from wheat, rye, barley, triticale, and some varieties of oats (Comino et al., 2011) must be eliminated from the diet of individuals suffering from celiac disease.

Cereal products, especially breads, are basic components of the diet in many countries due to their sensory characteristics or/and nutritional quality. However, the manufacture of bread-like products without gluten results in major technological problems for bakers. In fact, many gluten-free products available on the market are often of poor technological quality, exhibiting low volume, poor color, and crumbling crumb, besides great variation in the nutrient composition, with low protein and high fat contents (Matos & Rosell, 2011). A range of bread-like gluten-free products has been designed to provide coeliac disease sufferers or wheat free diet eaters with bread substitutes. The term gluten-free bread is generally used for referring to a gluten-free bakery product that is eaten as bread substitute, but has different characteristics than wheat bread, because of that, the term gluten-free bread-like products was preferred in this manuscript. The gluten-free bread recipes contain mainly rice or maize flours combined with potato, maize or wheat starches (Gujral & Rosell, 2004; Gallagher et al., 2004; Demirkesen et al., 2010; Matos & Rosell, 2011).

Rice flour is one of the most suitable cereal flours for preparing gluten-free products due to its several significant properties such as natural, hypoallergenic, colorless, and bland taste. In addition, it has also hypoallergenic proteins, and low content of sodium and fat and high amount of easily digested carbohydrates (Gujral & Rosell, 2004). The relatively small amount of prolamin in rice, forces to use some sort of gum, emulsifier, enzymes or dairy products, together with rice flour, for obtaining some viscoelastic properties (Demirkesen et al., 2010). Several studies had reported the use of rice flour for making good-quality gluten-free bread-like products (Kadan et al., 2001; McCarthy et al., 2005; Ahlborn et al., 2005; Moore et al., 2006; Lazaridou et al., 2007; Marco & Rosell, 2008 a,b; Pruska-Kędzior et al., 2008; Sciarini et al., 2010; Demirkesen et al., 2010). Those studies were mainly focused on bread instrumental and/or sensory characteristics.

Scarce information has been presented about the rheological characteristics of the gluten-free doughs, which greatly vary in consistency, going from batter to dough. Gluten free dough is referred to a semisolid system that can be manually handled, whereas when very high water content is added in the recipe, the rheological properties of the dough resemble a semiliquid system named batter. Some studies reported information about gluten-free dough behavior using rheometers. Pruska et al. (2008) compared the rheological properties of gluten-free dough formulations (maize flour, maize starch, rice flour) concluding that they can be defined as physical gels of different viscoelasticity and structural networking. Rice flour based dough or even protein enriched rice flour dough behaves as a viscoelastic solid with storage modulus (G′) higher than loss modulus (G″) (Gujral & Rosell 2004; Marco & Rosell, 2008b). The incorporation of resistant starch increases storage (G′) and loss (G″) moduli of gluten-free doughs, increasing their elastic behaviour (Korus et al., 2009). Other researchers have studied the rheological properties of different gluten-free doughs by extrusion and penetration tests using a Texture Analyzer (Moore et al., 2006; Sciarini et al., 2010; Onyango et al., 2011) and the average force after reaching a plateau was used as indicator of batter firmness or consistency. Rapid Visco Analyzer (Kim & Yokoyama, 2011) and Viscoamylograph (Sciarini et al., 2010) also gave information about the pasting properties of the batters. Additionally, mixing and pasting behaviour of different rice flour based doughs were studied using the Mixolab® (Marco & Rosell, 2008a).

Nevertheless, the information about dough or batter rheological properties has rarely been exploited when designing or developing gluten-free bread like products, neither it has been used for predicting bread characteristics. The main objective of this study was to define predictors of the quality of gluten-free bread-like products at dough level. With that aim, different gluten-free rice based doughs were defined to cover a range of gluten-free doughs with different rheological features, and in consequence, to obtain gluten-free bread like products with diverse technological and sensorial quality. The Mixolab® was used to obtain a complete characterization of the gluten-free dough behaviour by recording the mechanical changes during mixing and heating simulating the mechanical work as well as the heat conditions that might be expected during the baking process. Different correlations between rheological dough properties and quality parameters of gluten-free bread-like products were established.

2. Materials and methods

2.1. Materials

Commercial gluten-free blend (corn starch, whole egg powder, sugar, xanthan gum and salt) was generously donated by Huici-Leidan SA (Huarte, Spain). Commercial rice flour, supplied by Harinera Los Pisones (Zamora, Spain), had moisture and protein contents of 11.5g/100g and 6g/100g, respectively. Soybean protein isolate was from Trades SA (Barcelona, Spain). The soybean protein isolate had moisture, protein, lipid, ash and carbohydrates (calculated by difference) contents of 6.9, 80.8, 0.2, 3.6 and 8.5 g/100g, respectively. Composition of the different ingredients was determined following the ICC Standard Methods (1994). Corn starch, potato starch, skim milk powder and whole egg powder were obtained from EPSA, (Valencia, Spain). HPMC (Methocel K4M) was obtained from Dow Chemical (Pittsburg, USA). Xanthan gum food grade from Jungbunzlauer (Ladenburg, Germany) has an apparent viscosity of 6.0 mPas at 24ºC. Pectin (GENU®pectin 150 USASAG type Baking, PKelco) was provided by Puratos (Groot-Bijgaarden, Belgium). Vegetal seed oil, compressed yeast, commercial sugar and salt were purchased from local market. All reagents were of analytical grade.

2.2. Mixolab® Measurements

Mixing and pasting behaviour of the gluten-free flour blends were studied using the Mixolab® (Chopin, Tripette et Renaud, Paris, France), which allows mixing the dough under controlled temperature and also a temperature sweep until 90ºC followed by a cooling step. It measured in real time the torque (expressed in Nm) produced by passage of dough between the two kneading arms, thus allowing the study of its physico-chemical behaviour. All ingredients used on each formulation (Table 1), with the exception of yeast, were introduced into the Mixolab® bowl and mixed. The settings used in the test were 8 min for initial mixing, temperature increase at 2.3 ºC/min until 90 ºC, 7 min holding at 90 ºC, temperature decrease at 4ºC/min until 50ºC, and 5 min holding at 50ºC; and the mixing speed during the entire assay was 80 rpm. Three replicates were carried out for each formulation. The following parameters were obtained from the recorded curve: initial consistency (C1), stability (min) or elapsed time at which the torque produced is kept constant, minimum torque (Nm) or the minimum value of torque produced by dough passage subjected to mechanical and thermal constraint (C2), peak torque (Nm) or the maximum torque during the heating stage (C3), the minimum torque during the heating period (Nm) (C4) and the torque obtained after cooling at 50ºC (C5). Additionally, derived parameters were calculated: cooking stability range (C4-C3) and cooling setback or gelling (C5-C4). Detailed description of physical changes that occurred along Mixolab® measurement (mixing, pasting and

Table 1. Gluten-free dough formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7
Commercial GF blend, g	1000	-	-	-	-	-	-
Rice flour, g	-	1000	1000	350	400	696	500
Corn starch	+	-	-	225	200	130	-
Potato starch	-	-	-	300	400	174	500
Fresh yeast, g	50	30	28	20	50	22	50
Salt, g	-	18	24	17	15	17	20
Sugar, g	10	30	120	10	60	78	50
Vegetal oil, g	-	60	56	-	30	52	60
Skim milk powder, g	-	-	-	-	-	39	100
Whole egg powder, g	+	-	-	-	-	174	-
Soy protein, g	-	-	-	125	-	-	-
Xanthan gum, g	+	-	-	10	-	16	-
HPMC, g	-	40	28	-	-	4	22
Pectin, g	-	-	-	-	50	-	-
Water (mL)	600	1100	1060	1050	900	565	790

+ Ingredient present in the commercial blend gelling) was gathered by Rosell et al. (2007). Recently, detailed information about Mixolab® parameters has been reported by Marco & Rosell (2008a) and Rosell et al. (2010).

2.3. Breadmaking Process

Different gluten-free rice formulations were initially selected to cover a range of gluten-free doughs with different rheological features, and in consequence, gluten-free bread like products with diverse technological and sensorial quality. Bread formulations were based on reported recipes (Marco & Rosell, 2008a; McCarthy et al., 2005; Kadan et al., 2001; Moore et al., 2006; Pruska-Kędzior et al., 2008; Ahlborn et al., 2005; Sciarini et al., 2010; Demirkesen et al., 2010), which were modified according to preliminary rheological results. Seven formulations were used to obtain gluten-free bread-like products (BF), one was based on corn starch (commercial blend) and in the other, rice flour was the major ingredient, present individually or blended with potato or corn starch. They contained different ingredients (starches, proteins, other hydrocolloids) widely used in the design of gluten-free bread type products. The formulations used are showed in Table 1, which were based on the following: 1000g of corn starch (F1); 1000g of rice flour (F2, F3); 1000g of blend of rice flour + corn and potato starches (F4, F5, F6); and 1000g of blend of rice flour + potato starch (F7). Gluten-free batters or doughs were prepared in a spiral mixer (AV18/2, Vimar Industries 1900, S.L., Sabadell, Spain) by mixing all or part of the flour and the other ingredients with the water determined in preliminary test (Table 2). Dough pieces (400g) or batters (400g) were placed into regular metallic, lard coated pans and proofed in a cabinet at 85% relative humidity during the time (min) and temperature (ºC) detailed in Table 2. The batter or dough pieces were baked in an electric convection oven (Eurofours, Gommegnies, France) as described in Table 2. After baking, loaves were removed from the pans and kept at room temperature for 2 hours to cool down. Loaves packed in polyethylene bags to prevent drying were stored at 24 ºC for 24 hours and then used for bread quality assessment. Four loaves were obtained from each formulation. Duplicates were carried out in different days.

2.4. Quality Assessment of Gluten-free Bread-like Products

2.4.1. Instrumental quality parameters

The moisture content of gluten-free bread-like samples was determined following the ICC (1994). Volume was determined by the rapeseed displacement method. Specific volume (cm^3 /g) of the individual loaf was calculated by dividing volume by weight. Water activity of samples was measured using an Aqua Lab Series 3 (Decagon devices Pullman, USA) at 22ºC. The colour of the crumb samples was measured at three different locations by using a Minolta colorimeter (Chromameter CR-400/410, Konica Minolta, Tokyo, Japan) after standardization with a white calibration plate (*L**= 96.9, *a**= -0.04, *b**=1.84). The colour was recorded using CIE-*L*a*b** uniform colour space (CIE-Lab) where *L** indicates lightness, *a** indicates hue on a green (-) to red (+) axis, and *b**

Table 2 Breadmaking process conditions for each gluten-free dough formulations

Breakmaking		F1	F2	F3	F4	F5	F6	F7
Mixing	Procedure	Mix all ingredients	a) Mix 500g rice flour with 550ml boiling water for 5min, cool down till 35ºC. b) Add the rest of ingredients	a) Mix water, rice flour and oil b) Mix other dry ingredients c) Mix (a+b)	a)Mix yeast in a solution of sugar and water b)Add the rest of ingredients	Mix all ingredients	a)Mix yeast in a solution of sugar and water b) Add slowly xanthan gum and HPMC during 3min mixing c) Add rest of ingredients	a)Mix yeast with water and then oil b) Mix dry ingredients for 1 min c) Mix a+b
	Time (min)	5	5	10	10	3	5 (then hold 10 min), 3	2
Fermentation	Time (min)	45	60	40	30	40	50	35
	Temperature (ºC)	30	30	35	30	35	30	40
Baking	Time (min)	25	60	45	45	30	50	25
	Temperature (ºC)	210	175	190	190	200	190	230

indicates hue on a blue (-) to yellow (+) axis. Data from three slices per sample were averaged.
The crumb hardness was measured on uniform slices of 10mm thickness. Three slices from the centre of each loaf were used for texture evaluation. Texture profile analysis (TPA) was performed using a universal testing machine TAXT2i (Stable Micro Systems, Surrey, UK) equipped with a 30-Kg load cell and 25-mm aluminium cylindrical probe. Crumbcharacteristics were assessed using a texture analyser (TAXT2i texture analyser Stable Micro Systems, Surrey, UK). The settings used were a test speed of 2.0 mm/s with a trigger force of 5 g to compress the middle of the bread crumb to 50% of its original height at a crosshead speed of 1mm/s. Values were the mean of at least three replicates.

2.4.2. Chemical Composition

The chemical composition of the samples was determined according to ICC corresponding standard methods (ICC, 1994), namely, the moisture content (ICC standard 110/1), fat (ICC 136), proteins (N x 6.25) (ICC 105/2) and ash (ICC 104/1). Total carbohydrates were determined by difference subtracting 100 g minus the sum of protein, ash and fat expressed in grams/100 grams FAO (2003). Determinations were carried out in triplicate.

2.4.3. Sensorial Analysis

A descriptive sensory analysis was performed for evaluating the sensory characteristics of the gluten-free bread-like products. Sensory analysis was carried out with ten trained panellists under normal lightening conditions and at room temperature. The range of time that the test panellist had participated in descriptive analysis and scale rating of a wide range of bread products varied from 3 to 20 years. Samples were presented in slices (1cm thick) on plastic dishes coded and served in a randomised order. Preliminary training test was performed to define the best descriptors for characterizing the product. Panellists were sat in a round table and after evaluating the sample, an open discussion was initiated to define the best descriptors for characterizing the product. Evaluation included perception at first glance of the bread slice (crust and crumb included) and mastication with the molar teeth up to swallowing. The attributes assessors finally agree were appearance (by observing the product slice), odour, colour, taste, texture attributes during chewing and springiness (ability to regain original shape after pressing down the crumb with the middle finger). The descriptors for each attributes were appearance (visually liking or disliking), odour (scale goes from high when typical of bread or bakery products to low, uncharacteristic of bakery products), colour (scales goes from high yellow/beige to low when brown or grey), taste (scale goes from high when typical taste of bread or bakery products to low, uncharacteristic of bakery products), texture attributes during chewing (scales goes from hard-soft, crumbly-cohesive). Attribute intensity was scored on a scale varying from 1 to 5. Samples were considered acceptable if their mean score for overall acceptance was above 3.0 (neither like nor dislike).

2.5. Statistical Analysis

For each quality parameter, a one way analysis of variance (ANOVA) was applied using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). Fisher's least significant differences (LSD) test was used to assess significant differences (P<0.05) among samples that might allow discrimination among them. Additionally, Pearson correlation analysis was applied to establish possible relationships between the rheological dough properties and both instrumentals and sensorial quality parameters of the gluten-free bread-like products.

3. Results and discussion

3.1. Mixing and Pasting Properties of Gluten-free Doughs

Figure 1 show the curves obtained from the Mixolab® corresponding to the seven gluten-free dough formulations evaluated. Plots reflected the dough changes due to both the mixing force and the temperature. The patterns obtained during mixing, overmixing, pasting and gelling greatly varied with the mixture composition, which was expected considering the complex blend of ingredients (Table 1). The presence of different proteins and starches modifies protein-protein interactions and also the starch gelatinization and the gelling processes (Rosell et al., 2007; Marco & Rosell, 2008a; Rosell et al., 2010). All Mixolab® parameters significantly (P<0.05) discriminated among the formulated dough tested (Table 3). During the mixing and overmixing, significant variation was observed in the dough maximum consistency, time to reach that consistency and the stability (Table 3). Some formulations yielded

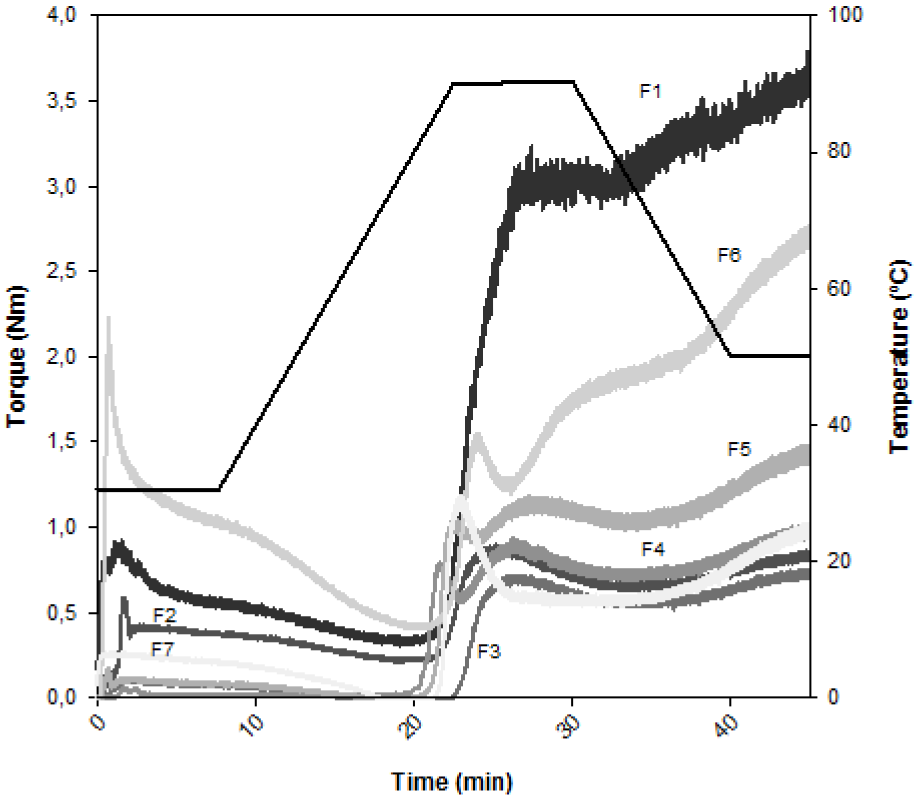

Figure 1. Curves Mixolab® from the different formulations

mixtures with dough consistencies (with C1 higher than 0.5 Nm), whereas F3, F4, F5 and F7 led to mixtures with batter consistencies (C1 lower than 0.3 Nm) that were difficult to handle. F6 showed the highest C1 value and the lower time to C1 value, indicating that this dough reached major consistency in minor time, likely due to its major amount of proteins (egg, milk). Regarding stability, F7 showed the highest value followed by F1, while F5 presented the lower dough stability value. The

Table 3 Rice-based dough characteristics during mixing and heating determined by using the Mixolab®

Dough Codes	Time to C1, min	C1, Nm	Stability, min	Amplitude, Nm	C2, Nm	C3, Nm	C4, Nm	C5, Nm	Cooking stability range,C4-C3, Nm	Gelling, C4-C3, Nm
F1	1.37±0.05 bc	0.88±0.10 d	2.49±0.30 e	0.07±0.01 b	0.33±0.01 b	3.07±0.03 e	2.99±0.04 d	3.64±0.6 e	-0.08±0.00 d	0.65±0.05 d
F2	1.79±0.03 c	0.56±015 c	0.51±0.08 b	0.07±0.00 b	0.22±0.01 b	0.87±0.01 b	0.65±0.06 ab	0.84±0.08 a	-0.22±0.00 b	0.19±0.02 a
F3	1.01±0.10 ab	0.14±0.20 ab	1.29±0.15 d	0.01±0.00 a	0.01±0.00 a	0.69±0.05 a	0.56±0.07 a	0.74±0.07 a	-0.13±0.00 c	0.18±0.02 a
F4	1.70±0.11 c	0.05±0.18 a	1.00±0.21 c	0.01±0.00 a	0.02±0.01 a	0.77±0.03 ab	0.70±0.04 b	1.00±0.05 b	-0.08±0.00 d	0.30±0.05 b
F5	0.75±0.19 a	0.14±0.15 ab	0.09±0.13 a	0.04±0.02 ab	0.01±0.00 a	1.05±0.07 c	1.03±0.05 c	1.45±0.04 c	-0.02±0.00 e	0.42±0.05 c
F6	0.67±0.21 a	1.77±0.13 e	0.48±0.03 b	0.29±0.01 c	0.23±0.01 b	1.30±0.06 d	1.07±0.03 c	2.61±0.07 d	-0.23±0.01 b	1.54±0.06 e
F7	1.03±0.15 ab	0.26±0.09 b	5.46±0.27 f	0.02±0.01 a	0.00±0.00 a	1.15±0.05 c	0.57±0.03 a	1.00±0.06 b	-0.58±0.02 a	0.43±0.04 c
p-value	0.0024	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000

Values are means ± standard deviation. Different letters within a column mean significant differences (*P*<0.05).
C1: initial consistency; C2: minimum torque; C3: maximum torque during the heating; C4: minimum torque during the heating period; C5: torque obtained after cooling at 50ºC.

amplitude, indicative of the role of water in the lubrication during mixing (Rosell & Collar, 2009) showed also significant differences, and thus different extensional properties of the evaluated doughs. The simultaneous mechanical shear stress and temperature led to a minimum torque that has been related to protein unfolding or protein weakening (Rosell et al., 2007). The values for C2 were quite low compared with the ones detected for wheat dough (0.4-0.5 Nm). That result might be ascribed to the protein thermal properties rather than to the amount of proteins, since some gluten-free doughs had very high protein content (F4 and F6). As temperature increases, starch gelatinization occurs and therefore viscosity increases, which is detected as an increase in torque (Rosell et al., 2007). As was expected F1 showed the highest C3 value, likely due to its highest starch content, specifically corn starch (Table 1). In the case of F2 and F3 (only with rice flour as starch source), a delayed peak corresponding to starch gelatinization was observed, derived from the high gelatinization temperature of the rice starch. It should be remarked that two gelatinization peaks were observed in F4, F5 and F6. Those peaks resulted from the presence of different starches (rice, corn and potato) with diverse pasting temperatures, being 65.4ºC for potato starch, 69.9ºC for corn starch and 70.2ºC for rice flour. Furthermore, it must be taken into account that hydrocolloids like xanthan gum, HPMC or pectin, contained in the doughs can retain water, competing with the starch for the available water, limiting the starch granule swelling and, therefore promoting a delay in the pasting process (Rosell et al., 2011).

During temperature holding at 90ºC, a reduction in consistency occurred, which is related to the physical breakdown of the starch granules. F1 showed the highest value, likely due to the high content of corn starch in this dough.

After cooling, F1 presented the highest C5 value followed by F6 and F5. The cooling process was accompanied by an enhancement of dough consistency associated to starch gelling, due to amylose chains crystallization, which is greatly dependent on the starch type and the presence of gelling additives or ingredients with water binding ability (Rosell et al., 2007; Rosell et al., 2010). Regarding the secondary parameters, all doughs showed very low cooking stability range (C4-C3); whereas the cooling setback (C4-C5) was only significantly higher for F1 and F6 (Table 3). High setback value suggests that dough presents high retrogradation tendency and, consequently the baked product prepared from this dough would undergo high staling rate over storage.

Some studies have been published about the effect of gelling agents and proteins on the mechanical properties of wheat dough due to dual mixing and temperature constraint using the Mixolab® (Collar et al., 2007; Marco & Rosell, 2008a, Rosell & Collar, 2009; Rosell et al., 2010). Those studies concluded that the effect of gelling or thickening agents on the mechanical properties greatly depends on the nature of the added polymer and the type of interaction among them. Moreover, the addition of proteins to wheat or rice flour also led to changes on the mechanical and baking properties, depending on the protein source (Bonet et al., 2006; Marco & Rosell, 2008a).

3.2. Bread Quality Assessment

Gluten-free bread-like products (BF1-BF7) obtained from the formulated doughs (F1-F7) presented important crumb differences regarding colour, appearance, shape, size and volume (Figure 2). The values obtained for specific volume, crumb colour, moisture content, water activity, height/width ratio and hardness are showed in Table 4. All instrumental quality parameters tested significantly (P<0.05) discriminated among samples. Specific volume values ranged from 1.44 to 3.03 cm^3/g, except for BF2 (4.48 cm^3/g) and BF7 (5.07 cm^3/g), which showed the highest alues of specific volume. In general, values of specific volume obtained in this study agree with previous reports (Hathorn et al., 2008, Marco & Rosell, 2008a; Sabanis et al., 2009, Sciarini et al., 2010).

The $L*$, $a*$ and $b*$ values for crumb colour showed significant (P<0.05) differences among gluten-free bread-like products (Table 4). The lower values of $L*$ (lightness) were obtained for BF4 and BF6, which had in common the presence of xanthan gum, and proteins blend (soybean protein in BF4 or skim milk powder and whole egg powder in BF6). Likely, soybean proteins and egg powder could be responsible of decreasing lightness, since BF7, containing only skim milk powder as protein source showed the highest $L*$ value. Regarding $a*$, all showed negative (green hue) values, with exception of BF6. The b* scale showed positive value (yellow hue) for all samples evaluated. BF6 exhibited significantly higher b* value than the other samples, derived from the original yellow pigment of the egg powder added as ingredient in this formulation.

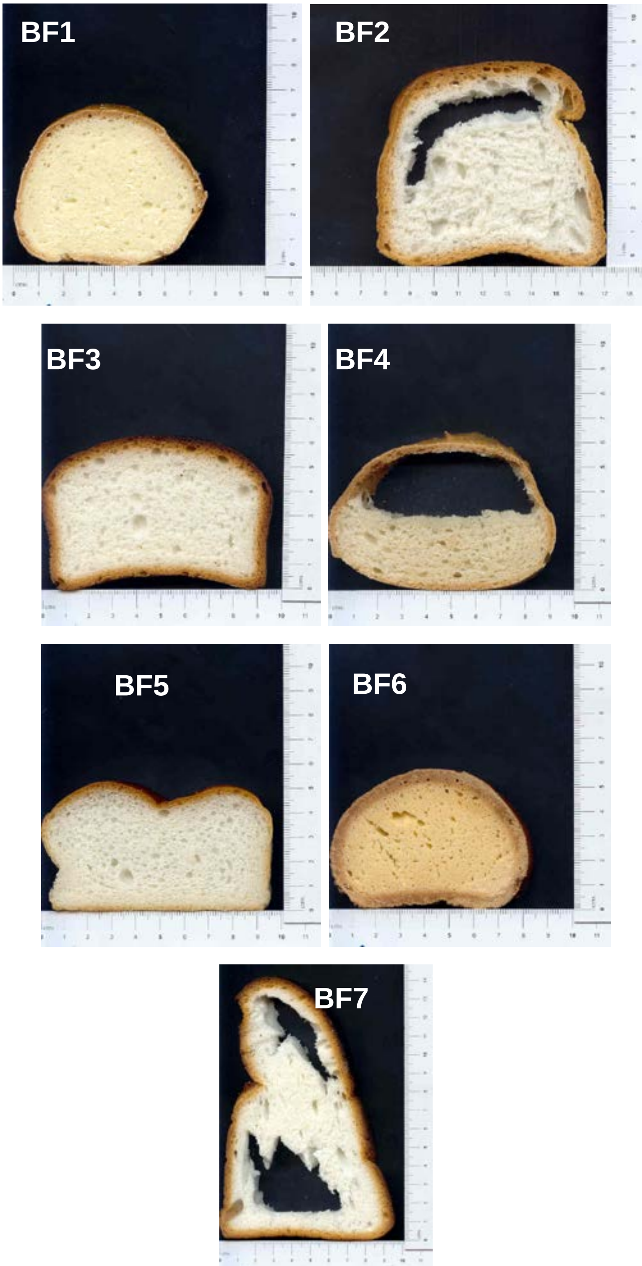

Figure 2. Digital Images of gluten free bread slices prepared from different formulations.

Table 4. Instrumental quality parameters of the gluten-free bread-like products

Samples codes	Specific Volume, cm^3/g	Crumb colour parameters *L**	*a**	*b**	Moisture content, %	Water activity	TPA-Hardness, N
BF1	1.91 ± 0.05 b	78.31 ± 0.76 d	-2.59 ± 0.17 a	14.47 ± 0.79 d	37.17 ± 0.07 c	0.96 ± 0.00 c	84.90 ± 3.07 c
BF2	4.48 ± 0.02 f	72.17 ± 1.01 c	-1.21 ± 0.20 bc	7.13 ± 1.02 b	37.97 ± 0.04 d	0.96 ± 0.00 c	1.33 ± 0.33 a
BF3	3.03 ± 0.04 e	73.79 ± 2.87 c	-0.89 ± 0.16 cd	6.30 ± 0.25 b	37.40 ± 0.17 c	0.95 ± 0.00 b	2.30 ± 0.30 a
BF4	2.52 ± 0.04 d	62.24 ± 0.81 a	-0.80 ± 0.15 d	12.15 ± 0.54 c	43.53 ± 0.32 f	0.97 ± 0.00 d	36.27 ± 2.93 b
BF5	2.41 ± 0.04 c	65.77 ± 0.27 b	-1.22 ± 0.02 bc	5.06 ± 0.12 a	39.30 ± 0.08 e	0.97 ± 0.00 d	7.53 ± 0.46 a
BF6	1.44 ± 0.03 a	63.40 ± 0.62 a	1.72 ± 0.43 e	21.89 ± 0.37 e	25.67 ± 0.30 a	0.92 ± 0.00 a	147.50 ± 11.12 d
BF7	5.07 ± 0.08 g	81.50 ± 0.09 e	-1.53 ± 0.04 bc	6.47 ± 0.15 b	33.33 ± 0.06 b	0.95 ± 0.00 b	5.43 ± 0.51 a
P- value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Values are means ± standard deviation. Different letters within a column mean significant differences ($P<0.05$).

TPA-Hardness: Crumb hardness measured with a texturometer.

Significant differences (P<0.05) in crumb moisture and water activity were found among the different gluten-free bread-like samples (Table 4). Differences in water activity and moisture content could be attributed to differences in the recipes. In fact, BF6 showed the lowest water activity and moisture content, which can be ascribed to the presence of whole egg powder in the formulation. The highest moisture content was observed in BF4 that contained soy protein, which agrees with results of Marco & Rosell (2008a) when incorporating soybean proteins to gluten-free breads. Overall, the crumb moisture contents were lower than those reported by other researchers (Sabanis et al., 2009; Marco & Rosell 2008a; Matos & Rosell, 2011).

Wide variation in the crumb hardness (1.3 N to 147.5 N) was observed among the gluten-free bread-like samples (Table 4). These results reflect large differences depending on type of formulation used for obtaining the experimental gluten-free baked products. Frequently, gluten-free bread-like products due to their complex formulation, mainly based in carbohydrates (Matos & Rosell, 2011), present high crumb hardness when compared to standard wheat bread.

Table 5 shows the macronutrients compositions of the seven gluten-free bread specialities evaluated in this study. Analysis of data collected using ANOVA showed that all chemical composition significantly (P<0.05) discriminated between the baked samples. Protein and fat content ranged between 3.30-14.97 g/100g, and 0.20-9.57 g/100g, respectively. In regard to protein content, it was high in the gluten-free bread-like samples BF4 and BF6 which contained more proteins, while BF6 and BF7 were the

Table 5. Proximate composition of the gluten-free bread-like products

Sample Codes	Protein, g/100g, dm	Fat, g/100g, dm	Minerals, g/100g, dm	Total Carbohydrate* g/100g, dm
BF1	3.30 ± 0.00 a	0.97 ± 0.02 b	1.37 ± 0.12 bc	94.36 ± 0.16 f
BF2	7.57 ± 0.12 e	3.40 ± 0.01 d	1.13 ± 0.08 a	87.90 ± 0.08 d
BF3	7.10 ± 0.04 c	3.70 ± 0.00 e	1.31 ± 0.00 b	87.89 ± 0.18 d
BF4	14.97 ± 0.00 g	0.20 ± 0.02 a	1.47 ± 0.03 c	83.36 ± 0.31 b
BF5	3.63 ± 0.03 b	1.87 ± 0.01 c	1.03 ± 0.06 a	93.47 ± 0.06 e
BF6	12.33 ± 0.03 f	9.57 ± 0.00 g	1.46 ± 0.01 c	76.64 ± 0.29 a
BF7	7.43 ± 0.03 d	4.77 ± 0.04 f	1.41 ± 0.14 bc	86.39 ± 0.17 c
P- value	0.0000	0.0000	0.0001	0.0000

(*)Total Carbohydrate (dm) by difference: 100 – (weight in grams [protein + fat + ash] in 100 g of food) (FAO. 2003).
Values are means ± standard deviation. Different letters within a column mean significant differences ($P<0.05$).

specialties with higher fat content. Total carbohydrate was the major component in gluten-free bread-like products based on flours and/or starches. These results agree with those recently reported by Matos & Rosell (2011) who evaluated in detail the chemical composition of many types of gluten-free bread like products.

Sensory analysis of the different types of gluten-free bread-like samples is presented in Table 6. According to ANOVA results, these bread-like products differed significantly (P<0.05) in crumb appearance, taste, colour, springiness, hardness and crumbliness. Conversely, no significant differences were observed in odour. The highest score for crumb appearance, colour and perceived hardness was obtained for BF3 and BF5. Additionally, the best taste was perceived in BF3, and BF5 received the highest score for springiness, indicating major elasticity. In general, BF3, which did not contain any additional protein source, was scored high for most of the sensorial attributes evaluated, including hardness and crumblines. On the contrary, BF6 was scored low for most of the sensory attributes evaluated. It seems that the addition of whole egg powder as unique source of proteins affected negatively the sensory perception of this product. The results obtained from sensory test clearly revealed great variability on sensory quality of the gluten-free bread-like products tested.

3.3. Relationships among the Rheological Properties of Formulated Doughs and the Instrumental and Sensory Characteristics of the Gluten-free Bread-like Products.

Table 6. Sensorial analysis of the gluten-free bread like products

Sample Codes	Crumb appearance	Taste	Odour	Colour	Springiness	Hardness	Crumblines
BF1	2.67 ± 1.21 bc	1.33 ± 0.52 a	2.17 ± 1.17	3.00 ± 0.89 bc	1.50 ± 1.22 a	1.50 ± 1.22 a	1.67 ± 0.82 a
BF2	2.67 ± 0.52 bc	2.50 ± 0.84 b	3.17 ± 0.75	3.67 ± 1.03 bc	1.33 ± 0.52 a	3.83 ± 0.75 b	3.67 ± 1.37 bc
BF3	4.50 ± 0.55 d	3.67 ± 1.14 c	3.33 ± 1.48	4.33 ± 0.45 c	2.00 ± 0.71 ab	4.17 ± 0.84 b	4.00 ± 1.00 c
BF4	1.33 ± 0.89 a	1.17 ± 0.45 a	1.83 ± 0.84	2.67 ± 0.89 ab	3.00 ± 1.87 bc	2.00 ± 1.22 a	1.33 ± 0.55 a
BF5	4.50 ± 0.55 d	2.50 ± 0.55 b	3.33 ± 1.03	4.33 ± 0.82 c	3.33 ± 1.03 c	3.67 ± 0.52 b	2.17 ± 0.75 ab
BF6	1.83 ± 1.17 ab	2.50 ± 0.84 b	2.33 ± 1.21	1.67 ± 0.82 a	1.17 ± 0.41 a	1.50 ± 0.84 a	1.50 ± 0.84 a
BF7	3.17 ± 0.41 c	3.33 ± 1.21 bc	2.83 ± 1.33	3.67 ± 1.21 bc	2.33 ± 1.37 abc	4.33 ± 1.21 b	3.00 ± 0.63 bc
P-value	0.0000	0.0000	0.1218	0.0002	0.0089	0.0000	0.0000

Values are means ± standard deviation. Different letters within a column mean significant differences ($P<0.05$)

Relationship among the rheological properties of formulated doughs recorded from Mixolab®, and the product instrumental and sensory characteristics were analyzed. Table 7 illustrates the broad range of correlations found between parameters obtained during the heating and cooling cycles with the Mixolab® and the instrumental quality parameters (specific volume, water activity, moisture content and TPA-hardness) of the bread-like baked products. Water activity and moisture content were highly significant and negatively correlated with C1, amplitude and gelling (C5-C4) parameters. Specific volume showed high and negative correlation with cooking stability range (C4-C3) and C5 parameters, which are associated to the cooling stage of the Mixolab®. Presumably, high dough or batter consistencies limit the expansion during proofing, reducing the specific volume. Nevertheless, a positive correlation between apparent viscosity and loaf volume (r = 0.83, P<0.05) and also between porosity and loaf volume values (r = 0.81, P <0.05) in gluten free breads has been reported by Sabanis et al., (2009). There were good correlations between TPA-hardness values and Mixolab® parameters. The relationships between the TPA-hardness and C1, amplitude, C5 and gelling (C5-C4) parameters were found to be particularly highly significant (P <0.001) and positive. This could indicate that the TPA-hardness values are strongly correlated (r >0.70) with parameters characterising both protein and starch cooling behaviours. It is important to remark that wheat dough viscosity characteristics determined with the Rapid Viscoanalyzer (RVA) have been also correlated with wheat bread texture parameters (Collar 2003). The pasting profile during cooking and cooling of wheat dough has been highly correlated with bread staling

Table 7. Correlation matrix between instrumental quality parameters of gluten-free bread-like products and dough/batter rheological parameters determined with the Mixolab®

	Instrumental quality parameters			
Mixolab® parameters	Specific volume	Water activity	Moisture content	TPA-Hardness
Time to C1		0.5101*	0.5422*	
C1	-0.4816*	-0.7833***	-0.8193***	0.8969***
Stability	0.5579**			
Amplitude	-0.5151*	-0.7768***	-0.8113***	0.8671***
C2				0.5916**
C3				0.4880*
C4	-0.5112*			0.4868*
C5	-0.6594**			0.7849***
Cooking stab range C4-C3	-0.7016***		0.4749*	
Gelling C5-C4	-0.5906**	-0.8013***	-0.8355***	0.9287***

Correlations indicated by *r* values. ****P*-value <0.001, ***P*-value <0.01, **P*- value <0.05.
C1: initial consistency; C2: minimum torque; C3: maximum torque during the heating; C4: minimum torque during the heating period; C5: torque obtained after cooling at 50ºC.

kinetic parameters. Particularly, peak viscosity, pasting temperature, and setback during cooling can be considered predictors at dough level of bread firming behaviour during storage of wheat bread. Regarding gluten-free doughs, pasting behaviour of corn flour has been significantly correlated with dough textural parameters. Specifically, springiness and stickiness parameters were positively associated to gelatinisation and retrogradation phenomena (Brites et al., 2010).

Table 8 showed correlation coefficients and significance levels found among Mixolab® parameters, instrumental quality parameters and sensory characteristics obtained from formulated dough and the prepared gluten-free bread like products. Particularly, all sensory characteristics evaluated (appearance, colour, springiness, hardness and crumblines) showed significant negative correlations with *b** (hue on a yellow axis), although correlation coefficients only indicated strong linear relationship between *b** and perceived colour and perceived hardness. It seems that crumb structure has strong influence on the *b** parameter. Additionally, hardness perceived revealed high ($P < 0.001$) and positive correlation with specific volume (r = 0.7149) and high negative correlations with *b** (r = -0.7945), TPA-hardness (r = -0.7646) and C5 (r = -0.7005) Mixolab® parameter.

Hardness is a very important sensory characteristic when assessing bread quality. In this study, as it was mentioned, perceived hardness showed negative correlation with *b** and TPA-hardness. Apparently, the colour perception is closely related to crumb structure since breads presenting hue yellowness and packed crumb structure could be rated lowly. It has been reported that smaller loaves were denser and had tightly packed crumb

Table 8. Correlation matrix between sensory characteristics and instrumental parameters at dough and baked product level

	Sensorial characteristics				
Instrumental parameters	Crumb appearance	Colour	Springiness	Hardness	Crumblines
Mixolab® parameters					
C1			-0.6494**	-0.571**	
Amplitude			-0.5182*	-0.5444*	
C2			-0.6232**	-0.5332*	
C3				-0.5179*	
C4				-0.5639**	
C5				-0.7005***	-0.5584**
Gelling C5-C4				-0.5913**	-0.5217*
Quality parameters					
Specific volume				0.7149***	0.6242**
*L**					0.4852*
*a**		-0.4737*			
*b**	-0.6073**	-0.7636***	-0.4398*	-0.7945***	-0.6071**
Water activity			0.5362*		
Moisture content			0.5403*		
TPA-Hardness	-0.4904*		-0.4375*	-0.7646***	-0.6102**

Correlations indicated by *r* values. ****P*-value <0.001, ***P*-value <0.01, **P*- value <0.05.

C1: initial consistency; C2: minimum torque; C3: maximum torque during the heating; C4: minimum torque during the heating period; C5: torque obtained after cooling at 50ºC structure, resulting in higher crumb firmness (Sabanis et al., 2009); this drives to think that bread with compact crumb could be perceived as hard. Sabanis et al. (2009) reported a negative correlation between crumb firmness and loaf volume (r = -0.89, P >0.05).

In general, many relationships were found (Table 8), however the correlation coefficients were higher between dough properties and instrumental bread parameters (r >0.70) than among instrumental parameters and sensory characteristics (r < 0.70).

4. Conclusions

The patterns obtained during mixing, overmixing, pasting and gelling greatly varied depending on the gluten-free dough or batter composition. All Mixolab® parameters significantly (P<0.05) discriminated among the doughs evaluated. Additionally, differences found in the rheological dough properties from Mixolab® were mainly associated with the presence/ absence of protein and starch sources in the dough. Instrumental quality parameters evaluated in the gluten-free bread-like products significantly (P <0.05) discriminated among the samples.

Several relationships were found among the rheological properties of formulated gluten-free dough/batter, the instrumental quality parameters and sensory characteristics of the bread-like products. In general, the highest correlation coefficients (r >0.70) were obtained between the Mixolab® rheological properties at dough level and the instrumental quality parameters of the fresh baked products. Conversely, lower correlation coefficients (r <0.70) were found when correlations were established with sensory characteristics. Particularly, dough/batter consistency during mixing (C1), amplitude and dough consistency after cooling (C5) would be useful predictors of TPA crumb hardness of baked product; and C5 would be also predictor of perceived hardness of gluten-free bread-like products.

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CAPÍTULO 4

UNDERSTANDING THE FUNCTION OF PROTEINS ON THE RHEOLOGICAL AND QUALITY PROPERTIES OF RICE BASED BATTERS AND MUFFINS

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Food Hydrocolloids

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Abstract

The incorporation of proteins has been long established in the bakery industry to obtain enriched products, but they also take active part on the making process of sweet baked goods. This study was focused on assessing the role of proteins on the rheology and quality of wheat free muffins by using rice flour. Six rice based formulations were used: without added protein (Control) and with different protein sources: soy protein isolate (SPI), pea protein isolate (PPI), egg white protein (EWP), casein(C), and for comparing purposes vital wheat gluten (VWG) was included. Proteins effects were established by evaluating the rheological behaviour of batters measuring the storage modulus (G') and the loss modulus (G''), and the technological characteristics of the muffins obtained (specific volume, colour, and texture). The overall results indicated that both the rheological properties of the batters and the technological characteristics of the muffin are dominated by the presence of the type of protein used in the formulations. The addition of SPI, PPI and C significantly ($P<0.05$) increased G', but it was not modified in batters containing EWP. Casein and EWP increased the specific volume of the muffins. SPI did not have effect on hardness, springiness, cohesiveness, chewiness, and resilience of the muffin, while PPI containing muffins were softer and springier. This study has allowed the development of whole egg-free and milk-free muffins; however the optimization of this type of formulations is fundamental to ensure the proper texture and good taste of these products.

Keywords: Gluten-free; Muffins; Rice flour; Protein sources; Batter, Rheology; Quality**.**

1. Introduction

Muffin is a popular breakfast or afternoon snack food, which is sold in many bakeries. Muffins are sweet, high-calorie baked products highly appreciated by consumers due to their good taste and soft texture. Muffins batter is a complex fat-in-water emulsion composed of an egg-sugar-water-fat mixture as the continuous phase and bubbles as the discontinuous phase in which flour particles are dispersed. Muffins are characterized by a typical porous structure and high volume, which confer a spongy texture. To obtain such a final structure, a stable batter lodging many tiny air bubbles is required (Martínez-Cervera, Sanz, Salvador, & Fiszman, 2012). Therefore, a large number of small cells provide high volume if the continuous phase of the batter is capable of retaining them during the baking process (Gómez, Ronda, Caballero, Blanco, & Rosell, 2007).

Traditionally, muffins recipe is mainly based on wheat flour, sugar, vegetal oil, egg and milk (Sanz, Salvador, Baixauli, & Fiszman, 2009). For this reason, persons with celiac disease (CD) are unable to consume this type of baked product since they are made with wheat flour. Gluten-free products were initially designed for people who have celiac disease. Nevertheless, there is an increasing number of people interested in wheat-free foods motivated by health concern or because they want to avoid wheat in the diet. However, the manufacture of baked goods products without gluten results in major technological problems for bakers. In fact, many gluten-free products available on the market are often of poor technological quality, exhibiting low volume, poor colour and crumbling crumb, besides great variation in the nutrient composition, with low protein and high fat contents (Matos & Rosell, 2011), particularly when compared to their wheat counterparts (Mariotti, Lucisano, Pagani, & Ng, 2009). Like bread, gluten-free muffins, cakes and other gluten-free baked goods have been commercially manufactured trying to resemble those made from wheat flour. However, these types of gluten-free baked products often present quality defects and low nutritional value.

Consumers adhered to gluten free products demand having gluten free counterparts for all the gluten baked goods. As consequence, in recent years, there has been extensive research for the development of gluten-free sweet bakery products aimed to improve the structure, mouth feel, acceptability, shelf-life and nutritional quality of the finished products (Turabi, Sumnu, & Sahin, 2008a,b; Gularte, de la Hera, Gómez, & Rosell, 2012a,b; Park, Ha, & Shin, 2012). The gluten-free muffins, cake or cupcakes recipes contain rice flour as principal ingredient (Turabi et al., 2008a,b; Gularte et al., 2012a,b; de la Hera, Martinez, Oliete, & Gómez, 2012; Park et al., 2012), or different starches sources, such as rice, corn, potato and wheat (Ronda, Oliete, Gómez, Caballero, & Pando, 2011). Additionally, other ingredients such as sugar, egg white powder or egg white liquid, milk, baking powder, salt, vegetal oil, hydrocolloids and emulsifiers, can be incorporated on their formulations to improve the final quality product (Turabi et al., 2008a,b; Ronda et al., 2011; de la Hera et al., 2012; Park et al., 2012).

It is well known that rice (Oryza sativa L) is seek out by people adhered to gluten-free diet, because it contains neither gluten nor gliadin and that makes rice hypoallergenic and easily digestible (Marco & Rosell, 2008). Rice also has low levels of sodium, fat and protein, and rice flour milled from white rice is very bland in taste. Thus, rice is one of the most convenient cereals in designing gluten-free products. However, regardless of its numerous advantages, rice flour shows an important drawback from the technological point of view, since its proteins do not develop the appropriate viscoelastic network necessary to retain gas produced during the fermentation process, resulting in low-quality products (Marco & Rosell, 2008). Therefore, some food additives such starch, gums, hydrocolloids, and dairy products are required for obtaining high volume, desired texture, colour, and crumb structure (Turabi et al., 2008b). The incorporation of dairy proteins has been long established in the bakery industry, but legumes such as soybean, can be also a good supplement for cereal based foods since they increased the protein content and complement the nutritional value of cereal proteins (Mariotti et al., 2009; Ronda et al., 2011; Gularte et al., 2012b). However, nutrition is not the only aim when adding proteins; they play a functional role, especially in muffins. In fact, Geera, Reiling, Hutchison, Rybak, Santha and Ratnayake (2011), when looking for egg replacers in wheat muffins, stated that egg is a critical ingredient in the muffins formulation to obtain expected product quality characteristics. Partial replacement of egg with commercial egg replacer changed product characteristics altering moisture retention, bulk volume, colour, texture and flavour, although those differences were not readily detected by sensory panellist.

Lots of people are strict vegans or vegetarians, and many others choose simply not to eat eggs, for myriad reasons – health, culture/religion, and likings. For this reason is also recommended the use of other proteins as egg substitutes when making muffins. Actuality, food manufacturers are seeking for alternatives to egg in baked product formulations to reduce production cost and to make health claims, such as to reduce fat and cholesterol content, and protein allergies. Most widely used egg replacers are whey protein isolates, soy ingredients, wheat gluten and different types of gums to obtain specific properties in targeted products (Geera et al., 2011). In general, research has been focused in the study of the effects of some proteins sources, usually of vegetal origin, on the technological or nutritional quality of the finished product. However, studies focused on the fundamental role of the proteins, as ingredients in the conventional muffins formulation and on the properties of the gluten-free muffins have not been found in the published literature.

The use of vegetable or animal proteins for total substitution of egg and milk in gluten-free-muffins formulations could be an interesting alternative, especially considering that there are people with specific dietary needs or retractions, as is the case of persons with celiac disease; vegans, vegetarian or high cholesterol people. For this reason, the present work was focused on the study of the role of proteins in the rheology and quality of muffins by using rice flour, in order to scientifically develop gluten free products. For other hand, the Rheometer was used to obtain a complete characterization of the gluten-free batter behaviour by recording the mechanical changes during mixing and heating simulating the mechanical work as well as the heat conditions that might be expected during the baking process.

2. Materials and Methods

2.1. Materials

Commercial rice flour was supplied by Harinera Derivats del Blat de Moro, S.L. (Parets del Vallés, Spain) had moisture and protein contents of 12.19 g/100g and 7.22 g/100g, respectively. Five commercial protein sources (all in dry powder form) were employed. Soybean protein isolate (Vicoprot) was from Trade, S.A (Barcelona, Spain). The soybean protein isolate had moisture and protein contents of 9.25 and 80.49 g/100g, respectively. Pea protein isolate (Pisane C9) from Cosucra Group Warcoing (Warcoing, Belgium) had moisture; protein contents 4.45 g/100g and 77.85g/100g, respectively. Vital Wheat Gluten from Roquette (Keokuk, IL) had moisture, protein contents 9.23g/100g and 72.4 4 g/100g, respectively. Casein from Cargill (Spain) had moisture and protein contents of 5.43g/100g and 84.54 g/100g, respectively. Egg white protein (EWP) from EPSA Aditivos Alimentarios (Valencia, Spain) had moisture, protein contents 6.83 g/100g and 79.38 g/100g, respectively. Composition of the different ingredients was determined following the AACCI Approved Methods (2000). Xanthan gum (Satiaxane CX-91) food grade was supplied from Cargill (Spain). Sodium bicarbonate and citric acid were purchased from Martínez SA (Valencia, Spain). Refined

sunflower oil was acquired from Coosur (Jaen, Spain). Sugar and salt were purchased from local market. All reagents were of analytical grade.
Batters containing both rice flour and different vegetal protein sources (VPS): Vital wheat gluten (VWG), Soy protein isolate (SPI) and Pea protein isolate (PPI); and batters containing rice flour and different animal protein sources (APS): Egg white protein (EWP), and Casein(C) were prepared.

2.2 Methods

2.2.1 Batter preparation

Rice flour-based batters were prepared without protein source (Control) and with five different protein sources: Vital wheat gluten (VWG), Soy protein isolate (SPI), Pea protein isolate (PPI), Egg white protein (EWP), and Casein (C). The ingredients (g/100g flour) used in the preparation of the muffins batters were based on traditional Spanish recipe. The amount of added protein was calculated based on the percentage of protein provided by both milk and egg in the traditional formulation (13%). On the other hand, it was considered a contribution of 75% of protein for the selected protein sources. In this way, the amount of protein that should be added to each formulation was obtained [(13 x 100)/75 = 17.3 g]. In addition, this amount of added protein kept the same solid content in all formulations. The samples were identified as Control, VWG, SPI, PPI, EWP, and C, according to the type of protein added.
The rice flour-based batters were prepared by the modified method of Sanz et al. (2009). The formulation of batters included 114.4g rice flour

(10.8% moisture, 6.69 % protein); 100g water; 17.3 g protein added (75% protein); 75g sugar; 46g refined sunflower oil; 4g sodium bicarbonate; 3g citric acid; 1.5g salt; 0.5g xanthan gum.

The batters were prepared in a mixer (Kenwood major Classic, UK), in which the rice flour, protein (depending on the formulation), sodium bicarbonate, sugar, citric acid, salt and xanthan gum, were incorporated in the first place, and sunflower oil was gradually dripped in; finally the water was added. The batter was beaten for 10 min to speed 4 (380 rpm) until smooth. The batter was used for both the rheological test and to prepare the gluten-free muffin. Each formulation was prepared twice (two replicates), on different days.

2.2.2. Batter properties

The specific gravity (SG) of batter was measured as the ratio of the weight of a standard container filled with batter (W2) to that of the same container filled with water (W1). Two different batches were employed and each formulation was measured in triplicate.

The rheological behaviour of the batter was evaluated. Properties of the rice flour-based batter were studied using an AR G2 controlled-stress rheometer (TA Instruments, Crawley, UK). The batters were all kept at 25°C for 60 min after batter preparation before the rheological test. The samples were allowed to rest in the measurement cell for 5 min as stabilization time. Parallel plate geometry (60 mm diameter) with 1 mm gap between the plates was employed.

Apparent viscosity at 25ºC was measured as a function of shear rate over the 0.01 to 100 s^{-1} range for 5 min; 100 points with a logarithmic distribution were recorded. Two replicates of each flow curve were run with samples prepared on different days. The flow curves were adjusted to the Oswald model: $\eta = K\dot{Y}^{n-1}$. Where η is the apparent viscosity, K is the consistency index, gamma dot ($\dot{Y}$) is the shear rate and "n" is the flow index. Results are means of two replications from different batches of each formulation.

An oscillatory stress sweep was made at a constant frequency of 1 Hz over an oscillatory stress range of 1.0×10^{-3} to 20 Pa for each batter sample. Frequency sweep test was performed from 0.01 to 10 Hz at a constant oscillatory stress within the linear viscoelastic range at 25°C. The oscillatory stress applied was selected to guarantee the existence of a linear viscoelastic range of each batter sample. The applied oscillatory stress varied among formulations and was between 0.12 and 0.32 Pa. To study the effect of heating in the batter structure, temperature sweeps were performed from 25°C to 95°C at a heating rate of 1.0°C/min and a constant strain. The strain applied was selected to guarantee the existence of linear viscoelasticity along the complete temperature range according to previous stress sweeps. The applied strain varied from 1.0×10^{-4} to 3.8×10^{-4}, depending on the specific batter sample. Vaseline oil (Panreac, Spain) was applied to the exposed surfaces of all the samples, in order to prevent evaporation during the measurements. The storage modulus (G'), loss modulus (G''), phase angle, and loss tangent (tanδ), were measured. Three replicates of each test were run with samples prepared on different days. Results are means of three replications from different batches of each formulation.

2.2.3. Rice flour-based muffins preparation

Rice flour-based muffins were prepared according to methods described by Sanz et al. (2009). Muffins without added protein (Control) and with different protein sources (VWG, SPI, PPI, EWP, and C) were prepared from the gluten-free muffin batters. The batter was poured into a dosing machine (Edhard Corp., Hackettstown, USA). Quantity of batter dispensed was of 65.0±0.2 g in each 60 mm diameter and 36 mm muffin paper cups. Twelve cups were arranged in three rows of four in a baking tray and baked for 20 min at 180 ºC in a conventional electric oven (Fagor Elegance 2H-114B, Guipúzcua, Spain) that had been preheated to this temperature for 10 min. The oven, the tray and the tray position in the oven were identical in each case.

The muffins were left to cool down at room temperature for 1h on rack. Then, they were packed in polypropylene bags (O_2 permeability at 23°C = 1650 cm^3/m^2.day; water vapour permeability at 38°C and 90% humidity = 9 g/m^2 day; thickness=65μm) and stored at 20ºC for 1day, until determinations were conducted. The muffins from each formulation were prepared twice, on different days, with 12 muffins in each batch.

2.2.4. Rice flour-based muffins properties

Samples were directly milled prior analytical determinations. Determinations were done in triplicate for obtaining mean values. The moisture and protein content were determined according to ICC corresponding standard methods (ICC, 1994). The muffins were weighed before baking (W3) and after baking and 1-h cooling (W4). The weight loss upon baking was calculated (W3-W4). Height was measured with a digital calliper from the highest point of the muffin to the bottom of the paper cup after cooling for 1-h cooling at room temperature. Volume was determined by rapeseed displacement. Specific volume of individual muffins was calculated by dividing volume by weight. Images of the muffins were captured using a flatbed scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP Scanjet 4400C, Hewlett–Packard, USA). Values were the mean of at least three replicates for each formulation.

A Konica Minolta CM-3500 spectrocolorimeter was used to measure the crumb colour parameters (L^*, a^*, b^*) of the muffins. The results were expressed in accordance with the CIELAB system (D65 illuminant and 10° viewing angle). The measurements were made with a 30 mm diameter diaphragm inset with optical glass. The parameters measured were L^* (L^*= 0 [black], L^*=100[white] indicates lightness, a^* indicates hue on a green ($-a^*$) to red ($+a^*$) axis, and b^* indicates hue on a blue ($-b^*$) to yellow ($+b^*$) axis. Additionally, hue or hue angle (h) and Chroma (C^*) values were obtained. Hue angle is the angle for a point calculated from a^* and b^* coordinates in the colour space. Chroma is the quantitative component of the colour, which reflected the purity of colour in the CIELAB space. The muffins were cut in half on a plane parallel to its base and the colour of crumb was measured at several points on the cut surface. Data from three slices per sample were averaged.

The instrumental texture measurements of the muffin samples were made with a TA.XT.plus Texture Analyzer (Stable Microsystems, Godalming, UK) provided with Texture expert software. The muffins were cut horizontally at the height of the cup, the upper half was discarded and the 1.5 cm high lower halves were removed from paper cup. A double compression test (texture profile analysis) was performed with a 75 mm diameter flat-ended cylindrical probe (P/75) and compression to 50% of the initial height at a speed of 1 mm/s with 5s waiting time between the two cycles. The parameters obtained from the curves were hardness, springiness, cohesiveness, chewiness, and resilience. Values were the mean of at least three replicates for each formulation, which were prepared twice (two batch), on different days.

2.2.5. Statistical analysis

For each parameter evaluated, a one way analysis of variance (ANOVA) was applied using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). Fisher's least significant differences (LSD) test was used to assess significant differences ($P<0.05$) among samples that might allow discrimination among them.

3. Results and Discussions

To determine the role of proteins in gluten free batters and muffins making, several proteins from different sources were selected and wheat gluten was used for comparison purposes.

3.1. Effect of protein source on specific gravity, flow properties and dynamic viscoelastic properties of rice flour-based batters

3.1.1. Specific gravity

According to the ANOVA results, it was observed that SG was significantly affected ($P<0.05$) by the protein type (Table 1). A significant increase in SG was found with VWG, SPI, PPI, and C batter formulations when compared with control (without protein added). This increase in SG implies a reduction in the retained amount of air into those batters and the intensity of this effect depended on the protein source. Batters containing SPI and PPI did not show significant differences between them, indicating a similar behaviour in relation to the retention of air of these batters. Nature of the leguminous proteins might be responsible of that effect. The highest SG value was obtained in the batter prepared with casein protein (C). On the contrary, batter in presence of egg white protein (EWP) had the lowest SG of all the batters, which showed that more air was incorporated and retained during mixing. In general, lower batter density means high air quantity into batter (Turabi et al., 2008; Ronda et al., 2011; Martínez-Cervera et al., 2011). Although EWP and C are from animal origin, they showed a completely distinct effect. Conversely VWG, PPI and SPI all from vegetable sources, showed similar effect on the SG. Differences observed ascribed to the protein origin could be attributed to the functional properties of the proteins, like emulsifying activity or foam stability. The

Table 1. Consistency index (*K*), flow behaviour index (n) and specific gravity (SG) for muffin batters prepared with different protein sources

Sample	*K* (Pa.s^n)				n				SG (g/mL)			
Control	46.51	±	3.23	a	0.36	±	0.01	a	1.03	±	0.01	b
VWP	73.45	±	1.86	b	0.37	±	0.00	a	1.05	±	0.01	d
SPI	122.32	±	11.5	c	0.41	±	0.00	b	1.04	±	0.01	c
PPI	166.10	±	7.04	d	0.39	±	0.01	b	1.04	±	0.01	c
EWP	52.78	±	4.28	a	0.41	±	0.01	b	0.97	±	0.00	a
C	nd				nd				1.08	±	0.00	e
P- value	0.0000				0.0003				0.0000			

Means ± standard deviation values followed by different letters within a column denote significantly different levels (P<0.05) (n=4); n.a.: not available
Control: rice flour; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein

presence of egg albumen or whey proteins increased the emulsifying activity of rice flour, while pea and soybean proteins hardly modified this parameter. The stability of the emulsion significantly decreased when egg albumen and whey proteins were present (Marco & Rosell, 2008).

3.1.2. Flow behaviour

The viscosity versus shear rate values showed a shear thinning (pseudoplastic) behaviour for all batters, which means that the apparent viscosity decreases as the shear rate increases. Experimental data in the studied shear rate range provided a good fit (R^2= 0.9939-0.9987) for the Ostwald model (Table 1). Flow index (n) of all batters ranged from 0.36 to 0.41. Control and VWG batters did not show significant differences between them, whereas SPI, PPI and EWP batters increased the n values significantly (P<0.05) (values closer to 1). The addition of casein produced a large increase in the batter viscosity, because of that the viscosity versus shear rate values are not available.

The consistency index (*K*), was significantly affected (P<0.01) by the type of protein source. For the vegetal proteins, higher increase in *K* was found when compared with the control batter. The presence of PPI increased the batter consistency, with a more noticeable increment than the one observed with SPI; while the batter prepared with EWP showed similar consistency than the batter made from rice flour (control). Similarly, other authors have found an increase in the consistency of layer cake batter with proteins and this effect was more evident in the presence of soy protein isolate than when using wheat protein (Ronda et al., 2011). In our study, the increase in *K* in the presence of pea and soybean protein isolates could be attributed to their high water binding capacity (data not showed). It has been reported that the increase in the water binding capacity of ingredients reduces the amount of free water available to facilitate the movement of particles in batters and consequently it gives high apparent viscosity values (Ronda et al., 2011).

3.1.3. Batter Viscoelastic behaviour

The viscoelastic properties of the rice-based muffins batter containing different protein sources were studied by dynamic oscillatory test. The mechanical spectra of all the batters (Figure 1 and 2) revealed the typical behaviour of soft gels with values of the storage modulus (G') higher than the values of loss modulus (G'') and slight dependence of both moduli with frequency (Figure 1). The same behaviour has been described for rice dough containing different protein isolate (Gujral & Rosell, 2004). Marco and Rosell (2008) reported that the mechanical spectra of rice flour dough samples (without and with protein isolate) showed G' values higher than G'' at the frequency range tested (0.1-1 Hz), suggesting a viscoelastic solid behaviour of the dough.

The addition of the proteins affected the batter viscoelastic behaviour and the extent of the effect was protein source dependent. The presence of all vegetable proteins modified the elastic and viscous component of the rice-based muffins batter, inducing a hardening effect (increase in G' and G'') on the batters. Batters containing PPI and SPI showed the highest increase in G′ and G″ values, whereas VWG batter only showed values

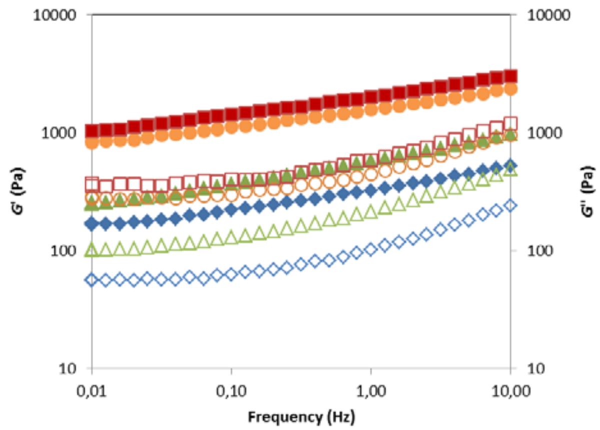

Figure 1. Dynamic mechanical spectra of different rice based batters. Control (♦) and various vegetal protein sources (▲VWG; ● SPI; and ■ PPI) measured 25°C. Closed symbols referred to storage modulus (*G′*) and open symbols designated loss modulus (*G″*).

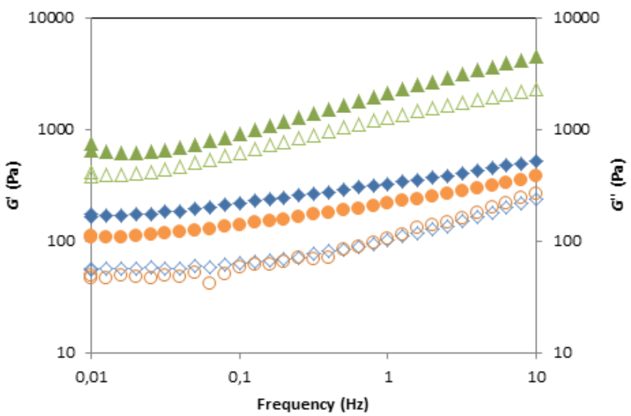

Figure 2. Dynamic mechanical spectra of different rice based batters. Control (♦) and various animal protein sources (● EWP and ▲C) measured at 25°C. Closed symbols referred to storage modulus (*G′)* and open symbols designated loss modulus (*G″).*

of G' and G'' slightly higher to those obtained with the control batter. Therefore, leguminous proteins induced a major hardening effect on the batter structure.

The animal proteins also modified the dynamic mechanical spectra of the rice based muffin batter, with a clear different trend between egg white powder and casein (Figure 2). The addition of casein induced a very noticeable change in the batter viscoelastic behaviour. In C batter both moduli showed higher frequency dependence than in the control and EWP batters. Also the predominance of G' over G'' was lower in the C batter indicating a more viscous and less elastic behaviour of this batter in comparison to control and EWP. However, values of both moduli in the C batter were higher than the control.

Viscoelastic data at a frequency of 1 Hz were submitted to analysis of variance to determine the main effects of the protein isolates on viscoelastic properties of rice based muffin batters (Table 2). The presence of the different protein types significantly ($P<0.05$) changed the viscoelastic properties of the batter. As already mentioned, values of G' were always higher than values of G''. The presence of SPI, PPI and C, significantly ($P<0.05$) increased the G' modulus, and the other proteins tested did not modify it. The extent of the effect of the added protein was greatly dependent on the nature of the added protein. Batters containing vegetable proteins had higher G' value, although in the case of cereal protein it was not significant, indicating similarities between the gluten protein and the rice proteins. The presence of leguminous proteins induced a large increase of the G′ modulus, being higher with PPI. Those

Tabla 2: Viscoelastic parameters at 25ºC and 1 Hz (6.28 rad/s) of muffin batters prepared with different protein sources

Sample	*G′* (Pa)	*G″* (Pa)	*G** (Pa)	Loss tangent	Phase angle (°)
Control	294.40 ± 52.34 ab	101.86 ± 20.71 a	311.53 ± 56.21 ab	0.34 ± 0.01 b	19.02 ± 0.59 b
VWG	579.60 ± 16.96 b	216.27 ± 8.82 a	618.63 ± 19.01 b	0.37 ± 0.00 c	20.46 ± 0.23 c
SPI	1576.67 ± 101.30 c	446.70 ± 12.73 b	1638.67 ± 99.29 c	0.28 ± 0.01 a	15.85 ± 0.78 a
PPI	2020.50 ± 105.36 d	587.75 ± 24.68 b	2104.50 ± 108.19 d	0.29 ± 0.00 a	16.22 ± 0.16 a
EWP	233.53 ± 30.30 a	98.81 ± 16.21 a	253.57 ± 34.19 a	0.42 ± 0.01 d	22.88 ± 0.68 d
C	2086.00 ± 347.39 d	1284.00 ± 185.95 c	2450.00 ± 392.17 d	0.62 ± 0.02 e	31.67 ± 0.74 e
P- value	0.0000	0.0000	0.0000	0.0000	0.0000

Means ± standard deviation values followed by different letters within a column denote significant differences ($P<0.05$) (n=4).
Control: rice flour; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein

results agree with Ronda et al. (2011) and Marco and Rosell (2008).

Regarding animal proteins, C induced a significant increase of G', whereas it was not significantly modified by EWP. The same tendency was observed for the G''. Complex modulus (G^*) significantly increased due to the addition of proteins, and it showed the same trend observed in G', indicating low contribution of the viscous component (G'') to the viscoelastic properties of the batter systems.

The loss tangent (tanδ) was also significantly ($P<0.05$) modified by the presence of the protein isolates. Considering that all batter showed $G'>G''$, the loss tangent was lower than 1. Both animal proteins significantly decreased the batter viscoelasticity (values of tanδ closer to 1), being the effect much more evident for casein. Contrarily, the vegetable proteins, SPI and PPI induced a significant reduction in the loss tangent with no significant differences between them.

Therefore, EWP and specially C led to structures with less solid like character than the rice batter alone, whereas leguminous protein isolates led to more structured and solid like (lower tanδ) batters. In general, bread dough, even those gluten-free which are usually more fluid, showed tanδ values lower than 1. In cake batters made of wheat flour, also values of tanδ lower than 1 has been reported (Baixauli, Sanz, Salvador, & Fiszman, 2007). The presence of protein in layer cake batter decreased significantly the loss tangent, with a major diminution when using the SPI than the wheat protein (Ronda et al., 2011). In all batters evaluated, phase angle was lower than 45°, which indicates that the material behaves more like a solid (Rosell & Foegeding, 2007). SPI and

PPI batters showed the lowest values of the phase angle, without significant differences between them. Nevertheless, the presence of the other protein significantly (*P*<0.05) increased the phase angle, with a major increase in the batter containing C (31.67), reflecting, as already mentioned that in the presence of casein the rice based batter increases its viscous component.

3.2. Effect of protein source on the viscoelastic properties of batters during heating

In order to understand the effect of protein type in the changes occurred during the thermal treatment of the rice-based batters, the viscoelastic properties were studied during the application of a temperature sweep. The storage modulus (*G′*) values during heating from 25 °C to 95°C are shown in Figures 3 and 4.

The presence of vegetable proteins produced changes in the slope of the heating curves that have been associated with starch gelatinization and protein coagulation processes in different muffin batter formulas (Martinez-Cervera et al., 2011; 2012). As expected, control batter exhibited an early onset of starch gelatinization (61-78 °C). A similar behaviour was displayed by the batter containing gluten protein, but in this case the onset of gelatinization was reached in the range 70 and 83°C. It is well known that the gelatinization of rice starch occurs at around 70-71°C; while the protein denaturation occurs at temperature above 60 °C, depending of each protein type. Rosell and Foegeding (2007) reported that when heating gluten a decrease of G′ is produced,

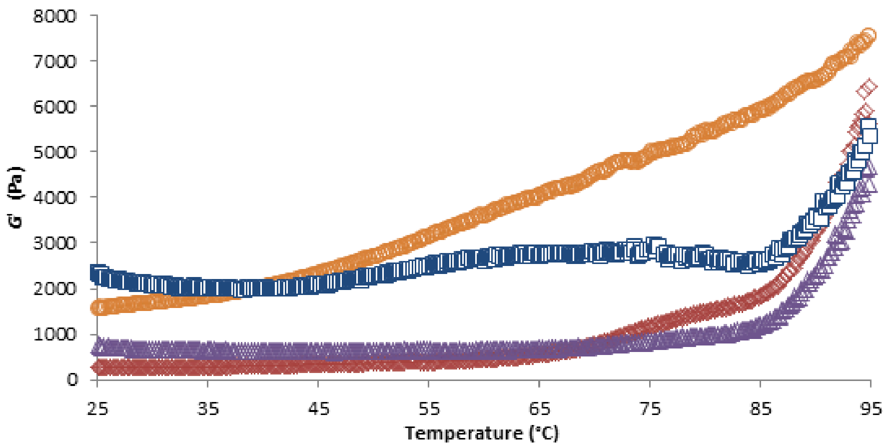

Figure 3. Storage modulus (*G′*) as a function of increasing temperature in different rice flour batters. Control (◊) and various vegetal proteins (Δ VWG ;○ SPI; and □ PPI)

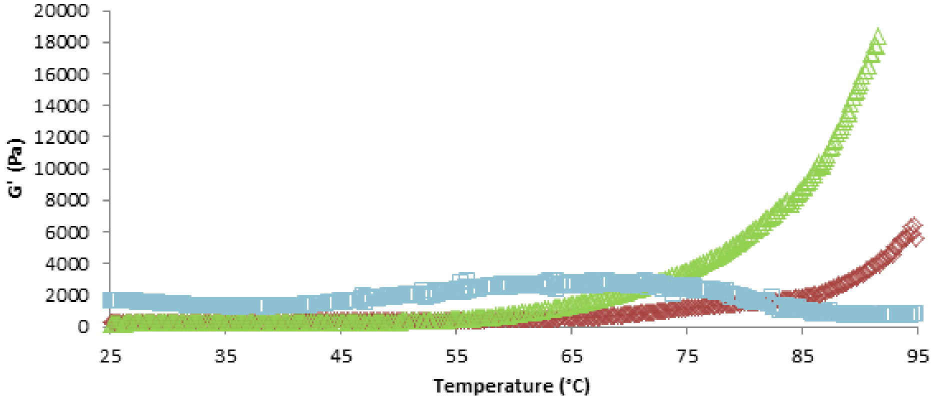

Figure 4. Storage modulus (*G′*) as a function of increasing temperature in different rice flour batters. Control (◊) and various animal proteins (Δ EWP and □ C).

reaching a minimum at 57°C, and further increase of the temperature induced the formation of a more elastic gluten network, as indicated the increase of *G′*. These authors explained that gluten proteins show a progressive loss of strength due to protein unfolding, resulting in a decrease of the elastic modulus and undergoes a thermal transition around 60°C.

It is well known that rheological behaviour is directly related to food formulation. In this study, the conformational changes experimented by both the rice starch and the added proteins were largely responsible for the predominant elastic behaviour of the batters. The addition of wheat proteins did not drastically affected the rheological properties of the batter at temperatures lower than 70°C; however at higher temperatures that batter showed less elastic behaviour, reflecting the development of hindered rice starch three-dimensional internal structure. Additionally, the underlining phenomena that determine the observed reduction in rigidity would be the dissociation and denaturation of the proteins (Sorgentini, Wagner, Arrese, & Añón, 1991) in this case wheat protein, and consequently the formation of a less elastic protein network (Kokini, Cocero, Madeka, & de Graaf, 1994). The starch dilution effect also would explain the storage modulus decrease of the batter containing gluten protein.

SPI batter showed a progressive increase of *G′* as the temperature rises, indicating the formation of a more rigid network (Figure 3). In general, *G′* increased with SPI, which can be associated with the development of an internal SPI structure. The heating of SPI dissociated the compact glycinin (11S) and β-conglycinin (7S) oligomers into monomers and therefore, the hydrophobic group are exposed (Tseng, Xiong, & Boatright, 2008), leading to an aggregation process and later the formation of a gel. Particularly, in this curve was not detected any point of inflection, probably the commercial SPI used could be greatly denatured, which allows greater capacity for interaction within active groups that may be present in the system. Thus, the denaturation of the proteins produced the formation of high molecular weight aggregates that are capable of forming strong elastic gels, reflected in the progressive increase of G'.

In regard to PPI batter, the thermal profile revealed different stages (Figure 3), in which G' upward or downward were detected along the temperature increase. The different stages observed could be indicating the effect of the distinct protein fraction present in the pea protein isolate, since they have different structures, molecular properties and different functional properties. Pea proteins, similarly to soybean proteins, are mainly storage proteins comprised of albumins and two globulins (11S and 7S). The globulins (>80% of total proteins) consist of legumin, vicilin and convicilin. Legumin is usually the major, and vicilin is the second major globulin fraction (Choi & Han, 2001; Andrade, Azevedo, Musampa, & Maia, 2010). Protein fraction 11S has greater tendency to form insoluble aggregates when it reaches an advanced extent of denaturation; instead, protein 7S can form this type of aggregate even if it is not completely denatured. It is stated that protein 7S has higher tendency to aggregated than protein 11S (Sorgentini et al., 1991). Batter containing PPI showed a marked inflection peak around 88°C, which could be associated with the pea protein coagulation, which ranged from 88.9 to 94.5°C (Choi & Han, 2001). The results indicate that the behaviour of batter containing mainly SPI and PPI is notably dominated by the presence of the protein network. Though both SPI and PPI are leguminous proteins, these proteins yielded different response on heating, likely due to the distinct thermal stability of the protein fractions (Sorgentini et al., 1991; Sirtori, Isak,, Resta, Boschin, & Arnoldi, 2012).

The animal proteins also influenced the development of storage modulus of the rice-based muffin batters (Figure 4). At 25°C only the batter containing casein, showed G' values higher than those obtained in the control batter. EWP containing batter showed similar trend than control at temperature lower than 65°C and a rapid increase was observed from 84°C until the end of the experiment, indicating the formation of a more rigid network. This increase might result from the progressive formation of higher molecular weight products (Kokini et al., 1994). The thermal profile revealed that, again the process of protein denaturation governs the evolution of the storage modulus. Egg white contains as many as 40 different proteins, among them; the major proteins imparting functionality are ovalbumin (54%), conalbumin (12%), ovomucoid (11%) and lysozyme (3.5%). Ovalbumin is the main constituent responsible for the egg white functionality. It has been reported that, the denaturation temperature of ovalbumin is close to 84°C, while conalbumin (ovotransferrin) denaturation occurs about 60°C and the denaturation temperature of lysozyme is around 70-75°C (Arzeni, Pérez,

& Pilosof, 2012). Therefore, the changes observed in G' behaviour clearly can be associated with the coagulation phenomena of the different egg white proteins. Regarding to batter containing casein, it showed a completely different behaviour. As heating progresses, the storage modulus value rose until approximately 70°C, where a maximum was detected, then decreased rapidly indicating that the structure was highly prone to weakening. Clearly, in this case, the presence of denatured casein could be inducing a drastic effect on the structure of the batter, yielding a weak gel. However, G' has a plateau value from 85°C until the end of the experiment, indicating that the gel structure behaves stable in this temperature range.

3.3. Effect of protein source on quality characteristics of rice flour-based muffins.

3.3.1. Protein and moisture contents of the gluten-free muffins

As it was expected, the addition of the different protein sources increased the protein content of the muffins. Muffins containing SPI, EWP and C showed the highest protein content (11.55 g/100g, dm); VWG and PPI containing muffins had 10.43 and 10.96 g/100g dm, respectively. Significant differences were also observed in the moisture content of the muffins (results not showed).

3.3.2. Height, weight loss, and specific volume

Rice flour-based muffins obtained from different recipes presented important differences in relation to height, weight loss, and specific volume (Table 3). Muffin height was significantly (*P*<0.001) affected by the protein type. The biggest effect on height was found with EWP, which caused a significant increase in this parameter. The incorporation of proteins did not significantly affect the weight loss parameter, with exception of the decrease observed when casein was added, indicating that casein containing muffin was more capable of binding water during baking. The control sample and the muffins containing vegetal protein source (VWG, SPI and PPI) did not differ significantly (*P*<0.01) in specific volume. Conversely, muffins with the highest specific volume were those prepared with animal protein sources, and the greatest effect was observed with EWP, likely due to that more air was incorporated and retained during mixing and baking. Geera et al. (2011) reported that muffins made with dry whole egg formulation had the highest height and volume and the lowest density. Park et al. (2012) found that the specific volume of the rice cupcakes ranged from 2.97 to 3.25 mL/g; while Turabi et al. (2008a) found specific volume ranged from 1.08 to 1.66 mL/g in rice cake formulated with different gums and an emulsifier blend. In another study, Gularte et al. (2012b) found that the incorporation of legume flour (chickpea, pea, lentil and bean) did not significantly affect the weight loss of the cake; but with the exception of chickpea cake, all legumes flour increased the specific volume. Ronda et al. (2011) evaluated layer rice cake made with SPI and wheat protein reporting that SPI did not modify volume but wheat proteins improved volume.

Table 3. Physical characteristics of protein enriched muffin prepared with different protein sources

Sample	Height (mm)				Weight loss (g)				Specific volume (mL/g)			
Control	37.10	±	1.08	bc	7.51	±	0.33	b	1.56	±	0.04	a
VWG	38.16	±	1.04	c	7.57	±	0.21	b	1.54	±	0.05	a
SPI	35.25	±	1.29	a	7.55	±	0.29	b	1.54	±	0.04	a
PPI	36.38	±	0.86	b	7.54	±	0.24	b	1.54	±	0.05	a
EWP	43.19	±	2.23	d	7.43	±	0.28	b	2.19	±	0.05	c
C	36.74	±	1.33	b	7.19	±	0.37	a	1.74	±	0.05	b
P- value	0.0000				0.0220				0.0000			

Means ± standard deviation values followed by different letters within a column denote significant differences ($P<0.05$) (n=6). Control: rice flour; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein.

3.3.3. Colour parameters

Results from the crumb colour parameters are presented in Table 4. The *L**, *a** and *b** values for crumb colour showed significant ($P<0.05$) differences among the different protein enriched muffins. Lightness of muffin crumb was significantly ($P<0.05$) decreased by VWG, SPI, PPI and C proteins; while the EWP addition increased *L** value. The lowest *L** was obtained for PPI containing muffin, which was due to the darker colour of the protein isolate (data no showed). Consequently, the *L** values can be associated to the original colour of both rice flour and protein isolates. Colour in baked goods comes from two sources: intrinsic colour imparted by individual ingredients (Gularte et al., 2012b) and developed colour resulting from the interaction of ingredients (Acosta, Cavender, & Kerr, 2011), since the increase in temperature is not high enough to give Maillard or caramelization reactions (Gómez, Moraleja, Oliete, Ruiz, & Caballero, 2010). Regarding *a** values, all samples showed positive *a** values, indicating hue on red axis, and all were higher than those of the control, with the exception of EWP sample that showed negative *a**. The *b** scale showed positive values (yellow hue) for all samples evaluated. However, EWP muffin did not exhibite significant ($P<0.05$) differences when compared to control sample. PPI, followed by SPI showed higher *b** value than the other samples, it could be derived from the original yellowish pigment of the pea and soy protein powder added as ingredient in each formulation. Results agree with previous studies (Gómez et al., 2010; Gularte et al., 2012b). In relation to hue angle (h) and chroma (C*) colour attributes, great variation was

Table 4. Crumb colour parameters of protein enriched muffins.

	Colour parameters				
Sample	*L**	*a**	*b**	*C**	*h* (º)
Control	78.13 ± 0.59 e	0.38 ± 0.10 b	15.88 ± 0.37 a	15.88 ± 0.37 a	88.62 ± 0.34 e
VWG	73.82 ± 0.29 c	1.82 ± 0.08 d	20.29 ± 0.26 c	20.37 ± 0.26 c	84.87 ± 0.19 c
SPI	73.27 ± 0.40 b	2.57 ± 0.12 e	21.36 ± 0.36 d	21.52 ± 0.36 d	83.15 ± 0.27 b
PPI	72.83 ± 0.60 a	3.87 ± 0.30 f	26.28 ± 0.55 e	26.57 ± 0.59 e	81.64 ± 0.45 a
EWP	86.40 ± 0.30 f	-0.60 ± 0.04 a	15.66 ± 0.27 a	15.67 ± 0.27 a	92.20 ± 0.16 f
C	77.18 ± 0.48 d	0.66 ± 0.13 c	17.22 ± 0.51 b	17.23 ± 0.52 b	87.83 ± 0.37 d
P- value	0.0000	0.0000	0.0000	0.0000	0.0000

Means ± standard deviation values followed by different letters within a column denote significantly different levels (P<0.05) (n=9)
Control: rice flour; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein

observed (Table 4). All the muffins presented positive hue angle values (81.64 - 92.29°) reflecting their yellow-orange hue. Additionally, the PPI and SPI muffins increased chroma compared with all other samples, which revealed their higher purity of colour related to major intensity of the yellow component.

3.3.4. Global appearance of the muffins

Muffin images clearly revealed differences among crumb muffins samples, mainly related to shape, crumb porosity, crumb colour and degree of collapse on surface of muffins by effect of type of protein added (Figure 5). Great variation in the appearance of the crumb structure between the samples was observed. Control and VWG containing muffin showed denser matrix, indicating more compact crumb than other muffins samples. Contrarily, muffins containing EWP and C protein showed higher number of air bubbles than control, showing more spongy and light structure. Addition of casein produced muffins with stable network structure with homogeneous air cell but showed higher degree of collapse on surface, in addition these muffins showed a soft and humid appearance. SPI and PPI muffins did not show collapse during baking, but presented compact crumb.

3.3.5. Instrumental texture

The effect of protein on the texture parameters of rice flour-based muffins is shown in Table 5. According to ANOVA results, muffins differed significantly ($P<0.05$) in crumb hardness, springiness,

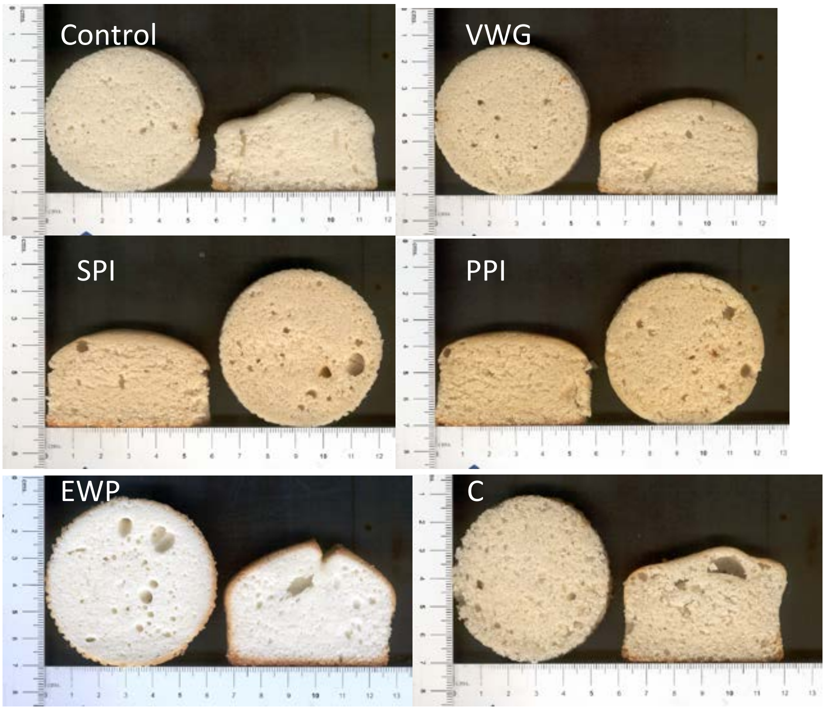

Figure 5. Photographs of cross and longitudinal sections of control and protein enriched muffins. VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; and C: casein.

Table 5. Texture parameters of protein enriched muffins prepared with different protein sources

	TPA parameters				
Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
Control	103.83 ± 10.56 ab	0.56 ± 0.04 a	0.41 ± 0.01 a	23.99 ± 2.07 a	0.19 ± 0.01 a
VWG	103.83 ± 8.00 ab	0.64 ± 0.05 c	0.46 ± 0.01 b	30.63 ± 4.29 b	0.19 ± 0.01 a
SPI	114.17 ± 14.25 bc	0.57 ± 0.04 ab	0.41 ± 0.01 a	27.07 ± 4.57 ab	0.18 ± 0.01 a
PPI	96.91 ± 15.44 a	0.61 ± 0.03 bc	0.45 ± 0.03 b	26.58 ± 4.32 ab	0.19 ± 0.01 a
EWP	113.71 ± 11.33 bc	0.82 ± 0.05 e	0.67 ± 0.06 d	62.51 ± 9.71 d	0.28 ± 0.04 c
C	122.56 ± 5.19 c	0.69 ± 0.03 d	0.49 ± 0.01 c	41.53 ± 2.67 c	0.21 ± 0.00 b
P- value	0.0013	0.0000	0.0000	0.0000	0.0000

Means ± standard deviation values followed by different letters within a column denote significant differences ($P<0.05$) (n=4)
Control: rice flour; VWG: vital wheat gluten; SPI: soy protein isolate; PPI: pea protein isolate; EWP: egg white protein; C: casein

cohesiveness, chewiness and resilience. The incorporation of protein sources increased significantly (*P*<0.05) springiness and cohesiveness of muffins samples, except with addition of SPI, which showed the same values as the control sample. The hardness significantly (*P*<0.05) increased only in presence of casein. It was also observed that hardness and chewiness showed similar trend for all samples, with exception of muffins containing EWP, which had the highest chewiness value.

In general, the addition of vegetal protein sources did not induce a clear tendency on crumb hardness. However, PPI containing muffins showed the lowest hardness, and the highest springiness value among the samples made from vegetable proteins. A significant (*P*<0.05) increase in the springiness and cohesiveness was observed in VWG and PPI containing muffins, while only the sample containing VWG showed a significant (*P*<0.05) increase in the chewiness, indicating more difficulty in chewing the sample. All muffins containing vegetal proteins showed low resilience value; however no significant differences were observed in this parameter when compared with control. Dense masses with lower number of gas cell led to lower resilience values, implying that it will take more time for the structure of the muffins to recover after compression (Martinez-Cervera et al., 2011). It has been reported that the incorporation of legumes flour (chickpea, pea, lentil and bean) significantly (*P*<0.05) increased the hardness and chewiness in rice based cakes, except with the addition of lentil (Gularte et al., 2012b).

Regarding the animal proteins, a significant (*P*<0.01) increase in the hardness was observed in C containing muffins. Additionally, a significant (P<0.05) increase in the springiness, cohesiveness, and resilience was observed in the presence of EWP and C muffins, indicating more elasticity. The increase in springiness, cohesiveness and resilience values could be also reflecting higher specific volume values, and more aerated structure, which was found for these samples. It is known that, springiness is associated to fresh, aerated and elastic product, and in the case of muffins high springiness values are linked to high quality (Sanz et al., 2009).

In general, muffins made from animal proteins were springier, more cohesive and chewy than those made from vegetal protein source. Results clearly revealed great variability on texture quality of the rice-based muffins made from different protein sources.

4. Conclusions

Results obtained allow concluding that both the rheological properties of the batters and the technological characteristics of the muffins obtained are notably dominated by the type of protein used in the formulations. All vegetal protein sources had similar effect on specific gravity of the batters, and increase the consistency index (K), while EWP decreased the specific gravity. The presence of SPI, PPI and C significantly (P<0.05) increased the storage modulus. In general, G' showed large increase with the temperature when SPI, PPI and EWP were added. These differences can be attributed to the nature and the denaturation pattern of the protein fractions comprised within each protein isolate. Regarding the muffins quality, EWG increases the height and specific volume, and muffins colour was dominated by the colour of the added proteins. Concerning texture, PPI containing muffins were the softest and springier than the control and casein gave the hardest muffin. In general, muffins with best visual appearance were those containing egg white protein or casein.

The development of whole egg-free and milk-free muffins by using different proteins as an alternative of sweet-baked gluten-free product is possible. However, it is necessary to keep in mind that the use of other proteins as egg and milk replacements, like soybean protein isolate, pea protein isolate, affects texture of baked goods. Therefore, the optimization of this type of formulations is fundamental to ensure the proper texture and good taste of these new products. Additionally, future studies will be undertaken to determine the sensory quality and consumer acceptance of gluten-free muffins.

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DISCUSIÓN GENERAL

El diseño y desarrollo de productos horneados a base de cereales sin gluten, que presenten un perfil funcional y sensorial aceptable, ha constituido una necesidad para la ciencia y la tecnología de los cereales y para el sector industrial a nivel mundial. Tradicionalmente, los productos dirigidos a la población celiaca se han diseñado atendiendo únicamente a la ausencia de alérgenos, utilizando mezclas de polímeros que pudieran originar productos con características sensoriales similares a los que contienen gluten. Sin embargo, como se ha puesto de manifiesto en la introducción de la presente tesis doctoral, el valor nutricional de los productos libres de gluten derivados de cereales ha recibido escasa atención por parte de la comunidad científica y empresas.

Las pautas terapéuticas o recomendaciones nutricionales para su tratamiento restringen la alimentación de los celiacos a productos libres de gluten. La adherencia a dietas libres de gluten afecta significativamente al consumo de productos alimenticios derivados de granos (Bardella y col., 2000). Esta afirmación adquiere grandes dimensiones cuando se considera que los productos alimenticios derivados de granos proporcionan alrededor del 30% de los requerimientos diarios de fibras, hierro, ácido fólico, niacina, riboflavina y tiamina (Subar y col., 1998). Thompson (2000) describió que los alimentos libres de gluten aportan distintos niveles de vitaminas del grupo-B, hierro y fibra dietética que sus homólogos con gluten, detectándose deficiencias en alguno de estos nutrientes (Thompson, 1999).

Por tanto, la sustitución de productos que contengan gluten por otros libres de gluten no asegura una correcta ingesta de todos los nutrientes, lo que puede provocar graves consecuencias tras periodos prolongados (Thompson y col., 2005). Investigaciones realizadas en este sentido alcanzan conclusiones contradictorias. Collins y col. (1986) describieron niveles normales en adultos celiacos. En contraposición, Mariani y col., (1998) encontraron elevados niveles de ingesta de proteínas y grasas, y bajos niveles de calcio y fibra dietética en pacientes adolescentes. Un estudio posterior realizado en Suecia con pacientes celiacos adultos concluyó que los pacientes celiacos ingieren la misma energía que la población sana, pero tienen una menor ingesta de fibras, niacina, folatos, vitamina B12, calcio, fósforo y zinc (Grehn y col., 2001). Por tanto, es necesario mejorar los hábitos dietéticos de los pacientes celiacos para asegurar una ingesta adecuada de todos los nutrientes y conocer el aporte de nutrientes que proporcionan los alimentos libres de gluten.

Dado que los productos de panadería son de consumo básico frecuente, en el presente estudio se evaluó la calidad nutricional de panes libres de gluten de origen comercial, con miras a definir sus características en cuanto al aporte nutricional que se deriva de su consumo. Para tal fin se adquirieron 11 tipos de panes sin gluten, todos provenientes de las principales marcas comerciales disponibles en el mercado español. La calidad nutricional de las muestras se estableció en términos de la determinación de la composición química, con énfasis en el contenido de proteínas, carbohidratos y grasas, y del contenido de fibra dietética. Adicionalmente se determinó la contribución del consumo de cada tipo de pan a la ingesta dietética de referencia (DRIs) de los principales macronutrientes (proteínas y carbohidratos), considerando una porción de consumo de 200g (cantidad recomendada de pan por la Organización Mundial de la Salud).

Los resultados derivados de este estudio han permitido caracterizar desde un punto de vista nutricional los diferentes tipos de panes comerciales disponibles en el mercado español, y han contribuido a corroborar la existencia, en la mayoría de los casos, de deficiencias nutricionales en términos del contenido de proteínas y/o del desbalance entre los demás nutrientes (carbohidratos, grasa y minerales). Las marcadas diferencias encontradas en el patrón nutricional de los panes libres de gluten de origen comercial, pueden estar determinadas principalmente por la diversidad de ingredientes utilizados como base en sus formulaciones (almidones y rara vez harinas, hidrocoloides, aceites y/o margarina). Lo cual conlleva a que exista entre las mismas gran variabilidad en sus características nutricionales; pudiendo encontrar tanto panes libres de gluten que tienen un contenido de proteína considerablemente alto (hasta 15g/100g) debido a la incorporación de ingredientes con alto valor proteico como soja, huevo o lupino, y otros panes que prácticamente carecen de este nutriente; pero que contienen grandes cantidades de carbohidratos (más específicamente almidón), y de grasas (hasta 26 g/100g). En general, el consumo de la mayoría de estos panes está lejos de contribuir a la ingesta recomendada de proteínas. En este estudio solamente una muestra presentó alta contribución a la IDRs para las proteínas debido a su mayor contenido proteico. Sin embargo con la excepción de dos de las muestras, todos los panes evaluados son ricos en carbohidratos, presentando alta contribución de estos nutrientes a la IDRs. Como aspecto positivo, la mayoría de los panes libres de gluten evaluados presentaron buen contenido de fibra dietética total (3,6 g/100g - 7,20 g/100g), debido principalmente a la contribución de los hidrocoloides y gomas utilizados como ingredientes en sus formulaciones.

El estudio de la digestibilidad *in vitro* del almidón permitió derivar aportes interesantes. La fracción de almidón predominante fue la del almidón rápidamente digerible (ARD), lo cual refleja la rápida degradación enzimática favorecida por el alto grado de gelatinización del almidón y la prevalencia de la estructura porosa propia de estos productos. Todas las muestras presentaron altos valores para la concentración de equilibrio (C_∞) lo cual está asociado con los altos niveles de almidón rápidamente hidrolizado, adicionalmente la constante cinética (k) mostró diferencias significativas entre las muestras, indicando la existencia de variaciones en la velocidad de la hidrólisis durante la etapa temprana de la digestión enzimática que dependen del tipo de pan. Todos los panes libres de gluten evaluados presentaron valores de IGe altos (83.3 – 96.1). El número y la variedad de ingredientes que conforman un producto son factores importantes que podrían estar determinando la digestibilidad del almidón (Bernal y col., 2002), así como su composición.

En general, muchas de las formulaciones comerciales utilizadas en la elaboración de panes libres de gluten, todavía están basadas en el uso de almidones puros y/o sus mezclas combinados con algún tipo de hidrocoloides, en consecuencia los productos finales resultantes se caracterizan por presentar bajos contenidos de proteínas, fibra y minerales, y altos contenidos de grasas y carbohidratos. Estos resultados coinciden con los obtenidos al evaluar muestras de panes libres de gluten comerciales vendidos en Italia (Pagliarini y col., 2010).

Tomando en cuenta los resultados obtenidos en la caracterización nutricional de los panes libres de gluten comerciales y principalmente en lo relativo al bajo aporte proteico que se deriva de su consumo, en la presente investigación se extendió el estudio de la caracterización nutricional a una serie de panes libres de gluten "formulados" basados en harina de arroz y enriquecidos en proteínas (aislado proteico de soja, huevo y leche entera). En general la composición química de los panes experimentales mejoró en comparación con la de los panes de origen comercial. El contenido de proteína y de grasa varió considerablemente en función de los ingredientes y de las proporciones utilizadas en cada una de las formulaciones (Tabla 1, Capítulo 2). En general los panes libres de gluten que contienen soja y huevo presentaron altos contenidos de proteína (>12 g/100g), pero el huevo incrementa el aporte de grasa. Es importante considerar que la sola incorporación de la harina de arroz en las formulaciones mejoró el balance en nutrientes de los panes, resultando en intervalos moderados de proteínas (7,6 g/100g) y de grasa (3,70 g/100g). Los resultados obtenidos para la composición química de los panes libres de gluten experimentales, reflejan claramente la influencia de los ingredientes presentes en las distintas formulaciones sobre la composición de los productos finales.

La combinación "fundamentada" de los distintos ingredientes para formular panes sin gluten permite mejorar su calidad nutricional. No obstante, muchas veces la adición de algunas proteínas y otros aditivos puede alterar tanto la textura como características sensoriales de sabor y/u olor de los panes, haciéndolos poco apetecibles a la hora de consumirlos. Este comportamiento ha sido referido también por Marco y Rosell (2008a), quienes encontraron que la incorporación de aislado proteico de soja en masas libres de gluten basadas en harina de arroz, incrementó la dureza de la miga y disminuyó el volumen específico de los panes. Por ello resulta necesaria la evaluación tecnológica de los productos resultantes.

La complejidad de las formulaciones de panes libres de gluten ha obligado a investigar las características de las masas y de los productos horneados resultantes. Sin embargo, pocos estudios han sido enfocados en evaluar la conexión entre las propiedades de la masa y las características tecnológicas del producto horneado. Por otra parte, muy pocas correlaciones han sido establecidas entre los parámetros instrumentales y los atributos sensoriales en este tipo de productos (Pagliarini y col., 2010), lo cual podría ser muy útil para definir cuáles son los mejores atributos de calidad de los productos finales. Es por ello que, como parte importante de esta investigación se realizó la caracterización tecnológica y sensorial de diversos tipos de productos libres de gluten tipo pan, comerciales y formulados, y la reología de las masas, con la finalidad de establecer posibles correlaciones entre los parámetros descriptivos de las características de los panes libres de gluten determinados a través de métodos instrumentales y del análisis sensorial, o bien para establecer relaciones entre los parámetros que permitan predecir la calidad de los panes a nivel de la masa.

Los panes libres de gluten se caracterizan por tener baja capacidad de conservar la humedad durante el almacenamiento es por ello que se determinaron las propiedades de hidratación de la miga en la caracterización de los panes. En el presente estudio se encontraron diferencias significativas (P<0,05) entre las muestras al evaluar las propiedades de hidratación de la miga. De los resultados se deriva, que las propiedades de hidratación medidas en las muestras de pan, están fuertemente gobernadas por la complejidad de las formulaciones y en particular por la presencia de diferentes tipos de proteínas e hidrocoloides (Rosell y col., 2001; Moore y col., 2004; Gallagher y col., 2004; Arendt y Moore, 2006; Lazaridou y col.,2007; Sabanis y col., 2009; Crockett y col., 2011; Houben y col., 2012), los cuales al parecer le confieren al producto final sus propiedades de hidratación y muy particularmente su alta capacidad de enlazar agua. Todas las características fisicoquímicas evaluadas discriminaron significativamente (P<0,05) entre las muestras. Tanto las muestras de origen comercial como los panes experimentales presentaron valores de volumen específico que se encuentran dentro de los rangos reportados para este tipo de productos (Sabanis y col., 2009; Marco y Rosell, 2008a). La mayoría de los panes de origen comercial presentaron valores más bajos para el contenido de humedad de la miga que los encontrados en la mayoría de las muestras experimentales. Estos resultados coinciden con los reportados por Marco y Rosell (2008a) y Sabanis y col. (2009), quienes describieron valores muy altos para el contenido de humedad en panes libres de gluten enriquecidos en proteínas y en fibras.

El color también es un parámetro importante en la caracterización de panes libres de gluten. Los parámetros de color (*L**, *a** y *b**) obtenidos para el color de la miga mostraron diferencias significativas ($P<0.05$) entre las muestras y la mayor variabilidad la presentó la luminosidad (*L**). En general los valores más bajos para el parámetro *L** fueron obtenidos en los panes provenientes de formulaciones donde uno o varios ingredientes aportaron su color natural, principalmente proteínas de soja, polvo de huevo y la goma xantana; en consecuencia estos ingredientes podrían ser los responsables de la disminución en la claridad o luminosidad de los panes. Valores más altos de *L** se observaron en aquellas muestras basadas en almidones o en harina de arroz y almidón sin la adición de huevo o soja como ingredientes. Sin embargo, el obscurecimiento del color de miga podría ser una característica deseable, ya que en general los panes libres de gluten tienden a presentar un color más claro que los panes de trigo (Gallagher y col., 2003).

Los resultados obtenidos en relación a los parámetros fisicoquímicos indicaron que las muestras de panes libres de gluten evaluadas además de discriminar entre ellas y presentar claras diferencias significativas entre los parámetros fisicoquímicos determinados, también cubren una buena gama de las características reportadas previamente en la literatura para este tipo de panes (Brites y col., 2010, Onyango y col., 2011; Sciarini y col, 2010; 2012).

La dureza de la miga es uno de los parámetros de calidad más importantes en la evaluación y caracterización de los panes libres de gluten, ya que a menudo puede representar un factor de aceptación rechazo por parte de los consumidores. En este estudio la dureza de la miga discriminó significativamente (*P*< 0,05) entre las muestras. Los resultados obtenidos mostraron considerables diferencias dependiendo del origen de las muestras y también del tipo de formulación utilizada en la elaboración de los respectivos panes libres de gluten. Los resultados obtenidos ratifican que los panes de origen comercial presentan baja calidad tecnológica, caracterizándose principalmente por presentar migas duras y secas, fácilmente disgregables y en consecuencia poco elásticas y con corta vida de almacenamiento (Moore y col., 2004; Gallager y col., 2004). En relación a los panes experimentales, la mayoría presentaron valores de dureza bajos (1,33N a 7,53N), sin embargo los valores obtenidos fueron más altos cuando se incorporó aislado proteico de soja o huevo entero en polvo en las formulaciones. En las muestras comerciales no se reflejó esta tendencia. En general las grandes diferencias observadas en las características tecnológicas de las diversas muestras evaluadas dependieron del tipo de formulación usada para la obtención de los panes libres de gluten. Tanto los panes de origen comercial, como los panes obtenidos a partir de las masas formuladas presentaron importantes diferencias en relación a color, apariencia, forma, tamaño, volumen y dureza de la miga. Resultados similares en variabilidad han sido reportados en otros estudios al evaluar la calidad tecnológica de diferentes tipos de panes libres de gluten (Kadan y col., 2001; Moore y col., 2004; Lazaridou y col., 2007; Marco y Rosell, 2008a, Pruska-Kędzior y col., 2008; Brites y col., 2010, Onyango y col., 2011; Sciarini y col, 2010; 2012; Sabanis y Tzia, 2011; Mariotti y col., 2013).

Los resultados del análisis descriptivo cuantitativo realizado para la evaluación sensorial de los panes libres de gluten, permitieron establecer que todas las muestras evaluadas se diferenciaron significativamente (P<0,05) para los atributos apariencia de rebanada, capacidad de recuperación y disgregabilidad; lo que sugiere que estos atributos particularmente podrían ser de utilidad en la caracterización de panes libres de gluten. Adicionalmente los atributos olor y color (P<0,01) permitieron diferenciar entre las muestra comerciales y gusto, color, y dureza entre las muestras experimentales. Contrariamente, no se observaron diferencias significativas (P<0,05) en la percepción del gusto, el regusto y la dureza entre las diferentes muestras comerciales; ni para el olor entre las muestras experimentales. Otros investigadores han descrito que en la evaluación de panes libres de gluten la preferencia de los consumidores tanto celiacos como no celiacos está positivamente influida por los atributos sensoriales suavidad, uniformidad en la porosidad de la miga y sabor dulce, y parcialmente por aroma y sabor a maíz (Pagliarini y col., 2010).

Algunos de los resultados obtenidos en la evaluación sensorial de las muestras comerciales presentaron discrepancias con aquellos obtenidos en la evaluación tecnológica a través de análisis instrumentales. Por un lado la dureza de la miga medida instrumentalmente presentó diferencias significativas que permitieron discriminar entre las muestras; que no fueron percibidas en la evaluación sensorial. Un resultado similar se observó en relación al color de los panes comerciales. Esto sugiere, que en panes almacenados estos dos atributos al parecer no permiten discriminar sensorialmente entre muestras.

Correlaciones significativas fueron observadas dentro de los parámetros usados para caracterizar los panes libres de gluten de origen comercial, pero estas correlaciones fueron obtenidas principalmente con los parámetros instrumentales. Fuertes correlaciones lineales fueron observadas entre los diferentes parámetros de color, pero también se observó una fuerte correlación lineal entre L^* y cohesividad ($P<0,001$) y la resiliencia ($P<0,001$). Presumiblemente, la estructura de la miga tiene gran influencia sobre las propiedades de textura y luminosidad de la miga. En tal sentido ha sido reportado que panes más pequeños resultaron más densos y con estructura más apretada de la miga, produciendo migas con mayor firmeza (Sabanis y col., 2009). Esto lleva a pensar que los panes con migas compactas podrían ser percibidos como duros. Sabanis y col. (2009) también reportaron una correlación negativa entre la firmeza de la miga y el volumen del pan (r = -0,89, $P>0,05$). En este tipo de productos, la actividad de agua muestra una significante relación positiva con el contenido de humedad. Es importante destacar las relaciones observadas entre las características de la hidratación de la miga y algunos otros parámetros, puesto que esas características no se han determinado previamente en pan. Las características de hidratación de la miga (hinchamiento, WHC y WBC) fueron significativa y positivamente relacionadas entre ellas. Por otra parte, fuertes relaciones positivas fueron observadas entre WHC con resiliencia (r<0,7020) y entre WBC con cohesividad (r<0,7633) y resiliencia (r<0,7901). Algunas relaciones entre los parámetros sensoriales y los parámetros instrumentales resultaron significativas, aunque los coeficientes de correlación fueron bajos, indicando correlaciones lineares muy débiles o bajas ($r \leq 0,35$), probablemente debido a la complejidad de las formulaciones.

Adicionalmente se encontraron relaciones altamente significativas (P<0,001) y positivas entre la dureza-TPA y los parámetros reológicos de las masas libres de gluten caracterizadas mediante el Mixolab. Esto podría indicar que los valores de dureza-TPA están fuertemente correlacionados (r>0,70) con parámetros que caracterizan el comportamiento durante el enfriamiento tanto de almidones como de proteínas. Es importante destacar que las características de la viscosidad de masas de trigo determinadas con el Viscoanalizador Rápido (RVA) también han sido correlacionadas con los parámetros de textura del pan de trigo (Collar 2003). El perfil de formación de pasta durante la cocción y el enfriamiento de la masa de trigo han sido altamente correlacionados con los parámetros de la cinética del envejecimiento del pan. Particularmente, la viscosidad pico, la temperatura de formación de pasta y el setback durante el enfriamiento pueden ser considerados predictores a nivel de la masa reafirmando el comportamiento de pan de trigo durante el almacenamiento. También se han descrito correlaciones positivas entre la viscosidad aparente de la masa y el volumen de pan (r = 0,83, P< 0,05) y también entre la porosidad y el volumen del pan (r = 0,81, P< 0.05) (Sabanis y col., 2009). Con respecto a las masas libres de gluten, el comportamiento de la pasta de harina de maíz ha sido significativamente correlacionado con parámetros de textura de la masa (Brites y col., 2010).

En general, los coeficientes de correlación más altos se obtuvieron entre las propiedades de la masa y los parámetros instrumentales de los panes (r >0,70), en comparación con los obtenidos entre los parámetros instrumentales y las características sensoriales (r < 0,70). No se encontraron relaciones coincidentes con coeficiente altos (r> 0,70) entre los parámetros instrumentales y las características sensoriales. Las pocas correlaciones comunes encontradas entre los parámetros que caracterizaron los panes libres de gluten evaluados fueron correlaciones negativas y bajas (r< 0,60) entre uno o varios de los parámetros de color (L^*, a^*,b^*, croma) con la apariencia de la miga, el color percibido, y la capacidad de recuperación percibida; y otras positivas y bajas con la disgregabilidad percibida.

El pan es el producto libre de gluten de mayor consumo, no obstante en los últimos años también se ha generado la demanda de productos libres de gluten que se consumen por placer, sin buscar satisfacer necesidades nutricionales. A este tipo de productos también se les conoce como “alimentos indulgentes” o “alimentos hedónicos”, y entre ellos se encuentran los productos dulces de bollería como las magdalenas.

Las magdalenas, además de ser uno de los productos dulces de bollería más consumidos, podrían considerarse un sistema ideal de estudio ya que desde un punto de vista tecnológico su desarrollo requiere procesos diferentes a los utilizados en la elaboración del pan. Por tanto a través de la formulación y el desarrollo de este tipo de productos es posible evaluar un comportamiento reológico distinto de las mezclas libres de gluten. En esta investigación se utilizó la harina de arroz con miras a ampliar el conocimiento de su aplicación en la elaboración de productos libres de gluten dulces horneados no fermentados y enriquecidos con proteínas de distintas fuentes; desde un enfoque científico, para avanzar en el conocimiento del rol de las proteínas en el comportamiento reológico de las masas-batidas y sobre las características de calidad del producto final.

El estudio reológico de las mezclas formuladas para elaborar magdalenas a base de harina de arroz demostró que las propiedades reológicas de las masas-batidas estuvieron gobernadas en gran medida por el tipo de proteína utilizada en cada formulación. En general, las formulaciones que contenían proteínas de origen vegetal (gluten vital de trigo, aislado proteico de soja y aislado proteico de guisantes) presentaron comportamientos reológicos diferentes a las de aquellas que contenían proteína de origen animal (clara de huevo y caseína). Adicionalmente, se observaron comportamientos distintos entre las masas-batidas que contenían proteínas del mismo origen. Particularmente los efectos de la adición de la clara de huevo y de la caseína resultaron prácticamente opuestos al compararlas entre ellas; mientras que a diferencia de las proteínas de legumbres, la presencia del gluten vital de trigo presentó un comportamiento similar a la masa-batida control en la mayoría de las características reológicas evaluadas.

La adición de proteína de legumbres en la masa-batida basada en harina de arroz produjo un gran incremento en el índice de consistencia, siendo este efecto más marcado en presencia de aislado proteico de guisantes. De forma similar, otros autores han encontrado incremento en la consistencia de las masas-batidas de bizcochos por efecto de la adición de proteínas, siendo mayor en presencia de aislado proteico de soja que en presencia de proteína de trigo (Ronda y col., 2011). En el presente estudio, el incremento observado en la consistencia de la masa-batida en presencia de aislado proteico de guisante y de soja podría ser atribuido a la gran capacidad de enlazar agua de estas proteínas, la cual al parecer es conferida a la masa-batida. La gravedad específica de la masa-batida también se vio afectada por la presencia de las proteínas vegetales, y entre ellas el mayor incremento fue observado en presencia de gluten vital de trigo. Sin embargo, las masas-batidas que contenían aislado proteico de soja o aislado proteico de guisantes no presentaron diferencias significativas entre sí para la gravedad específica, lo cual indica que las mismas tiene un comportamiento similar en relación a la capacidad de retención de aire; esto puede ser atribuido a la naturaleza de las proteínas de legumbres. Por otra parte, un comportamiento completamente diferente fue observado entre las proteínas de origen animal. La adición de clara de huevo incorporó y retuvo más aire dentro de la masa durante el mezclado. En general, las masas-batidas con menor densidad suelen tener mayor cantidad de aire incorporado (Turabi y col., 2008a,b; Ronda y col., 2011; Martínez-Cervera y col., 2011), lo cual es deseable en la elaboración de magdalenas.

Las diferencias observadas entre las proteínas del mismo origen pueden ser atribuidas a las propiedades funcionales de las proteínas, tales como actividad emulgente o estabilizador de espuma. En este sentido, Marco y Rosell (2008b) encontraron que la presencia de proteínas de albumen de huevo o proteína de suero incrementa la actividad emulgente de harina de arroz, mientras que las proteínas de soja o de guisantes modificaron fuertemente este parámetro. Adicionalmente, indicaron que la estabilidad de la emulsión disminuyó significativamente en presencia de las proteínas de huevo o de suero.

Los valores obtenidos para los parámetros de viscoelásticidad indicaron que la presencia de los diferentes tipos de proteínas produjo cambios significativos (P<0,05) sobre las propiedades viscoelásticas de la masa-batida control. Una vez más se pone de manifiesto que la extensión del efecto de la adición de proteínas sobre las propiedades viscoelásticas de la masa-batida depende en gran medida de la naturaleza de la proteína añadida. Las masas-batidas que contenían proteína vegetal produjeron valores más altos de G' en comparación con los del control, aunque en el caso del gluten vital de trigo el incremento no fue significativo, indicando similitudes entre las proteínas del gluten y las del arroz. Resultados similares han sido reportados por Ronda y col. (2011) y Marco y Rosell (2008b). La adición de caseína produjo un incremento significativo (P<0,05) de G', similar al producido por efecto de la adición de aislado proteico de guisante. Sin embargo, G' no fue significativamente modificado por la presencia de clara de huevo, indicando un comportamiento similar al de la masa-batida control.

En general, la adición de proteínas también modificó significativamente (*P*<0,05) las características tecnológicas de las magdalenas obtenidas a partir de las masas-batidas formuladas y la dimensión del efecto estuvo influida por el tipo de proteína. Las magdalenas preparadas con proteínas de origen animal presentaron el volumen específico más alto, y el mayor efecto fue observado con clara de huevo; al parecer debido a que esta masa-batida fue capaz de incorporar y retener más aire durante la mezcla y el horneado (Geera y col., 2011). En consecuencia, las magdalenas que contenían huevo o caseína presentaron un mayor número de burbujas de aire, resultando en una estructura más esponjosa y ligera. La dureza aumentó significativamente (*P*<0,05) sólo en presencia de la caseína. Además, se observó un aumento significativo (*P*< 0,05) de la capacidad de recuperación, la cohesividad y la elasticidad por efecto de la presencia de proteínas de origen animal, indicando más elasticidad. Se sabe que la elasticidad está asociada al producto fresco, aireado y elástico y en el caso de las magdalenas valores altos de elasticidad están vinculados a alta calidad de producto final (Sanz et al., 2009). Mientras que masas densas con menor número de células de aire conducen a valores bajos de elasticidad, lo que implica que se requiere más tiempo para que la estructura se recupere después de la compresión (Martínez-Cervera y col., 2011).

En general, las magdalenas elaboradas a partir de proteínas de origen animal fueron más elásticas y cohesivas que las elaboradas utilizando proteínas de origen vegetal. Los resultados de este estudio revelaron claramente gran variabilidad en la calidad de la textura de las magdalenas elaboradas a partir de harina de arroz y enriquecidas con diferentes fuentes de proteínas.

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CONCLUSIONES

A continuación se presentan las conclusiones más relevantes de esta Tesis Doctoral:

- La evaluación nutricional de diferentes tipos de pan libres de gluten de origen comercial reveló que estos son alimentos amiláceos con gran divergencia en la composición de proteínas y grasas; y de alto índice glucémico.

- De acuerdo al análisis de componentes principales los atributos sensoriales, así como las propiedades de hidratación y parámetros de textura descritos en este estudio serían los más adecuados para caracterizar productos libres de gluten tipo pan.

- El diseño de formulaciones de mezclas libres de gluten basadas en harina de arroz y enriquecidas en proteínas condujo a la obtención de distintos tipos de pan, los cuales discriminaron ampliamente en el contenido de proteínas y grasa. Adicionalmente, los parámetros instrumentales de calidad evaluados y las características sensoriales discriminaron entre los diferentes panes libres de gluten formulados. Tales diferencias pueden ser atribuidas a la complejidad y la composición de las formulaciones.

- Entre todos los panes libres de gluten diseñados, el formulado a base de harina de arroz y HPMC presentó buen balance de nutrientes (7,10 g/100g de proteínas, 3,70 g/100g de lípidos; minerales 1,31 g/100g, y 87,89 g/100g de carbohidratos, bs), las mejores características tecnológicas y la mayor aceptación sensorial. En general los panes enriquecidos en proteínas presentaron mayor dureza y menor aceptación global.

-El estudio reológico de la amplia gama de masas/masas-batidas formuladas a base de harina de arroz y enriquecidas en proteínas reveló que todos los parámetros obtenidos a partir del Mixolab® discriminaron significativamente (P<0,05) entre las masas evaluadas. Adicionalmente, las diferencias encontradas en las propiedades reológicas de las masas fueron asociadas principalmente a la presencia o ausencia de proteínas y almidón.

-Las correlaciones con los coeficientes más altos fueron obtenidas entre las propiedades reológicas de las masas y los parámetros instrumentales de calidad de los productos horneados frescos. Particularmente, la consistencia de la masa/masa-batida durante el mezclado (C1), la amplitud y la consistencia de la masa/masa-batida después del enfriamiento (C5) podrían ser predictores adecuados del parámetro dureza de la miga en los panes libres de gluten.

-En lo concerniente al estudio del rol de las proteínas en el desarrollo de productos tipo magdalenas, los resultados obtenidos permitieron concluir que tanto las propiedades viscoelásticas de las masas-batidas como las características tecnológicas del producto horneado final están dominadas por la naturaleza de las proteínas utilizadas en la formulación. La adición de clara de huevo disminuyó la gravedad específica de la masa-batida. Por otra parte, la presencia de aislado proteico de soja, de aislado proteico de guisantes y de caseína incrementó el módulo de almacenamiento (G') modificando fuertemente el comportamiento viscoelástico de la masa-batida basada en harina de arroz. En general, la adición de aislado proteico de soja, de aislado proteico de guisantes y de clara de huevo produjo un gran incremento en G' por efecto del calentamiento. Estas diferencias pueden atribuirse a la naturaleza y al patrón particular de la desnaturalización que presentan las diferentes fracciones de proteínas presentes en cada tipo de proteína utilizada.

-Con respecto a la calidad tecnológica de las magdalenas la adición de aislado proteico de guisantes produjo magdalenas más suave y elásticas que las elaboradas con la masa-batida control y las proteínas de origen animal; mientras que las proteínas de origen animal produjeron magdalenas con mejor apariencia.

-Los resultados obtenidos en la presente investigación permiten concluir que es viable elaborar productos horneados (panes y magdalenas) libres de gluten basados en harina de arroz y enriquecidos en proteínas con características nutricionales, tecnológicas y sensoriales mejores que los disponibles en el mercado. Sin embargo, es necesario tener en cuenta que el uso de harina de arroz como sustituto de la harina de trigo, y de proteínas de origen vegetal como soja o guisantes en sustitución del huevo en este tipo de productos, produce cambios notables en la calidad tecnológica y sensorial de los productos finales. Por lo tanto, la optimización de este tipo de formulaciones y la selección fundamentada de los ingredientes y su combinación es necesaria para garantizar la adecuada textura y aceptación de estos productos libres de gluten alternativos.