Universidad de Valladolid

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍAS AGRARIAS DE PALENCIA

PROGRAMA DE DOCTORADO:

CIENCIA E INGENIERÍA AGROALIMENTARIA Y DE BIOSISTEMAS

TESIS DOCTORAL:

NUTRITIONAL AND FUNCTIONAL IMPROVEMENT OF GLUTEN-FREE BREADS: ADDITION OF BETA-GLUCANS OF DIFFERENT ORIGINS AND MOLECULAR WEIGHTS ACCORDING TO THE HEALTH CLAIMS APPROVED BY THE EFSA

Presentada por Sandra Pérez Quirce para optar

al grado de Doctor con mención "Doctor Internacional"

por la Universidad de Valladolid

Dirigida por: Dra. Felicidad Ronda Balbás y Dra. Athina Lazaridou

Palencia, 2017

Universidad de Valladolid

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍAS AGRARIAS DE PALENCIA

PROGRAMA DE DOCTORADO:
CIENCIA E INGENIERÍA AGROALIMENTARIA Y DE BIOSISTEMAS

TESIS DOCTORAL:

MEJORA NUTRICIONAL Y FUNCIONAL DE PANES SIN GLUTEN: ADICIÓN DE BETA-GLUCANOS DE DIFERENTES ORÍGENES Y PESOS MOLECULARES ATENDIENDO A LAS DECLARACIONES DE SALUD APROBADAS POR LA EFSA

NUTRITIONAL AND FUNCTIONAL IMPROVEMENT OF GLUTEN-FREE BREADS: ADDITION OF BETA-GLUCANS OF DIFFERENT ORIGINS AND MOLECULAR WEIGHTS ACCORDING TO THE HEALTH CLAIMS APPROVED BY THE EFSA

Memoria presentada por Sandra Pérez Quirce para optar al grado de Doctor con mención internacional por la Universidad de Valladolid

Fdo: Sandra Pérez Quirce

Palencia, 18 de Mayo de 2017

Universidad de Valladolid

AUTORIZACIÓN DE LOS DIRECTORES DE LA TESIS PARA SU PRESENTACIÓN

Las Dras. Dña. Mª Felicidad Ronda Balbás y Dña. Athina Lazaridou, como directoras de la Tesis Doctoral titulada "NUTRITIONAL AND FUNCTIONAL IMPROVEMENT OF GLUTEN-FREE BREADS: ADDITION OF BETA-GLUCANS OF DIFFERENT ORIGINS AND MOLECULAR WEIGHTS ACCORDING TO THE HEALTH CLAIMS APPROVED BY THE EFSA" realizada por Dña. Sandra Pérez Quirce en la Escuela Técnica Superior de Ingenierías Agrarias de la Universidad de Valladolid dentro del Programa de Doctorado en Ciencia e Ingeniería Agroalimentaria y de Biosistemas, autorizan su presentación dado que reúne las condiciones necesarias para su defensa.

Y para que conste a los efectos oportunos, lo firman en
Palencia a __ de _____ de 2017.

Fdo: Dra. Dña. Mª Felicidad Ronda Balbás
Profesora Titular de Universidad
Universidad de Valladolid

Fdo.: Dra. Dña. Athina Lazaridou
Proffessor
Aristotles University (Greece)

Este trabajo se ha realizado en la Universidad de Valladolid y ha sido financiado por el Ministerio de Economía y Competitividad y el Fondo Europeo de Desarrollo Regional (FEDER) (Proyectos AGL2012-35088 y AGL2015-63849-C2-2-R). La autora de esta Tesis Doctoral ha sido beneficiaria de un contrato laboral con cargo al primer proyecto y de una ayuda Erasmus para la realización de una Estancia Breve en la Universidad de Aristóteles (Tesalónica, Grecia).

Agradecimientos

Llegar hasta aquí no ha sido fácil. Pero, con la ayuda de muchas personas que he ido encontrando a mi paso, este proyecto ha visto la luz y es el momento de agradecer su apoyo a todos ellos:

En primer lugar, a Felicidad Ronda, por darme la oportunidad de trabajar en este proyecto. Por la confianza que ha depositado en mí desde el primer día y por haber generado en mí esa inquietud por investigar e inculcarme el buen hacer en el laboratorio.

Extiendo este agradecimiento a Athina Lazaridou, que aceptó codirigir esta Tesis, y a Costas Biliaderis. Ambos han supuesto un gran apoyo para mí durante mi estancia en Grecia, y su colaboración en el desarrollo del trabajo experimental y en la publicación de algunos de los artículos ha sido decisiva. Agradecerles también por su orientación, enseñanzas y dedicación a este trabajo.

A todos los investigadores que han contribuido en el desarrollo de esta Tesis, agradecerles su ayuda y el apoyo recibido durante estos años, especialmente a Concha Collar y Pedro Caballero por sus aportaciones.

A todos mis compañeros de batallas. A mis compañeros de Universidad, gracias por todo este tiempo compartido y por hacer más amenas las largas jornadas de laboratorio y de redacción. Han sido muchos los que han pasado por el laboratorio en este tiempo; algunos de ellos por varios años, como Workineh o Marina, y otros tan sólo por unos meses, pero todos ellos han sido piezas clave. Me llevo la amistad de muchos de vosotros que me acompañará siempre.

Agradecer también a otros grupos de investigación con los que he tenido la suerte de coincidir. Siempre se puede aprender algo nuevo de cada uno de ellos. Y también a los compañeros de laboratorio de la Universidad de Aristóteles por su buena acogida y su amabilidad.

Gracias también a todos aquellos amigos que han compartido conmigo mis alegrías y tristezas durante estos años, especialmente a Hugo y a Paula, a pesar del poco tiempo que he podido dedicarlos.

Por último, y no menos importante, agradecer y dedicar esta tesis a mi familia y a David por estar en todo momento conmigo, por sus consejos, su confianza, su paciencia y sus esfuerzos para conseguir que esta Tesis se convierta en realidad. Por saberme escuchar y celebrar conmigo los buenos momentos y ser mi gran apoyo y aliento en los malos. A ellos y a otras personas que ya no están les debo gran parte de este logro. Sin vosotros no hubiera sido posible.

ÍNDICE DE CONTENIDOS

Contenido

RESÚMENES

RESUMEN

A pesar de los avances realizados en las últimas décadas, los productos sin gluten horneados continúan siendo en su mayoría de baja calidad físico-química y sensorial. También presentan carencias a nivel nutricional que ejercen un efecto de empeoramiento en la dieta de los pacientes con enfermedad celíaca. A menudo son productos que contienen un alto contenido en grasas y carbohidratos y bajos contenidos en fibra, vitaminas y otros nutrientes esenciales. El enriquecimiento de panes sin gluten con β-glucanos (BG), una fibra dietética soluble con efectos fisiológicos beneficiosos probados en la salud humana, supone un especial interés para esta población de salud vulnerable.

Esta tesis doctoral aborda el empleo de (1→3)(1→4)-β-glucanos procedentes de avena o cebada, en dosis conformes con las declaraciones de salud aprobadas por la EFSA. Además, explora, las posibilidades de enriquecimiento de este pan con (1→3)(1→6)-β-glucanos procedentes de levadura y hongos.

Se ha comprobado que es posible el enriquecimiento de panes sin gluten con BG de cereal cumpliendo con las alegaciones de EFSA con una calidad física y nutricional mejorada.

Los factores de estudio que han resultado determinantes son el origen del BG, su concentración y su peso molecular. La primera parte de este estudio se centra en la evaluación de la capacidad estructurante del BG. De acuerdo a los resultados obtenidos, el BG no es capaz de sustituir al Hidroxi-Propil-Metil-Celulosa (HPMC), hidrocoloide comúnmente empleado en panificación sin gluten, por lo que se requiere la adición simultánea de ambos ingredientes. Entre los diferentes tipos de HPMC, aquel que exhibió mejores resultados fue el de fuerza de gel débil. El HPMC de fuerza media dio lugar a grandes defectos en la miga del pan que no se consideran aceptables en el producto final. Al mismo tiempo se concluyó la necesidad de una optimización de la hidratación de la masa en función de la dosis de BG como requisito para alcanzar una adecuada calidad del producto, persiguiendo como parámetros más destacados de calidad el máximo volumen específico y la mínima dureza del pan.

La segunda parte del estudio se centró en la comparación del efecto de los concentrados de BG de cereal comerciales según su origen y dosis, en las propiedades de las masas y panes enriquecidos. Se obtuvieron grandes diferencias en función del origen del BG. A igual contenido en BG puro, los efectos del BG de cebada, de bajo Peso Molecular (PM) y pureza elevada, resultaron más notables comparados con el control, dando lugar a masas de consistencias más elevadas, un volumen específico de los panes menor y durezas de la miga mayores que los panes con BG de avena, de alto Peso Molecular y baja pureza. Esto se relacionó con la baja pureza del BG de avena (entorno al 30%) y, en consecuencia, su alto contenido en excipientes que se añadían junto con el hidrocoloide, que previsiblemente podían debilitar las masas. Por este motivo, se continuó el estudio planteando la obtención de purificados de BG de avena para reducir el efecto de estas sustancias interferentes (fundamentalmente maltodextrinas), al objeto de poder comparar el efecto del Peso Molecular de los BG sin la interferencia. También se planteó la obtención de preparaciones con rangos de pesos moleculares diferenciados, más allá de los disponibles comercialmente.

Tras sucesivas etapas de purificación y aislado de los BG se obtuvieron tres fracciones claramente diferenciadas en sus respectivos pesos moleculares y de similar pureza, que han sido utilizadas en las siguientes etapas de estudio. Se dedujo el importante efecto del peso molecular y de la dosis de incorporación del BG sobre las propiedades reológicas de las masas y la calidad de los panes sin gluten elaborados con harina de arroz. La incorporación de BG de alto peso molecular tuvo el mayor efecto sobre las masas y condujo al mayor volumen específico y menor dureza del pan, al tiempo que un mayor potencial en los beneficios para la salud. Los BG de medio y bajo peso molecular también mostraron un impacto significativo en los parámetros reológicos de la masa y en los atributos de calidad del pan, aunque ligeramente menores. El BG de alto peso molecular reveló la mayor capacidad para reducir la glucosa rápidamente digerible del pan, a pesar de que se observó una importante degradación de su peso molecular en el producto final. Se consideró necesario conservar en el pan el elevado PM del BG añadido ya que los efectos beneficiosos para la salud de esta fibra soluble están asociados con el aumento de viscosidad que aportan al contenido del tracto intestinal que a su vez es estrechamente dependiente de su peso molecular. Se estudió y comprobó la

viabilidad de un tratamiento térmico de la harina de arroz, con objeto de eliminar su actividad β-glucanásica endógena responsable de la hidrólisis de los BG en el proceso de panificación. Para ello se aplicó energía de microondas para el tratamiento térmico. El tratamiento con microondas demostró ser efectivo para la inactivación enzimática cuando se aplicó a harinas con humedad elevada. Tratamientos cortos de tan sólo 4 minutos en harinas al 25% de humedad fueron suficientes para alcanzar la destrucción enzimática total sin modificar las propiedades funcionales de la harina. Gracias al empleo de estas harinas modificadas a través de un método apenas investigado, fue posible obtener panes sin gluten enriquecidos con BG en los cuales el peso molecular del BG se preservó después de la panificación.

Por último se realizó una valoración de las posibilidades de incorporación de BG de otras procedencias, como hongos y levaduras, a panes sin gluten. Aunque por el momento no están reconocidos por EFSA los efectos beneficiosos para la salud del (1→3)(1→6)-β-glucano, existe mucha literatura científica que demuestra su actividad inmuno-estimulante, antiinflamatoria, antimicrobiana y antitumoral. Los resultados evidenciaron la viabilidad de su empleo en este tipo de productos mejorando la calidad física y sensorial de los panes, en particular los enriquecidos con el producto comercial soluble obtenido a partir de las paredes celulares de Saccharomyces.

ABSTRACT

Despite the advances made in gluten-free bakery technology during the last decade, the resultant baked products continue to display poor sensorial and physicochemical attributes. Additionally, these products are often high in fat and carbohydrate and low in fiber, vitamin and other essential nutrients content; therefore, their consumption can exert a worsening effect on the diet of celiac patients resulting in nutritional deficiencies. Therefore, the enrichment of gluten-free breads with β-glucans (BG), a soluble dietary fiber having various health benefits, is of special interest to this vulnerable population group.

This doctoral thesis addresses the inclusion of (1→3)(1→4)-β-glucans from oat and barley into gluten-free rice-based bread formulations at levels that fulfil the health claims approved by EFSA. The effect of origin, concentration, and molecular weight of BG on quality parameters of the bread dough and final products have been studied. Elimination of β-glucan degradation during breadmaking by thermal pretreatment of rice flour was also investigated. Finally, this thesis explores the potential use of (1→3)(1→6)-β-glucans from yeast and fungi in gluten-free bread was explored.

The findings of this thesis showed that incorporation of cereal BG into gluten-free breads fulfilling the EFSA claims and simultaneously possessing improved physical and nutritional properties can be feasible.

The first part of this study was focused on the evaluation of the BG structuring ability. Experimental data showed that BG cannot substitute the Hydroxy-Propyl-Methyl-Cellulose (HPMC), a hydrocolloid commonly used in gluten-free breadmaking, and thus the simultaneous addition of both ingredients to the bread formulation was required. Among the two tested types of HPMC differing in gel forming capacity, the polymer that gave the optimum bread quality was that gives a dough with a weak gel network. The medium gel strength HPMC resulted in bread crumb defects that the final products could not considered acceptable. Additionally, it was concluded the necessity of optimization of the dough hydration levels as a function of BG concentration, since both seem to be the most important parameters

for obtaining final products with maximum specific volume and minimum crumb hardness.

In the second part of the study, the effect of origin and concentration of commercial cereal BG concentrates added to the gluten-free formulations on the physicochemical properties of resultant doughs and breads was investigated. Large differences in these properties were observed depended on the origin of BG. For the same concentration of pure BG, a barley BG concentrate with low molecular weight and high purity (~70% BG) affected more remarkable the gluten-free formulations resulting in more consistent doughs, lower specific volume and higher crumb hardness than an oat BG concentrate having high molecular weight and low purity (around 30% BG). The low purity of the oat BG concentrate could be responsible as the addition of the same amount of BG results in the contribution of other substances, possibly maltodextrins, that can weaken the dough network structure. Therefore, the use of BG preparations with similar purity and different molecular weights, beyond the commercially available concentrates, was proposed in order to compare, without any interference, the impact of BG molecular weight on dough and bread quality parameters.

After successive purification and isolation steps, three fractions of BG, clearly differentiated in their respective molecular weights and with similar purity (~70% BG), were obtained for the following stages of the study. A large impact of the molecular weight and the concentration of BG added to gluten-free rice breads on the rheological behavior of dough and quality of the final product was found. The incorporation of high molecular weight BG had the greatest effect on the dough rheological properties and led to the highest specific bread volume and lowest bread hardness, as well as the greatest potential health benefits as indicated by *in vitro* starch digestibility assays. Medium and low molecular weight BGs also showed a significant impact on the dough rheological parameters and the bread quality attributes, although lower than the high molecular weight sample. The latter BG preparation revealed the greatest ability to reduce rapid digestible glucose of the bread, despite a significant degradation of its molecular weight in the final product. Thus, it was considered necessary to preserve the high MW of the added BG in the bread, since the health beneficial effects of this soluble fiber are associated with its ability to increase the viscosity of the gastrointestinal tract content, which in turn is closely dependent on BG molecular weight. The feasibility of a heat treatment of the

rice flour was studied for the elimination of flour endogenous β-glucanase activity responsible for the BG hydrolysis during the baking process. For this purpose, microwave energy was applied to the rice flour as a pretreatment. Microwave treatment proved to be effective in enzymatic inactivation when applied to flours with high water content. Short treatments of only 4 minutes in flours at 25% moisture content were sufficient to achieve total enzymatic destruction without modifying the functional properties of the flour. Using these pretreated flours it was possible to obtain gluten free breads enriched with BG, in which the molecular weight of the polysaccharide was preserved during the whole process of breadmaking.

Finally, an assessment of the possibility of incorporating BG from other sources, such as fungi and yeast, to gluten-free breads was carried out. Although the beneficial health effects of (1→3)(1→6)-β-glucans are not currently recognized by EFSA, there are many studies indicating their immune-stimulatory, anti-inflammatory, antimicrobial and antitumor activity. Findings evidenced the feasibility of the inclusion of microbial BGs in this type of products since they improved the physical and sensorial quality of the breads particularly, in those enriched with the soluble commercial BG concentrate obtained from *Saccharomyces* cell walls.

INTRODUCCIÓN

INTRODUCCIÓN

1. La enfermedad celiaca

La enfermedad celiaca es una enteropatía caracterizada por una lesión del intestino delgado que provoca la atrofia de las vellosidades intestinales y la consiguiente mala absorción de nutrientes (hierro, ácido fólico, calcio y vitaminas entre otros) en individuos genéticamente susceptibles (Sapone et al., 2012). Representa uno de los trastornos genéticos humanos más extendidos en la actualidad, con una prevalencia en aumento del 1 – 2 % (Cabrera-Chavez y Calderon de la Barca, 2010; Vici et al., 2016). No existe cura para la enfermedad celiaca. El único tratamiento es el seguimiento de una dieta estricta sin gluten de por vida.

La reacción inflamatoria causada por la enfermedad se desencadena por la ingestión de gluten y, específicamente, por la fracción de la gliadina del trigo y prolaminas de granos comunes tales como la cebada (hordeínas) y centeno (secalinas). Aunque la avena es naturalmente libre de gluten, su uso en la fabricación de productos sin gluten es controvertido debido a la posible contaminación de la avena que puede ocurrir durante su proceso productivo (Cabrera-Chavez y Calderon de la Barca, 2010; Kupper, 2005).

En los últimos años el mercado de productos libres de gluten está experimentando un crecimiento producido principalmente por el aumento del número de pacientes diagnosticados con la enfermedad celíaca, alergias u otras reacciones de sensibilidad al gluten en las que no intervienen mecanismos autoinmunes (Mir et al., 2016; Sapone et al., 2012; Rosell et al., 2014) y por una nueva tendencia seguida por personas que, a pesar de no padecer cualquier forma de intolerancia al gluten, adoptan la decisión de consumir estos productos, basada en la creencia, en muchos casos equivocada de que estos productos son más saludables que sus homólogos convencionales (Miranda et al., 2014).

Al mismo tiempo, la enfermedad celiaca viene asociada con frecuencia a otras patologías con una tasa de incidencia significativa, tales como obesidad y diabetes (Capriles et al., 2016; Cronin y Shanahan, 1997; Kupper, 2005). Por lo tanto, estos pacientes deben mantener una dieta exenta de gluten al tiempo que siguen un adecuado control glucémico. En consecuencia, se sugiere una dieta con alimentos de

bajo índice glicémico (IG), es decir, ricos en carbohidratos de baja velocidad de digestión. Los alimentos con alto IG que son rápidamente digeridos y absorbidos causan grandes incrementos de glucosa en sangre, mientras que los de bajo IG experimentan una liberación de glucosa gradual en el tiempo (Brand-Miller et al., 2009). El pan se ha considerado como un alimento con IG intermedio-alto que se tiende a reducir con la incorporación, por ejemplo, de ingredientes ricos en fibra a su formulación.

2. El pan sin gluten

La variedad disponible en el mercado de alimentos libres de gluten es limitada y son productos en general caros y con carencias nutricionales y sensoriales, además de otras propiedades físico-químicas indeseables como una vida útil relativamente corta (Capriles et al., 2016; Mir et al., 2016; Torbica et al., 2010).

En cuanto al pan, a pesar de los esfuerzos y avances, la mayoría de los panes libres de gluten comercialmente disponibles son de calidad inferior a sus homólogos con gluten (Gallagher et al., 2003; Torbica et al., 2010; Vici et al., 2016). La sustitución del gluten en productos de panadería representa, sin duda, un importante desafío tecnológico que aumenta en aquellos productos cuya elaboración depende estrechamente de la presencia de gluten. El gluten es el principal formador de estructura en el pan de trigo debido a su capacidad para constituir una masa cohesiva viscoelástica capaz de atrapar el gas durante la fermentación y la cocción y proporcionar una buena estructura a la miga resultante. Su eliminación conduce a una masa menos cohesiva y elástica y más pegajosa y difícil de manejar, y afecta a su capacidad de desarrollo durante el proceso de panificación (Gallagher et al., 2004). Ningún otro cereal muestra dichas capacidades.

El proceso de elaboración de masa sin gluten difiere del utilizado en los productos tradicionales como consecuencia de las diferencias asociadas a las necesidades de hidratación, que son responsables de la consistencia lograda por la masa durante el mezclado (Marco y Rosell, 2008), a la manipulación de la masa y sus propiedades de cocción (Gómez et al., 2013La harina sin gluten requiere una mayor cantidad de agua que la harina de trigo (Capriles y Areas, 2014; Marco y Rosell, 2008), logrando una viscosidad más parecida a la de masas batidas empleadas en repostería que a la masa

propiamente dicha de pan y requiriendo, generalmente, el empleo de moldes para su fermentación y cocción. La formación de masa con propiedades viscoelásticas comparables al trigo se puede obtener enriqueciendo las masa sin gluten con sustancias poliméricas.

Desde un punto de vista tecnológico, la mayoría de las investigaciones desarrolladas en la elaboración de pan sin gluten se han basado en la sustitución de harina de trigo por harinas sin gluten o una mezcla de estas con almidones (maíz, sorgo, patata, mandioca…) y con agentes estructurantes como proteínas o hidrocoloides (Demirkesen et al., 2014; Mancebo et al., 2015; Sciarini et al., 2010). Entre las materias primas, la harina de arroz es probablemente la más aceptable por su sabor suave, color blanco, y propiedades hipoalergénicas junto con unos niveles bajos en sodio y proteína y alta cantidad de hidratos de carbono de fácil digestión (Rosell et al., 2014). Entre los hidrocoloides, polímeros capaces de mimetizar las propiedades viscoelásticas del gluten y mejorar la calidad global de los productos finales, se incluyen diferentes polisacáridos y proteínas capaces de realizar funciones como gelificante, espesante y emulsionante, cuando se incorporan en alimentos (Demirkesen et al., 2014). Lahidroxipropil metilcelulosa (HPMC), derivado químico de la celulosa, parece ser el hidrocoloide que mejores resultados ha permitido alcanzar en panes de arroz (Gallagher et al., 2004; Houben et al., 2012; Marco y Rosell, 2008; Matos y Rosell, 2014; Sivaramakrishnan et al., 2004) Sin embargo, este ingrediente puede presentar distintas fuerzas de gel y, por tanto, aportar a la masa distinta consistencia, por lo que se consideraría necesario un estudio detallado del tipo y dosis de HPMC a emplear y de sus requerimientos de agua.

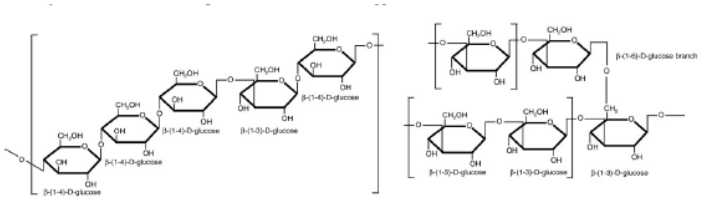
Por otra parte, otra preocupación actual con respecto a los panes sin gluten se relaciona con la mejora de sus propiedades nutricionales (Vici et al., 2016). Mejorar la calidad nutricional de los productos sin gluten de forma simultánea con la mejora de las propiedades tecnológicas y sensoriales sigue siendo una desafío importante. Sin embargo, entre alimentos enriquecidos con ingredientes funcionales, especialmente aquellos con declaraciones de salud reconocidas por la Autoridad Europea de Seguridad Alimentaria (EFSA), no suelen estar incluidos los alimentos sin gluten. Esto supone una contradicción, ya que se incrementa la distancia entre la calidad nutricional natural de los alimentos destinados a la población sana y la celiaca, a pesar de ser uno de los colectivos más necesitados de los beneficios

asociados a estos ingredientes. Por lo tanto, el desarrollo de alimentos aptos para enfermos celiacos de elevada calidad resulta de gran interés (Capriles et al., 2016). Los hidrocoloides se incluyen dentro del concepto de fibra y, por tanto, la adición de estas sustancias ejerce una función nutricional (Wood et al., 1994; Matos y Rosell, 2014). Así, la cantidad de fibra dietética en la dieta podría mejorarse mediante la adición de ingredientes con alto contenido de fibra o fibra funcional en alimentos como el pan.

Estudios previos en personas sanas mostraron que la ingesta de pan sin gluten aumentaba significativamente el área bajo la curva de glucosa en sangre, en comparación con la ingesta equivalente de carbohidratos de pan con gluten (Berti et al., 2004). Otros autores observaron diferentes respuestas glucémicas en función de la composición de los alimentos y el tipo de procesado, afectando de manera importante a la disponibilidad de los carbohidratos (Jenkins et al. 1987; Ronda et al. 2010, 2012). Por tanto el enriquecimiento de pan sin gluten con fibra podría cobrar un doble sentido, ya que la adición de fibra contribuiría reduciendo significativamente el IG de los alimentos que la contienen.

3. Los β-glucanos

Los β-glucanos (BG) son polisacáridos que están presentes de forma natural en los granos de cereal, hongos y levaduras e incluso bacterias y algas (Izydorczyk y Dexter, 2008; Wood, 2004; Zhu et al., 2015). Sus aplicaciones en medicina, industria farmacéutica, alimentos, cosmética, industria química y en la producción de piensos reflejan su gran potencial (Zhu et al., 2016). Dependiendo del origen, existen claras diferencias en la estructura macromolecular entre β-glucanos (Figura 1). Su estructura depende del origen botánico, de tal modo que están constituidos por D-glucopiranosas unidas entre sí por enlaces glucosídicos β (1→3) y (1→4) en el caso de los cereales o enlaces β (1→3) y (1→6) cuando su origen son los hongos o levaduras. Además de las diferencias en el tipo de enlace y ramificación, los β-glucanos pueden variar en solubilidad, peso molecular, estructura terciaria, grado de ramificación y carga polimérica. Todas estas características pueden influir en sus efectos fisiológicos y tecnológicos (Volman et al., 2008).



cereal β-glucan

Polymer of β-(1-4)-D-glycopyranosyl units separated by single β-(1-3)-D-glycopyranosyl units.

yeast β-glucan

Polymer of β-(1-3)-D-glycopyranosyl units with branching at β-(1-6)-D-glycopyranosyl units

β-Glucan type	Structure	Description
Bacterial		Linear β1,3 glucan (i.e. Curdlan)
Fungal		Short β1,6 branched, β1,3 glucan (i.e. Schizophyllan)
Yeast		Long β1,6 branched, β1,3-glucan (i.e. WGP β-glucan, Betafectin™)
Cereal		Linear β1,3/β1,4-glucan (i.e oat, barley, rye)

Fig. 2. Structure of cereal and yeast β-glucan and scheme of various β-glucan sources.

Figura 1. Estructura de los β-glucanos de acuerdo a su origen (extraído de Volman et al., 2008).

3.1. Los β-glucanos de cereal

Los β-glucanos se encuentran de forma natural en las paredes celulares del endospermo de los granos de cereal, especialmente en la cebada (2.5 – 11.3%), avena (2.2 – 7.8%) y, en menor medida, el centeno (1.2 -2.0%) y el trigo (0.4 -1.4%) (Lazaridou et al., 2007). A su vez, los β-glucanos de cereal presentan una gran diversidad de estructuras, tamaño y peso molecular, que resultan determinantes de sus propiedades físicas —solubilidad, viscosidad y propiedades de gelificación— (Lazaridou et al., 2003, 2004) y de su funcionalidad, así como de su acción fisiológica en el tracto gastro-intestinal (Wood, 2004). Los β-glucanos de cereales se clasifican como una fibra dietética que no es hidrolizada en el tracto digestivo humano, siendo un ingrediente no calórico (Borchani et al., 2016).

3.1.1. Funcionalidad fisiologica

Estos polisacáridos se clasifican como fibra dietética soluble con implicaciones nutricionales reconocidas. La Administración de Alimentos y Fármacos de Estados Unidos (FDA, 2005) aprobó una declaración de salud para los productos que contienen β-glucano de cebada por su capacidad de reducir el riesgo de enfermedad cardiaca coronaria. Este reconocimiento es una extensión de la declaración de propiedades saludables aprobada originalmente para el β-glucano de avena (FDA, 1997). Está basada en el consumo diario de al menos 3 g de β-glucano o 4 porciones

de un producto alimenticio que aporte por lo menos 0,75 g de β-glucano por porción (FDA, 2005) como parte de una dieta baja en grasas saturadas y colesterol. Años más tarde, la EFSA ha aprobado declaraciones saludables sobre los β-glucanos de avena y cebada según las cuales la ingesta diaria de 3 g de BG de avena o cebada reduce los niveles de colesterol sanguíneo, que supone un alto riesgo en enfermedades coronarias (EFSA, 2011a); También ha reconocido que la ingesta diaria de aproximadamente 4 g de BG de avena o cebada por cada 30 g carbohidratos en productos de panificación y pasta, es capaz de disminuir la respuesta glicémica postprandial (EFSA, 2011b) y que el consumo de alimentos que contienen fibra de cebada o avena en una concentración de al menos 6 g / 100 g de producto ó 3 g / 100 kcal incrementan el volumen fecal (EFSA 2011c).

Sus efectos beneficiosos dependen de la viscosidad, la cual está controlada por el peso molecular de los β-glucanos y su concentración (Wolever et al., 2010; Lazaridou y Biliaderis, 2007) y por consiguiente de su capacidad de aumentar la viscosidad del contenido intestinal (Wood et al., 1994). La disminución de la solubilidad del β-glucano o la aparición de factores que ocasionen su despolimerización pueden hacer que se reduzca su eficacia fisiológica, aunque no hay conclusiones claras al respecto (Wood et al., 1994, 2000). Wood et al. (1994), Östman et al. (2006) y Panahi et al. (2007) entre otros, demostraron que la capacidad del β-glucano para disminuir la glucemia postprandial está fuertemente correlacionada con su viscosidad. Wood et al (2000) comprobó que dicha respuesta de la glucosa en la sangre dependía del peso molecular y la concentración del β-glucano; mientras que otros autores destacaron la solubilidad β-glucano como un factor clave (Lan-Pidhainy et al., 2007). Por tanto, es necesario caracterizar estas variables con el fin de medir la eficacia fisiológica de β-glucano.

3.1.2. Extracción de β-glucano

El empleo de extractos concentrados de β-glucanos de cereal y el interés en la producción de alimentos enriquecidos con β-glucano es cada vez mayor. Actualmente están disponibles en el mercado concentrados de (1→3)(1→4)-β-glucanos extraídos de avena y cebada pero presentan un coste elevado derivado de los procedimientos para su aislamiento y concentración que requieren. Los procesos de separación en seco empleados comúnmente son métodos relativamente simples y

de bajo costo, aunque el contenido de β-glucano de la fibra concentrada es generalmente ≤30% (Zheng et al., 2000). Con el fin de obtener concentraciones más elevadas se emplean métodos de extracción en medio acuoso aplicados al salvado o a la harina del cereal que permiten obtener, tras una separación final de proteínas, grasas y almidón, un aislado que una vez seco puede alcanzar concentraciones muy elevadas de β-glucano (Lazaridou et al., 2004; Benito-Roman et al., 2011). La extracción acuosa de los β-glucanos, que permite eliminar selectivamente las proteínas desencadenantes de la intolerancia celiaca es un requisito imprescindible para poder aplicarlos en alimentos sin gluten, ya que ni la cebada ni la avena son seguros para los pacientes celiacos. Entre los diversos problemas técnicos asociados con estos procedimientos de extracción se incluyen la alta susceptibilidad del β-glucano a la degradación enzimática por las β-glucanasas endógenas, que llevaría consigo una reducción en el peso molecular del β-glucano y, por tanto, un descenso en la viscosidad de los extractos de β-glucano (Panahi et al., 2007). Sería de gran interés el desarrollo de un método de extracción o purificación que permita obtener BG de mayor pureza sin que exista degradación durante el procesado.

3.1.3. Funcionalidad tecnológica

A nivel tecnológico, el β-glucano es un hidrocoloide con gran potencial para la inclusión en los productos alimenticios. Posee una gran capacidad de retención del agua, debido a la abundancia de grupos hidroxilo dentro de su estructura (Lazaridou et al., 2007). Además, sus propiedades funcionales permiten su empleo como espesante, emulsionante y estabilizador en una variedad de productos alimenticios -salsas, sopas, postres y productos cárnicos entre otros-. Por ello, los β-glucanos son capaces de impartir una gran variedad de propiedades estructurales y texturales a los alimentos y su incorporación mejoraría también su valor nutritivo.

3.1.4. Enriquecimiento pan sin gluten con β-glucano

Al decidir sobre alimentos para fortificar con β-glucano es importante tener en cuenta aquellos productos capaces de lograr un mayor alcance de sus efectos beneficiosos en el mayor número de personas. El pan, como alimento básico, tiene un gran potencial para la incorporación de esta fibra y para llegar a un mayor segmento de la población.

Los efectos del peso molecular, la estructura y la concentración de los β-glucanos de cereal sobre la reología de sus disoluciones en agua, han sido ampliamente estudiados (Lazaridou et al. 2003, 2004). Sin embargo, trabajos previos que realizaron ensayos reológicos de masas de trigo enriquecidas con extractos concentrados de β-glucano de cereal mostraron resultados contradictorios (Cavallero et al., 2002; Skendi et al., 2010). Skendi et al. (2009; 2010) concluyeron que el efecto de estos polisacáridos sobre la consistencia y maquinabilidad de las masas y sobre la calidad del pan dependía de manera determinante del peso molecular de los β-glucanos, de su concentración y de la calidad de la harina utilizada. No se han encontrado trabajos que controlen y evalúen el efecto del peso molecular de los β-glucanos de cereal sobre las masas y panes sin gluten.

Los panes hechos con ingredientes ricos en β-glucano a menudo se caracterizan por un menor volumen del pan, menor suavidad de la miga y una miga y corteza más oscuras (Cavallero et al., 2002; Skendi et al., 2010; Trogh et al., 2004). Cleary et al. (2007) en su estudio de enriquecimiento de pan convencional informaron que la mayor pérdida en la calidad del pan enriquecido con β-glucano de alto peso molecular se debió a su capacidad para unirse a grandes cantidades de agua en comparación con la adición de β-glucano de bajo peso molecular. A pesar de los efectos negativos en la calidad, el β-glucano puede tener un efecto positivo mediante el aumento de la vida útil del pan, ya que los hidrocoloides ayudan a reducir la tasa de aumento de la firmeza y la deshidratación de la miga durante el almacenamiento (Guarda et al., 2004; Mohamed et al., 2008). Por lo tanto, para diseñar pan sin gluten de calidad adecuada, con dosis de β-glucanos conformes con las declaraciones de salud de la EFSA, es preciso realizar un estudio que incluya todas las variables de influencia, entre ellas la concentración y el peso molecular de este hidrocoloide, y analizar su interacción con la hidratación de la masa.

3.1.5. Conservación del peso molecular del β-glucano

A pesar de estas consideraciones, estudios previos han obtenido reducciones en el peso molecular y en la viscosidad del β-glucano tras su incorporación en pan (Frank et al., 2004; Wood et al., 1994, 2000) y la consecuente disminución en los efectos beneficiosos para la salud (Aman et al., 2004; Andersson et al., 2004, 2008; Cleary et al., 2007; Frank et al., 2004). Con el fin de retener el impacto fisiológico completo

del β-glucano, es esencial identificar la etapa o etapas durante el proceso de elaboración del pan que van en detrimento del β-glucano para minimizar su despolimerización. Se cree que la degradación más importante se produce durante las etapas de mezcla y fermentación, ya que Andersson et al (2004) informaron que no había diferencias significativas en el peso molecular del β-glucano entre la masa fermentada y el pan horneado.

La reducción sustancial en el peso molecular del β-glucano se relaciona con la actividad enzimática de β-glucanasas endógenas de las harinas, además del tiempo de contacto con el β-glucano durante las etapas de amasado y fermentación (Aman et al., 2004; Andersson et al., 2004, 2008; Moriartey et al., 2010; Trogh et al., 2004). A partir de estas observaciones se plantea necesaria la destrucción de la actividad β-glucanasica de la harina con el fin de obtener panes sin gluten enriquecidos con β-glucano de elevado peso molecular, ya que la adaptación de los tiempos de mezclado y fermentación no parecen ser modificaciones tecnológicamente viables, ya que irían en detrimento de los parámetros de calidad de los productos finales

La actividad β-glucanasica endógena en la harina de arroz no se había demostrado hasta el momento, aunque un estudio previo ya apuntó hacia la posible presencia de actividad β-glucanasa en la harina de arroz debido a la reducción del peso molecular del β-glucano observada en panes sin gluten enriquecidos (Hager et al., 2011). Algunos de los métodos utilizados para inactivar la actividad β-glucanasa han incluido el empleo de autoclave, escaldado y reflujo con etanol (Lazaridou et al., 2014; Moriartey et al., 2010; Rieder et al., 2015). Sin embargo, no hay información en literatura sobre el uso del calentamiento por microondas para la inactivación de la enzima β-glucanasica, a pesar de las grandes posibilidades que ofrece por ser un método altamente eficaz, rápido y limpio. En aplicaciones enzimáticas únicamente se conocen procesamientos de arroz con microondas para inactivar las enzimas lipasa y lipoxigenasa con el fin de aumentar su estabilidad durante el almacenamiento (Chang y El-Dash 1998; Zhong et al., 2013).

En tratamientos térmicos de harinas de cebada con autoclave, la hidratación se ha identificado como un parámetro crítico para el éxito de la inactivación β-glucanásica (Lazaridou et al., 2014). Sólo en las harinas con el más alto contenido de humedad, cercano al 14%, hubo una destrucción completa de esta enzima. A partir de estas

apreciaciones, parece necesario el estudio del efecto del contenido de humedad en la cinética de inactivación β-glucanásica por calentamiento por microondas en la harina de arroz y la consiguiente repercusión del empleo de la harina inactivada en la elaboración de pan sin gluten enriquecido con β-glucanos. Los efectos de estos tratamientos sobre las propiedades físicas de la masa y del pan sin gluten elaborados con estas harinas son aún desconocidos. Al mismo tiempo, los tratamientos de microondas de la harina durante un tiempo prolongado pueden destruir otras enzimas como la α-amilasa, que conduciría a una disminución en la calidad del pan obtenido, por lo que es necesario su estudio detallado.

3.2. Los β-glucanos de hongos y levaduras.

Los (1→3)(1→6)-β-glucanos se encuentran ampliamente distribuidos en las paredes celulares de los microorganismos -particularmente de levadura *Saccharomyces cerevisiae*-, y hongos (Kittisuban et al., 2014; Liu et al. Al., 2008). El β-glucano más abundante en la pared celular de los hongos es el β-(1→3), que representa entre un 65 a un 90% del contenido total (Bowman y Free, 2006).

3.2.1. Funcionalidad fisiológica

Aunque aun no tienen declaraciones de salud reconocidas por EFSA, es habitual su empleo en aplicaciones farmacéuticas (Samuelsen et al., 2014). Numerosos estudios atribuyen a estos compuestos los efectos medicinales beneficiosos de ciertos hongos como *Ganoderma lucidum, Pleurotus ostreatus, Lentinus edodes* (Askin et al., 2010; Wasser, 2002) y levaduras (Harnack et al., 2011). Estudios clínicos atribuyen a estos β-glucanos propiedades cardiovasculares (Wasser, 2002), antiinflamatorias o protectoras del hígado (Lindequist et al., 2005), antibacterianas (Shittu et al., 2005; Beattie et al., 2010), inmunomoduladoras (Wasser, 2002), antivirales y antitumorales (Moradali et al., 2007; Tsukada et al., 2003; Zhang et al., 2007) y contra la obesidad (Zhang et al., 2013). La EFSA aún no ha aprobado una declaración de propiedades saludables sobre la función inmune de los preparados de β-glucano de levadura (EFSA, 2011; Samuelsen et al., 2014).

3.2.2. Aplicaciones en alimentos

En cuanto a sus aplicaciones alimentarias, éstas se encuentran todavía sin explorar y resulta imprescindible aportar conocimiento de sus posibilidades como ingrediente alimentario. La Autoridad Europea de Seguridad Alimentaria (EFSA) ha aprobado recientemente ciertos preparados de β-glucano derivado de levadura como nuevos ingredientes alimentarios (EFSA 2010 y 2011c) y la Administración de Alimentos y Fármacos de Estados Unidos ha reconocido su empleo como "seguro".

Como se apunta en estudios previos, los β-glucanos procedentes de hongos parecen mostrar actividades muy diferentes a aquellas mostradas por los β-glucanos derivados de avena y cebada (Zhu et al., 2015). La diferencia en las características moleculares y estructurales según el origen de BG conduce a diferencias en sus propiedades físicas y, por tanto, efecto diferente sobre la funcionalidad de los sistemas alimentarios (Banchathanakij & Suphantharika, 2009).

Aunque muy escasos, hay disponibles algunos extractos concentrados comerciales de (1→3)(1→6)-β-glucanos y es de gran interés conocer la viabilidad de su uso en los alimentos. Los únicos precedentes de enriquecimiento de alimentos con estos β-glucanos se refieren a yogurt (Hozova et al. 2004), masas y pan de trigo (Seguchi et al. 2001; Kim et al. 2011), y su empleo como espesante y estabilizante, agente absorbente de aceite o de retención de agua, sustituto de la grasa en emulsiones alimentarias (Santipanichwong y Suphantharika, 2009; Worrasinchai et al, 2006) y como modificador de la textura de geles de almidón (Satrapai y Suphantharika, 2007). La mayoría de ellos utilizan directamente los hongos tras operaciones de preparación como secado/trituración y lavado, en lugar de concentrados de BG. En cuanto a productos sin gluten, algunos estudios han centrado el uso de BG en el enriquecimiento de bizcochos (Kim et al., 2011), en fideos de arroz (Heo et al., 2014) y panes (Kittisuban et al., 2014). Kim et al. (2011) empleó extractos de BG de *Lentinus edodes* (fibra insoluble) con el objetivo de obtener un alto contenido en fibra y reducir las calorías en bizcochos de harina de trigo, concluyendo una mejora en las propiedades elásticas de las masas pero un empeoramiento en el volumen y dureza del producto final. Heo et al. (2014) lograron fideos de arroz con mayor extensibilidad y firmeza en presencia de *Lentinus edodes* para las tres concentraciones analizadas (4, 8 y 12%). Kittisuban et al. (2014) utilizaron la

metodología de superficie de respuesta para analizar los efectos del BG de levadura insoluble junto con hidroxi-propil-metil-celulosa (HPMC), y aislado de proteína de suero sobre masas y panes sin gluten basados en almidón de arroz. Encontraron un efecto perjudicial del BG sobre el volumen y la dureza del pan que atribuyeron al aumento de la consistencia de la masa asociado a la adición de BG, que dificultó su desarrollo durante la fermentación y horneado. Queda pendiente estudiar el efecto del BG en masas a hidratación adaptada, considerado como uno de los factores más relevantes en las fortificaciones con fibra, con el objeto de obtener masas de la consistencia adecuada que posibiliten su buen desarrollo.

Los BG de hongos y levaduras también exhiben diferencias en la solubilidad, que pueden dar lugar a diferencias en los comportamientos en las matrices alimentarias (Martínez et al., 2014) y que no se han considerado hasta ahora. En general, fibras solubles como inulina y polidextrosa disminuyeron la consistencia de la masa, al tiempo que favorecieron un mayor desarrollo del pan, panes más oscuros y con menor dureza de la miga y mayor densidad alveolar que el pan control (Martínez et al., 2014). En cambio, las fibras insolubles, como las de avena, bambú, patata y guisante, particularmente las de gran tamaño de partícula, disminuyeron el volumen específico del pan y aumentaron notablemente la firmeza del pan. Por lo tanto, el estudio del efecto de la fortificación con extractos (1→3)(1→6)-β-glucanos de derivados de levaduras y hongos en función de su solubilidad y a distintas concentraciones sobre las características de manejo de masas y calidad de los panes a base de harina arroz supondría un gran avance para la posterior elaboración de nuevos alimentos funcionales(Zhu et al., 2015).

INTRODUCTION

1. Coeliac disease

Coeliac disease (CD) is an immune enteropathy of the small intestine that causes intestinal villi atrophy and consequent malabsorption of several nutrients such as iron, folic acid, calcium and vitamins in genetically susceptible individuals (Sapone et al., 2012). At present, it represents one of the most common human genetic disorders, with an increasing prevalence of 1-2% worldwide (Cabrera-Chavez and Calderon de la Barca, 2010; Vici et al., 2016). There no cure for coeliac disease that is a lifelong condition; the only treatment is a strict gluten-free (GF) diet for life.

The inflammatory reaction caused by the CD is generated by the ingestion of gluten and, mostly, the gliadin fraction of wheat and the prolamins from common grains such as barley, rye and oat. Although oat is naturally gluten-free, its use in gluten-free breadmaking is controversial because of concerns of potential contamination of commercial oats that may occur during the productive process (Cabrera-Chavez and Calderon de la Barca, 2010; Kupper, 2005).

In recent years, the gluten-free market is attracting much research interest motivated by the rising number of patients diagnosed with CD, allergies or other gluten sensitivity reactions (Mir et al., 2016; Sapone et al., 2012; Rosell et al., 2014) and an emerging trend followed by people who, despite not having any form of gluten intolerance, adopt the decision to consume these products, based on the often misconception belief that gluten-free products are healthier than their conventional counterparts (Miranda et al., 2014).

At the same time, CD is frequently associated with a high incidence of type I (insulin dependent) diabetes mellitus (Capriles et al., 2016; Cronin and Shanahan, 1997) and obesity (Kupper, 2005). Therefore, patients should keep a gluten-free diet along with an adequate glycemic control. Thus, diet with low glycemic index (GI) food is suggested, i.e., foods rich in slow-digesting carbohydrates. Foods with high GI values have been shown to be more rapidly digested and absorbed, causing greater fluctuations in blood glucose per unit of carbohydrate than foods with lower GI values, which represent a gradual glucose release over time (Brand-Miller et al.,

2009). Bread is considered as a high-intermediate GI food, which tends to be reduced by incorporating, for example, high-fiber ingredients into its formulation.

2. Gluten-free bread

Gluten-free products available on the market are limited, expensive and present nutritional and sensory deficiencies, as well as other undesirable physico-chemical properties, such as a relatively short shelf-life (Capriles et al., 2016; Mir et al., 2016; Torbica et al., 2010).

Despite the recent efforts and advances in gluten-free breadmaking, most of the commercially available gluten-free breads are lower in quality to their gluten counterparts (Gallagher et al., 2003; Torbica et al., 2010; Vici et al., 2016). Gluten substitution in bakery products represents an important technological challenge that increases in those products whose processing and quality parameters depends very closely on the presence of gluten, i.e. leavened baked products. Gluten is the main structure-former in wheat bread due to its ability to form cohesive viscoelastic dough capable of entrapping gas during fermentation and baking and to provide a good structure in crumb breads. Its removal leads to a gluten-free dough less cohesive and elastic and more sticky and difficult to handle, and affects their expansion during the baking process (Gallagher et al., 2004). No other cereal shows such capabilities.

The process of making gluten-free dough differs from that used in traditional products as a result of different dough hydration requirements, which govern its consistency during mixing (Marco and Rosell, 2008), dough handling and behaviour duirng baking (Gómez et al., 2013). Gluten-free flour requires a higher amount of added water than wheat flour (Capriles and Areas, 2014; Marco and Rosell, 2008), leading to a dough system having viscosity resembled to that of cake batters rather than to a bread dough, and therefore requiring use of pans during its fermentation and baking. The formation of a dough with viscoelastic properties comparable to wheat can be obtained by enriching the GF bread formulations with hydrocolloids.

From a technological point of view, most of the research efforts in GF breadmaking have focused on the substitution of wheat flour with gluten-free flours or a mixture of these with starches (maize, sorghum, potato, cassava) and structuring agents such as proteins or polysaccharides (Demirkesen et al., 2014; Mancebo et al., 2015;

Sciarini et al., 2010). Among raw materials, rice flour is probably the most commonly used because of its bland taste, white color, and hypoallergenic properties along with low content of protein and sodium and high amounts of readily digestible carbohydrates (Rosell et al., 2014). Among the hydrocolloids that can mimic the gluten viscoelastic properties and improve the overall quality of the end product, different polysaccharides and proteins having gelling, thickening and emulsifying properties when incorporated into foods have been included (Demirkesen et al., 2014). The hydroxypropyl methylcellulose (HPMC), a chemical derivate of cellulose, seemed to be the hydrocolloid with the highest improving effect on sensory attributes of rice breads (Gallagher et al., 2004; Houben et al., 2012; Marco and Rosell, 2008; Matos and Rosell, 2014; Sivaramakrishnan et al., 2004). However, the different types of this ingredient may vary in gel strength of their structured dispersions and, therefore, provide different dough consistency of GF formulations. Therefore, a comprehensive study must be considered on the type and level of used HPMC as well as the amount of added water to the dough when this hydrocolloid is present in the bread formulations.

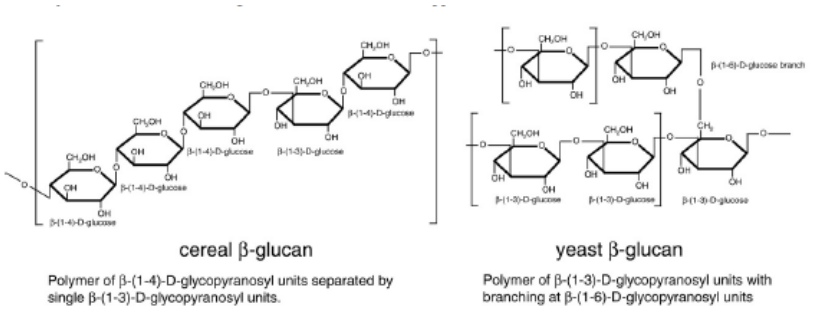
On the other hand, another current concern regarding gluten-free bread is related to the improvement of their nutritional profile (Vici et al., 2016). Enhancement of the nutritional quality of GF products simultaneously with the improvement of their sensory properties and other quality parameters remains a major challenge. However, among foods enriched with functional ingredients, especially those with health claims approved by the European Food Safety Authority (EFSA), GF breads are not usually included. Additionally, food products developed for celiac patients have usually lower nutritional quality than those consumed by healthy individuals. Therefore, the development of gluten-free food products for celiac patients with proved health benefits deserves great awareness (Capriles et al., 2016). Hydrocolloids are included in the group of dietary fibers from nutritional viewpoint and, therefore, further to their viscosity enhancement and gelling properties, the addition of these substances exerts a physiological action as well (Wood et al., 1994; Matos and Rosell, 2014); thus, the amount of dietary fiber in the diet of celiac patients could be improved by adding these functional ingredients in GF products such as bread. Previous *in vivo* studies in healthy people showed that GF bread intake significantly increased the area under the curve of blood glucose response compared

to wheat bread (Berti et al., 2004). Other authors observed that composition and type of processing of the various food products, significantly affect their glycemic responses (Jenkins et al., 1987; Ronda et al., 2010, 2012).

In general, the enrichment of gluten-free bread with dietary fibers could improve functionality of GF breads from both technological and nutritional point of view.

3. β-Glucan

β-Glucans are polysaccharides naturally present in cereal grains, fungi and yeasts, and even bacteria and algae (Izydorczyk and Dexter, 2008, Wood, 2004; Zhu et al., 2015). Their applications on medicine, pharmaceutical industry, foods, cosmetics, chemical industry and feed production reflect their great potential (Zhu et al., 2016). They are homopolysaccharides of β-D-glucose, but there are clear differences in molecular structure between β-glucans from different sources (Fig. 1). Thus, they exist in the form of linear mixed-linked (1→3), (1→4) β-D-glucans in cereals or branched (1→3), (1→6) β-D-glucans in fungi and yeasts. Furthermore, besides differences in type of linkage and branching, β-glucans can vary in solubility, molecular mass, tertiary structure, degree of branching, polymer charge and conformation in solution. All these molecular characteristics govern their physiological and technological properties (Volman et al., 2008).



cereal β-glucan

Polymer of β-(1-4)-D-glycopyranosyl units separated by single β-(1-3)-D-glycopyranosyl units.

yeast β-glucan

Polymer of β-(1-3)-D-glycopyranosyl units with branching at β-(1-6)-D-glycopyranosyl units

β-Glucan type	Structure	Description
Bacterial		Linear β1,3 glucan (i.e. Curdlan)
Fungal		Short β1,6 branched, β1,3 glucan (i.e. Schizophyllan)
Yeast		Long β1,6 branched, β1,3-glucan (i.e. WGP β-glucan, Betafectin™)
Cereal		Linear β1,3/β1,4-glucan (i.e oat, barley, rye)

Figure 2. Structure of β-glucans from various sources (Volman et al., 2008).

3.1. Cereal β-glucans

β-Glucans are present in the starchy endosperm cell walls of cereal grains, especially in barley (2.5–11.3%), oat (2.2–7.8%) and, to a lesser extent, in rye (1.2–2.0%), and wheat (0.4–1.4%) (Lazaridou et al., 2007). At the same time, cereal β-glucans exhibit a wide diversity in primary structure and molecular weight, which determine their physical properties, such as solubility, viscosity and gelling capacity and their functionality in formulated products (Lazaridou et al., 2003; 2004), as well as their physiological action in the gastro-intestinal tract (Wood, 2004). β-Glucans from cereals are classified as dietary fibers and thus, they can lower the calories of foods that are present, since they have 2 kcal/g (Borchani et al., 2016).

3.1.1. Physiological functionality

Cereal β-glucans display well recognized nutritional benefits. The Food and Drug Administration of the United States (FDA, 2005) approved a health claim for the products containing barley β-glucan for its capacity of reducing the risk of coronary heart disease. This recognition is an extension of the health claim originally approved for oat β-glucan (FDA, 1997). It is based on the daily intake of, at least, 3 g of β-glucan or 4 servings of a food product that provides at least 0.75 g of β-glucan per serving (FDA, 2005) as part of a diet low in saturated fat and cholesterol. Years later, the European Food Safety Authority (EFSA) has also authorized health claims for oat and barley β-glucans according to which the recommended daily intake is 3 g of oat or barley β-glucan ingestion leads to the reduction of blood plasma cholesterol levels, that is a major risk factor for the development of coronary heart disease (EFSA 2011a). It has been also recognized the that about 4 g of β-glucans per 30 g of available carbohydrates in bread and pasta products are able to reduce the post-prandial glycemic response (EFSA 2011b), and foods containing barley or oat grain fiber, at least 6/100 g product or 3 g/100 kcal, can increase the faecal bulk (EFSA 2011c).

The hypocholesterolemic and hypoglycaemic effects of cereal β-glucans depend on their ability to increase the viscosity of the gastrointestinal (GI) content, which is controlled by the molecular weight and the amount (concentration) of the solubilized β-glucans in GI tract (Lazaridou and Biliaderis, 2007; Wolever et al., 2010; Wood et al., 1994). Many studies have shown that there is an inverse relationship between the

viscosity of aqueous extracts of foods fortified with β-glucans and the postprandial glycemic responses and blood LDL-cholesterol levels after a meal with these products (Panahi et al., 2007; Östman et al., 2006; Wood et al., 1994). Additionally, Wood et al. (2000) verified that the glucose response in blood depended on the product of molecular weight and concentration of β-glucans, while other authors highlighted the β-glucan solubility as a key factor for their hypoglycaemic effects (Lan-Pidhainy et al., 2007). Any factor that can decrease the solubility or/and cause depolymerization of β-glucans could reduce their physiological impact (Wood et al., 1994, 2000). Therefore, it is necessary to determine these variables and study their possible changes (e.g. degradation) during food processing and storage in order to evaluate the physiological efficacy of cereal β-glucans.

3.1.2. β-Glucan extraction

The use of β-glucan-concentrated extracts from cereal grains and the interest for the production of foods enriched with β-glucan is growing. Concentrates of (1→3)(1→4)-β-D-glucans extracted from oats and barley are currently available on the market, but they are expensive because they require isolation and concentration processes with many steps. Dry-separation processes commonly used are relatively simple and cheap methods, although the β-glucan content in the concentrated preparations is generally ≤30% (Zheng et al., 2000). Thus, aqueous extraction of β-glucans for the bran flour of cereal grains followed by sequential removal of fats, starch and protein has been employed, that allow to obtain isolates with very high β-glucan concentrations (>90%) (Lazaridou et al., 2004; Benito-Roman et al., 2011). Additionally, the aqueous extraction of β-glucans could allow the selective elimination of the proteins trigger the celiac intolerance (i.e. storage proteins); the latter is an essential requirement for β-glucan inclusion in gluten-free foods, since neither barley nor oats themselves are safe for celiac patients. However, the extraction procedure could lead to a molecular weight reduction of β-glucan due to the high susceptibility of β-glucan to enzymatic degradation by endogenous β-glucanases, and therefore, to a decrease in the viscosity of β-glucan extracts (Panahi et al., 2007). The development of an extraction or purification method able to obtain β-glucan concentrates with high purity without degradation during processing would be of great interest.

3.1.3. Technological functionality

From a technological point of view, β-glucan is a hydrocolloid with great potential for its incorporation into foodstuffs. It has high water retention capacity, due to the presence of hydroxyl groups within its structure (Lazaridou et al., 2007). In addition, functional properties β-glucan allow its use as a thickener and stabilizer in a wide variety of food products, such as sauces, soups, desserts and meat among others. Therefore, β-glucans could provide a great variety of structural and textural properties to foods and their incorporation would also improve nutritional value of the products.

3.1.4. Enrichment of gluten-free bread with β-glucan

When deciding on foods to fortify with β-glucan it is important to take into account those products capable of achieving greater scope of its beneficial effects in a higher number of people. Bread, as a staple food, exhibit a great potential as a carrier of β-glucans that could deliver the proved health benefits of this fiber to a large group of population.

The effects of molecular weight, structure and concentration of cereal β-glucans on the rheological behavior of their aqueous dispersions have been extensively studied showing clear trends (Lazaridou et al., 2003, 2004). On the other hand, previous works focusing on the rheology of wheat doughs enriched with cereal β-glucan extracts showed contradictory results (Cavallero et al., 2002; Skendi et al., 2010). Skendi et al. (2009; 2010) concluded that the effect of these polysaccharides on the consistency and handling of the dough and on the bread quality depended on the molecular weight and concentration of β-glucans as well as the breadmaking quality of wheat cultivar used as a base flour. However, there are no studies that evaluate the effect of molecular weight of cereal β-glucans on gluten-free dough and bread quality parameters.

Breads made with preparations rich in β-glucan are often characterized by lower loaf volume, lower crumb softness and darker crumb and crust (Cavallero et al., 2002; Trogh et al., 2004; Skendi et al., 2010). Cleary et al. (2007), found greater loss in the quality of wheat breads enriched with a high molecular weight β-glucan preparation due to its ability to bind larger amounts of water compared to bread fortified with a low molecular weight β-glucan sample. Further to the adverse impact on bread

quality, β-glucan may have a positive effect by increasing product shelf life, since hydrocolloids can decrease the hardening rate and dehydration of the crumb during bread storage (Guarda et al., 2004; Mohamed et al., 2008). Hence, in order to develop gluten-free breads having acceptable sensory attributes and containing β-glucans in levels that fulfil EFSA's health claims, a study that would include all variables that affect the product quality and stability, such as concentration and molecular weight of this hydrocolloid and the dough hydration requirements should be carried out.

3.1.5. Preservation of the molecular weight of β-glucan

In spite of these considerations, previous studies found a significant reduction in molecular weight of β-glucans during production of leavened baked products and in the viscosity of product aqueous extracts and thus, a possible decrease in β-glucan physiological action (Aman et al., 2004; Andersson et al., 2004, 2008; Cleary et al., 2007; Frank et al., 2004; Lazaridou et al., 2014 Wood et al., 1994; 2000). The identification of the step(s) that result in depolymerization of β-glucans during breadmaking and the changes in processing to minimize the polysaccharide hydrolysis is crucial for retaining the full physiological impact of β-glucan. It was reported that the most important degradation occurs during the mixing and fermentation stages of the breadmaking, since Andersson et al. (2004) found that there were no significant differences in the molecular weight of β-glucans between those extracted from the fermented dough and the baked bread.

The substantial reduction in the molecular weight of β-glucan is related to the activity of endogenous β-glucanases in the flour when both substrate and enzyme are in contact during the kneading and fermentation stages (Aman et al., 2004; Andersson et al., 2004, 2008; Trogh et al., 2004, Moriartey et al., 2010). Therefore, the inactivation of β-glucanase activity of flour is required for obtaining gluten-free breads enriched with high molecular weight β-glucan, since the alternative large reduction in mixing and fermentation times do not seem to be technologically viable, because it could be detrimental for the quality parameters of the final product.

No endogenous β-glucanase activity in rice flour has been measured to date, although a previous study has already pointed to the possible presence of β-glucanase activity in rice flour due to the molecular weight reduction of β-glucan in gluten-free breads

enriched with cereal β-glucan concentrates (Hager et al., 2011). Some of the methods used to inactivate β-glucanase activity included the use of autoclaving, scalding and ethanol refluxing (Lazaridou et al., 2014; Moriartey et al., 2010; Rieder et al., 2015). However, there is no information in the literature on the application of microwave heating for the inactivation of β-glucanase, despite the great possibilities it offers for being a highly efficient, fast and clean method. In the past, microwave processing in rice flours has been used only, for inactivation of lipase and lipoxygenase to increase their stability during storage (Chang and El-Dash 1998; Zhong et al., 2013).

Additionally, flour water content has been identified as a critical parameter for the successful β-glucanase inactivation during autoclaving heat treatments of barley flour (Lazaridou et al., 2014); complete enzyme inactivation has been found only in flours with the highest moisture content (approximately 14%). These findings show that a study on the effect of flour moisture content on kinetics of β-glucanase inactivation by microwave heating of rice flour and the consequent impact of the use of inactivated flour in the production of gluten-free bread enriched with β-glucan is necessary. The effects of this thermal treatment on rheological properties of dough and quality parameters of gluten-free breads made by the treated flours are still unknown. Additionally, microwave treatments of flour over a long time can inactivate other enzymes such as α-amylase, which would lead to a decrease in the quality of the obtained bread and thus, a study of those factors in detail is required as well.

3.2. Fungi and yeast β-glucans

(1→3)(1→6)-β-D-glucans from fungi and yeast are widely distributed in the cell walls of these microorganisms particularly, of the baker's and brewer's yeast *Saccharomyces cerevisiae*, and mushrooms (Kittisuban et al., 2014; Liu et al., 2008). They have recently been authorized as a novel food ingredient by the European Commission based on safety reports have been issued by EFSA (2010 and 2011c). The most abundant β-glucan in the cell wall of fungi is (1→3)-β-D-glucan, which represents around 65-90% of the total content (Bowman and Free, 2006).

3.2.1. Physiological functionality

Although there is no any health claim allowance by EFSA for these polysaccharides, yet, their use in pharmaceutical applications is common (EFSA, 2011; Samuelsen et al., 2014). Numerous studies have attributed health beneficial effects to certain fungi, such as *Ganoderma lucidum, Pleurotus ostreatus, Lentinus edodes* (Askin et al., 2010; Wasser, 2002) and yeasts (Harnack et al., 2011). Clinical studies showed that these β-glucans exhibit cardiovascular protective (Wasser, 2002), anti-inflammatory or liver protective (Lindequist et al., 2005), antibacterial (Shittu et al., 2005; Beattie et al., 2010) immunomodulatory (Wasser, 2002), anti-viral and antitumor properties (Moradali et al., 2007; Tsukada et al., 2003; Zhang et al., 2007) and can contribute to prevention of obesity (Zhang et al., 2013).

3.2.2. Food applications

Food applications of microbial β-glucans are mostly unexplored, so it is essential to provide knowledge of their potential uses as food ingredient. Certain preparations of yeast-derived β-glucans have been recently approved as novel food ingredients by the European Food Safety Authority (EFSA) and given "Generally Recognized as Safe" status by US Food and Drug Administration (EFSA 2010 y 2011c).

Previous studies pointed out that fungi β-glucans showed a large difference in the physical properties than those exhibited by oat and barley β-glucans (Zhu et al., 2015). The differences in molecular and structural features (e.g. molecular weight and degree of branching) depending on the origin of microbial β-glucans lead to differences in their physical properties and thereby a large diversity in function properties of the food systems formulated with these polysaccharides can be obtained (Banchathanakij & Suphantharika, 2009).

Although very few commercial extracts of (1→3)(1→6)-β-D-glucans are available on the market, it is of great interest to know their potential use in food. The few studies on food product enrichment with β-glucan, concern yogurt (Hozova et al., 2004), dough and wheat bread (Seguchi et al. 2001; Kim et al. 2011) fortification, and use of branched β-glucans as thickener, oil absorbing or water retention agent, fat substitute in food emulsions (Santipanichwong y Suphantharika, 2009; Worrasinchai et al, 2006) and as a texture modifier of starch gels (Satrapai y Suphantharika, 2007). In most of the studies, fungi following simple preparation

protocols such as drying /trituration and washing, instead of β-glucan concentrates were used. Regarding gluten-free products, few studies have explored the use of these polysaccharides on the fortification of gluten-free cakes (Kim et al., 2011), rice noodles (Heo et al., 2014) and bread (Kittisuban et al., 2014). Kim et al. (2011) used β-glucan extracts from *Lentinus edodes* (insoluble fiber) as a high-fiber and low-calorie ingredient in wheat flour cakes and found an increase in batter viscosity with more pronounced shear-thinning behavior and enhanced elastic properties. Although no significant differences were observed between the control and cakes containing 1 g of yeast β-glucan per serving, a further increase of polysaccharide level resulted in decrease of the volume and increase of the hardness of cakes. Heo et al. (2014) obtained rice noodles with greater extensibility and firmness with *Lentinus edodes* fortification for all concentrations tested (4, 8 and 12%). Kittisuban et al. (2014) used response surface methodology to analyze effects of hydroxypropylmethylcellulose (HPMC), yeast β-glucan (insoluble), and whey protein isolate (WPI) on physical properties of gluten-free rice breads and found a decrease in bread volume and a increase in crumb hardness with increasing β-glucan concentration (from 1 g / 100 g to 2 g / 100 g). However, in this work the optimum level of added water to the dough was not investigated, which is considered one of the most important factors for optimization of the quality of the bread fortified with any fiber. In addition, fungi and yeast β-glucans exhibit differences in solubility, which may lead to different behavior in complex food matrices (Martinez et al., 2014); this fact has also to be considered in food fortification with microbial β-glucans. In general, incorporation of soluble fibers into bread formulations, such as inulin or polydextrose decrease the dough consistency, and favour a better bread development, darker breads, lower crumb hardness and higher cell density compared to control breads. In contrast, insoluble fibers from oat, bamboo, potato and pea, particularly those with coarse particle size, decreased the bread specific volume and increased largely the crumb firmness (Martinez et al., 2014).

Therefore, the study of the effect of the fortification with (1→3)(1→6)-β-D-glucans extracts from yeast and fungi, differing in their solubility and concentrations on dough handling and quality characteristics of gluten-free rice breads would be a great advance in development of novel functional foods.

OBJETIVOS

OBJETIVOS

El objetivo principal de este proyecto fue la mejora nutricional y funcional de pan destinado a enfermos celiacos mediante la adición de (1→3)(1→4)-β-glucanos procedentes de cereales, en dosis conformes con las declaraciones de salud aprobadas por la EFSA. Adicionalmente se estudió el enriquecimiento con β-glucanos (1→3)(1→6) de distinta procedencia como son levaduras y hongos**.**

Para conseguir el objetivo principal se plantearon los siguientes **OBJETIVOS CONCRETOS**:

1. **Estudio de la capacidad estructurante del β-glucano. Optimización de la dosis de otros hidrocoloides estructurantes habitualmente empleados como mimético del gluten, como la hidroxi-propil-metil-celulosa, y de las condiciones de hidratación de la masa en presencia de β-glucano.**

2. **Comparación del efecto de los concentrados de β-glucano de cereal comerciales sobre las propiedades reológicas de las masas y la calidad de los panes enriquecidos resultantes, elaborados en base harina de arroz.**

3. **Estudio del efecto del peso molecular del β-glucano sobre la reología de las masas sin gluten y la calidad de los panes resultantes. Empleo de β-glucanos purificados e hidrolizados en el laboratorio a partir de los extractos comerciales de avena.**

   El empleo de productos comerciales puede limitar las conclusiones obtenidas sobre el efecto del peso molecular debido a la baja pureza de los extractos disponibles que obligará a aportar cantidades importantes de sustancias que pueden interferir en el efecto del propio β-glucano. Por ello fue preciso obtener y aplicar concentrados de elevada pureza.

4. **Estrategias para la conservación del peso molecular del β-glucano en el producto final.**

   La presencia de la enzima β-glucanasa degrada los (1→3)(1→4)-β-glucanos. Los tratamientos térmicos sobre las harinas son capaces de reducir y destruir esta actividad enzimática. En concreto, los tratamientos con microondas supondrían

un importante avance por tratarse de un método apenas estudiado y más rápido, limpio y menos agresivo que los tratamientos convencionales. Es necesario comprobar que se pueden elaborar panes sin gluten en los que se conserven tanto el contenido de β-glucanos como su peso molecular. También fue preciso establecer las consecuencias tecnológicas que el empleo de estas harinas tratadas térmicamente puede generar en la calidad del pan.

5. **Efecto de la adición de concentrados de β-glucanos de levadura y hongos sobre las masas y panes sin gluten, en función de su procedencia y solubilidad.**

OBJECTIVES

The main objective of this project was the nutritional and functional improvement of bread suitable for celiac patients by adding (1→3)(1→4)-β-D-glucans from cereals at levels able to fulfil the EFSA approved health claims. In addition, the enrichment of gluten-free bread with (1→3)(1→6)-β-D-glucans from different origin such as yeast and fungi was studied.

To achieve the main objective, the following specific objectives were proposed:

1. Preliminary study of the structuring capacity of cereal β-glucans. Optimization of the level of other structurant hydrocolloids commonly used as gluten mimetics, such as HPMC, and of the dough hydration conditions in the presence of β-glucans.

2. Comparison of the effect of commercial cereal β-glucan concentrates on dough rheological properties and quality of the resultant enriched breads made from rice flour.

3. Study of the effect of molecular weight of β-glucan on the rheology of gluten-free doughs and the quality of the resultant breads. Use of high molecular weight and hydrolyzed β-glucans isolated from commercial concentrates from oats.

The use of commercial β-glucan concentrates for bread enrichment may limit the derived findings on the effect of polysaccharide molecular weight due to the low purity of the available concentrates, which results in inclusion of significant amounts of other substances that may interfere with the effect of β-glucan itself. Therefore, it was necessary to obtain and fortify the gluten-free breads with β-glucan concentrates of relatively high purity.

4. Strategies for the preservation of β-glucan molecular weight in the final product.

The presence of endogenous β-glucanases in flour mixes degrades the cereal β-glucans. Heat treatment applied to flours are able to reduce or eliminate this enzymatic activity. More specifically, microwave treatments would be an important

advance because it is a barely studied method and faster, cleaner and less aggressive than the conventional flour processing methods. It is also necessary to confirm that both β-glucan content and molecular weight are preserved in gluten-free breads by applying heat treatments in rice flour. Additionally, a study on the effect of flour processing on the bread quality is required.

5. Effect of yeast and fungi β-glucan concentrates on gluten-free dough and bread properties as they depend on polysaccharide origin and solubility.

LISTADO DE ARTÍCULOS

El contenido de esta tesis doctoral se divide en siete capítulos constituidos por artículos publicados (o en proceso de publicación) en revistas científicas de alto impacto. Estos capítulos se corresponden con los objetivos anteriormente planteados.

I. Ronda, F., Pérez-Quirce, S., Angioloni, A., Collar, C. (2013) "Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: a fundamental and empirical rheological approach". Food Hydrocolloids 32: 252-262.

II. Pérez-Quirce, S., Collar, C., Ronda, F. (2014) "Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads". Food Science and Technology 49 (5): 1375-1382.

III. Ronda, F., Perez-Quirce, S., Lazaridou, A., Biliaderis, C.G. (2015) "Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics". Food Hydrocolloids, 48, 197-207.

IV. Perez-Quirce, S., Lazaridou, A., Biliaderis, C.G., Ronda, F. (2017). "Effect of β-glucan molecular weight on rheological properties of gluten-free rice-based dispersions and doughs, physical characteristics and in vitro starch digestibility of breads". LWT - Food Science and Technology. DOI: 10.1016/j.lwt.2017.04.065.

V. Perez-Quirce, S., Ronda, F., Melendre, C., Lazaridou, A., Biliaderis, C.G. (2016) "Inactivation of endogenous rice flour β-glucanase by microwave radiation and impact on physico-chemical properties of the treated flour". Food and Bioprocess Technology, 9 (9): 1562–1573.

VI. Perez-Quirce, S., Ronda, F., Lazaridou, A., Biliaderis, C.G. (2017) "Effect of microwave radiation pre-treatment of rice flour on gluten-free breadmaking and molecular size of β-glucans in the fortified breads". Food and Bioprocess Technology DOI: 10.1007/s11947-017-1910-7.

VII. Perez-Quirce, S., Caballero, P.A., Villanueva, M., Ronda, F. (2017) "Impact of yeast and fungi (1→3)(1→6)-β-glucan concentrates on viscoelastic behavior and breadmaking performance of gluten-free rice-based doughs". Submitted to Food Hydrocolloids.

PLAN DE TRABAJO

PLAN DE TRABAJO

La tesis doctoral se fundamentó en la necesidad de investigar la mejora nutricional y organoléptica de los panes sin gluten destinados a la población celiaca. Para lograr este propósito, se pretendió evaluar la viabilidad del enriquecimiento con β-glucanos, tanto los procedentes de cereales como los de hongos y levaduras, cuyos beneficios para la salud han sido ampliamente demostrados.

El estudio se dividió en **cinco secciones diferentes** encaminadas a la consecución de los objetivos específicos descritos anteriormente.

I. La primera de las líneas descrita en los **capítulos I y II** consistió en la realización de ensayos preliminares. En el capítulo I se incluyó un estudio reológico empírico y fundamental de las mezclas de fibras dietéticas -HPMC de diferente fuerza de gel y β-glucano de cebada- en matrices de harina de arroz bajo distintos niveles de hidratación. Resultó necesario esclarecer la viabilidad de la adición de BG, las necesidades de hidratación y las dosis de fibras a emplear, así como evaluar la capacidad de manejo de las masas sin gluten. A continuación, el capítulo II detalló el efecto de la combinación de fibras con diferentes niveles de hidratación en la calidad del pan sin gluten y en su envejecimiento (vida útil). Se llevó a cabo un estudio de correlación de ambos capítulos para conocer las relaciones entre las propiedades reológicas y físico-químicas de masa y pan y la valoración sensorial de los panes sin gluten enriquecidos con β-glucano resultantes.

   Con estos dos capítulos se localizaron los factores de mayor influencia en la calidad del pan, sus niveles de empleo y la existencia de interacciones significativas entre ellos. En función de los resultados obtenidos se estableció la capacidad del BG para sustituir otros hidrocoloides. Para llevarlo a cabo se aplicó un diseño de experimentos muy optimizado (Draper-Lin) donde los factores de estudio fueron: tipo de HPMC (media y baja fuerza de gel), dosis de HPMC, dosis de BG y dosis de agua. Para cumplir con la alegación de salud de la EFSA relacionada con el mantenimiento de los niveles de colesterol en sangre, la concentración de BG de cereal en el pan de estableció como mínimo

en 1,5 % en base harina (suponiendo un consumo de pan de 4 raciones de 50 g diarios).

II. La finalidad de la segunda sección (**capítulo III**) fue el estudio del efecto del enriquecimiento de pan sin gluten elaborado con harina de arroz con concentrados comerciales de β-glucanos derivados de cebada y de avena, en la reología de las masas y las características de calidad del pan. Partiendo de los resultados alcanzados en capítulos previos, se mantuvo constante el contenido y tipo de HPMC. Sin embargo, se varió el contenido de agua en la formulación, de modo que se pudiera optimizar el nivel de hidratación de los panes fortificados para maximizar la calidad del pan. Los factores de estudio fueron: tipo de BG, dosis de BG y dosis de agua.

Se decidió trabajar con los extractos concentrados de β-glucanos de cereal disponibles en el mercado. Se emplearon tres β-glucanos comerciales, dos de ellos de marcas Barliv^{TM} (Cargill) y GlucagelTM, procedentes de cebada y con purezas cercanas al 70% aunque pesos moleculares relativamente bajos, y un tercero procedente de avena, PromOat®, caracterizado por alto peso molecular pero baja pureza (entorno al 30%). El primero de ellos dejó de fabricarse durante la realización de esta Tesis, por lo que se continuó con el segundo. Se analizó el contenido de gluten en los extractos comerciales para asegurar que no estuvieran contaminados con las prolaminas que desencadenan la intolerancia de los enfermos celiacos. Los resultados obtenidos mostraron un contenido de gluten en BG de avena y decebada de marca Barliv^{TM} por debajo del límite de detección(<6,2 mg / kg), mientras que la concentración de gluten en el BG de cebada (Glucagel^{TM}),fue de 1,76 g / kg que ocasiona que este concentrado comercial de BG no esté libre de gluten. Sin embargo, se incluyó como factor de estudio ya que se consideró factible la obtención de un preparado libre de gluten y no se estimaron variaciones en cuanto a su empleo desde un punto de vista tecnológico.

III. En el **capítulo IV** se presentaron los resultados del empleo de extractos concentrados de BG de cereal de elevada pureza y peso molecular controlado sobre las propiedades reológicas de las masas y las propiedades físicas de los panes, considerando los aspectos nutricionales como el índice glucémico en el

producto final. Los factores de estudio del diseño de experimentos fueron: peso molecular y dosis de BG, partiendo de las hidrataciones de masa optimizadas en estudios anteriores.

Con objeto de estudiar el efecto del peso molecular y reducir o eliminar los efectos de los excipientes, se optó por la obtención a escala laboratorio de BG purificados a partir del BG de avena de alto peso molecular y alta pureza. Este procedimiento se llevó a cabo durante mi estancia en la Universidad de Tesalónica (Grecia). Los procesos realizados fueron:

a) Purificación del extracto comercial de BG de avena- hasta alcanzar un contenido de β-glucano ~ 72%.

b) Hidrólisis controlada del BG de avena purificado para obtener un segundo BG, de peso molecular intermedio, tras una purificación sobre una porción del BG obtenido en el apartado anterior (ver diagrama de ambos procesos en el capítulo IV).

IV. En el capítulo anterior se observó una degradación del peso molecular del BG en el producto final, aunque se conservaba el contenido de BG incorporado. Con el fin de retener el impacto fisiológico completo del BG en el producto final se consideró crucial minimizar su despolimerización (hidrólisis) durante el proceso de panificación. Ello abrió la cuarta línea de investigación, recogida en los **capítulos V y VI,** donde se presentó el estudio de la inactivación de la enzima β-glucanasa endógena de la harina de arroz y su aplicación en la elaboración de panes enriquecidos con β-glucano:

a) Estudio del efecto de la aplicación de energía microondas sobre la harina de arroz (capítulo V). Los factores de estudio del diseño de experimentos fueron: humedad de la harina y tiempo de tratamiento en microondas.

b) Efecto de la adición de las harinas tratadas térmicamente en panes sin gluten enriquecidos con BG (capítulo VI). Se procedió a sustituir la harina de arroz nativa por la tratada térmicamente con el propósito de comprobar la conservación tanto del contenido como del peso molecular del BG en el producto final.

V. La última línea de investigación abordada en esta Tesis Doctoral (recogida en el capítulo VII) se centró en el enriquecimiento del pan sin gluten con concentrados comerciales de (1→3)(1→6)-β-glucano (BGHL) procedentes de levadura (*Saccharomyces cerevisiae)* y hongos (*Pleurotus Ostreatus*). Se evaluó el efecto de su adición sobre las propiedades de masas y panes sin gluten de forma análoga a los estudios realizados para el BG de cereal.

Todas las líneas de investigación y sus capítulos correspondientes llevaron consigo un análisis estadístico de los resultados, que representó una herramienta de gran utilidad para la comparación y discusión de los resultados obtenidos y para la implantación tecnológica del BG como componente de alimentos y en el diseño de nuevos productos. Una descripción detallada de los métodos empleados y de los resultados derivados se presentaron en los respectivos subapartados de cada capítulo de esta Tesis Doctoral.

WORK PLAN

The doctoral thesis was aimed at investigating the nutritional and organoleptic improvement of gluten-free breads that are appropriate for the needs of celiac patients. To this direction, the feasibility of enrichment of rice breads with β-glucans, from cereal grains, fungi and yeast having well recognized health benefits was explored.

The study was divided into five different sections aimed at achieving the specific objective described above.

I. The first part of research described in **chapters I and II**, consists of preliminary investigations. Chapter I includes a study of rheological behaviour using empirical and fundamental methods of rice doughs fortified with two HPMC types differing in their gel strength, and barley β-glucan under different levels of dough hydration. It was important to clarify the effect of the addition of cereal β-glucans to gluten-free dough handing ability, the dough hydration requirements and the concentration of fibers that can be used. Chapter II describes in details the effect of fiber mixtures (two HPMC types and barley β-glucans) at different levels of dough hydration on gluten-free bread quality and shelf life. A correlation study was carried out in both chapters to determine the relationship between the rheological and physico-chemical properties of dough and the bread sensory attributes of β-glucan enriched breads.

   In these two chapters, the factors and their levels that had the greatest influence on bread quality were also discussed. The ability of β-glucan to substitute other hydrocolloids, such as HPMC in gluten-free dough systems was examined using an optimization experimental design (Draper-Lin) where the studied factors were: HPMC type (medium and low gel strength), and HPMC, β-glucan and water levels. In order to fulfil the EFSA's health claim related to the maintenance of blood cholesterol levels, the cereal β-glucan concentration in the bread was set at a minimum level of 1.5% assuming a daily consumption of 4 servings of 50 g bread).

II. The purpose of the second part of the research (**Chapter III**) was to investigate the effect of the enrichment of rice bread formulations with commercial barley and oat β-glucan concentrates on dough rheology and bread quality characteristics. Based on the findings obtained in the previous chapters, certain level and type of HPMC were used for breadmaking. However, the amount of added water in the dough formulation was varied, for determining the optimum level at which the quality of the final product is maximized. Type of β-glucan, and β-glucan and water levels were the studied factors.

Three commercial β-glucans concentrates were used, two of them were Barliv™ (Cargill) and Glucagel™, derived from barley with β-glucan content close to 70% and relatively low molecular weights. The third β-glucan sample from oat, PromOat®, was characterized by high molecular weight although had low purity (around 30% β-glucans). The gluten content in the commercial extracts was analyzed to ensure that they were not contaminated with the prolamins that trigger the coeliac gluten sensitivity. Results showed a gluten content in oat and barley β-glucan, Barliv™, under the limit gluten levels (<6.2 mg / kg), while the gluten concentration in barley β-glucan, Glucagel™, was 1.76 g / kg showing that this commercial β-glucan concentration is not gluten-free. However, this preparation was included in the study, since it was considered feasible to obtain a gluten-free preparation and by using of this samples in gluten-free breads no particular effect would be expected from a technological point of view.

III. **Chapter IV** presents the findings of the effect of concentrated cereal β-glucans with high purity and tailor made molecular weight on the dough rheological properties and bread physical properties, considering nutritional aspects such as the glycemic index in the final product. According to the experimental design factors that were explored were molecular weight and level of β-glucan, while the used water levels added to the dough based on the optimization studied given in the previous chapters.

In order to study the effect of β-glucan molecular weight and reduce or eliminate the effects of the other substances present in the commercial oat β-glucan preparation, this sample was further concentrated at laboratory scale resulting in an oat β-glucan preparation with high molecular weight and relatively high

purity. This procedure was carried out during stay of the PhD student at the University of Thessaloniki (Greece). The isolation processes performed involve:

a. Purification of the commercial concentrate of β-glucans from oats until reaching a content of β-glucan ~ 72%.

b. Acid hydrolysis performed on a portion of the isolated oat β-glucans from the previous step under controlled conditions (hydration level and time) to obtain a second β-glucan sample with intermediate molecular weight (see diagram of both processes in Chapter IV).

IV. In chapter III a degradation of the molecular weight of β-glucans in the final product was observed, although the content of β-glucans added was preserved. In order to retain the full physiological impact of β-glucan in the final product, it was considered crucial to minimize its depolymerization (hydrolysis) during the baking process. This was carried out in the fourth part of research included in **Chapters V and VI** which describe the study of endogenous β-glucanase inactivation in rice flour and its application in the production of breads enriched with cereal β-glucans:

a. Firstly, the effect of microwave energy application on rice flour was investigated by studying the impact of water content in the rice flour and microwave treatment time on enzyme residual activity (Chapter V).

b. Secondly, the effect of the addition of the microwave treated flours to gluten free breads enriched with β-glucans was explored to confirm the preservation β-glucan content and molecular weight in the final product (Chapter VI).

V. The last part of this PhD Thesis discussed in **chapter VII** is focused on the enrichment of gluten-free bread with (1→3)(1→6)-β-D-glucan commercial concentrates (BGHL) extracted from yeast, *Saccharomyces cerevisiae*, and fungus, *Pleurotus Ostreatus*. The effect of their addition on the properties of gluten-free doughs and breads was evaluated in an analogous way to the studies performed for cereal β-glucans.

All parts of this research and their corresponding chapters include statistical analysis of the results, that were a useful tool for the comparison and discussion of the findings and for the technological implementation of β-glucans as a food ingredient in new product formulations. A detailed description of the used statistical methods and the subsequent results were presented in the following sections of this PhD Thesis.

BIBLIOGRAFÍA

BIBLIOGRAFÍA

1. Aman, P., Rimsten, L., Andersson, R. (2004). Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 81(3):356-360.

2. Andersson, A.A.M., Armö, E., Grangeon, E., Fredriksson, H., Andersson, R., Åman, P. (2004). Molecular weight and structure units of (1-3, 1-4)-β-glucans in dough and bread made from hull-less barley milling fractions. J Cereal Sci. 40:195-204.

3. Andersson, A.A., Rüegg, N., Åman, P. (2008). Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. J Cereal Sci. 47:399-406.

4. Askin, R.; Sasaki, M.; Goto, M. (2010) Recovery of water-soluble compounds from Ganoderma lucidum by hydrothermal treatment. Food and Bioproducts Processing. 88: 291-297.

5. Beattie, K.D., Rouf, R., Gander, L., May, T.W., Ratkowsky, D., Donner, C.D., et al., (2010). Antibacterial metabolites from Australian macrofungi from the genus Cortinar-ius. Phytochemistry 71, 948–955.

6. Banchathanakij R.; Suphantharika M. (2009). Effect of different β-glucans on the gelatinisation and retrogradation of rice starch. Food Chemistry 114, 5–14.

7. Benito-Román, E. Alonso, S. Lucas (2011) Optimization of the β-glucan extraction conditions from different waxy barley cultivars. Journal of cereal science. 53: 271-276.

8. Berti, C.; Riso, P.; Monti, L.; Porrini, M. (2004) In vitro starch digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts. European Journal of Nutrition. 43, 198:204.

9. Borchani, C.; Fonteyn, F.; Jamin, G.; Paquot, M.; Thonart, P.; Blecker, C. (2016). Physical, functional and structural characterization of the cell wall fractions from baker's yeast *Saccharomyces cerevisiae*. Food Chemistry, 194, 1149–1155.

10. Bowman, S.M., Free, S.J. (2006). The structure and synthesis of the fungal cell wall. BioEssays 28 (8), 799–808

11. Brand-Miller, J.C., Stockmann, K., Atkinson, F., Petocz, P., & Denyer, G. (2009).Glycemic index, postprandial glycemia, and the shape of the curve in healthy subjects:analysis of a database of more than 1000 foods. The American Journal of Clinical Nutrition, 89, 97-105.

12. Cabrera-Chávez, F.; Calderón de la Barca, AM (2010) Trends in wheat technology and modification of gluten proteins for dietarytreatment of coeliac disease patients. Journal of Cereal Science. 52: 337-341.

13. Capriles, V.D., & Areas, J.A.G. (2014). Novel approaches in gluten-free breadmaking: interface between food science, nutrition, and health. Comprehensive Reviews in Food Science and Food Safety, 13, 871-890.

14. Capriles, V.D., dos Santos, F.G., Areas, J.A.G., (2016). Gluten-free breadmaking: Improving nutritional and bioactive compounds. Journal of Cereal Science, 67, 83-91.

15. Cavallero, A., Empilli, S., Brighenti, F. & Stanca, A. M. (2002) High (1→3,1→4)- b-Glucan Barley Fractions in Bread Making and their Effects on Human Glycemic Response. Journal of Cereal Science 36, 59–66.

16. Chang, Y. K. & El-Dash, A. A. (1998). Effect of microwave energy on lipase inactivation and storage stability of brown rice. Acta Alimentaria, Vol. 27, No.2, 193-202.

17. Cleary, L.J., Andersson, R., Brennan, C.S. (2007). The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and in vitro digestion. Food Chem. 102:889-897.

18. Cronin, C. & Shanahan, F. (1997) Insulin-dependent diabetes mellitus and celiac disease. Lancet, 349 1096-1097.

19. Demirkesen, I., Kelkar, S., Campanella, O.H., Sumnu, G., Sahin, S., Okos, M.. (2014). Characterization of structure of gluten-free breads by using X-ray Microtomography. Food Hydrocolloids, 36, pp. 37–44.

20. EFSA (2010) Scientific Opinion on the safety of "*Lentinus edodes* extract" (Lentinex®) as a Novel Food ingredient. EFSA Journal 2010; 8(7):1685.

21. EFSA (2011a) Scientific Opinion on the substantiation of a health claim related to barley β-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/20061. EFSA Journal, 9(12),2470.

22. EFSA (2011b) Scientific Opinion on the substantiation of health claims related to β-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 EFSA Journal, 2011;9(6):2207.

23. EFSA (2011c) Scientific Opinion on the safety of 'yeast β-glucans' as a Novel Food ingredient. EFSA Journal, 9(5),2137.

24. Food and Drug Administration. (1997). Food labeling: health claims; oat and coronary heart disease; Final Rule Federal Register Doc. 97-1598, filed 1-22-97.

25. Food and Drug Administration. (2005). Food labeling: health claims; soluble dietary fibre from certain foods and coronary heart disease; Final Rule Federal Register Doc. 2004P-0512, filed 12-23-05.

26. Frank, J., Sundberg, B., Kamal-Eldin, A., Vessby, B., Åman, P. (2004). Yeast-leavened oat breads with high or low molecular weight β-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J Nutr. 134:1384-1388.

27. Gallagher, E., Gormley, T. R. and Arendt, E. K. (2003). Crust and crumb characteristics of gluten-free breads. Journal of Food Engineering, 56, 153-161.

28. Gallagher, E., Gormley, T.R., & Arendt, E.K. (2004). Recent advances in the formulation of gluten-free cereal-based products. Trends in Food Science and Technology, 15, 143–152.

29. Gomez, M., Talegon, M., & dela Hera, E. (2013). Influence of mixing on quality of gluten-free bread. Journal of Food Quality, 36, 139-145.

30. Guarda, A., Rosell, C.M., Benedito, C., Galotto, J. (2004). Different hydrocolloids as bread improvers and antistaling agents. Food Hydrocoll. 18(2):241-247.

31. Hager, A.S., Ryan, L.A.M., Schwab, C., Ganzle, M.G., O'Doherty, J.V., & Arendt E.K. (2011). Influence of the soluble fibres inulin and oat β-glucan on quality of dough and bread. European Food Research and Technology, 232, 405-413.

32. Harnack U.; Kellermann U.; Pecher G. (2011) Yeast-derived β-(1→3)(1→6)-D-glucan Induces Up-regulation of CD86 on Dectin-1-(positive) Human B-Lymphoma Cell Lines Anticancer Research , 31: 4195-4199.

33. Heo, S.; Jeon, S. & Lee, S. (2014). Utilization of *Lentinus edodes* mushroom β-glucan to enhance the functional properties of gluten-free rice noodles. Food Science and Technology 55, 627 – 631.

34. Houben, A., Hochstotter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. European Food Research and Technology,235, 195-208.

35. Hozová, B., Kuniak L. & Kelemenová, B. (2004) Application of β-D-Glucans Isolated from Mushrooms *Pleurotus ostreatus* (Pleuran) and *Lentinus edodes* (Lentinan) for Increasing the Bioactivity of yoghurts. Czech Journal of Food Science., 22, 204-214.

36. Izydorczyk, M.S., Dexter, J.E. (2008). Barley b-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products–a Review. Food Research International 41 (2008) 850–868.

37. Jenkins, D.J.A.; Thorne N.J.; Wolever, T.M.S.; Jenkins, A.L.; Venketschwer, R.; Thompson L.U. (1987) The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. Am. J. Clin. Nutr. 45, 946-951.

38. Kim, J. Lee, S.M., Young Bae, I., Park, H.G., Gyu Lee, H., & Lee S. (2011). (1–3)(1–6)-β Glucan-enriched materials from *Lentinus edodes* mushroom as a high-fibre and low-calorie flour substitute for baked foods. Journal of the Science of Food and Agriculture. 91: 1915-1919.

39. Kittisuban, P.; Ritthiruangdej, P. & Suphantharika, M. (2014). Optimization of hydroxypropylmethylcellulose, yeast β-glucan, and whey protein levels based on physical properties of gluten-free rice bread using response surface methodology. Food Science and Technology 57, 738 – 748.

40. Kupper, C. (2005). Dietary guidelines and implementation for celiac disease. Gastroenterology, 128, 121-127.

41. Lan-Pidhainy, X., Brummer, Y., Tosh, S., Wolever, T.M., Wood, P. (2007). Reducing β-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. Cereal Chem. 84(5):512-517.

42. Lazaridou, A., Biliaderis, C.G. (2007). Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. J Cereal Sci. 101-118.

43. Lazaridou, A., Biliaderis, C.G., Izydorczyk, M.S. (2003). Molecular size effects on rheological properties of oat b-glucans in solution and gels. Food Hydrocolloids, 17, 693–712.

44. Lazaridou, A., Biliaderis, C.G., Micha-Screttas, M., Steele, B.R. (2004) A comparative study on structure–function relations of mixed-linkage (1 -3), (1- 4) linear b-D-glucans. Food Hydrocolloids 18, 837–855.

45. Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., Biliaderis, C.G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. J Food Eng. 79:1033-1047.

46. Lazaridou, A., Marinopoulou, A., Matsoukas, N. P., & Biliaderis, C. G. (2014). Impact of flour particle size and autoclaving on β-glucan physicochemical properties and starch digestibility of barley rusks as assessed by in vitro assays. Bioactive Carbohydrates and Dietary Fibre, 4 (1), 58-73.

47. Lindequist, U., Niedermeyer, T.H.J., Ju¨ lich, W.D., (2005). The pharmacological poten-tial of mushrooms. Evidence-Based Complementary and Alternative Medicine 2, 285–299.

48. Liu, X.; Wang, Q.; Cui, S. & Liu, H. (2008). A new isolation method of β-D-glucans from spent yeast Saccharomyces cerevisiae. Food Hydrocolloids 22, 239 – 247.

49. Mancebo, C. M., Miguel, M. A. S., Martinez, M. M., & Gomez, M. (2015). Optimization of rheological properties of gluten-free doughs with HPMC, psyllium and different levels of water. Journal of Cereal Science, 61, 8-15.

50. Marco, C. & Rosell, C.M. (2008) Breadmaking performance of protein enriched, gluten-free breads. European Food research and Technology, 227, 1205-1213.

51. Martínez, M. M.; Díaz, A.; Gómez, M. (2014). Effect of different microstructural features of soluble and insoluble fibres on gluten-free dough rheology and bread-making. Journal of Food Engineering 142, 49–56.

52. Matos, M.E., & Rosell, C.M. (2014). Understanding gluten-free dough for reaching breads with physical quality and nutritional balance. Journal of the Science of Food and Agriculture, 95, 653-661.

53. Mir, S.A., Shah, M.A., Naik, H.R., Zargar, I.A. (2106). Influence of hydrocolloids on dough handling and technological properties of gluten-free breads. Trends in Food Science & Technology, 51, 49-57.

54. Miranda, A., Lasa, A., Bustamante, M.A., Churruca, I., & Simon, E. (2014). Nutritional differences between a gluten-free diet and a diet containing equivalent products with gluten. Plant Foods of Human Nutrition, 69, 182–187.

55. Mohamed, A., Rayas-Duarte, P., Xu, J. (2008). Hard Red Spring wheat/C-TRIM 20 bread: formulation, processing and texture analysis. Food Chem. 107:516-524.

56. Moradali, M. F., Mostafavi, H., Ghods, S., & Hedjaroude, G. A. (2007). Immunomodu-lating and anticancer agents in the realm of macromycetes fungi (macrofungi).International Immunopharmacology, 7, 701–724.

57. Moriartey, S., Temelli, F., & Vasanthan, T. (2010). Effect of formulation and processing treatments on viscosity and solubility of extractable barley β-glucan in bread dough evaluated under in vitro conditions. Cereal Chemistry, 87(1), 65-72.

58. Östman, E., Rossi, E., Larsson, H., Brighenti, F., Björck, I. (2006). Glucose and insulin responses in healthy men to barley bread with different levels of (1-3, 1-4)-β-glucans; predictions using fluidity measurements of in vitro enzyme digests. J Cereal Sci. 43:230-235.

59. Panahi, S., Ezatagha, A., Temelli, F., Vasanthan, T., Vuksan, V. (2007). β-Glucan from two sources of oat concentrates affect postprandial glycemia in relation to level of viscosity. J Am College Nutr. 26(6):639-644.

60. Rieder, A., Ballance, S., Knutsen, S.H. (2015). Viscosity based quantification of endogenous β-glucanase activity in flour. Carbohydrate Polymers, 115, 104–111.

61. Ronda F., Rivero, P.; Caballero, P.A.; Quilez, J. (2012) High insoluble fiber content increases in vitro starch digestibility in partially baked breads. Submitted to International Journal of Food Science and Nutrition.

62. Ronda, F.; Gómez, M.; Quílez, J. (2010) Prolonged frozen storage of partially-baked wheat bread increases in vitro slowly digestible starch after final bake. International Journal of Food Sciences and Nutrition, 61, 624–629.

63. Rosell C.M., Barro, F.; Sousa C., Mena, M.C. (2014), Cereals for developing gluten-free products and analytical tools for gluten detection. Journal of Cereal Science, 59, 354-364.

64. Samuelsen, A.; Schrezenmeir, J. & Knutsen, H. (2014). Effects of orally administered yeast-derived β-glucans: A review. Molecular Nutrition & Food Research 58 (1) 183 – 193.

65. Santipanichwong, R., & Suphantharika, M. (2009). Influence of different b-glucans on the physical and rheological properties of egg yolk stabilized oil-in-water emulsions. Food Hydrocolloids, 23, 1279–1287.

66. Satrapai, S., & Suphantharika, M. (2007). Influence of spent brewer's yeast b-glucan on gelatinization and retrogradation of rice starch. Carbohydrate Polymers, 67, 500-510.

67. Sapone, A., Bai, J.C., Ciacci, C., Dolinsek, J., Green, P.H.R., Hadjivassiliou, M., Kaukinen, K., Rostami, K., Sanders, D.S., Schumann, M., Ullrich, R., Villalta, D., Volta, U., Catassi, C., & Fasano, A. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Medicine, 10, 1-12.

68. Sciarini, L.S., Ribotta, P.D., Leon, A.E., Perez, G.T.. Effect of hydrocolloids on gluten-free batter properties and bread quality. International Journal of Food Science and Technology, 45 (2010), pp. 2306–2313.

69. Seguchi, M., Morimoto, N., Abe, M., Yoshino Y. (2001). Effect of Maitake (Grifola frondosa) Mushroom Powder on Bread Properties. Food Chemistry and Toxicology, 66, 261-264.

70. Shittu, O.B., Alofe, F.V., Onawunmi, G.O., Ogundaini, A.O., Tiwalade, T.A., (2005). Mycelial growth and antibacterial metabolite production by wild mushrooms. African Journal of Biomedical Research 8, 157–162.

71. Sivaramakrishnan, H. P., Senge, B., & Chattopadhyay, P. K. (2004). Rheological properties of rice dough for making rice bread. Journal of Food Engineering, 62, 37-45.

72. Skendi, A., Biliaderis, C.G., Papageorgiou, M., Izydorczyk, M.S. (2010) Effects of two barley b-glucan isolates on wheat flour dough and bread properties. Food Chemistry 119, 1159–1167.

73. Skendi, A., Papageorgiou, M, Biliaderis. C.G. (2009) Effect of barley b-glucan molecular size and level on wheat dough rheological properties. Journal of Food Engineering 91, 594–601.

74. Torbica, A., Hadnađev, M., &Dapčević, T. (2010). Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour. Food Hydrocolloids, 24, 626-632.

75. Trogh, I., Courtin, C., Andersson, A.A., Åman, P., Sørensen, J., Delcour, J. (2004). The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→3,1→4)-β-D-glucan levels. J Cereal Sci. 40:257-267.

76. Tsukada C; Yokoyama H; Miyaji C; Ishimoto Y; Kawamura H; Abo T. (2003) Inmunopotentiation of intraepithelial lymphocytes in the intestine by oral administration of b-glucan. Cellular Immunol. 221: 1-5.

77. Vici, G., Belli, L., Biondi, M., Polzonetti, V. (2016) Gluten free diet and nutrient deficiencies: A review. Clinical Nutrition 35(6): 1236-1241. doi: 10.1016/j.clnu.2016.05.002.

78. Volman, J. J.; Ramakers, J. D.; Plat, J. (2008). Dietary modulation of immune function by β-glucans. Review. Physiology & Behavior, 94, 276–284.

79. Wasser S. (2002) Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol., 60: 258-274.

80. Wolever, T. M. S., Tosh, S. M., Gibbs, A. L., Brand-Miller, J., Duncan, A. M., Hart, V., et al. (2010). Physicochemical properties of oat β-glucan influence its ability to reduce serum LDL cholesterol in humans: A randomized clinical trial. The American Journal of Clinical Nutrition, 92, 723–732.

81. Wood, P.J. (2004). Relationships between solution properties of cereal β-glucans and physiological effects — a review. Trends in Food Science & Technology; 15, 313-320.

82. Wood, P.J., Beer, M.U., Butler, G. (2000). Evaluation of the role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 84:19-23.

83. Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., Collins, M.W. (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit J Nutr. 72:731-735.

84. Worrasinchai, S., Suphantharika, M., Pinjai, S., & Jamnong, P. (2006). β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. Food Hydrocolloids, 20, 68–78.

85. Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Antitumor polysac-charides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. Trends in Food Science and Technology, 18,4–19.

86. Zhang, Y., Konga, H., Fang, Y., Nishinari, K., & Phillips, G. O. (2013). Schizophyllan: A review on its structure, properties, bioactivities and recent developments.Bioactive Carbohydrate and Dietary Fiber, 1, 53–71.

87. Zheng, G.H., Rossnagel, B.G., Tyler, R.T., Bhatty, R.S. (2000). Distribution of β-glucan in the grain of hull-less barley. Cereal Chem. 77:140-144.

88. Zhong, Y., Tu, Z., Liu, C., Liu, W., Xu, X., Ai, Y., et al. (2013). Effect of microwave irradiation on composition, structure and properties of rice (O*ryza sativa* L.) with different milling degrees. Journal of Cereal Science, 58 (2), 228-233.

89. Zhu, F. M., Du, B., Bian, Z. X., & Xu, B. J. (2015). β-glucans from edible and medicinal mushrooms: characteristics, physicochemical and biological activities. Journal of Food Composition and Analysis, 41, 165-173

90. Zhu, F. M., Du, B., Xu, B.J. (2016). A critical review on production and industrial applications of β-glucans. Food Hydrocolloids 52, 275-288.

CAPÍTULO I

Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: a fundamental and empirical rheological approach *

*Ronda, F., Pérez-Quirce, S., Angioloni, A., Collar, C. (2013) "Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: a fundamental and empirical rheological approach". Food Hydrocolloids 32: 252-262.

Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: a fundamental and empirical rheological approach

Felicidad Ronda[1], Sandra Pérez-Quirce [1], Alessandro Angioloni[2], Concha Collar[2*]

[1] Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

[2] Cereal Group, Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Avenida Catedrático Agustín Escardino 7, Paterna 46980, Valencia, Spain.

Abstract

The significance of the incorporation of associated viscous dietary fibers (hydroxypropylmethlcellulose semi-firm –SFE- and weak –NE- gel like forming, and barley ß-glucan, BBG) at different amounts (1.6–7.5%, flour basis) into gluten-free rice-based dough formulations, on the rheological profile of hydrated (70-110%, flour basis) fibre-flour composite blends has been investigated. A dual fundamental (dynamic oscillatory and creep-recovery tests) and empirical (consistency, and viscometric profile) rheological approach was adopted to assess the viscoelasticity of fibre-enriched rice-based dough matrices. Retrieved functional variables were analysed for dependence on dough hydration and on viscous dietary fibres, and for correlations within parameters from both small and large deformation tests. The water competition of fibre macromolecules expliciting different water binding and gelling abilities resulted in additive, synergistic and/or antagonistic effects on major rheological features. Higher consistent doughs corresponded to those with larger dynamic moduli with poorer frequency dependence, lower elastic deformation at a constant stress, and higher viscosity at steady state. In addition, doughs exhibiting higher viscous component (oscillatory dynamic test) showed concomitantly lower viscosity profiles during cooking (mimetic viscometric test).

Keywords: Gluten-free, hydroxypropylmethylcellulose, β-glucan, dough rheology

1. Introduction

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible persons. CD is one of the most common human genetic disorders, with a prevalence of 1-2% worldwide that is apparently increasing in incidence (Cabrera-Chavez & Calderon de la Barca, 2010). Despite advances in the understanding of CD pathogenesis and the potential development of novel therapies, at present the only safe and effective treatment for CD sufferers is a lifelong avoidance of gluten-containing foods. One of the major issues for CD sufferers to completely adhere to a gluten-free diet is finding good quality gluten-free foods. Despite several gluten-free (GF) products are nowadays available on the market, baked products from gluten-free ingredients are generally of poor physic-chemical and sensory quality, and lack fibre, vitamins and nutrients, which results in a worsening effect on the already nutritionally unbalanced diet of CD sufferers (Thomson, 2009). Although the development of functional and nutritionally enhanced foods for a healthier population is currently highly prioritised, GF products often deserve only a marginal attention. The enrichment of GF breads with β-glucan, a dietary fibre with demonstrated physiological benefits, holds a special interest to this vulnerable population who has a significant incidence of some associated diseases, such as diabetes (Cronin & Shanahan, 1997).

The European Food Safety Authority (EFSA) has recently approved health claims for foods that contribute to the diet 3 g per day of β-glucan from oat or barley for its ability to reduce the LDL-cholesterol level in blood, and for foods that provide at least 4 g / 30 g carbohydrate, for reducing the postprandial glycaemic response (EFSA, 2011). Incorporation of β-glucans from barley in baked goods represents a technological challenge since the protein fraction, hordein, triggers the allergic reaction in CD patients. A selective solubilisation of β-glucans free of water insoluble allergenic proteins (prolamins) is mandatory to avoid intolerances in susceptible individuals. Nor oats are totally safe.

In breadmaking applications, a careful selection of dietary fibres with suitable physic-chemical properties preventing permanent disruption of the protein matrix that encompasses excessive weakening of the protein/starch networks, is a pre-

requisite to obtain processable doughs, particularly for gluten-free systems lacking the endogenous viscoelastic biopolymer.

To date, the main approach for the development of gluten-free breads has been the addition of structural macropolymers such as hydroxylpropylmethylcellulose (HPMC) to mimic gluten viscoelastic properties (Ahlborn, Pike, Hendrix, Hess, & Huber, 2005). Isolates of cereal β-glucan are hydrocolloids with thickening properties (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007) that could replace or supplement the action of HPMC, besides increasing nutritional value of GF bread in terms of dietary fiber content with proven health promoting effects. Associations of soluble dietary fibres in wheat bread formulations affect dough viscoelasticity in variable trend and extent depending on the nature, the physic-chemical and molecular features of added fibres (Collar, Andreu, Martinez, & Armero, 1999) through synergistic, antagonistic and additive effects.

In this work, a fundamental end empirical rheological study of viscous dietary fibres blends -HPMC of different gel strength and barley β-glucan- in hydrated rice flour matrices is envisaged prior to the development of GF bread enriched with β-glucan to meet the EFSA health claims.

2. Material and methods

2.1 Materials

Rice flour (12.5% moisture, 0.46 % ash, 7.5% protein, 0.49 % fat and 79.1 % starch, particle size distribution: 6% > 150 μm, 150 μm > 63.2% > 100 μm, 30.8% < 100μm) was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar, and sunflower oil were purchased from the local market. Two types of hydroxypropylmetylcellulose (HPMC) (E464) from Shin-Etsu Chemical Co Ltd. (Japan) were used: HPMC SFE-4000 (27 – 30 % methoxyl content, 4.0 – 7.5 % hydroxypropoxyl content) coded SFE and HPMC NE-4000 (19 – 24 % methoxyl content, 4.0 – 12 % hydroxypropoxyl content) coded NE. According to manufacturer's application notes, both types of HPMC (10 % moisture content) develop the same apparent viscosity (4300 ± 1300 cP) in 2% aqueous solution at

20ºC, but different gel strength after thermal treatment. HPMC-SFE 4000 forms a semi-firm gel (gelation temperature 58-63ºC) while HPMC-NE 4000 forms a weak and sticky gel (gelation temperature 61-65ºC). Preliminary laboratory breadmaking tests performed with increased single addition of either SFE and NE revealed larger volume but uneven crumb structure for SFE-breads, while discrete volume but even crumb grain for NE-breads at water addition above 90% on flour basis. Visible holes could be probably related to the easier crust forming ability of SFE (a semi-firm gel forming) than NE (a weak gel forming) helping to retain the large bubbles formed inside the crumb. In this paper both HPMC were simultaneously tested for rheological performance at different hydration level of doughs. (1-3)(1-4) β-glucan (BBG) was obtained from barley Barliv™ and supplied as free sample by Cargill (Barcelona, Spain). 2% aqueous solution of BBG developed an apparent viscosity of 28 cP at 20ºC. The characteristics of BBG were: 6 % moisture, 2.2 % soluble protein, 2.6 % ash and 0.9 % fat (commercial data); 70% purity (Megazyme® kit); 5.6 % starch (Megazyme® kit); 140 kDa molecular weight (size exclusion HPLC). BBG extract was analysed for gluten content and a concentration under the detection limit was obtained (< 6.2 mg/kg of Gluten) using the ELISA test based on the R5 antibody.

2.2. Dough preparation

A straight dough process was performed using the following formula on a 100 g flour basis: 6% oil, 5% sucrose, 2 % salt, and 70% water. Combinations of fibres according to a Draper-Lin small composite design for sampling (Draper & Lin, 1990) were added to the basic formula at different hydration levels (Table 1). Design factors (quantitative independent factors) tested at five levels (-1.4142, -1, 0, 1, 1.4142), included SFE (from 0.10 to 2.50 g/100 g flour basis), NE (from 0.10 to 2.50 g /100 g flour basis), BBG (from 0.10 to 3.90 g/100 g flour basis), and WATER (from 0 to 40 mL extra water with respect to 70mL/100 g flour basis). The model resulted in 19 different combinations of fibre-enriched hydrated rice-based samples, including three central point replicates. Gluten-free doughmaking was achieved by blending first solid ingredients in a kitchen-aid professional mixer (KPM5) for 10 s at speed 2. Then, liquid ingredients (oil and water at 20 ± 2 ºC) were added and mixed for 5 min at speed 6.

Table 1: Draper-Lin small composite design for sampling

Run	SFE	NE	BBG	WATER
1	0	-1.4142	0	0
2	-1	-1	-1	-1
3	-1.4142	0	0	0
4	1	-1	1	1
5	0	0	-1.4142	0
6	1	1	1	-1
7	0	0	0	0
8	-1	-1	1	-1
9	1.4142	0	0	0
10	0	1.4142	0	0
11	0	0	0	-1.4142
12	-1	1	1	1
13	0	0	1.4142	0
14	0	0	0	1.4142
15	0	0	0	0
16	1	-1	-1	1
17	-1	1	-1	1
18	1	1	-1	-1
19	0	0	0	0

Design factors are: hydroxypropylmethylcellulose semi-firm gel forming HPMC SFE 4000 (SFE), hydroxypropylmethylcellulose weak gel forming HPMC NE 4000 (NE), Barley Beta-Glucan (BBG), and water addition (WATER). -1.4142, -1, 0, 1, and 1.4142 indicate coded levels of design factors; axial distance from the center points: 1, 1.4142.

2.3. Dough rheological assessment

2.3.1 Large deformation mechanical test: Forward extrusion test

Forward extrusion assays of formulated rice doughs were performed in a TA-XTplus texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 30 kg-load cell and operating at 10 mm/s head speed. The test measures the compression force required for a piston disc to extrude the dough through a specific size outlet (10 mm) in the base of the sample container. The extrusion cell and the compression plunger were 2.55 and 2.50 cm in diameter, respectively. Samples were carefully scooped into acrylic cylindrical containers (20 cm^3 volume) with help of spatula. The complete sample container was located into a centralising insert fitted into the Heavy

Duty Platform, and the plunger was attached to the load cell using a probe adapter. Compression force-time curve allowed evaluating maximum force, determined as the force at which the slope changed The change of slope was visually detected, and the force at this point calculated using the Texture Analyser software. The curve plateau representing the force necessary to continue with the extrusion process and the area under the curve were both used to define the sample consistency. All measurements were performed in triplicate.

2.3.2. Large deformation thermoviscous test: Viscometric profile

Viscometric profiles (gelatinization, pasting, and setback properties) of formulated rice doughs were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC Standard 162. Freeze-dried samples (3.5 g, 14% moisture basis) were transferred into canisters and ≈25 ± 0.1 mL of distilled water were added (corrected to compensate for 14% moisture basis). The slurry was heated to 50°C and stirred at 160 rpm for 10 s for thorough dispersion. The slurry was held at 50°C for up to 1 min, and then heated to 95°C over 3 min 42 s and held at 95°C for 2 min 30 s, and finally cooled to 50°C over 3 min 48 s, and held at 50°C for 2 min. The pasting temperature (when viscosity first increases by at least 25 cP over a 20 s period), peak time (when peak viscosity occurred), peak viscosity (maximum hot paste viscosity), holding strength or trough viscosity (minimum hot paste viscosity), breakdown (peak viscosity minus holding strength or trough viscosity), viscosity at 95°C, viscosity at the end of the 95°C holding period, viscosity at 50°C, final viscosity (end of test after cooling to 50°C and holding at this temperature), setback (final viscosity minus peak viscosity), and total setback (final viscosity minus holding strength) were calculated from the pasting curve (Collar, 2003) using Thermocline v. 2.2 software. For each viscometric measurement, 3 samples were used.

2.3.3. Small deformation mechanical test. Oscillatory and creep recovery tests

Oscillatory and creep-recovery tests were carried out with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) with parallel plate geometry (60 mm diameter) of serrated surface and with 2 mm gap. The excess of dough was removed and vaseline oil was applied to cover the exposed sample surfaces. Before the measurement, the dough was rested for 10 min to allow relaxation. Frequency

sweeps were carried out from 10 to 0.1 Hz in the linear viscoelastic region (LVR) previously established for each dough by means of stress sweeps from 0.1 to 60 Pa. The frequency sweeps of all doughs were carried out at stress values between 1.5 Pa and 35 Pa. Temperature was 25 ºC. Frequency sweep data were fitted to a potential equation as in previous works (Ronda, Oliete, Gomez, Caballero, & Pando, 2011):

$$G'(\omega) = G_1' \cdot \omega^a \qquad (1)$$

$$G''(\omega) = G_1'' \cdot \omega^b \qquad (2)$$

$$\tan\ \delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''}{G'}\right)_1 \cdot \omega^{(b-a)} = (\tan\ \delta)_1 \cdot \omega^c \qquad (3)$$

The coefficients G_1', G_1'', and *(tan δ)1*, represent the elastic and viscous moduli and the loss tangent at a frequency of 1Hz. Fittings were done in the frequency range (0.1–10 Hz), where a linear double logarithm curve was systematically obtained. The a, b and c exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency, ω. Creep tests were performed by imposing a sudden step shear stress in the LVR for 60 s. In the recovery phase the stress was suddenly removed and the sample was allowed for 180 s to recover the elastic (instantaneous and retarded) part of the deformation. Each test was performed in triplicate. Creep data are described in terms of creep compliance, J, which is defined as the strain divided by the stress applied (maintained constant during the creep test). The data from creep tests were modelled to the 6-parameter Burgers model (Lazaridou et al., 2007; Van Bockstaele, De Leyn, Eeckhout, & Dewettinck, 2011) given by:

$$J_c(t) = J_0 + J_1\left(1 - \exp\left(\frac{-t}{\lambda_1}\right)\right) + J_2\left(1 - \exp\left(\frac{-t}{\lambda_2}\right)\right) + \frac{t}{\mu_0} \qquad (4)$$

In the equation, J_c (t) is the creep compliance, J_0 is the instantaneous compliance, J_1 and J_2 are the retarded elastic compliance or viscoelastic compliances, λ_1 and λ_2 are the retardation times and μ_0 gives information about the steady state viscosity. In case of Burgers model, steady state compliance is the sum of J_0, J_1 and J_2 (Van Bockstaele et al., 2011).

Similar equations were used for the recovery compliance $J_r(t)$. As there is no viscous flow in the recovery phase, equations consist only of parameters describing the elastic response after removal of the shear stress. The data from creep tests were modelled to the 5- parameter Burgers model given by:

$$J_r(t) = J_{\max} - J_{r0} - J_{r1}\left(1 - \exp\left(\frac{-t}{\lambda_1}\right)\right) - J_{r2}\left(1 - \exp\left(\frac{-t}{\lambda_2}\right)\right) \qquad (5)$$

J_{max} is the maximum creep compliance obtained at the end of the creep step. The steady-state compliance in recovery step, J_{steady}, was also calculated by subtracting the compliance value at the terminal region of curve (where dough recovery reached equilibrium) from the J_{max}.

2.4. Statistical analyses

Multivariate statistical analysis of data (non-linear regression, stepwise regression analysis, and Pearson correlation analysis) was performed by using Statgraphics Centurion v.6 program (Bitstream, Cambridge, MN, USA).

3. Results and discussion

A dual fundamental and empirical rheological approach was adopted to assess the viscoelasticity of fibre enriched rice-based dough matrices. Dynamic oscillatory and creep-recovery rheological behaviour, consistency, and viscometric profile during cooking and cooling were evaluated in gluten-free doughs according to a Draper-Lin design (Table 1). Retrieved instrumental physical parameters were analysed for dependence on dough hydration and on viscous dietary fibres (Tables 2, 3, and 4), and for correlations within parameters from both small and large deformation tests (Table 5).

3.1 Effects of viscous dietary fibres on gluten-free dough rheological properties

Preliminary tests were carried out to establish the levels of the different design factors. Analytical data on dough fundamental and empirical rheological characteristics were fitted to multiple regression equations using added principles (SFE, NE, BBG, WATER) as independent factors in order to estimate response

surfaces of dependent analytical variables. Significant coefficients (95% confidence interval) obtained from the stepwise regression fitting model are included in Tables 2 and 4.

3.1.1 Fundamental measurements

The viscoelasticity of gluten-free doughs was examined by oscillatory and creep measurements.

Oscillatory measurements

Oscillatory measurements in the LVR performed without disturbing or destroying the inherent structure, have proven to be useful in studying the influence and the significance of structural ingredients such as hydrocolloids in dough systems (Angioloni & Collar, 2008; Weipert, 1990) since dynamic mechanical parameters are highly sensitive to changes in polymer type and concentration, and on water content (Ferry, 1980).

LVR for gluten-free dough samples was established by stress sweep experiments, from 0 to 60 Pa (curves not shown). The drop of elastic modulus, G', started to occur at stress values that ranged from 3 Pa to above 60 Pa, indicating different resistance to the rupture by the action of stress for gluten-free formulated dough structures. Samples with the highest water content (runs 14-16-17) had the lowest limit stress in the LVR. Doughs with low water content (runs 6 and 11) showed the wider LVR. Adjustment of values for dynamic moduli G' and G'' *vs* frequency (0.1 – 10 Hz) to a potential equation, allowed to estimate $G_1^{'}$ and $G_1^{''}$ coefficients and *a* and *b* exponents, with R^2 values ranging from 0.99 to 0.9999 (data not shown).

Table 2: Significant coefficients (95% confidence interval) of design factors (independent variables) of the stepwise regression fitting model for fundamental dough characteristics (dependent analytical variables)

Factor	G_1' (Pa)	a (10^{-3})	G_1'' (Pa)	b (10^{-3})	tan δ_1 (10^{-3})	c (10^{-3})	J_0 (10^{-5} Pa^{-1})	J_1 (10^{-5} Pa^{-1})	λ_1 (s)	J_2 (10^{-5} Pa^{-1})	μ_0 (10^5 Pa.s)	J_{steady} (10^{-5} Pa^{-1})	J_{max} (10^{-5} Pa^{-1})	Recovery %
CONSTANT	22821	95.1	4308	125.9	125.3	15.9	6.72	5.54	-9.70	4.64	16.90	17.84	20.10	88.91
SFE	17626	44.2	6127		93.8	-55.7	-10.05		20.70					
NE	17910		6185		62.2				18.59					
BBG	8056		1663	-16.2										-9.43
WATER	-3315	3.3	-865	8.6	4.0	5.0	0.55				-0.76			-0.51
SFE^2					-13.9									
NE^2					-14.9									
BBG^2	1510							2.02	2.68		2.31			1.56
$WATER^2$	48.4		9.5	-0.055	0.050		0.026	0.055	-0.0076	0.042	0.013	0.13	0.18	
SFE*NE	-7918		-1887	-5.2			4.22							-6.63
SFE*BBC								-3.03	-5.85		-1.08			2.23
SFE*WATER								0.41						
NE*BBG					-6.2				-4.78		-1.10			
NE*WATER		1.5			1.6	-1.8	-0.33							0.29
BBG*WATER	-275	-1.3		-0.65	-1.7		-0.18	-0.74		-0.38	-0.15	-1.14	-1.47	
R-SQ	0.99	0.97	0.99	0.98	0.99	0.90	0.96	0.95	0.93	0.94	0.92	0.94	0.92	0.83

Independent variables: SFE: HPMC SFE 4000; NE: HPMC NE 4000; BBG: Barley Beta-Glucan; WATER: water addition;
J_{max}: Maximum compliance in creep phase; $J_{staeady}$: steady state compliance (instantaneous plus retarded compliance. calculated as $J_0+J_1+J_2$)
Blanks correspond to non significant effects at level of significance of 5%; R-SQ adjusted square coefficient of the fitting model

Coefficients $G_1^{'}$ and $G_1^{''}$, that respectively represent the elastic and viscous moduli at a frequency of 1 Hz, and the exponents a and b, that quantify the variation of dynamic moduli with frequency, were greatly dependent on the presence of SFE, NE, BBG and water. Values for $G_1^{'}$ and $G_1^{''}$ moduli that ranged from 3 to 80 kPa and from 1.4 to 23 kPa, respectively fitted regression equations with R^2 well above 0.90 (Table 2). Single addition of hydrocolloids promoted elastic and viscous moduli in different extent (Table 2). BBG addition conferred major effects, particularly on the elastic modulus $G_1^{'}$, through quadratic and linear positive effects. The effect is in good accordance with the BBG behaviour under dynamic rheological measurements that approaches that of solid-like materials at higher frequencies, with G' being greater than G'' (Lazaridou & Biliaderis, 2007). At flour hydration level of 70%, increases ranged from 143% at 2.7% of BBG addition to 240% at maximum BBG level tested (3.9%, flour basis). In highly hydrated doughs (110%, flour basis), effects of BBG on $G_1^{'}$ were counteracted due to the negative interaction BBG*WATER. A low, nearly null, effect of β-glucan on G' at 1Hz on gluten-free doughs was observed by Lazaridou et al. (2007). Little effects found could be due to the additional amount of water used in dough when hydrocolloids were added, to the marked effect of water on G' and to the significant negative BBG*WATER interaction, as it was confirmed in the present study. SFE and NE added singly provided similar linear effects on both $G_1^{'}$ and $G_1^{''}$ leading to a promotion up to 200% in $G_1^{'}$ when added at maximum dosage (2.5%, flour basis) to dough. Dynamic moduli increase, already found in HPMC added rice flour doughs (Gujral, Guardiola, Carbonell, & Rosell, 2003; Sivaramakrishnan, Senge, & Chattopadhyay, 2004) could be expected because of the enhanced viscoelastic properties of polysaccharides in aqueous medium, probably due to the low viscosity of BBG solutions. On the contrary, the elastic and viscous moduli decreased with increasing water content in dough, fast from 70% to 100%, and slowly from 100% to the maximum water content tested, 110%. This phenomenon is well documented for wheat flour doughs (Autio, Flander, Kinnunen, & Heinonen, 2001), including durum wheat dough (Edwards, Dexter, Scanlon, & Cenkowski, 1999) and for gluten-free dough (Lazaridou et al., 2007), simply attributed to the dilution of constituents. Combination of HPMC and an additional amount of water did not affect dough consistency further than the sum of individual

effects. However the negative coefficient of the interaction SFE*NE indicates a sharp depletion in dynamic moduli in presence of both cellulose derivatives, particularly for the elastic modulus, probably ascribed to the strong water competition of both hydrocolloids. Edwards et al. (1999) reported dynamic mechanical measurements as means of discriminating durum wheat cultivars according to dough strength; for stronger and least extensible samples, higher G' values were found than for their counterparts from weaker cultivars. Frequency sweep showed that for all gluten-free doughs the elastic (or storage) modulus, G', was greater than the viscous (or loss) modulus, G'', in the whole range of frequencies. Both moduli slightly increased with frequency. This suggests a solid elastic-like behaviour of all gluten-free doughs. Therefore, tan δ = (G''/G') values for all dough formulations were lower than 1. Similar observations on dynamic rheological studies was previously reported for wheat flour doughs (Angioloni & Collar, 2009; Dobraszczyk & Morgenstern, 2003; Edwards, Mulvaney, Scanlon, & Dexter, 2003), as well as for rice flour dough without or with HPMC (Gujral et al., 2003; Lazaridou et al., 2007; Sivaramakrishnan et al., 2004).

The $(\tan \delta)_1$, or the loss tangent at a frequency of 1Hz, that ranged between 0.18 and 0.49, was significantly affected by hydrocolloids and water concentration. The individual effect of the two types of HPMC and water was to increase $(\tan \delta)_1$. The three factors also showed significant quadratic terms but of opposite sign: While the effect of HPMC on $(\tan \delta)_1$ showed a maximum (the quadratic coefficient was negative), the effect of water showed a minimum. The individual effect of BBG on tan δ was not significant. However, in presence of water amounts above 70%, BBG decreased significantly $(\tan \delta)_1$ indicating an increasing contribution of the elastic component with respect to the viscous one. A significant interaction NE*Water of opposite sign was also confirmed. The increase of water increased the effect of NE on the loss tangent further than the sum of individual effects. The effect of SFE on the loss tangent was greater irrespective of water content. This may indicate that NE, as BBG, has higher water requirements for acting on dough structure. In breadmaking, flours producing doughs with balanced tensile and elastic properties are required to ensure optimal baking performance. Weipert (1990) attempted to relate the elastic and tensile properties of wheat doughs determined by extensigraph and alveograph, as well as sensorial analysis with the dynamic rheological

parameters of dough. He demonstrated that a dough with small tan δ reflects a rigid and stiff material, and doughs characterized as moist and slack exhibited higher tan δ values than those described as short and dried surface appearance. Edwards et al. (1999) found no significant correlation between tan δ values and dough strength of durum wheat as measured by empirical methods, while the G' values strongly correlated with the dough strength, as already mentioned. Other authors have also described important hydrocolloid effects on the G''/G' ratio in gluten-free systems, depending on the type and dose of hydrocolloid (Lazaridou et al., 2007). This influence seems to be related to the molecular structure and chain conformation of the polysaccharides that determine the physical intermolecular associations of the polymeric chains.

a and b exponents ranged from 0.11 to 0.33. Individual increase of SFE/NE and water content led to significantly higher "a" exponents. Conversely, the individual addition of BBG did not show significant effect on the exponent "a", but in presence of water contents above 70% the addition of BBG decreased the "a" exponent, according to the negative sign of the interaction BBG*WATER. Low values of the a exponent, near zero, mean that the storage modulus, G', is not dependent on the frequency. On the opposite, high values indicate a high increase of the storage modulus with frequency. Given that both HPMC and BBG increased the storage modulus at 1Hz, $G_1^{'}$, its opposite effect on the exponent "a" means that the dependence between the storage modulus and the "a" exponent is opposite for each hydrocolloid. The increase in the storage modulus associated to an increase in HPMC encompasses a severe frequency dependence. On the contrary, when the increase is due to an increase of BBG or a decrease of water, the dependence on frequency is not relevant.

Creep measurements

Creep-recovery tests were also conducted on formulated gluten-free doughs. Stresses applied in the LVR ranged from 1.5 Pa to 35 Pa, and were maintained for 60 s, sufficient for the sample to reach the steady-state flow. Creep-recovery curves of gluten-free doughs exhibited a typical viscoelastic behaviour combining both viscous fluid and elastic components, similar to the corresponding curves obtained previously for wheat doughs (Rouille, Della Valle, Lefebvre, Sliwinski, & vanVliet, 2005; Van

Bockstaele et al., 2011), rice flour (Sivaramakrishnan et al., 2004) and other gluten-free doughs (Lazaridou et al., 2007).

Representative creep-recovery curves of such doughs are presented in Fig. 1 to show the effect of the addition of increasing amounts of BBG and water to dough formulation. The incorporation of BBG and the reduction in dough water content increased the resistance of dough to deformation as shown by the reduction of maximum creep compliance (compliance at the end of creep phase).

Creep parameters for all gluten-free dough formulations are summarized in Table 3. As expected, a strong correlation was found between all creep compliance parameters and the equivalents for the recovery phase ($p<0,001$), since the creep-recovery tests were carried out in the LVR. Besides, it was observed that factors providing an increase in viscosity at the steady state, μ_0, decreased all creep and recovery compliance parameters, J_0, J_1 and J_2. Similar trends were observed by Lazaridou et al. (2007) when tests performed out of the LVR.

Major impact on creep parameters was associated to water content. In absence of hydrocolloids, increasing dough water content led to significantly greater instantaneous and retarded elastic compliance (positive linear regression coefficient), which means a higher dough deformation submitted to a constant stress. On the opposite, the incorporation of SFE into formulation, decreased Jo (negative linear regression coefficient), regardless dough water content which indicates a lower instantaneous elastic deformation in terms of compliance. NE did not show significant effect on Jo in firmer doughs (70% water, flour basis). However, with higher amount of water, NE addition also reduced elastic compliance. This fact confirms that both hydrocolloids explicit different water requirements to strengthen dough structure. This could explain the different results found in the literature on creep-recovery parameters for HPMC supplemented rice doughs (Lazaridou et al., 2007; Sivaramakrishnan et al., 2004).

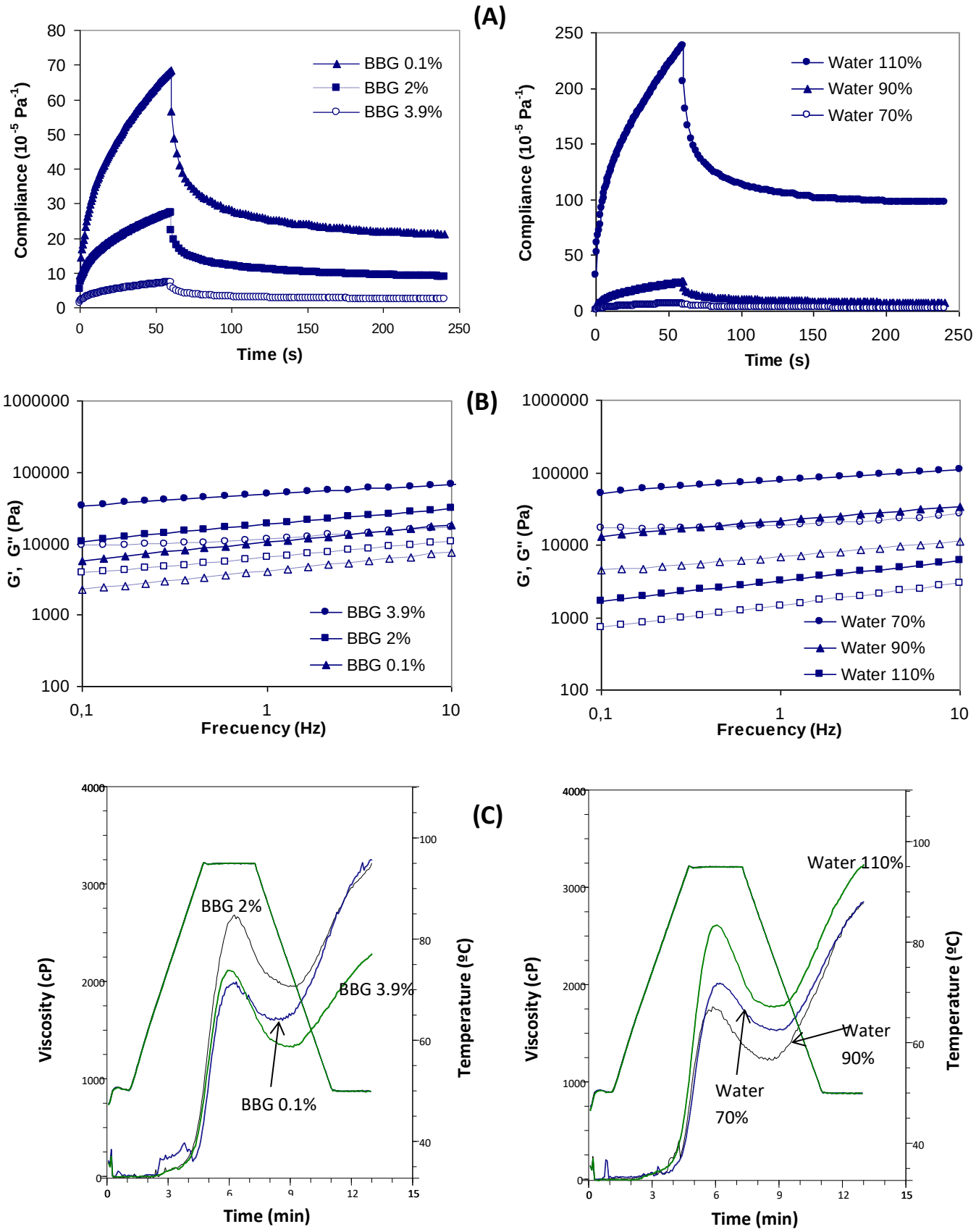


Figure 1: Effect of barley beta-glucan (BBG) addition (left) and water content (right) on creep test curves (A), mechanical spectra (where G' is represented by solid symbols and lines and G'' by open symbols and discontinuous lines) (B) and pasting profiles (C) of gluten-free doughs. Intermediate doses (0 level) of design factors (1.3% SFE; 1.3% NE; 90% WATER equivalent to 20% extra water) (left) and (1.3% SFE; 1.3% NE; 2% BBG) (right) were respectively used.

Table 3: Effect of dietary fibre addition on creep-recovery parameters of gluten-free doughs. See Table 1 for coded run formulation.

Run	Creep phase									Recovery phase							
	J_0 (10^{-5} Pa^{-1})	J_1 (10^{-5} Pa^{-1})	λ_1 (s)	J_2 (10^{-5} Pa^{-1})	λ_2 (s)	μ_0 (10^5 Pa·s)	R^2	J_{steady} (10^{-5} Pa^{-1})	J_{max} (10^{-5} Pa^{-1})	J_0 (10^{-5} Pa^{-1})	J_1 (10^{-5} Pa^{-1})	λ_1 (s)	J_2 (10^{-5} Pa^{-1})	λ_2 (s)	R^2	J_{steady} (10^{-5} Pa^{-1})	Recovery %
1	8.73	9.63	8.3	1.88	0.3	5.02	0.9996	20.24	32.00	8.56	6.98	47.2	7.65	5.6	0.9984	23.19	72.5
2	6.07	2.87	3.0	5.26	18.2	12.62	0.9996	14.20	18.79	6.02	5.45	64.5	4.25	5.3	0.9995	15.72	83.7
3	12.81	12.55	16.6	7.89	2.9	3.80	0.9996	33.25	48.67	13.73	10.19	34.9	10.73	3.5	0.9994	34.65	71.2
4	7.92	9.69	14.1	5.74	2.7	5.04	0.9996	23.34	35.03	7.87	9.22	55.2	8.84	4.7	0.9990	25.94	74.0
5	10.81	30.99	42.9	17.11	4.9	3.46	0.9997	58.91	68.57	12.27	16.12	50.7	19.17	4.6	0.9990	47.55	69.3
6	1.46	2.96	30.3	1.57	4.8	19.00	0.9998	5.99	8.75	1.29	1.99	62.5	2.20	6.5	0.9992	5.48	62.6
7	5.71	8.62	19.9	4.68	3.2	6.79	0.9997	19.02	27.42	5.40	6.50	57.4	7.15	4.6	0.9990	19.04	69.5
8	1.89	1.84	23.4	0.92	3.6	38.34	0.9997	4.65	6.07	1.84	1.55	58.7	1.32	6.0	0.9995	4.71	77.6
9	3.93	6.85	38.0	7.96	6.2	4.65	0.9998	18.74	30.25	3.66	7.67	56.9	7.16	5.1	0.9991	18.50	61.2
10	3.79	10.20	30.5	5.35	4.4	5.50	0.9999	19.34	28.83	3.70	7.98	62.0	7.17	6.0	0.9994	18.86	65.4
11	1.40	2.71	25.9	1.32	4.6	16.59	0.9998	5.43	8.75	1.48	2.22	50.5	2.00	6.1	0.9994	5.70	65.1
12	9.35	10.50	16.4	7.86	2.9	4.94	0.9996	27.70	39.57	9.28	10.95	71.2	10.04	5.0	0.9990	30.27	76.5
13	2.50	3.88	23.6	1.90	3.9	16.13	0.9998	8.28	11.71	2.43	3.06	46.1	2.65	5.1	0.9993	8.14	69.5
14	35.25	50.79	14.7	45.02	3.1	0.64	0.9997	131.06	223.33	28.39	54.53	51.2	58.38	4.6	0.9990	141.29	63.3
15	5.36	8.57	19.7	6.07	4.0	5.67	0.9998	20.00	30.15	5.53	8.12	60.1	7.69	5.2	0.9992	21.33	70.7
16	29.42	87.44	25.0	53.68	4.2	0.92	0.9998	170.54	227.89	28.04	61.30	67.5	65.17	5.3	0.9991	154.51	67.8
17	25.95	58.60	22.4	41.05	3.5	1.45	0.9997	125.60	163.00	27.52	55.62	78.8	54.65	4.9	0.9991	137.78	84.5
18	1.95	10.03	57.1	3.25	5.5	11.12	0.9999	15.23	17.10	1.79	4.47	59.7	3.98	6.4	0.9993	10.24	59.9
19	6.65	11.36	15.0	6.09	2.9	4.28	0.9997	24.09	37.88	6.49	8.82	62.0	9.95	5.1	0.9991	25.26	66.7

J_0 Instantaneous Compliance; J_1. J_2: Retarded Elastic Compliances; λ_1; λ_2: retardation times; μ_0: Steady state viscosity; J_{steady}: Steady state compliance (instantaneous plus retarded compliance, calculated as $J_0+J_1+J_2$); J_{max}: Maximum compliance in creep-recovery curve

Single BBG addition to increasing hydrated doughs reduced significantly the instantaneous elastic compliance J_0 through the negative regression coefficient of the interaction BBG*WATER, in agreement with the high BBG requirements for water to create an elastic structure that resists deformation. The cellulose-like segments of cereal beta-glucans might contribute to the stiffness of the molecules in solution; while blocks of adjacent b-(1-4) linkages may exhibit a tendency for interchain aggregation (and hence lower solubility) via strong hydrogen bonds along the cellodextrin portions (Lazaridou & Biliaderis, 2007).

Similar results were obtained for J_1, J_2, $J_{elastic}$ and J_{max} from the regression analysis, although only BBG, water and BBG*WATER attained significant effects (Table 2). This reinforces that water is a key ingredient for optimizing gluten-free formulations, acting in two ways: by direct influence on the rheological properties of doughs, attributed to constituent concentration effects, and indirectly by interacting with hydrocolloids, and specifically with β-glucan through secondary forces.

Retardation time λ_1, was significantly affected by all hydrocolloids. Both SFE and NE linearly increased λ_1 in a similar extent. BBG increased λ_1 more slowly than HPMC did. Higher retardation times indicate a slower retarded elastic response. The effect of water was really low, as can be seen by the very low coefficient of the quadratic term of water. Lazaridou et al. (2007) found that generally λ_1 values were higher for the hydrocolloids-supplemented gluten free doughs compared with the control formulation (without hydrocolloid). β-glucan was the only hydrocolloid that hardly affected retardation time, while the cellulose derivative (carboxymethylcellulose) showed the maximum effect. No significant coefficients were obtained for λ_2, probably due to the great uncertainty obtained in this variable.

Empirical measurements

Analytical data from Draper-Lin small composite design samples (Table 1) on dough consistency during forward extrusion and viscometric characteristics during pasting and gelling (Fig. 1) were fitted to multiple regression equations using added fibres as independent variables to estimate response surfaces of dependent empirical dough quality variables along extrusion, cooking and cooling (Table 4). Stepwise regression equations included only significant coefficients ($P < 0.05$). Response surface plots of

main rice dough imitative viscoelastic parameters during extrusion process, and cooking and cooling cycles *vs* fibre formulations are shown in Fig. 2.

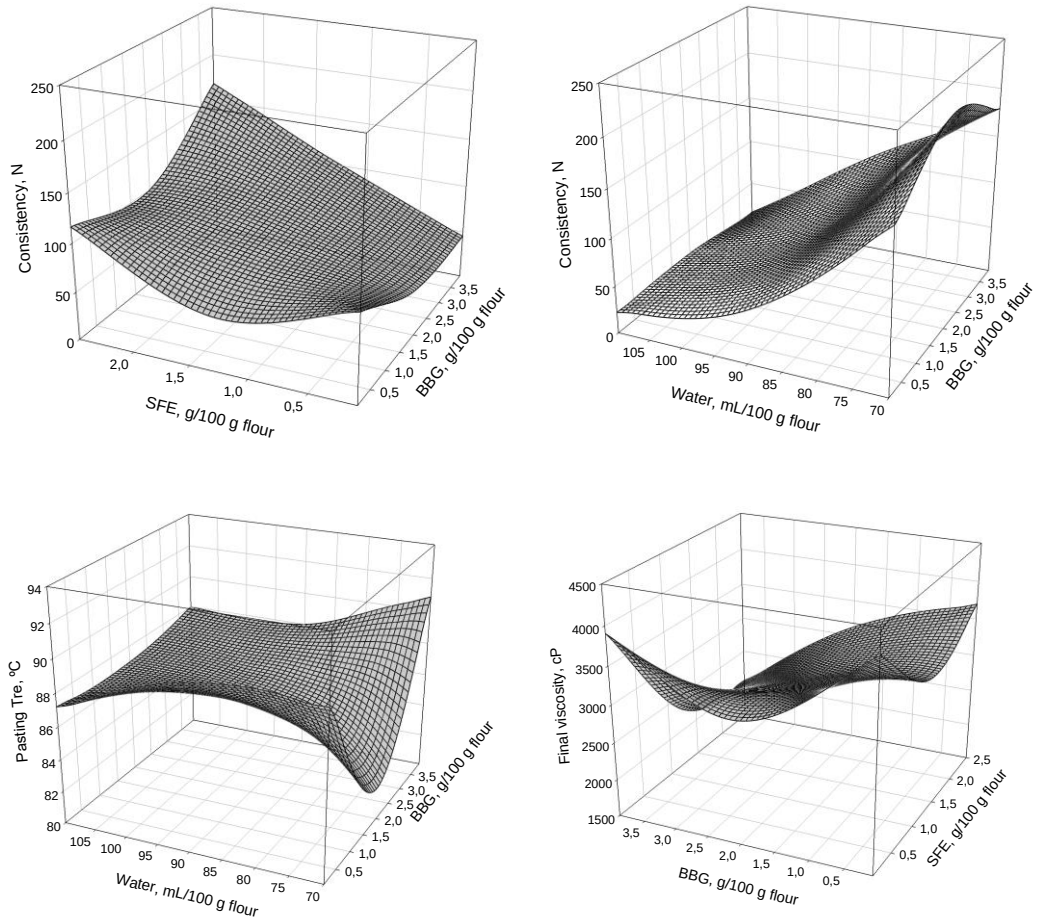


Figure 2: Response surface plots of selected empirical parameters (forward extrusion and rapid viscoanalyser tests) *vs* design factors of formulated rice-based doughs. SFE: HPMC SFE 4000; NE: HPMC NE 4000; BBG: Barley Beta-Glucan; WATER: water addition.

Effects of structuring dietary fibre addition into hydrated rice flour on the extrusion resistance, and cooking (pasting and gelatinization) and cooling (gelling) starch properties of fibre enriched rice doughs were studied. Incorporation of fibre blends at different amounts (1.6–7.5%) into rice dough formula (Table 1) significantly changed the qualitative and quantitative extrusion and viscometric patterns of hydrated fibre flour blends (Table 4, Figs. 1–2). Dependence of extrusion, pasting and gelling parameters on flour-fibre hydrated blends was particularly significant for consistency both force and area peak viscosity (R^2 0.97), viscosity at 50ºC (R^2 0.95), final viscosity (R^2 0.94), total setback on cooling (R^2 0.87), and pasting temperature (R^2 0.79), (Table 4).

Table 4: Significant coefficients (95% confidence interval) of design factors (independent variables) of the stepwise regression fitting model for empirical dough characteristics (dependent analytical variables)

Factor	Consistency (Forward extrusion test)		Viscometric parameters on cooking and cooling (Rapid Visco Analyser test)							
	Force	Area	Peak viscosity	Pasting Temperature	Holding strength	Viscosity at end 95ºC	Viscosity at 50ºC	Setback	Total Setback	Final Viscosity
	(N)	(N.s)	(cP)	(ºC)	(cP)	(cP)	(cP)	(cP)	(cP)	(cP)
CONSTANT	191	63	3127	86.2	2300	2767	3244	1266	1886	3979
SFE			-255				-651			-739
NE			-344		-541	-673	-696		-366	-852
BBG	-27.6	-90.8		-3.6			274			363
WATER	-8.8	-29.6		0.34				-56.8	-18.9	
SFE^2				0.99			182			212
NE^2				-1.35						
BBG^2				0.85						
WATER^2	0.11	0.38	0.40							
SFE*NE				1.62			274		190	371
SFE*BBG	12.7	42.5					-106		-68.1	-157
SFE*WATER				-0.197				11.5		
NE*BBG	13.8	45.7					-126	-132	-61.5	-158
NE*WATER					13.0	15.8	17.7	23.4	14.8	22.9
BBG*WATER			-4.8		-6.9	-6.9	-8.4			-10.8
R-SQ	0.97	0.97	0.73	0.79	0.77	0.73	0.95	0.78	0.87	0.94

Independent variables: SFE: HPMC SFE 4000; NE: HPMC NE 4000; BBG: Barley Beta-Glucan; WATER: water addition
Blanks correspond to non significant effects at level of significance of 5%; R-SQ adjusted square coefficient of the fitting model

Forward extrusion assays of formulated rice doughs measure the compression force required for a piston disc to extrude a dough through a specific size outlet. From the compression force-time curves, the curve plateau representing the force necessary to continue with the extrusion process and the area defined under the curve were used to indirectly assess the sample consistency. Consistency primarily depended on dough hydration (Water) and on the level of viscous dietary fibre (BBG) added to dough formulation (Table 4). The chemical features of cereal ß-glucans are reflected by their solubility in water and their extended, flexible chain conformation. A significant dough softening effect was observed at higher hydration levels and/or increasing amounts of BBG addition through a decrease up to 92% and 55%, respectively in dough consistency parameters. Addition of structuring dietary fibres either SFE or NE to doughs formulated with viscous BBG provided a restoration of initial consistency values and even 15% harder doughs when fibres were incorporated at maximum levels (Fig. 2, Table 4), attributed to the high water binding capacity of dietary fibres.

When heated above a characteristic temperature in an excess of water, native starch granules undergo gelatinization, regarded as the disruption of the molecular order within the granule that results in the swelling of the starch granules and the leaching of amylose. In concentrated aqueous suspensions of native starch, temperature-induced swelling and amylose leaching lead to the formation of viscous pastes, regarded as composite materials built up from a continuous polysaccharide phase with swollen starch granules as fillers. A sharp increase of the suspension viscosity takes place at the pasting temperature and characterizes the onset of the pasting process. Granule swelling and amylose leaching, which are the processes that lead to the viscosity increase (pasting), are nonequilibrium processes. In hydrated fibre-flour blends, the pasting parameters significantly depended on both the presence of water and on dietary fibres, particularly for pasting temperature (Fig. 2). When water was added from 70 to 110% into dough formulation, an increase in both pasting temperature (16%) and peak viscosity (20%) were obtained (Table 4). The presence of BBG significantly decreased values for pasting temperature through single and quadratic effects. Minimum pasting temperature (82.5ºC) was reached with a BBG dose of 2.10 g/100 g flour (Fig. 2), that induced a concomitant decrease in peak viscosity by 13% at the highest hydration level (110% water). Single addition of

structuring dietary fibres SFE or NE linearly decreased peak viscosity up to 20% and 28%, respectively. Whereas, they provided variable effects on the pasting temperature depending on the hydrocolloid: increase up to 7.2% (SFE) and decrease up to 9.8% (NE) when respectively added singly at maximum dosages. HPMC undergo thermoreversible sol:gel phase transition at elevated temperatures, in which a phase separated structured gel is formed on heating (Bajwa, Sammon, Timmins, & Melia, 2009). HPMC changes in sol:gel state can explain differences in starch pasting temperature. At low temperatures it was suggested that the residual crystallinity causes polymer chains to be dispersed in bundles. During heating, these gradually disintegrate (stage 1) before hydrophobic association of chains leads to gelation (stage 2) at elevated temperatures. The extent of methoxyl and hydroxypropyl substitution markedly affects the sol:gel transition temperature (Donges, 1990). Increasing methoxyl substitution (SFE) lowers the incipient gelation temperature, whereas increased hydroxypropyl substitution (NE), raises it. The latter effect is explained by the hydroxypropyl groups forming a stable solvate shell in water, but also by the bulkiness of these groups, which sterically hinders intermolecular association. This can favour water availability for the starch swelling and further gelatinization, resulting in lower pasting temperatures for NE doughs. Simultaneous presence of both cellulose derivatives encompasses a positive interaction on pasting temperature leading to values 8.9ºC higher when added at highest dosages (Table 4), mainly adscribed to water restrictions. The reduction in peak viscosity, in good accordance with a reduced starch concentration, can also indicate a reduced degree of starch granule swelling as stated before (Symons & Brennan, 2004). The pasting temperature of starch-based suspensions can be considered as a parameter directly linked to the swelling and amylose leaching processes (Mira, Eliasson, & Persson, 2005). The effect of fibres on the pasting temperature could be interpreted on the basis of the changes induced on the swelling-amylose leaching process responsible for starting the pasting process. Higher pasting temperatures would result from delayed or restricted swelling and amylose leaching as observed before for the effects of surfactants in starch suspensions (Mira et al., 2005). Major effects for the viscosity of the hot paste during the cooking cycle were provided by both NE and water (Table 4). Single addition of NE at maximum dose (2.5 g/100 g flour) and firmer doughs (70% of water, flour basis) led to a decrease of both holding strength and viscosity at end of 95ºC of 60%, in good agreement with

the weak gel forming ability (see experimental section). At maximum hydration levels (110% of water, flour basis), a restoration of initial values characterizing the hot paste was observed, associated to the higher water availability to solvate the polymer. Breakdown of viscosity during the cooking cycle is caused by rupture of the swollen granules. The observed decrease in hot paste viscosity following the addition of fibres to a restricted water hydrated flour mixture can be attributed to an increased rate of starch granule rupturing during RVA processing. The process reversed in excess of water.

Added fibres compete for water with starch and showed preferential water binding, especially for cellulose derivatives that account for major effects. Lower values for pasting viscosities are an indication of a reduction in starch available for gelatinization. This reduction is likely due to a general reduction in the starch concentration of the pastes because of addition of soluble dietary fibres that can additionally retain water from the starch granules. The reduction of available water in the system would reduce initial starch granule swelling and, hence, add to the explanation of lower peak viscosities of the pastes. In addition to the retention of the integrity of the starch granules, it is suggested that a reduction in pasting characteristics may be associated with a reduced enthalpy of starch gelatinization as observed in dietary enriched biscuits (Brennan & Samyue, 2004) and β-glucan fibre enriched starch-water dispersions (Symons & Brennan, 2004).

Upon subsequent cooling, a gel is formed that consists of an amylose matrix in which amylopectin enriched granules are embedded (Miles, Morris, Orford, & Ring, 1985). Effects of hydrated fibre blends on the parameters characterizing the gelling process were particularly significant for viscosity of the cold paste (Table 4, Figure 2). Complex effects on cooling parameters were provided by hydrocolloids SFE and NE through single and quadratic effects and second-order interactions within gums, with BBG and water (Fig. 2), giving complex cooling profiles with no conclusive tendencies defined by single factors (Figure 1C). Single addition of SFE at maximum level induced an increase by 15% in the viscosity of the cold paste, while NE added singly led to a net depletion at about 50% at 70% water addition. NE action during cooling can be associated to the weakening of the separated structured gel formed on heating through the formation of the water cages, shells and intermolecular hydrogen bonding (Liu, Joshi, Lam, & Tam, 2008). Minimum values for cold paste viscosity

correspond to 1.8 % of SFE addition (Fig. 2). Incorporation of NE to SFE formulated doughs at maximum level tested did not encompass any additional effect providing doughs with a viscosity of the cold paste similar to those of SFE doughs. Single addition of BBG provided a higher viscosity profile of the cold paste, reaching viscosity values 33 % higher when added at 3.9%, flour basis. Cereal ß-glucans have shown to form physically crosslinked hydrogels whose three-dimensional structure is stabilised mainly by multiple inter- and intrachain hydrogen bonds in the junction zones of the polymeric network (Lazaridou & Biliaderis, 2007). Stabilisation of a three-dimensional network by secondary forces might explain the higher viscosity profile of gelled BBG-containing doughs. In presence of either SFE or NE, viscosity enhancement by BBG incorporation at maximum dosage was reduced to 16%. Extra water addition (up to 110%) with respect to basic dough formula (70%) modulated NE and BBG effects: leading to promoted (NE) or depleted (BBG) values for cold paste viscosity, in accordance with different water requirements for the different hydrocolloids.

The incorporation of soluble dietary fibres hinders the intermolecular association that takes place in the macromolecular starch network upon cooling by water competition, physical interference, and disruption of secondary forces of intertwined amylose molecules incorporating dispersed swollen and ruptured starch granules in the three-dimensional network.

Table 5: Correlations between fundamental and empirical rheological variables.

	G'_1	a	G''_1	b	tan δ_1	c	$J_{0\text{-creep}}$	$J_{1\text{-creep}}$	$J_{2\text{-creep}}$	μ_0	Consistency	Peak viscosity	Holding strength	Viscosity at end 95ºC	Viscosity at 50 ºC	Total Setback	Final Viscosity
SFE	ns	ns	ns	ns	ns	-0.53^{*}	ns	ns	ns	ns	ns	-0.45^{*}	-0.43^{*}	-0.44^{*}	ns	ns	ns
NE	ns	ns	ns	ns	ns	-0.51^{*}	ns	ns	ns	ns	ns	-0.61^{**}	-0.61^{**}	-0.63^{**}	ns	ns	ns
BBG	ns	-0.53^{**}	ns	-0.45^{*}	-0.53^{*}	ns		-0.51^{*}	-0.46^{*}	0.45^{*}	ns	ns	ns	ns	-0.56^{**}	-0.71^{***}	-0.61^{**}
WATER	-0.79^{***}	0.56^{**}	-0.74^{***}	0.86^{***}	0.62^{**}	0.60^{**}	0.76^{***}	0.64^{**}	0.68^{***}	-0.69^{***}	-0.82^{***}	ns	ns	ns	ns	ns	ns
G'_1		ns	0.95^{***}	-0.91^{***}	-0.50^{*}	-0.80^{***}	-0.66^{**}	-0.55^{*}	-0.56^{*}	0.76^{***}	0.91^{***}	ns	ns	ns	ns	ns	ns
a			ns	0.70^{***}	0.99^{***}	ns	0.60^{**}	0.74^{***}	0.73^{***}	-0.67^{***}	ns	ns	ns	ns	ns	ns	ns
G''_1				-0.84^{***}	ns	-0.92^{***}	-0.66^{**}	-0.52^{*}	-0.53^{*}	0.57^{**}	0.94^{***}	-0.60^{**}	-0.51^{*}	-0.57^{**}	ns	ns	ns
b					0.75^{***}	0.64^{**}	0.83^{***}	0.79^{***}	0.79^{***}	-0.78^{***}	-0.83^{***}	ns	ns	ns	ns	ns	ns
tan δ_1						ns	0.69^{***}	0.79^{***}	0.79^{***}	-0.68^{***}	ns	ns	ns	ns	ns	ns	ns
c							0.51^{*}	ns	ns	ns	-0.85^{***}	0.63^{**}	0.51^{*}	0.58^{**}	ns	ns	ns
$J_{0\text{-creep}}$								0.90^{***}	0.95^{***}	-0.55^{**}	-0.62^{**}	0.46^{*}	ns	ns	ns	ns	ns
$J_{1\text{-creep}}$									0.98^{***}	$-0,49^{*}$	-0.50^{*}	ns	ns	ns	ns	ns	ns
$J_{2\text{-creep}}$										-0.49^{*}	-0.50^{*}	ns	ns	ns	ns	ns	ns
μ_0											0.51^{*}	ns	ns	ns	ns	ns	ns
Consistency												-0.53^{*}	ns	-0.50^{*}	ns	ns	ns
Peak viscosity													0.95^{***}	0.99^{***}	0.81^{***}	ns	0.74^{***}
Holding strength														0.98^{***}	0.93^{***}	ns	0.88^{***}
Viscosity at end 95ºC															0.86^{***}	ns	0.81^{***}
Viscosity at 50ºC																0.70^{***}	0.99^{***}
Total Setback																	0.79^{***}

SFE: HPMC SFE 4000; NE: HPMC NE 4000; BBG: Barley Beta-Glucan; WATER: water addition; J_0 Instantaneous Compliance; J_1. J_2: Retarded Elastic Compliances; μ_0: Steady state viscosity; Consistency: Force in forward extrusion test; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; ns: not significant

3.2. Correlation between fundamental and empirical rheological variables.

Multivariate data handling of rheological variables supplied useful information on the significantly correlated viscoelastic characteristics of gluten-free dough samples. Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.43 to 0.99) was obtained for the relationships between fundamental and empirical properties of fibre-supplemented rice-based matrices (Table 5). A significant interdependence ($0.50<r<0.99$) within both rheometer and mimetic measurements was found. This is especially true for parameters retrieved from the same fundamental (oscillatory measurements and creep-recovery features) and mimetic (pasting and gelling) tests. Storage and loss moduli, indicators of dough strengthened structure and solid-like behavior, strongly correlated ($p<0.001$, $r=0.95$). The loss tangent tan δ indicating solid-like or liquid like nature, is highly connected to the "a" exponent ($p<0.001$, $r=0.99$), while G' and G'' correlated with "b" exponent ($r=-0.91$, $r=-0.84$). The higher the dough consistency, the greater is the elastic component, the stronger is the solid-like behavior and the poorer is the dependence of dynamic moduli on the frequency. From creep measurements, it was observed that viscosity at the steady state, μ_0, negatively correlated with creep compliance parameters, J_0, J_1 and J_2 ($r= -0.55, -0.49, -0.49$, respectively), in good agreement with lower instantaneous and retarded elastic deformations at higher reached viscosity values. In addition, μ_0 explicited a positive correlation with $G_1^{'}$ and $G_1^{''}$ and a negative correlation with $(\tan \delta)_1$. Within mimetic parameters, consistency estimated on the extrusion test and pasting parameters during cooking and cooling set at RVA of fibre-enriched rice-based doughs significantly and positively correlated, with some exceptions. Consistency, strongly dependent on dough hydration and BBG addition (Table 4), showed no correlation with gelling parameters during cooling, and observed negative relationships with peak viscosity parameters ($r = –0.53$) and viscosity at the end of the pasted state ($r =- 0.50$), in agreement with a poorer viscosity profile during cooking for harder doughs. In general, most parameters derived from pasted and gelled states closely dependent on the presence of dietary fibres and water, strongly correlated ($r > 0.9$). The dependence was particularly relevant for peak viscosity *vs* holding strength and viscosity at end of 95°C, holding strength *vs* viscosity at end of 95°C and final viscosity, and cold paste viscosity *vs* final viscosity (Table 5).

Some significant correlations (-0.54 < r < 0.94) were observed between fundamental and imitative rheological measurements of gluten-free samples, particularly for those directly/indirectly assessing dough strength characteristics. Higher consistent doughs corresponded to those with larger dynamic moduli ($G_1^{'}$, $G_1^{''}$) with poorer frequency dependence (b), lower elastic deformation at a constant stress (J) and higher viscosity at steady state (μ_0). In addition, doughs expliciting higher viscous component G''(oscillatory dynamic test) showed concomitantly lower viscosity profiles during cooking (mimetic RVA test).

These data endorse that either fundamental rheological parameters recorded at small deformation or empiric characteristics encompassing large deformation may be useful to characterize gluten-free dough samples in terms of their viscoelastic behaviour in supplemented rice flour-fibre blends. The water competition of fibre macromolecules expliciting different water binding and gelling abilities resulted in additive, synergistic and/or antagonistic effects on rheological features. Viscoelastic fundamental properties and consistency are closely linked to protein network performance while pasting/gelling profiles are strongly associated to starch (Dobraszczyk & Morgenstern, 2003). The observation can be explained on the basis that both fundamental microscopic measurements and mimetic macroscopic tests take into account not only the individual performance of the main biopolymers involved in dough structure, but also the functionality derived from interactions between endogenous macropolymers and with the non-starch-polysaccharide entities added as dietary fibres in variable amounts of water.

Acknowledgement

Authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad (Projects AGL2012-35088 and AGL2011-22669) and Comunidad de Castilla y León (Project VA252A12-2), the laboratory of Pharmacy (laboratory accredited according to ISO 17025) of the University of Basque Country for the gluten analysis in barley β-Glucan extracts, and Cargill Company for the delivery of Barley Beta-glucan.

References

Ahlborn, G., Pike, O., Hendrix, S., Hess, W., & Huber, C. (2005). Sensory, mechanical, and microscopic evaluation of staling in low-protein and gluten-free breads. *Cereal Chemistry, 82*(3), 328-335.

Angioloni, A., & Collar, C. (2008). Functional response of diluted dough matrixes in high-fibre systems: A viscometric and rheological approach. *Food Research International, 41*(8), 803-812.

Angioloni, A., & Collar, C. (2009). Small and large deformation viscoelastic behaviour of selected fibre blends with gelling properties. *Food Hydrocolloids, 23*(3), 742-748.

Autio, K., Flander, L., Kinnunen, A., & Heinonen, R. (2001). Bread quality relationship with rheological measurements of wheat flour dough. *Cereal Chemistry, 78*(6), 654-657.

Bajwa, G. S., Sammon, C., Timmins, P., & Melia, C. D. (2009). Molecular and mechanical properties of hydroxypropyl methylcellulose solutions during the sol:Gel transition. *Polymer, 50*(19), 4571-4576.

Brennan, C., & Samyue, E. (2004). Evaluation of starch degradation and textural characteristics of dietary fiber enriched biscuits. *International Journal of Food Properties, 7*(3), 647-657.

Cabrera-Chavez, F., & Calderon de la Barca, A. M. (2010). Trends in wheat technology and modification of gluten proteins for dietary treatment of coeliac disease patients. *Journal of Cereal Science, 52*(3), 337-341.

Collar, C. (2003). Significance of viscosity profile of pasted and gelled formulated wheat doughs on bread staling. *European Food Research and Technology, 216*(6), 505-513.

Collar, C., Andreu, P., Martinez, J., & Armero, E. (1999). Optimization of hydrocolloid addition to improve wheat bread dough functionality: A response surface methodology study. *Food Hydrocolloids, 13*(6), 467-475.

Cronin, C., & Shanahan, F. (1997). Insulin-dependent diabetes mellitus and coeliac disease. *Lancet, 349*(9058), 1096-1097.

Dobraszczyk, B., & Morgenstern, M. (2003). Rheology and the breadmaking process. *Journal of Cereal Science, 38*(3), 229-245.

Donges, R. (1990). Nonionic cellulose ethers. *British Polymer Journal, 23*(4), 315-326.

Draper, N. R., & Lin, D. K. J. (1990). Small response-surface designs. *Technometrics, 32*(2), 187-194.

Edwards, N., Dexter, J., Scanlon, M., & Cenkowski, S. (1999). Relationship of creep-recovery and dynamic oscillatory measurements to durum wheat physical dough properties. *Cereal Chemistry, 76*(5), 638-645.

Edwards, N., Mulvaney, S., Scanlon, M., & Dexter, J. (2003). Role of gluten and its components in determining durum semolina dough viscoelastic properties. *Cereal Chemistry, 80*(6), 755-763.

Scientific Opinion on the Substantiation of Health Claims Related to Beta-Glucans from Oats and Barley and Maintenance of Normal Blood LDL-Cholesterol Concentrations (ID 1236, 1299), Increase in Satiety Leading to a Reduction in Energy Intake (ID 851, 852), Reduction of Post-Prandial Glycaemic Responses (ID 821, 824), and "digestive Function" (ID 850) Pursuant to Article 13(1) of Regulation (EC) no 1924/2006, 2207 (2011).

Ferry, J. D. (1980). *Viscoelastic properties of polymers*. New York: Wiley.

Gujral, H., Guardiola, I., Carbonell, J., & Rosell, C. (2003). Effect of cyclodextrinase on dough rheology and bread quality from rice flour. *Journal of Agricultural and Food Chemistry, 51*(13), 3814-3818.

Lazaridou, A., & Biliaderis, C. G. (2007). Molecular aspects of cereal beta-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science, 46*(2), 101-118.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering, 79*(3), 1033-1047.

Liu, S. Q., Joshi, S. C., Lam, Y. C., & Tam, K. C. (2008). Thermoreversible gelation of hydroxypropylmethylcellulose in simulated body fluids. *Carbohydrate Polymers, 72*(1), 133-143.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research, 135*(2), 271-281.

Mira, I., Eliasson, A., & Persson, K. (2005). Effect of surfactant structure on the pasting properties of wheat flour and starch suspensions. *Cereal Chemistry, 82*(1), 44-52.

Ronda, F., Oliete, B., Gomez, M., Caballero, P. A., & Pando, V. (2011). Rheological study of layer cake batters made with soybean protein isolate and different starch sources. *Journal of Food Engineering, 102*(3), 272-277.

Rouille, J., Della Valle, G., Lefebvre, J., Sliwinski, E., & vanVliet, T. (2005). Shear and extensional properties of bread doughs affected by their minor components. *Journal of Cereal Science, 42*(1), 45-57.

Sivaramakrishnan, H. P., Senge, B., & Chattopadhyay, P. K. (2004). Rheological properties of rice dough for making rice bread. *Journal of Food Engineering, 62*(1), 37-45.

Symons, L., & Brennan, C. (2004). The effect of barley beta-glucan fiber fractions on starch gelatinization and pasting characteristics. *Journal of Food Science, 69*(4), C257-C261.

Thomson, T. (2009). The nutritional quality of gluten-free foods. In E. Gallagher (Ed.), *Gluten-free food science and technology* (pp. 45-51). New Delhi: Wilet-Blackwell.

Van Bockstaele, F., De Leyn, I., Eeckhout, M., & Dewettinck, K. (2011). Non-linear creep-recovery measurements as a tool for evaluating the viscoelastic properties of wheat flour dough. *Journal of Food Engineering, 107*(1), 50-59.

Weipert, D. (1990). The benefits of basic rheometry in studying dough rheology. *Cereal Chemistry, 67*(4), 311-317.

CAPÍTULO II

Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads*

*Pérez-Quirce, S., Collar, C., Ronda, F. (2014) "Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads". Food Science and Technology 49 (5): 1375-1382.

Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads

Sandra Pérez-Quirce [1], Concha Collar[2], Felicidad Ronda[1*]

[1] Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

[2] Cereal Group, Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Avenida Catedrático Agustín Escardino 7, Paterna 46980, Valencia, Spain.

Abstract

The impact of associated viscous dietary fibers (hydroxypropylmethylcellulose semi-firm –SFE- and weak –NE- gel forming, and barley ß-glucan, BBG) incorporated at different amounts (1.6–7.5%, flour basis) into gluten-free rice-based dough formulations, on the breadmaking performance and staling behaviour of hydrated (70-110%, flour basis) fibre-flour composite blends has been investigated.

Single BBG addition fails to mimic gluten visco-elasticity properly, but simultaneous incorporation of either SFE or NE contribute to bread improvement in terms of bigger volume and smoother crumb. 3.3g of BBG (70% purity) and 104mL of water addition to 100g rice flour provided sensorially accepted breads (7.6/10) with a theoretical ß-glucan content of 1.24g/100g GF bread that would allow a daily ß-glucan intake of 3 g provided a bread consumption of 240g/day. Complementary tests should be carried out to know the amount and molecular weight of ß-glucan in the final bread before assuring the nutritional benefit of this addition.

Keywords: Gluten-free; Hydrocolloids; β-glucan; bread quality

1. Introduction

The increased consumer demand for healthy foods has driven to address concerted efforts from both research and industry to develop breads that combine properly health benefits with good physico-chemical and sensory properties. This goal is specially challenging in gluten-free (GF) breadmaking where the lack of gluten biopolymer seriously constrains dough visco-elastic character, leads to a failure in carbon dioxide entrapment, and hence deteriorates the techno-functional quality of resulting breads. In addition, a poor nutritional balance often characterises the multi-ingredient GF matrices (Thomson 2009). GF baked goods are often low in fibre, both soluble and insoluble; consequently its enrichment with dietary fibre seems to be necessary (Sabanis et al. 2009).

The natural, synthetic and biotechnological hydrocolloids, because of their high water-binding capacity and their structure-creating behaviour, are mostly used in the different recipes for replacing the gluten network and its functionality (Houben, Hoechstoetter, and Becker 2012). Water availability plays a crucial role in the functionality of hydrocolloids by binding to the macromolecules in three different ways: via hydrogen bounds, embedded in inter- or intramolecular openings or immobilized by structuring (Anton 2008). The modified cellulose derivative hydroxypropyl-methyl-cellulose -HPMC- (linear and neutral polymer) has, because of its hydrophilic character, a high water-binding capacity and also has, in its structure, hydrophobic methyl and hydrophilic hydroxypropyl groups located, which makes HPMC an interface activity in the dough system during the resting period promoting dispersion and preventing coalescence of the gas bubbles. HPMC can create a reversible, heat-set gel network (Haque et al 1993) that leads to an increase in dough viscosity and stabilization of the boundaries of the expanding gas cells. During baking, the gas-binding capacity is increased and higher volume can be achieved (Bell 1990; Collar et al 1999).

The positive effects of isolates of cereal ß-glucans have been recently reviewed by Wood (2010), with most of the data deriving from studies with oat ß-glucans, followed by barley and rye (Kinner et al 2011). The European Food Safety Authority (EFSA) has recently approved health claims for foods that contribute to the diet 3 g per day of ß-glucan (BG) from oat or barley for its ability to reduce the LDL-

cholesterol level in blood, and for foods that provide at least 4 g / 30 g carbohydrate, for reducing the postprandial glycaemic response (EFSA 2011). Isolates of cereal ß-glucan are hydrocolloids with thickening properties (Lazaridou et al 2007) that could replace or supplement the action of HPMC when added in appropriate amounts, besides increasing nutritional value of GF bread in terms of dietary fiber content with proven health promoting effects. It is also stressed that high concentration of ß-glucan decreases the water availability for the protein network and thus impairs the baking properties of wheat breads (Gill et al 2002). Molecular mass and structure, chain length, bonds and chemical modification, added doses, raw materials and process parameters used -pH value, temperature, shearing, ionic bonds and the attendance of ions- account for some factors determining the significance of associated hydrocolloids in bread performance (Houben, Hoechstoetter, and Becker 2012).

Previous studies have shown a great difference in the effect of ß-glucan and HPMC of different gel strengths on gluten-free rice dough viscoelastic behaviour (Collar et al 1999; Collar, Santos, and Rosell 2005; Collar, Santos, and Rosell 2007; Ronda et al 2013). The water competition of the fibre macromolecules expliciting different water binding and gelling abilities resulted in additive, synergistic and/or antagonistic effects on major rheological features. The present study aims to establish the effect of viscous dietary fibres blends at different hydration levels on GF bread quality and staling. A correlation study between dough and bread properties was carried out to know the relationships between rheological performance and physico-chemical and sensory pattern of ß-glucan-enriched GF breads.

2. Material and methods

2.1 Materials

Rice flour (12.5% moisture, 0.46 % ash, 7.5% protein, 0.49 % fat and 79.1 % starch) was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar, and sunflower oil were purchased from the local market. Two types of hydroxypropylmetylcellulose (HPMC) (E464) from Shin-Etsu Chemical Co Ltd.

(Japan) were used: HPMC SFE-4000 (27 – 30 % methoxyl content, 4.0 – 7.5 % hydroxypropoxyl content) coded SFE and HPMC NE-4000 (19 – 24 % methoxyl content, 4.0 – 12 % hydroxypropoxyl content) coded NE. According to manufacturer's application notes, both types of HPMC (10 % moisture content) develop the same apparent viscosity (4300 ± 1300 cP) in 2% aqueous solution at 20ºC, but different gel strength after thermal treatment. HPMC-SFE forms a semi-firm gel (gelation temperature 58-63ºC) while HPMC-NE forms a weak and sticky gel (gelation temperature 61-65ºC). (1-3)(1-4) β-glucan (BBG) was obtained from barley, Barliv™, and supplied as free sample by Cargill (Barcelona, Spain). The characteristics of BBG were: 6 % moisture, 2.2 % soluble protein, 2.6 % ash and 0.9 % fat (commercial data); 70% purity (Megazyme® kit); 5.6 % starch (Megazyme® kit); 140 kDa molecular weight (size exclusion HPLC). BBG extract was analysed for gluten content and a concentration under the detection limit was obtained (< 6.2 mg/kg of gluten) using the ELISA test based on the R5 antibody.

2.2. Dough preparation and breadmaking

A straight dough process was performed using the following formula on a 100 g rice flour basis: 6% oil, 5% sucrose, 2 % salt, 3**%** dried yeast**,** and 70% water**.** Combinations of fibres according to a Draper-Lin small composite design for sampling (Draper and Lin 1990) were added to the basic formula at different hydration levels (Table 1). Design factors (quantitative independent factors) tested at five levels (-1.4142, -1, 0, 1, 1.4142), included SFE (from 0.10 to 2.50 g/100 g flour basis), NE (from 0.10 to 2.50 g /100 g flour basis), BBG (from 0.10 to 3.90 g/100 g flour basis), and WATER (from 0 to 40 mL extra water with respect to 70mL/100 g flour basis, that was the minimum amount added). The model resulted in 19 different combinations of fibre-enriched hydrated rice-based doughs, including three central point replicates. These replicates were made in order to know the repeatability and accuracy of results. GF dough-making was achieved by blending first solid ingredients in a kitchen-aid professional mixer (KPM5) for 10 s at speed 2. Then, liquid ingredients (oil and water at 20 ± 2 ºC) were added and mixed for 5 min at speed 6. The dough, 200 g, was placed into an alumnium oil coated pan and was proofed at 30ºC and 90% relative moisture for 40 min. Subsequently, baking was carried out in a Salva oven (Lezo, Spain) at 190ºC for 40 min. After baking, breads were removed from the pan and left for one hour at room temperature before

analysis. To study the effect on staling, breads were stored in hermetic polypropylene bags for 0, 1, 2, 5, 7 and 9 days at (4 ± 2) ºC. Seven breads were made per run (one batch).

Table 1: Draper-Lin small composite design for sampling

Run	SFE	NE	BBG	WATER
1	0	-1.4142	0	0
2	-1	-1	-1	-1
3	-1.4142	0	0	0
4	1	-1	1	1
5	0	0	-1.4142	0
6	1	1	1	-1
7	0	0	0	0
8	-1	-1	1	-1
9	1.4142	0	0	0
10	0	1.4142	0	0
11	0	0	0	-1.4142
12	-1	1	1	1
13	0	0	1.4142	0
14	0	0	0	1.4142
15	0	0	0	0
16	1	-1	-1	1
17	-1	1	-1	1
18	1	1	-1	-1
19	0	0	0	0

Design factors are: hydroxypropylmethylcellulose semi-firm gel forming HPMC SFE 4000 (SFE), hydroxypropylmethylcellulose weak gel forming HPMC NE 4000 (NE), Barley Beta-Glucan (BBG), and water addition (WATER). -1.4142, -1, 0, 1, and 1.4142 indicate coded levels of design factors; axial distance from the center points: 1, 1.4142.

2.3. Evaluation of bread quality

Bread volume was determined in duplicate using a volume analyzer BVM-L370 TexVol Instruments (Viken, Sweden). The bread was weighed immediately after removal from the pan once cooled. A digital calibre was used to measure bread height and width.

Crumb texture was determined in triplicate with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) provided with the software "Texture Expert". An

Aluminium 20 mm diameter cylindrical probe was used in a "Texture Profile Analysis" double compression test (TPA) to penetrate to 50% depth, at 1 mm/s speed test, with a 30 s delay between first and second compression (Collar, Bollain, and Angioloni 2005). Hardness (N), chewiness (N), cohesiveness, springiness and resilience were calculated from the TPA graphic. Analysis was carried out at (20 ± 2) ºC for bread slices of 20 mm thickness taken from the centre of the loaf.

Colour was measured with a Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Japan). Results were obtained in the CIE L*a*b* coordinates using the D65 standard illuminant, and the 2º standard observer (CIE 1931). The hue (h) and the chroma (C*) were calculated from them with the equations $h=atan(b^*/a^*)$ and $C^*=((a^*)^2+(b^*)^2)^{1/2}$ (Ronda et al 2005). L* ranges from 0 (black) to 100 (white). The hue scale extends from 0º (red), 90º (yellow), 180º (green) to 270º (blue). The chroma informs about the purity of the colour: a near zero C* value corresponds to a colour of low purity, near grey. On the opposite high C* values mean colours of high purity near the pure spectral colours. Colour determinations were made 5x5 times: bread crumb and crust colours were checked at five different points on each bread and every point was measured five times.

2.4 Sensory analysis

Sensory analysis was performed by a panel of ten trained judges (four males and six females aged 25–52) from the baking laboratory, very familiar with the instrumental and sensory evaluation of breads. Ratings were given to breads wrapped in plastic bags and stored at room temperature for 24 hours. An intensity (except overall acceptance) semi-structured scale from 1 to 10 was used. The attributes were defined in such a way that the highest score (very much=10) was given to the situation usually preferred by the consumer. This allowed correlating easily higher scores with better sensorial properties of breads. The attributes tested were: crumb grain (1=very large and inhomogeneous cells; 10= very small and homogeneous cells), crumb softness (1= very hard; 10= very soft), crumb chewiness (1=very rubbery, requiring many bites to swallow; 10= very little rubbery), crust crumbliness (1= very soft and annealed crust; 10=very crunchy crust) taste and flavour intensity (1: very little; 10: very much). Additionally, a hedonic, overall acceptability test was also included in

the sensory evaluation with a scale from 1 (dislike very much) to 10 (like very much). The samples were analysed in four sessions.

Panellists were trained in the sensory evaluation of breads in four sessions by evaluating the intensity of the sensory attributes of different commercial breads from the Spanish market. They were explained the specific intensity scale to be used for each sensory parameter. The references used in training were: Crumb grain 1: rustic Spanish bread, 10: pan bread from refined wheat flour; Crumb softness 1: two-day stored pan bread from refined wheat flour, 10: fresh pan bread from refined wheat flour; Crumb chewiness 1: fresh pan bread from refined wheat flour, 10: whole grain rye bread; Crust crumbliness 1: the crust of pan bread made from refined wheat flour, 10: the crust of a fresh baguette type bread; Intensity of taste 1: Water, 10: sourdough bread from refined wheat flour; Intensity of flavour 1: Water, 10: sourdough bread from refined wheat flour.

2.5. Statistical analyses

Multivariate statistical analysis of data (non-linear regression, stepwise regression analysis, and Pearson correlation analysis) was performed by using Statgraphics Centurion v.6 program (Bitstream, Cambridge, MN, USA).

Table 2: Effect of dietary fibre addition on physical properties of gluten-free breads. See Table 1 for coded run formulation.

Run	Specific Volume (mL/g)	Height/ Width	Loss of Weight (g)	Firmness (N)	Chewiness (N)	Resilience	Cohesiveness	Springiness	Crum Colour			Crust Colour			ΔFirmness 1 day (N)	ΔFirmness 9 days (N)
									L*	h	C*	L*	h	C*		
1	4.00 e	0.75 fg	39.0 ij	1.0 a	0.28 a	0.21 ab	0.48 abc	0.61 a	76 bc	91 gh	11 de	64 cde	73 h	30 fgh	1.0 ab	7 ab
2	2.90 c	0.54 c	34.6 c	2.3 cd	0.80 ab	0.26 defgh	0.52 cdef	0.70 bcde	75 bc	90 bcdefg	12 f	70 ij	75 i	28 bcd	13 f	20 f
3	2.94 c	0.60 c	35.0 cd	1.6 abcd	0.66 ab	0.24 cde	0.53 defgh	0.76 efg	73 a	90 bcdefgh	12 ef	69 ghij	75 i	27 bc	4.0 e	14 e
4	4.12 ef	0.70 ef	38.0 ghi	1.1 ab	0.36 a	0.23 bcd	0.52 defg	0.64 abcd	77 c	91 defgh	11 de	65 def	70 g	32 h	0.9 ab	7 ab
5	4.42 h	0.84 ij	38.5 ghi	1.1 ab	0.34 a	0.23 bc	0.51 bcde	0.62 ab	74 ab	91 h	9 b	64 cdef	68 de	30 defg	2.1 bcd	7 ab
6	2.00 b	0.41 b	26.7 a	13 f	6.0 d	0.26 efgh	0.55 fghi	0.8 efg	77 cd	89 bc	12 ef	63 bcd	66 bcd	30 defg	14.3 fg	45 g
7	4.31 gh	0.80 hi	37.0 def	1.4 abc	0.44 ab	0.25 cdef	0.51 cdef	0.63 abc	76 bc	89 bcde	10 bcd	60 a	67 bcd	29 cdef	1.5 abc	9 bc
8	1.42 a	0.28 a	31.4 b	17.0 g	5.80 d	0.19 a	0.45 a	0.78 fg	78 cde	90 bcdefgh	11 ef	72 jk	85 j	19 a	21.3 h	175 i
9	4.80 i	0.79 ghi	39.3 ij	1.0 a	0.27 a	0.22 abc	0.47 ab	0.57 a	73 a	90 cdefgh	7 a	61 abc	65 abc	31 gh	0.7 a	4 a
10	4.80 i	0.87 j	39.2 hij	1.1 ab	0.32 a	0.24 bcde	0.50 bcde	0.60 a	76 bc	90 cdefgh	9 b	60 ab	64 a	30 efgh	1.4 abc	7 ab
11	1.97 b	0.42 b	26.0 a	11.1 e	3.9 c	0.21 ab	0.50 bcd	0.72 def	77 c	88 b	12 f	64 cde	67 cd	29 def	15.2 g	73 h
12	3.69 d	0.66 de	41.0 k	1.3 ab	0.60 ab	0.22 abc	0.56 hi	0.70 cdef	79 def	89 b	11 de	67 efghi	70 fg	30 defg	0.9 ab	4 a
13	2.99 c	0.63 d	32.3 b	1.9 abcd	0.80 ab	0.28 fgh	0.58 ij	0.72 def	72 a	87 a	9 b	68 fghi	68 def	28 bcd	1.9 abc	14 e
14	5.05 j	0.77 gh	40.8 jk	1.6 abcd	0.80 ab	0.26 defg	0.61 j	0.81 gh	80 ef	90 bcdef	10 cd	67 fghi	69 efg	29 def	2.1 bcd	13 de
15	4.25 fg	0.80 gh	36.3 cdef	1.4 abc	0.56 ab	0.25 cdefg	0.56 ghi	0.70 bcdef	76 c	89 bcd	10 cd	65 def	65 ab	28 bcde	2.0 abc	9 bcd
16	3.56 d	0.87 j	36.9 efg	1.2 ab	0.49 ab	0.25 cdef	0.59 ij	0.70 bcdef	80 def	91 cdefgh	9 b	69 hij	69 efg	30 efgh	1.3 ab	7 ab
17	2.83 c	0.54 c	35.2 cde	2.0 bcd	1.1 b	0.28 gh	0.61 j	0.86 h	80 f	91 fgh	9 bc	74 k	73 h	27 b	3.3 de	16 e
18	3.50 d	0.63 d	31.7 b	2.6 d	0.90 ab	0.28 gh	0.54 efgh	0.65 abcd	80 ef	90 cdefgh	10 bc	66 defgh	68 de	30 fgh	2.6 cd	13 cde
19	4.20 fg	0.79 ghi	37.5 fgh	1.4 abc	0.56 ab	0.29 h	0.58 ij	0.69 bcde	76 bc	91 efgh	10 cd	66 defg	69 efg	32 h	2.1 bcd	9 b
SD	0.06	0.02	0.6	0.4	0.2	0.01	0.01	0.03	1	1	1	1	1	1	0.4	1

L*: Lightness; h: hue; C*: Chroma. ΔFirmness 1 day: Firmness increase in 1 storage day; ΔFirmness 9 day: Firmness increase over 9 storage days.
Values followed by the same letter in the same column are not significantly ($p < 0.05$) different. SD: Standar deviation obtained form ANOVA

Table 4: Significant coefficients (95% confidence interval) of design factors (independent variables) of the stepwise regression fitting model for bread characteristics (dependent analytical variables).

Factor	Specific volume (ml/g)	Height/ width	Loss of weight (g)	Firmness (N)	Sprin-giness	Chewiness (N)	Crust L*	Crust h	Crust C*	Crumb C*	ΔFirmness 1 day (N)	ΔFirmness 9days (N)	Crum grain (Score)	Crumb softness (Score)	Overall Acceptance (Score)
CONSTANT	1.41	0.30	31.1	2.52	0.71	0.71	71.6	86.4	86.4	11.7	20.7	10.7	10.8	1.8	2.3
SFE	1.54	0.29			-0.054		-8.69	-16.0	-16.0		-5.6				
NE								-9.2	-9.2						
BBG	0.14		-2.1	4.98		1.97						74.0	-1.8		
WATER	0.10	0.017	0.51	-0.50		-0.18					-1.1	-6.5	-0.10	0.35	0.29
SFE^2	-0.48	-0.096					2.22	2.9	2.9						
NE^2								1.7	1.7						
BBG^2	-0.24	-0.025			0.017		0.67	0.82	0.82		0.87		0.52	-0.21	-0.12
$WATER^2$	-0.0026	-0.0006	-0.011	0.015	0.00018	0.0057	0.0070	0.0046	0.0046	0.0043	0.020	0.10		-0.0045	-0.0038
SFE*NE	0.15							3.3	3.3						
SFE*BBG		-0.031										-13.1		0.24	
SFE*WATER		0.0056								-0.079	0.16	1.2	-0.045		
NE*BBG		0.028					-0.71	-1.4	-1.4	0.23		-14.3			
NE*WATER										-0.086		1.3			
BBG*WATER	0.029	0.0028	0.10	-0.17	-0.0031	-0.068	-0.11	-0.058	-0.058		-0.13	-1.3			
R-SQ	0.91	0.99	0.77	0.91	0.70	0.92	0.75	0.95	0.95	0.81	0.94	0.95	0.88	0.91	0.76

Independent variables: SFE= HPMC SFE 4000; NE= HPMC NE 4000; BBG: Barley Beta-Glucan; WATER= water content above 70%. Blanks correspond to no significant effects at level of 5%; R-SQ adjusted square coefficient of the fitting model

3. Results and discussion

3.1 Effect on physical properties of fresh breads

Analytical data on bread characteristics (Table 2) were fitted to multiple regression equations using added principles (SFE, NE, BBG, WATER) as independent factors in order to estimate response surfaces of dependent analytical variables. Significant coefficients (95% confidence interval) obtained from the stepwise regression fitting model are included in Table 4.

Specific volume and height/width rate

Loaf specific volume of the breads, that varied between 1.4 and 5.1 mL/g, and the loaf height/width ratio, that ranged 0.28 – 0.87, exhibited similar trends, as could be expected in pan breads (Table 2). The multiple regression equations obtained for height/width and specific volume explained the 99% and 91% of their variability, respectively (Table 4). Specific volume increased with WATER, SFE and BBG addition until a maximum. The positive coefficients of the linear terms and the negative coefficients of the quadratic ones of these three factors account for this evolution (Table 4). It should be noticed the non significant individual effect of NE ($p>0.05$) on both the height and the specific volume of breads, were probably masked by the greater effect of the remaining design factors. A significant positive interaction SFE*NE was observed, which means that NE enhanced the individual SFE effect on bread size. The effect of SFE on specific volume did not show a significant dependence on dough water content, being 1.6% the dose that led to the maximum size of the bread. From the multivariate regression equation the individual addition of 1.6% SFE to the dough with the minimum water content tested (70%, equivalent to 0 level in the design) would nearly double the initial volume of the bread. For this SFE dose, a water increase from 70% to 90% would lead to an additional specific volume increase by 37%. 90% of dough hydration led to the maximum bread specific volume, only dependent on BBG dose. The positive interaction BBG*WATER (Table 4) indicates that water favors the effect of BBG on specific volume. This fact establishes an important difference between the HPMC and BBG action on GF breads: BBG requires an important additional amount of water to show a beneficial effect on bread volume while HPMC acts even in adverse

conditions of low dough hydration showing an effect little dependent on dough water content. The individual addition of BBG to dough (70% water) decreased markedly the height and the volume of GF breads. The same effect was observed in wheat breads by Brennan & Cleary (2007) when β-glucan content varied from 2.5% to 5%. Some authors (Cavallero et al 2002; Gill et al 2002) relate the above mentioned effect to the high water binding capacity of β-glucan that restricts available water for the development of gluten network. The same reason might explain BBG effect on GF breads. Doughs with too low disposable water are probably too consistent to get a certain development during proofing and subsequent baking (Ronda et al. 2013). BBG added to reduced-water doughs is unable of establishing cross-links or entanglements in the dough and, thus, dough structure cannot be developed. It was previously shown that BBG was unable to decrease the elastic compliance, J_o, at dough water content of 70% as HPMC did (Ronda et al 2013). But, in these conditions, it conferred the major effects on dough G' modulus. So that, BBG addition to doughs only increased dough consistency, which made dough development even more difficult, leading finally to a lower bread volume. Simultaneous addition of water and BBG counteracted the single BBG effect leading to an increase of 34% in bread volume compared to the maximum value obtained in BBG absence. In a previous work (Ronda et al. 2013), a significant BBG*Water negative interaction on the elastic modulus G' was found for the same GF doughs, allowing to relate the increase in bread volume with a decrease in dough consistency. The additional amount of water required to get the maximum volume in bread increased at a rate of 5 - 6 % per 1% increase of BBG in dough formulation (flour basis in both cases): the dough hydration needed for 2% BBG would be 101%, and for 3.5% BBG it would be 109 %. However, the model predicts that without HPMC, even with the optimal dose of water, BBG addition above 2.5% would start to decrease specific volume attaining a reduction of 24% at the maximum dose tested (3.9%). Lazaridou et al. (2007) also observed a maximum on bread specific volume with water, and obtained an additional increase in bread volume by 1% - 2% β-glucan addition to dough (Lazaridou et al 2007). Significant bread volume increases were also obtained when oat and barley dietary fibres were added to GF doughs of adapted hydration (Sabanis et al. 2009). Hydrocolloids can improve dough development and gas retention by increasing dough viscosity (Houben,

Hoechstoetter, and Becker 2012), but there is an optimum value for the resistance to deformation. Too high values can cause a limited and slow expansion of the gas cells during proofing (Van Vliet et al 1992).

It is noteworthy that formulations yielding higher specific volume in breads correspond to high doses of both SFE and water that gave poor quality breads with large gas cells (pictures not shown), in agreement with previous works (Haque and Morris 1994; McCarthy et al 2005; Nishita et al 1976) on high water GF breads. Authors related pocket formation to a poor dough consistency due to an excess of water. The excessive low consistency seems to cause the bubbles to become unstable, coalescing and resulting in large holes, after the crust has formed. Owing to the differences between the two types of HPMC used in the present work, defects could be related to the facility of crust forming that could be higher in the case of SFE (a semi-firm gel forming) than in NE (a weak gel forming) helping retain the large bubbles formed inside the crumb. With no exception, defects were only observed at water amounts above 90%.

Loss of weight

The loss of weight during baking varied between 26 g and 41 g (Table 2), equivalent to 13% and 21% with respect to the initial amount of the baked dough. Table 4 shows that the loss of weight depended mainly on the water content of the dough. The significant ($p<0.05$) coefficients of the regression equation indicate that water loss increased up to a maximum with increased dough water content (Table 4). In absence of hydrocolloids the maximum loss of weight increase was 20% and took place at a dough hydration of 94%. The marked effect of water content on loss of weight during baking probably masked the effect of the remaining design factors.

The individual addition of BBG to dough decreased the loss of weight during baking as indicated by the negative linear significant coefficient. At a flour hydration level of 70%, the individual addition of 3.9 % BBG led to a 27% decrease in the loss of weight. This was probably due to the high water binding capacity of β-glucans in line with previous results (Brennan and Cleary 2007). However, the positive sign of the BBG*WATER interaction coefficient anticipates that an increase in dough water content decreased the individual ability of BBG to reduce the loss of weight during baking. Accordingly, the amount of water that led to the maximum loss of weight in

BBG absence, 94%, increased to 104 % for 2% BBG and to 112% for 3.9% BBG. The mentioned dough hydration values that maximized the loss of weight during baking also maximized the bread specific volume at each BBG addition.

Texture of the bread

Fresh bread crumb firmness varied from 1 N to 17 N (Table 2), which means a very wide range of crumb hardness among tested breads. From the multiple regression study, firmness was only significantly affected ($p < 0.05$) by both water and BBG (Table 4). These only two factors could explain 91% of the variability of fresh bread firmness, as indicates the R^2 value included in Table 4. The effect of cellulose derivatives was notably smaller than that associated to the other two ingredients. In absence of hydrocolloids, a significantly softer crumb was observed when the water content was increased from 70% to 90% as was reported earlier (McCarthy et al 2005). However, as can be predicted from the positive quadratic coefficient, additional amounts of water made crumb firmer again. Firmness evolution could be explained in parallel to specific volume evolution. In fact, both properties showed a significant ($p<0.001$) negative correlation (see section 3.3). Water dosage that led to a minimum crumb firmness led to a concomitant maximum specific volume. The same could be reported from the BBG effect.

The individual effect of BBG can be concluded from the high positive coefficient of the linear term of the regression equation (Table 4). However, the significant negative interaction between water and BBG explains that the simultaneous addition of BBG and water counteracted the individual effect of BBG. From the predictive model, addition of 4% BBG with water content above 95 % would lead to crumbs with a similar firmness to those obtained with doses of cellulose derivative around 1.5 %. Fig. 1 shows the firmness of fresh breads and their evolution during nine days of storage. It allows the comparison of runs where only varies a factor while the rest are constant in the intermediate level of the range studied. The comparison of runs 7 and 11 shows the importance of dough hydration on BBG-enriched GF bread development (Fig. 1c).

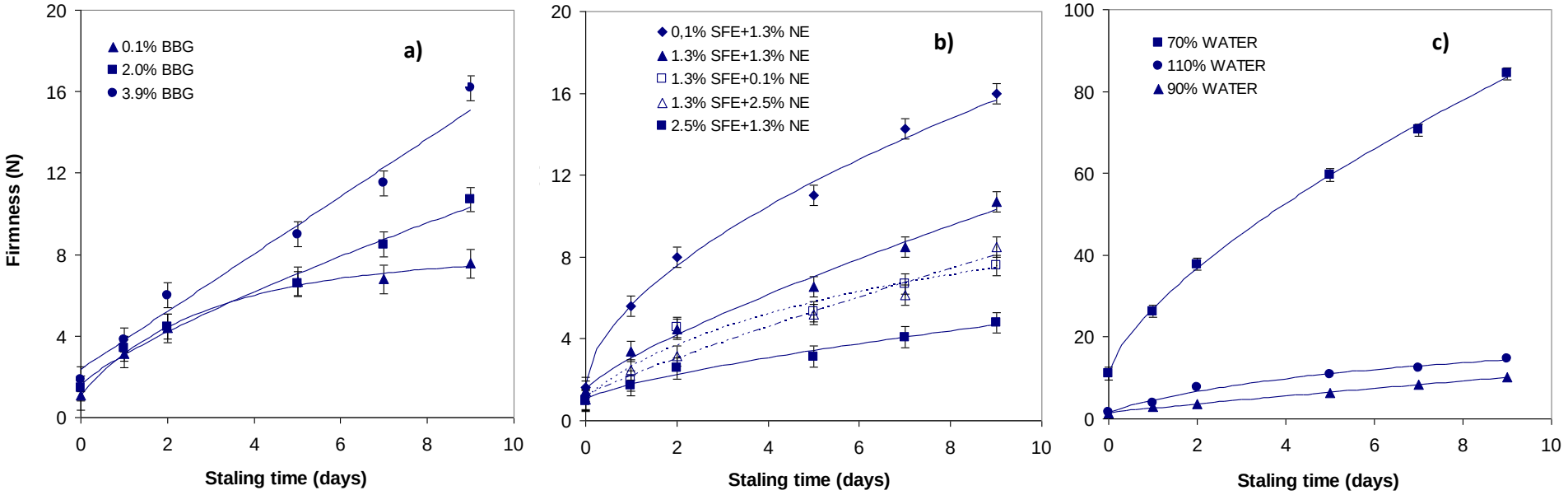


Figure 1: Crumb firmness evolution during 9 days of storage at 4ºC of breads with different amounts of BBG (runs 5, 7 and 13) (a) HPMC (runs 1, 3, 7, 9 and 10 (b) and Water (runs 7, 11 and 14) (c). Intermediate doses of dietary fibres (1.3% SFE; 1.3% NE; 2% BBG) and water (90% WATER) were used as constant factors. Values of firmness are the average of four replicates and error bars represent standard deviation.

Chewiness of breads ranged 0.3 – 5.8 N and varied in parallel as hardness did (Table 2). This could be expected as both textural properties are directly related. Cohesiveness (varied between 0.4 and 0.6) and springiness (ranged 0.6 – 0.9), also involved in chewiness equation, showed minor effects (Table 2). The resilience variation could not be correlated to the studied factors, probably due to the low values and the low variations observed between runs (0.19 – 0.29). Springiness, increased with single addition of BBG and/or water (see significant quadratic terms in Table 4). From the multivariate regression equation it could be predicted a 21% increase in crumb springiness obtained from low hydration dough (70% water) with 3% BBG and of 39% for 3.9% BBG. In absence of hydrocolloids the increase of dough hydration from 80% to 110% led to springiness increases around a 10% per each 10 % increase in dough water content. The negative interaction WATER*BBG explains that the simultaneous increase of water and BBG in dough did not lead to the expected springiness as result of the sum of the individual effect of Water and BBG.

Crumb and crust colour

Bread crust colour features showed bigger dependence on both hydrocolloid and water contents, than bread crumb. Bread crusts showed lightness values that ranged 60-74%. The a^* and b^* coordinates varied between 2–13 and 19–30 respectively leading to hue values of 64 – 84 degrees, and chromas of 19 – 32 (Table 2)). Crust colours had higher L^* and b^* coordinates and lower a^* one than other authors found (Lazaridou et al, 2007) probably due to the use of corn as starch source in that work. From multivariate regression equation (Table 4) it would be predictable a 10% increase in bread crust Lightness for the maximum dough hydration of 110% (40 level) with respect to the lowest one (70%). This was probably due to the reduction of Maillard reaction progress as consequence of this reaction precursor's dilution.

Single SFE addition to a dough with the minimum water content, 70%, would lead to a 12% of maximum decrease in crust L* at a dose of 2%. However, an additional dose of SFE did not showed additional effect (Table 4). Single BBG increased crust Lightness until 14% for the maximum dose tested, 3.9%, in accordance with the positive quadratic coefficient in the regression equation. The effect, also observed in previous works (Lazaridou et al 2007), could be explained by the already mentioned

BBG water retention capacity during baking. that led to breads with significantly lower weight loss. Simultaneous increase of BBG and Water led to darker bread crusts than expected for the individual action of BBG (significant BBG*Water coefficient), concomitant with the increase in baking water loss already mentioned in the previous section.

Bread crust hues, significantly affected by all the factors studied (Table 4) corresponded to more yellowish (h=90) than reddish (h=0) colours. It was observed that, in general, breads with lower crust hues (more reddish) had lower lightness values. The SFE increase and the water/BBG decrease led to reddish and darker bread crust. Lazaridou et al (2007) also observed a tendency to yellowish in 1% oat β-glucan-added GF-bread crusts (Lazaridou et al 2007). The crust hue was also significantly decreased by NE, although in less extent than SFE (Table 4).

Crumb colour, that mainly depends on the own ingredient colours, was not very affected by the design factors due to the white-slight cream colour of added fibers. Only the chroma showed significant linear dependence on dough hydration and on viscous dietary fibres with a R^2 of 81.4%. With no added hydrocolloids the maximum water content increase (40%) led to 59% increase in the crumb chroma (more vivid colours). The presence of both types of HPMC counteracted the individual effect of water.

Bread staling

Bread staling was assessed by means of hardness evolution over 9 days (Figure 1). Experimental data were fitted to the Avrami equation (Armero and Collar 1998; Ronda and Roos 2011), and adjusted values were plotted leading to the continuous lines. Hardening during storage is expected as a result of moisture loss and starch retrogradation (Ronda et al 2011). Water was the factor with more prominent effect on GF bread staling, as shown Fig. 1c. This figure shows the bread made with a water content of 70 % reached, after one storage day, a hardness ten times that of the bread with a 90% and the same hydrocolloid contents. After nine days of storage hardness was 84 N, which represents an extremely high value. This demonstrates that hydrocolloids, with water restrictions, lead to unsuitable bread characteristics. The different effect of SFE and NE on bread hardening was also remarkable. Fig. 1b shows that SFE increase from 0.1% to 2.5% led to significant depletion in bread

hardness during storage, in spite of 1.3% NE presence. On the contrary, same changes in the concentration of NE to a fixed concentration of 1.3% SFE hardly changed hardness curves.

As it can be seen in Fig. 1a, higher contents of BBG accelerated the aging of breads with 90% of water and 2.6% of HPMC (1.3% SFE + 1.3% NE). Other authors also observed this effect in breads added with β-glucan (Lazaridou et al 2007). This could be due to the higher water necessities of this high BBG dose (3.9%), near 110% as was shown before, instead of 90%. It is also noteworthy that 3.9% BBG delayed the achievement of the maximum hardness during storage at 4ºC, generally attaint in 6 – 8 days (Ronda and Roos 2011), probably due to the great water binding ability of BBG, controlling the loss of water during aging (Ronda et al 2011).

Table 4 shows the significant coefficients of the fitting model for the crumb hardening after one (short-term) and nine (long-term) days of storage at 4ºC. The regression study confirmed the individual effects of HPMC, BBG and Water on the already mentioned crumb hardening.

Table 3: Effect of dietary fibre addition on sensory properties of gluten-free breads. See Table 1 for coded run formulation.

Run	Crumb grain	Crumb softness	Crumb chewiness	Crust crumbliness	Intensity of Taste	Intensity of Flavour	Overall acceptability
1	6 bcd	8,1 fg	6 ghi	7 f	5,1 abcd	5 a	6,5 def
2	9,7 g	1,4 a	3,3 ab	2,8 a	7,1 i	6 bcde	2,4 ab
3	6 bcde	5,5 d	4,2 bcde	4 bcde	5 ab	5 abcd	5,5 cd
4	4 a	8,6 gh	7,4 hi	5 de	5,1 bcd	5,9 abcde	7 fg
5	6 bcd	8,0 fg	4,6 bcde	7 f	7 ghi	6,3 cde	7,5 gh
6	9,5 g	1,3 a	5 efg	3 a	6,8 ghi	6 cde	3 ab
7	7 cde	8 gh	5 cdefg	3,6 ab	6,1 efgh	5,7 abcde	7,0 fgh
8	9,4 g	1,8 abc	5 cdefg	4 bc	6,0 efg	5,3 abc	3 b
9	5 b	7,5 ef	5 defg	5 de	4,8 ab	5,5 abcd	6,8 fg
10	5 bc	7,2 e	5 defg	5,3 de	4,9 ab	6,2 cde	5,9 cde
11	9,1 fg	1,6 ab	4 bcd	4,8 cde	6,0 defg	6,1 bcde	2,6 ab
12	6 bcd	8,1 fg	7,4 i	4,3 bcd	6,1 efgh	6,9 e	7,6 h
13	9,7 g	2,2 bc	2,2 a	2,7 a	6,1 efgh	5 abc	2,2 a
14	2 a	8,4 gh	6 fgh	5 de	5,0 abc	6,4 cde	7 fgh
15	6 bcd	7,2 e	6,1 fghi	6 e	5,3 bcde	6,6 de	7,0 fgh
16	3 a	8,1 fg	4,0 bcd	3,5 ab	4,1 a	6 abcde	7 ef
17	7 de	8,9 h	4 bcde	7,5 f	6 cdef	5 abcd	6,9 fgh
18	8,9 fg	2,5 c	3,7 bc	3,7 ab	7,0 hi	4,9 ab	3,2 b
19	8 ef	6,9 e	5 bcdef	4,3 bcd	6,3 fghi	6,3 cde	5,6 cd
SD	0.5	0.3	0.6	0.5	0.4	0.5	0.4

Values followed by the same letter in the same column are not significantly ($p < 0.05$) different.
SD: Standar deviation obtained form ANOVA

3.2. Effect on bread sensory quality

The coefficients of the regression fitting models for the sensory attributes ($0.76 \leq R^2 \leq 0.90$) are compiled in Table 4. SFE, BBG and water markedly affected crumb grain scores that varied between 2.4±0.5 and 9.7±0.5 in the scale 1-10 (Table 3). Negative coefficients of single BBG and/or Water (Table 4) suggest both factors increased alveoli size and crumb grain heterogeneity, although BBG quadratic coefficient supports that from 1.7 % BBG addition crumb grain ratings became higher. SFE and Water showed a significant negative interaction on crumb grain scores, so that, high SFE dosages to softer doughs led to a prominent decrease in crumb grain ratings (Table 3). Lower crumb grain scores were concomitant with the presence of big holes (pockets) in bread crumb, and unaccepted by panelists. Ratings for crumb softness were dependent on Water, BBG and SFE (Table 4). Increased water amounts up to 95 -100% hydration in dough promoted significantly sensory ratings for crumb softness. Higher hydration levels did not provide any additional perception. BBG increasing single addition decreased softness regardless dough water content. Simultaneous SFE presence helped BBG increase crumb softness when added up to 2%, flour basis. In fact, SFE counteracted BBG deleterious effect on crumb smoothness, so that SFE appears as a key ingredient in BBG-enriched GF-breads.

Overall acceptance of GF-breads, that varied between 2.2±0.4, for Run 13, and 7.6±0.4, for Run 12, was only significantly affected ($p<0.05$) by water and BBG dosages. According to panelits' scores, overall acceptance was mainly/significantly dependent on crumb grain, and softness (Table 6). Breads with water formulation greater/equal than 90% (flour basis) deserved acceptance (≥ 5/9), with the exception of Run 13, including 3.9 % BBG. It should be noticed that the bread of Run 12 (3.34% BBG, 0.45% SFE, 2.15% NE, 104% WATER), was individually preferred (7.6±0.4/9). The ratio BBG/Water of this formulation coincided with that predicted from the regression equations as a ratio capable of optimizing the bread quality leading to minimum bread firmness and maximum specific volume.

Table 5: Pearson product moment correlations between pair of variables measured in dough and bread. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables. The rheological properties correspond to the fitting of experimental oscillatory measurements to power law model ($G' = G'_1 \cdot \omega^a$; $G'' = G''_1 \cdot \omega^b$; $\tan\delta = (\tan\delta)_1 \cdot \omega^c$). Creep test results to the 6-parameter Burgers model (where J_o and μ_o are the instantaneous compliance and the steady state viscosity respectively); Consistency is the firmness measured in an extrusion empirical test (Ronda et al. 2013).

	Specific volume	**Loss of weight**	**Firmness**	**ΔFirmness 1 day**	**ΔFirmness 9days**	**Cohesiveness**	**Chewiness**	**Crumb grain**	**Crumb softness**	**Overall Acceptability**
G'_1	-0.64**	-0.86***	0.79***	0.69***	0.59**	ns	0.79***	0.70***	-0.83***	-0.79***
a	0.53*	ns	-0.47*	-0.58**	-0.51*	0.49*	ns	-0.57**	0.65**	0.59**
G''_1	ns	-0.78***	0.61**	0.49*	ns	ns	0.63**	0.61**	-0.72***	-0.69**
b	0.56*	0.72***	-0.67**	-0.65**	-0.54*	0.54*	-0.65**	-0.74***	0.88***	0.81***
$\tan\delta_1$	0.56*	ns	-0.48*	-0.58**	-0.50*	0.54*	ns	-0.62**	0.68**	0.60**
c	ns	0.61**	ns	ns	ns	ns	ns	ns	0.53*	0.49*
$J_{0\text{-creep}}$	ns	ns	ns	ns	ns	0.62**	ns	-0.66**	0.62**	0.50*
$\mu_{0\text{-creep}}$	-0.76***	-0.68**	0.88***	0.85***	0.90***	-0.49*	0.84***	0.70***	-0.83***	-0.74***
Consistency	-0.50*	-0.82***	0.58**	0.56*	ns	ns	0.61**	0.67**	-0.77***	-0.75***
Peak Temperature	0.57*	ns	-0.50*	-0.49*	-0.46*	ns	-0.52*	ns	ns	ns

Asterisks indicate the P-value which tests the statistical significance of the estimated correlations. *p<0.05 (statistically significant non-zero correlations at the 95.0% confidence level); ** p<0.01 (at the 99.0% confidence level); *** p<0.001 (at the 99.9% confidence level). ns: Not significant (p > 0.05)

Table 6: Pearson product moment correlations between pair of variables measured in bread. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables.

	Loss of weight	**Firmness**	**ΔFirmness 1 day**	**ΔFirmness 9days**	**Chewiness**	**Crust Lightness**	**Crust hue**	**Crust Chroma**	**Crumb grain**	**Crumb softness**	**Overall Acceptance**
Specific volume	0.83***	-0.80***	-0.83***	-0.72***	-0.80***	-0.49*	-0.56*	0.62**	-0.72***	0.73***	0.73***
Loss of weight		-0.75***	-0.74***	-0.55*	-0.77***	ns	ns	ns	-0.75***	0.76***	0.83***
Firmness			0.92***	0.91***	0.99***	ns	ns	-0.61**	0.55*	-0.73***	-0.62**
ΔFirmness 1 day				0.87***	0.90***	ns	0.53*	-0.65**	0.64**	-0.81***	-0.71***
ΔFirmness 9 days					0.85***	ns	0.65**	-0.61***	0.46*	-0.64**	-0.51*
Chewiness						ns	ns	-0.57*	0.55*	-0.71***	-0.62**
Crust Lightness							0.72***	-0.56*	ns	ns	ns
Crust hue								-0.78***	ns	ns	ns
Crust Chroma									ns	ns	ns
Crumb grain										-0.83***	-0.83***
Crumb softness											0.91***

Asterisks indicate the P-value which tests the statistical significance of the estimated correlations. *p<0.05 (statistically significant non-zero correlations at the 95.0% confidence level); ** p<0.01 (at the 99.0% confidence level); *** p<0.001 (at the 99.9% confidence level). ns: Not significant (p > 0.05).

3.3. Correlation between variables

Multivariate data handling of dough visco-elastic and bread quality parameters supplied useful information on the significantly correlated dough and bread characteristics. Fundamental and empirical dough rheological properties were reported in a previous work (Ronda et al 2013). Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.46 to 0.90) was obtained for the relationships between dough viscoelastic parameters and bread properties of fibre-supplemented rice-based matrices (Table 5). Table 6 reports Pearson correlation coefficients for the relationships between bread properties. Bread specific volume strongly correlated with dough viscosity at the steady state, η_0, obtained from creep tests ($p<0.001$, $r = -0.76$). Specific volume explicited a negative correlation with the elastic modulus at 1 Hz, G_1', ($r = -0.64$) and positive with loss tangent, tan δ, and the exponents "a" and "b" ($r=0.56$, $r= 0.53$, $r=0.56$). The exponents, resulting from fitting power law to oscillatory frequency sweep data, quantify the dependence of dough elastic and viscous moduli on frequency. The higher the dependence of dynamic moduli on the frequency is, the higher tan δ and the greater the bread volume. This means that lower dough consistency and less marked solid-like behaviour (within the range $0.19<\tan\delta<0.48$) favoured dough development and bread volume. Baking weight loss was strongly correlated with dough visco-elastic moduli, G_1' and G_1'', ($p<0.001$; $r =-0.86$; $r =-0.78$), the viscosity at the steady state, η_0, ($p<0.01$; $r =-0.68$) and the dough consistency or the force obtained in a back extrusion test ($p<0.001$; $r=-0.82$). The greater the dough consistency means the higher the baking yields. This is probably due to the fact that fibres, substances responsible for the dough consistency increase, were also good water-binding macromolecules. Fresh bread crumb firmness and one- and nine-days stored bread crumb hardening were strongly correlated to all visco-elastic dough properties. The strongest positive correlation was obtained for fresh bread crumb firmness and the elastic modulus, G_1' ($p<0.001$; $r= 0.79$), and the viscosity at steady state, η_0, ($p<0.001$; $r =0.88$). The short- and long-term bread hardening exhibited similar relationships with dough properties than initial firmness. Sensory scores related to crumb grain, crumb softness and overall acceptance were strongly correlated ($p<0.001$) with fundamental and empirical dough rheological properties.

4. Conclusions

Draper-Lin small composite design for sampling allow to obtain useful information on the significance of different hydrocolloid and water contents on breadmaking performance and staling behaviour of GF rice-based complex/heterogeneous bread matrices. Dough and bread characteristics strongly correlated supporting the key influence of the restoration of dough visco-elasticity on the physico-chemical and sensory achievement of GF final baked goods.

Single BBG by itself fails to mimic gluten visco-elasticity properly, but simultaneous addition of either SFE or NE, in doses around 1.6 %, contribute to bread improvement in terms of bigger volume and smoother crumb. Weak gel forming NE led to harder and lower volume breads than semi-firm gel forming SFE did, probably ascribed to the ability of hydroxypropyl groups to form a stable solvate shell in water restricting available water for starch to swell. It should be noticed that the surplus of water needed to incorporate large amounts of BBG into bread favoured the formation of big holes (pockets) in bread crumb particularly when SFE was included into formulation. Visible holes could be probably related to the easier crust forming ability of SFE than NE helping retain the large bubbles formed inside the crumb. A dough hydration of 90% would be recommended to get the maximum volume of no BBG added breads. This optimal amount of water would increase at a rate of 5-6% per 1% increase in BBG.

3.3 g of BBG (70% purity) and 104mL of water addition to 100 g rice flour provided sensorially accepted breads (7.6±0.4 over 10) with a theoretical ß-glucan content of 1.24 g/100 g GF bread. It would allow a daily intake of 3 ß-glucan provided a bread consumption of 240 g/day. Complementary tests aimed at establishing the real content and molecular weight of ß-glucan in final bread are still pending to assure the expected nutritional benefit of BBG enrichement.

Acknowledgement

Authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad (Projects AGL2012-35088 and AGL2011-22669) and Comunidad de Castilla y León (Project VA252A12-2), the laboratory of Pharmacy (laboratory accredited according to ISO 17025) of the University of Basque Country for the gluten analysis in barley β-Glucan extracts.

References

Anton, A. A. (2008). Improving the nutritional and textural properties of wheat flour tortillas. *Cereal Research Communications*, 36, 301-311.

Armero, E. & Collar, C. (1998). Crumb firming kinetics of wheat breads with anti-staling additives. *Journal of Cereal Science*, 28, 165-174.

Bell, D. A. (1990). Methylcellulose as a structure enhancer in bread baking. *Cereal Foods World*, 35, 1001-&.

Brennan, C. S. & Cleary, L. J. (2007). Utilisation of Glucagel® in the beta-glucan enrichment of breads: A physicochemical and nutritional evaluation. *Food Research International*, 40, 291-296.

Cavallero, A., Empilli, S., Brighenti, F. & Stanca, A. (2002). High (1 - 3,1 - 4)-beta-glucan barley fractions in bread making and their effects on human glycemic response. *Journal of cereal science*, 36, 59-66.

Collar, C., Andreu, P., Martinez, J. & Armero, E. (1999). Optimization of hydrocolloid addition to improve wheat bread dough functionality: a response surface methodology study. *Food Hydrocolloids*, 13, 467-475.

Collar, C., Santos, E. & Rosell, C. M. (2005). Providing healthier baked goods through high fibre optimised formulations: Functional requirements. In: *Intradfood Innovations in Traditional Foods* (edited by P. Fito & F. Toldrá). Pp. 879-882. London: Elsevier.

Collar, C., Bollain, C. & Angioloni, A. (2005). Significance of microbial transglutaminase on the sensory, mechanical and crumb grain pattern of enzyme supplemented fresh pan breads. *Journal of Food Engineering*, 70, 479-488.

Collar, C., Santos, E. & Rosell, C. M. (2007). Assessment of the rheological profile of fibre-enriched bread doughs by response surface methodology. *Journal of Food Engineering*, 78, 820-826.

Draper, N. R. & Lin, D. K. J. (1990). Small response-surface designs. *Technometrics*, 32, 187-194.

EFSA. (2011). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal*, 9, 2207.

Gill, S., Vasanthan, T., Ooraikul, B. & Rossnagel, B. (2002). Wheat bread quality as influenced by the substitution of waxy and regular barley flours in their native and extruded forms. *Journal of cereal science*, 36, 219-237.

Haque, A. & Morris, E. R. (1994). Combined use of ispaghula and HPMC to replace or augment gluten in breadmaking. *Food Research International*, 27, 379-393.

Haque, A., Richardson, R. K., Morris, E. R. & Dea, I. C. M. (1993). Xanthan-like weak gel rheology from dispersions of ispaghula seed husk. *Carbohydrate Polymers*, 22, 223-232.

Houben, A., Hoechstoetter, A. & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology*, 235, 195-208.

Kinner, M., Nitschko, S., Sommeregger, J., Petrasch, A., Linsberger-Martin, G., Grausgruber, H., Berghofer, E. & Siebenhandl-Ehn, S. (2011). Naked barley-optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. *Journal of cereal science*, 53, 225-230.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N. & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*, 79, 1033-1047.

McCarthy, D., Gallagher, E., Gormley, T., Schober, T. & Arendt, E. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry*, 82, 609-615.

Nishita, K. D., Roberts, R. L., Bean, M. M. & Kennedy, B. M. (1976). Development of a yeast-leavened rice-bread formula. *Cereal Chemistry*, 53, 626-635.

Ronda, F., Perez-Quirce, S., Angioloni, A. & Collar, C. (2013). Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: A fundamental and empirical rheological approach. *Food Hydrocolloids*, 32, 252-262.

Ronda, F. & Roos, Y. H. (2011). Staling of fresh and frozen gluten-free bread. *Journal of cereal science*, 53, 340-346.

Ronda, F., Gomez, M., Blanco, C. A. & Caballero, P. A. (2005). Effects of polyols and nondigestible oligosaccharides on the quality of sugar-free sponge cakes. *Food Chemistry*, 90, 549-555.

Ronda, F., Caballero, P. A., Quilez, J. & Roos, Y. H. (2011). Staling of frozen partly and fully baked breads. Study of the combined effect of amylopectin recrystallization and water content on bread firmness. *Journal of cereal science*, 53, 97-103.

Sabanis, D., Lebesi, D., Tzia, C. (2009). Effect of dietary fibre enrichement on selected properties of gluten-free bread. *Food Science and Technology*, 42, 1380-1389.

Thomson, T. (2009). The nutritional quality of gluten-free foods. In: *Gluten-Free Food Science and Technology* (edited by E. Gallagher). Pp. 45-51. New Delhi: Wilet-Blackwell.

Van Vliet, T., Janssen, A. M., Bloksma, A. H. & Walstra, P. (1992). Strain-hardening of dough as a requirement for gas retention. *Journal of Texture Studies*, 23, 439-460.

Wood, P. J. (2010). Oat and Rye beta-glucan: Properties and function. *Cereal Chemistry*, 87, 315-330.

Zessner, M., Helmich, K., Thaler, S., Weigl, M., Wagner, K. H., Haider, T., Mayer, M. M. & Heigl, S. (2011). Ernährung und flächennutzung in Österreich. *Öwaw*, 5/6, 95-104.

CAPÍTULO III

Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics*

* Ronda, F., Perez-Quirce, S., Lazaridou, A., Biliaderis, C.G. (2015) "Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics". Food Hydrocolloids, 48, 197-207.

Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics

Felicidad Ronda[1*], Sandra Perez-Quirce[1], Athina Lazaridou[2], Costas G. Biliaderis[2]

[1]Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

[2]Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 235, Thessaloniki, Greece, 541 24.

Abstract

The impact of commercial oat or barley β-glucan concentrates incorporated at different levels (1.3 – 3.9 % actual β-glucan concentration, flour basis) into gluten-free (GF) rice-based dough formulations differing in water content (89–141%, flour basis) on dough rheology (empirical and fundamental tests) and breadmaking performance has been investigated. The effect of the baking process on the content and molecular weight of ß-glucan in the final bread has been also evaluated. The rheological properties of the dough were dramatically influenced by dough water content; i.e. optimization of dough hydration is indeed of primary importance on improving GF bread quality. The bread specific volume was negatively correlated with the dough elastic modulus (G') and the viscosity (η_0), and positively with the loss tangent. At optimum hydration, the rheological properties of barley β-glucans-enriched doughs and the quality attributes of breads derived therefrom were notably affected by the soluble fibre content; the G' at 1Hz increased up to ~100% and the bread volume decreased ~ 32% with respect to the values of the control dough and bread. In contrast, the impact of concentration of the oat-β-glucan preparation in the fortified doughs and bread was much less pronounced. These findings could be explained by the ability of the low molecular weight barley β-glucans to develop a gel network structure at higher concentrations, whereas the preparation of the high molecular weight oat β-glucan exhibits a more viscous-like rheological response. The added β-glucans to doughs were also quantitated in the bread crumbs, and a significant decrease in their molecular weight was noted, most likely due to the β-glucanase activity in the raw materials incorporated in the GF flour mixtures.

Consequently, although the EFSA claims are achievable in gluten-free breads enriched with commercial β-glucans concentrates, control of β-glucanase activity in the raw materials may be a critical issue in exerting all the physiological benefits associated with the consumption of these bioactive polysaccharides.

Keywords: Barley β-glucans; oat β-glucans; Gluten free formulations; dough rheology; bread; β-glucan molecular weight.

1. Introduction

The impact of dietary fibre on the maintenance and improvement of human health has attracted considerable scientific interest in the last decades (Brennan & Cleary., 2007). However, innovative DF-enriched products have to meet the main quality requirements for food products: nutritional added value, safety, texture-taste, palatability, convenience and easy handling during processing (Angioloni et al., 2011). This goal is particularly challenging in gluten-free (GF) breadmaking where the lack of the gluten protein matrix seriously constrains the dough visco-elastic character, leading to a failure in carbon dioxide entrapment during proofing and baking, and thereby a quality decline in the resulting breads. Moreover, a poor nutritional balance frequently characterises multi-ingredient (composite) GF matrices due to the higher content of readily-digestible carbohydrates (Thomson, 2009). The GF baked goods are often low in fibre, both soluble and insoluble; consequently their enrichment with dietary fibre seems to be necessary for nutritional improvement of these products (Sabanis et al., 2009). Cereal β-glucans (BG) are classified as soluble dietary fibre with well recognized ability for reducing blood serum LDL-cholesterol levels and attenuating the postprandial blood glucose and insulin levels (EFSA, 2011). The beneficial to health effects of cereal ß-glucan isolates have been recently reviewed by Wood and co-workers (Wood, 2010; Tosh, 2013; Whitehead et al., 2014), with most of the data derived from studies with oat ß-glucans (OBG), followed by barley (BBG) and rye (Kinner et al., 2011). Isolates of cereal BG are hydrocolloids with thickening properties (Lazaridou & Biliaderis, 2007) that can increase the nutritional value of GF bread in terms of soluble dietary fiber content with proven human health promoting effects. On the other hand, it has been

reported that a high concentration of BG decreases the water availability for the protein-starch network and thus impairs the quality of wheat breads (Gill et al., 2002). In this context, it must be noted that, as for all hydrocolloids, the fine structure, the molecular mass - chain length, the solution conformation and any chemical modification, the concentration added, the nature of raw materials and process parameters would be all important determinants of BG functionality during breadmaking (Houben et al., 2012).

Previous studies have shown a great variability in the effect of BG on the viscoelastic behaviour and bread quality of rice flour-based GF bakery products, depending on dough water and BG contents (Lazaridou et al., 2007; Ronda et al., 2013; Pérez-Quirce et al., 2014). Therefore, a systematic study to quantify the effect of water content in order to optimize the hydration level in the dough formulation depending on BG content and source is needed to evaluate and compare the effect of different BG prepartions on dough and bread properties. It should be also noted that farinographic tests, universally accepted to establish dough water requirements in wheat bread making, are not well adapted to GF dough formulations (Hager et al., 2011).

The potentially beneficial physiological activities of β-glucans have been largely attributed to their ability to increase the viscosity of the gut content and thereby modification of the absorption rates of nutrients and bile acids (Wood, 2010). In this respect, the importance of BG molecular weight (MW) on its nutritional and health-related benefits is well known (Wood, 2010). The technological effects of BG on wheat doughs and breads, also dependent on their molecular weight and concentration, have been recently studied (Cleary et al., 2007; Sabanis et al., 2009; Skendi et al., 2010). However, as far as we know, the impact of β-glucan MW on GF dough rheology and the resultant bread quality has not been explored yet. It is also important to explore the influence of the baking process on the content and molecular weight of the β-glucan present in the final GF product.

The aim of the present work was to study the effect of enrichment of GF flour formulation with commercial concentrates of β-glucans (BGs), derived from barley and oat, on dough rheology and bread quality characteristics; the water content of the dough was also varied to optimize the hydration level of the BG fortified systems to maximize the bread quality. The impact of the GF bread making process on the content and MW

of BG, was also examined in order to assess the nutritional implications of the fortification on the final baked product.

2. Materials and methods

2.1. Materials

Rice flour (12.5% moisture, 0.43 % ash, 7.5% protein, 0.47 % fat and 79.1 % starch) was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar and sunflower oil were purchased from the local market. Hydroxy-propyl-methyl-cellulose (HPMC) 4KM was a gift from Dow Chemical (Midland, EEUU). Barley (1→3)(1→4)-β-D-glucan (BBG) (Glucagel^{TM}) a preparation of low/medium molecular weight was given as a free sample from DKSH (Hamburg, Germany). The oat (1→3)(1→4)-β-D-glucan concentrate (OBG) (Promoat^{TM}) which was a high molecular weight β-glucan preparation, was supplied by Biovelop AB (Kimstad, Sweden). The proximate composition of these materials as given by the suppliers was: 2.52 % moisture, 4.75 % soluble protein, 1.43 % ash, 1.32 % fat, >85% total carbohydrates, and >72 % β-glucan for the BBG concentrate, and 6 % moisture, 54-56% carbohydrates (dextrin), <4.5% protein, 1-3 % ash and 0.5-1 % fat, and 33 – 36 % β-glucan for the OBG preparation. The gluten content of the commercial oat and barley BG samples was analysed by the ELISA test based on the R5 antibody; i.e. the gluten content in OBG was under the detection limit (< 6.2 mg/kg), while the gluten concentration of BBG was found 1.76 g/kg that rendered this commercial β-glucan concentrate not gluten-free. Nevertheless, the BBG was also included in this study for evaluation of the effect of BG MW on the rice flour-based gluten-free dough formulation since it is technically feasible to obtain a gluten-free barley β-glucan preparation (Ronda et al. 2013).

2.2. Dough preparation and breadmaking

A straight dough process was performed using the following formulation on a 100 g rice flour basis: 78% water, 6% oil, 5% sucrose, 2% HPMC, 1.8 % salt and 3% dried yeast. Table 1 summarizes the amounts of β-glucan concentrates and the amount of water added to the dough mixture, following an experimental design of 32 elaborations (2 BG types x 4 BG levels x 4 water levels). The dough water content, established with preliminary tests, was adapted to the BG content as it is well known the high water

absorption capacity of these hydrocolloids (Ronda et al., 2013). The GF dough- and bread-making procedures are described in detail elsewhere (Ronda et al., 2013; Pérez-Quirce et al., 2014). After baking, breads (around 200 g) were removed from the pans and left for one hour at room temperature before any analysis.

Table 1. Experimental design: amounts of commercial β-glucan concentrates from oat (OBG) and barley (BBG) dose and water contents in each elaboration.

Run	BBG (% rfb)	Run	OBG (% rfb)	WATER[1] (% rfb)
1	0	**17**	0	78
2	0	**18**	0	85
3	0	**19**	0	92
4	0	**20**	0	99
5	1.8	**21**	3.9	89
6	1.8	**22**	3.9	97
7	1.8	**23**	3.9	105
8	1.8	**24**	3.9	113
9	3.6	**25**	7.9	100
10	3.6	**26**	7.9	109
11	3.6	**27**	7.9	118
12	3.6	**28**	7.9	127
13	5.4	**29**	11.8	111
14	5.4	**30**	11.8	121
15	5.4	**31**	11.8	131
16	5.4	**32**	11.8	141

[1] The amount of water added for both the OBG and BBG fortified dough formulations on each raw, as specified by the respective run numbers, is common for the two concentrates.

2.3. Evaluation of dough properties

2.3.1. Large deformation mechanical test: Forward extrusion test

Forward extrusion assays were performed in a TA-XTplus texture analyser (Stable Micro Systems) equipped with a 25 kg-load cell and operating at 10 mm/s head speed as described in detail elsewhere (Ronda et al., 2013). This test measures the compression force or the work required for a piston disc to extrude the dough through a standard size outlet (1 cm) in the base of the sample container. The extrusion cell and the compression plunger were 2.55 and 2.50 cm in diameter, respectively (20 cm^3 volume). All measurements were performed at least in triplicate.

2.3.2. Small deformation mechanical test. Oscillatory and creep recovery tests

Dynamic and creep-recovery tests were carried out with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) equipped with a parallel plate geometry (60 mm diameter) having a serrated surface, at a 3 mm gap. The excess of batter was trimmed and vaseline oil was applied to cover the exposed sample surfaces to prevent sample drying during testing. Before the measurement, the batter was rested for 10 min to allow dough relaxation (a preliminary oscillatory test recording the elastic modulus, G', for 1 h at 1 Hz frequency (25 ºC) and under 1 Pa stress showed that a plateau G´ value was obtained in less than 5 min). Frequency sweeps were carried out from 0.1 to 20 Hz in the linear viscoelastic zone (LVR) identified for each batter by means of stress sweeps from 0.1 to 1000 Pa at 1Hz. The frequency sweeps of all batters were carried out at stress values in the range 0.5 - 8 Pa and 25 ºC. Frequency sweep data were fitted to a power law model as previously described by Ronda et al. (2013). The recorded viscoelastic parameters, G_1' and G_1'', and $(\tan \delta)_1$, represent the elastic and viscous moduli, and the loss tangent, respectively, at a frequency of 1Hz. The *a*, *b* and *c* exponents quantify the dependence of the dynamic moduli and the loss tangent on the oscillation frequency. Creep tests were performed by imposing a sudden step of shear stress (between 1-8 Pa in the LVR) for 150 s. In the recovery phase, the stress was suddenly removed and the sample was allowed for 300 s to recover the elastic (instantaneous and retarded) part of the deformation. Each test was performed in triplicate. Creep data are described in terms of creep compliance, J, which is defined as the strain divided by the stress applied (maintained constant during the creep test). The data from creep and recovery tests were modelled to the 4- and 3-parameter Burger's

models, respectively (Lazaridou et al., 2007; Ronda et al., 2013). J_0 is the instantaneous compliance, J_1 is the retarded elastic compliance or viscoelastic compliances, λ_1 is the retardation time and η_0 is the viscosity (estimated from the Burger's model using the creep data). Data modelling was performed with the Statgraphic Centurion v.6 software using the non-linear regression option (Bitstream, Cambridge, Massachusetts, USA).

2.4. Evaluation of bread quality

The volume, height and width of bread were determined by a Vol Scan analyser (Viken, Sweden); measurements were performed in four replicates. The breads were weighed immediately after removal from the pan once cooled. Crumb texture was determined in quadruplicate samples with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) using the software "Texture Expert". An aluminium 20 mm diameter cylindrical probe was employed in the "Texture Profile Analysis" double compression test (TPA) at 1 mm/s speed test, with a 30 s delay between first and second compression, at a deformation level of 50%. Hardness (N), chewiness (N), cohesiveness, springiness and resilience were calculated from the TPA curves; the analysis was carried out at 20 ± 3 ºC on bread slices, with 20 mm thickness, taken from the centre of each loaf. Moreover, the differences in hardness values of breads between the fresh products and those after storage for 1 and 7 days (ΔHardness) were taken as a staling index.

Colour was measured with a Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Japan); the results were obtained in the CIE L*, C*, h coordinates using the D65 standard illuminant, and the 2º standard observer. Colour determinations were made 4x4 times; i.e. bread crumb and crust colours were checked at four different points on each loaf and every point for colour assessment was measured four times.

Crumb grain characteristics of bread were calculated using a digital image analysis system using the ImageJ software. The analysis was performed on 30x30 mm squares taken from the centre of the loaf. This field of view represented approximately one-third of the cross-sectional area of the loaves. The crumb grain characteristics studied were the mean cell area (mm^2), the cell density ($cells/cm^2$; higher levels denote a finer structure) and the mean cell wall thickness (mm; calculated as the averaged mean intercellular distance of neighbouring cells sampled). Crumb grain parameters were measured in triplicate.

2.5. Evaluation of the content and molecular weight of β-glucan in breads

The β-glucan content in commercial barley and oat concentrates, crumb breads and β-glucan isolates from the oat concentrate and breads was determined using the mixed-linkage (1→3), (1→4) β-D-glucan assay kit purchased from Megazyme International Ltd.

The apparent peak molecular weight (Mp) of the commercial β-glucan concentrates and the β-glucan isolates derived from bread crumbs was estimated using a high performance size exclusion chromatography (HPSEC) system, which consisted of a single pump (Marathon IV, Rigas Labs, Thessaloniki, Greece), a guard TSKPWH column and two SEC columns in series, 7.5x300 mm TSK G6000 PW and 7.5x600 mm TSK G5000 PW (Tosoh Bioscience GmbH, Stuttgart, Germany), and a refractive index (RI) detector (ERC-7515A, ERC- Inc. Nishiaoki, Kawaguchi-City, Japan). The running conditions of the chromatograph and the sample preparation are described in detail, elsewhere (Lazaridou et al., 2004). The β-glucan standards (Mp: 14.9, 32.5, 83, 186, 340, and 466 x 10^3) employed for plotting of a standard curve were isolated as described elsewhere (Lazaridou, et al., 2004).

The β-glucans present in the oat concentrate (OBG) were further purified-concentrated until a ~ 72% β-glucan content was reached prior to evaluation of their molecular weight. Thus, OBG was firstly refluxed with 82% (v/v) ethanol (85^{o}C x 2 h) followed by aqueous extraction of the polysaccharide (45^{o}C x 1 h, 1:30 solids:water ratio), digestion (90^{o}C x 3 h, pH 4.5) with a preheated (90^{o}C x 30 min) thermostable α-amylase preparation (Megazyme), storage of the slurry overnight (pH 4.5), neutralization, centrifugation (2800 x g for 20 min), precipitation by ethanol, re-suspension in 2-propanol, filtration and air-drying. Bread crumb samples used for β-glucan MW content measurement were first lyophilized and then the β-glucans were isolated using additional purification steps as described in detail by Lazaridou et al. (2014) until a β-glucan content of about 75% was obtained in the isolates; this purification protocol included starch enzymic digestion and the complete removal of its hydrolyzates by combined exhaustive dialysis and ethanol precipitation.

2.6 Statistical methods

Response surface methodology (RSM) was used to study the simultaneous effects of BG (oat and barley) and dough hydration on GF dough and bread properties. The response surface study and the multivariate statistical analysis of the data (ANOVA, non-linear regression and Pearson correlation analysis) were performed by using the Statgraphics Centurion v.6 program (Bitstream, Cambridge, MN, USA); the LSD test was used to establish significant differences among means ($p<0.05$).

3. Results and discussion

Analytical data from the dough and bread characteristics were fitted to multiple regression equations using the added constituents (BG, WATER) as independent variables in order to derive response surfaces of the dependent (measured) variables (Tables 2 and 3). From these responses, the effect of BG depending on its MW and source, on dough and bread properties can be established and compared. The optimal dough hydration, depending on BG content and type, can also be estimated. As a non-random water content variation was used in the study, but adapted to the BG dose, special attention must be paid to not extrapolate the fitting equations out of the studied concentration ranges (i.e. either at high dough hydrations in absence of BG or at low dough hydrations in the presence of high BG contents). Thus, the water levels tested were chosen to optimize the dough hydration in order to get better bread quality as well as to evaluate and compare the effect of BG, content and source, on bread physical and nutritional characteristics.

3.1. Effect of dough hydration on properties of β-glucan-enriched GF doughs and breads

3.1.1. Effect of dough hydration on viscoelastic properties

Figure 1 shows the single effect of hydration on some dough rheological properties; i.e. the elastic modulus from oscillatory test, G_1', as indicator of dough consistency, and the instant elastic compliance from creep test, J_o, as a measurement of dough deformation capacity following application of a certain stress. Both parameters showed opposite trends with increase in dough hydration. The G_1' exhibited a continuous decline with

increasing dough hydration, following an exponential response of the type $y = a \cdot x^{-b}$, with different exponent values, *b*, and coefficients, *a*, (data not shown), depending on the BG content and its source. The J_0, increased with dough hydration and followed an evolution of the type $y = a \cdot x^{b}$, with *a* and *b* being also dependent on BG dose and its source. These single regression models demonstrated the best fitting to the experimental data versus dough hydration, in agreement with previous studies (Nunes et al., 2009).

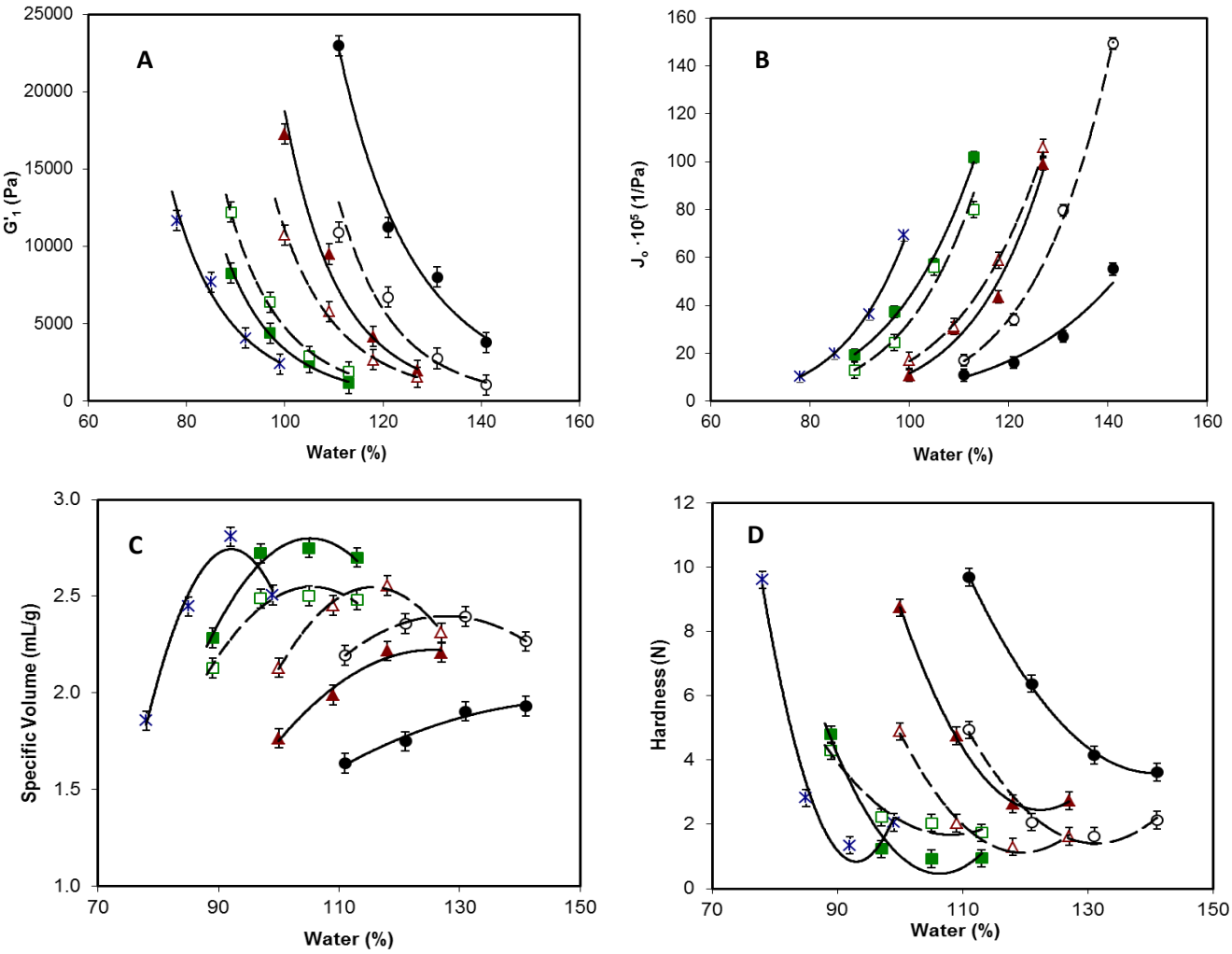


Figure 1. Effect of dough hydration as well as content and source of BG on: A) dough elastic moduli, G_1'; B) dough instant elastic compliance, J_o; C) bread specific volume; and D) bread crumb firmness. Filled symbols correspond to barley BG and empty symbols correspond to oat BG; BG content refers to concentration of pure β-glucan in the dough formulations. ✳: 0% BG (Control); ■: 1.3 % BG; ▲: 2.6 % BG; ●: 3.9 % BG. The lines correspond to potential ($y = a \cdot x^{-b}$ for G' and $y = a \cdot x^{b}$ for J_0) and parabolic (for specific volume and crumb hardness) equations that resulted from fitting the experimental data versus dough hydration for each BG level.

Table 2. Significant coefficients (95 % confidence interval) of studied factors (independent variables) of the polynomial surface response equation for dough characteristics (dependent analytical variables).

Factor	Consistency (N)	Consistency Area (N·s)	G_1' (Pa)	a (10^{-3})	G_1'' (Pa)	b (10^{-3})	tan δ_1 (10^{-3})	c (10^{-3})	J_0-creep (10^{-5} Pa^{-1})	J_1-creep (10^{-5} Pa^{-1})	$\lambda_{\text{-creep}}$ (s)	η_0 (10^5 Pa·s)	J_0-recov (10^{-5} Pa^{-1})	J_1-recov (10^{-5} Pa^{-1})
BARLEY BG														
CONSTANT	21.4	1672	11100	194	3432	251	289	54	4.4	2.1	21.4	10.2	5.7	7.7
BG	5.5	458	5277	-42	882	-29	-58	-1.9	9.6	-1.7		3.0	10.4	1.0
WATER	-1.04	-80	-714	5.1	-160	3.8	7.8	-0.091	1.4	2.8	-0.31	-0.68	2.6	2.5
BG^2			2783		289				5.6	11.6		2.1		8.2
BG x WATER			-349	-0.77	-37	-0.80	-1.3	0.49	-2.1	-2.9		-0.23	-1.6	-2.4
WATER^2	0.0070	0.50	15.4		2.2	0.037			0.089	0.10	0.0069	0.013	0.066	0.091
R-SQ	88.6	89.4	95.0	92.8	93.9	97.9	92.7	89.3	94.7	93.8	52.7	94.4	93.7	94.8
OAT BG														
CONSTANT	22.0	1599	11732	209	3681	257	308	45	11.7	14.5	19.3	10.2	15.2	16.8
BG	3.5	282	5268	-31	1533	-62	-22	-30	-4.2	-9.3	-1.0	2.2	-10.5	-19.5
WATER	-0.97	-69	-674	3.5	-185	4.0	5.3	0.58	0.38	0.48		-0.53	1.0	1.4
BG^2			760	7.2	190	13.3		7.7	7.1	14.5			6.0	9.9
BG x WATER			-169	-0.71	-44	-0.55	-0.68		-1.7	-2.9			-1.5	-1.7
WATER^2	0.0072	0.50	10.5		2.6				0.11	0.17		0.0041	0.10	0.10
R-SQ	90.3	84.1	98.7	71.6	97.3	91.3	92.9	74.5	99.0	95.1	32.3	90.3	97.8	95.1

Independent variables: BG: β-Glucan level expressed in terms of the actual polysaccharide content on a rice flour basis; WATER: additional water added to the minimum dough hydration of 78%. Blanks correspond to non-significant effects (significance level 5%). R-SQ adjusted square coefficient of the fitting model.

Table 2 shows the significant coefficients (95% confidence interval) of the polynomial response surface equations for dough characteristics versus BG and Water content and the respective R^2 of the fittings for each of the rheological parameters. The dough consistency data, estimated from the empirical forward extrusion tests, led to a simple dependence equation; the addition of BG linearly increased the force and the energy needed for dough extrusion, while water addition decreased both parameters in the range of dough hydration tested in spite of the positive quadratic term. The elastic modulus, G_1', was always above the viscous one, G_1'', resulting in tan δ <1 (Table 4), indicating that the batters had a solid like behaviour (Steffe, 1996), in agreement with previous studies (Lazaridou et al., 2007; Ronda et al., 2013; Hager et al., 2011). Both viscoelastic moduli, G_1' and G_1'', largely increased with BG addition (positive linear and quadratic terms), and to a greater extent for the elastic modulus than the viscous one (Table 2). Consequently, the loss tangent decreased with BG addition, implying an increase in the solid like behaviour. The negative sign of all BGxWater coefficients predicts a decrease in all dough rheological properties as a result of the interaction of these two factors beyond the sum of the individual addition of them. This interaction must be related to the high water binding capacity of beta-glucans (Brennan & Cleary 2007). The decrease of the G''/G' ratio was more marked for BBG than for OBG addition (Table 2); i.e. the BBG addition increased the dough solid like behaviour to a greater extent than OBG. The low purity of oat OBG concentrate (33% versus 72% of BBG) could be responsible for this behaviour as the addition of the same amount of BG results in the contribution of other substances, possibly maltodextrins, that may weaken the composite dough network structure (Witczak, et al., 2010); these constituents originate from starch enzymic digestion of oat flour during preparation of OBG concentrate. The empirical equations predict that the BG addition to low hydration doughs leads to an increase in compliance, both instant, J_o, and retarded, J_1 (see positive quadratic BG term in Table 2). The competition between BG and the rest of dough ingredients for water might weaken the dough and cause this effect. However, from the negative significant interaction (BG x Water), it can be seen that the simultaneous increase of water and BG allowed the formation of a structured network and the decrease of dough compliance, independently of the BG source; i.e. a lower dough deformation under the application of a certain stress. The individual

increase in dough hydration level always resulted in an important increase in dough compliance, as was previously reported (Ronda et al., 2013).

3.1.2. Effect of dough hydration on bread quality

Figure 1 depicts a parabolic evolution of bread specific volume and crumb hardness versus dough hydration for different BG contents from both sources. Table 3 includes the linear and quadratic significant coefficients of the surface response equations for predicting the bread physical characteristics. Different values for the coefficients were obtained depending on the source of BG used for bread enrichment, although similar tendencies were observed in both cases. The individual addition of BG to a low hydration dough decreased the bread volume, while water exerted the opposite effect, leading to an increase in the loaf volume until a maximum value, corresponding to the optimal dough hydration (note the positive linear coefficient and the negative quadratic coefficient for water), from where the bread specific volume started to decline again (Table 3 and Figure 1C). The significant positive interaction (BGxWater) gives account of the dependence of the optimal dough hydration level on BG content and explains the high water demand of BG-formulated doughs, as was already reported by other authors for wheat-based systems (Brennan & Cleary, 2007). The GF dough development during proofing and baking is also dependent on the availability of water. Firstly, because of the hydration requirements of the hydrocolloids used in GF breads, as HPMC, to develop the structure that mimics the gluten effects (Ahlborn et al.,2005; Perez-Quirce et al., 2014). Secondly, because of the important effect of water on dough viscoelastic properties, that determines breadmaking performance. From the bread volume regression equation it can be concluded that the addition, for example, of 2.6% BG to dough increases the dough hydration necessities from 94% (for control bread, without BG) to 116% and 119% for OBG and BBG, respectively. Considering the target of getting the maximum volume in BG-enriched breads, the optimal dough hydration could be calculated from the respective regression equations (Table 3):

$$W = 93 + 10.3 \cdot BBG \qquad (1)$$

$$W = 94 + 8.4 \cdot OBG \qquad (2)$$

where W represents the dough hydration (% flour basis) and BBG or OBG are the amounts of β-glucans (expressed as percentage of the actual polysaccharide concentration on a flour basis) from the barley or oat commercial concentrates, respectively. The water amount requirements, although similar, they seemed to be smaller for doughs fortified with the commercial product derived from oat. This could be attributed to the lower purity of this preparation. The main 'contaminant' component of OBG, maltodextrins, could be responsible for the reduction of GF dough consistency and for the lower water content requirements of these doughs to maximize bread specific volume (Witczak et al., 2010). The slightly different intercepts, 94 and 93, correspond to the optimal dough hydration levels of the formulations without BG. The equations (1) and (2) lead to optimal dough hydration values of (93, 106, 119, and 132) % and (93, 104, 116 and 127) % for addition of (0, 1.3, 2.6 and 3.9) % BG, from barley and oat, respectively.

Table 3. Significant coefficients (95% confidence interval) of studied factors (independent variables) of the polynomial surface response equation for bread characteristics (dependent analytical variables).

Factor	Specific volume (mL/g)	Loss of Weight (%)	Hardness (N)	Chewiness (N)	Resilience	Cohesiveness	ΔHardness-1d (N)	ΔHardness-7d (N)	Crust L*	Crust h	Crumb C*
BARLEY BG											
CONSTANT	2.5	15.7	3.9	2.0	0.30	0.52	16.6	29.0	52.0	58.2	10.0
BG	-0.34	0.53	3.4	1.6	-0.017	-0.024	7.6	10.0	-0.31	0.60	3.03
WATER	0.030	0.019	-0.33	-0.16	0.0012	0.0044	-1.2	-1.8	0.098	0.16	-0.27
BG^2	-0.14	0.15	1.44	0.63	-0.019	-0.016	1.7	3.8	0.83	0.96	0.46
BG x WATER	0.020	-0.036	-0.22	-0.099	0.0030	0.0023	-0.37	-0.59	-0.030	-0.053	-0.10
$WATER^2$	-0.00099	0.0014	0.010	0.0047	-0.00010	-0.00012	0.025	0.037			0.0059
R-SQ	86.1	78.4	92.8	90.7	71.9	62.0	84.2	80.2	76.7	89.5	71.8
OAT BG											
CONSTANT	2.2	15.4	4.0	2.1	0.25	0.51	8.0	18.8	53.6	59.4	10.3
BG	-0.42	0.33	2.7	1.6	0.098	0.043	4.5	7.9	5.0	3.9	2.82
WATER	0.043	0.079	-0.29	-0.16	0.0042	0.0055	-0.47	-1.1	-0.29	-0.12	-0.35
BG^2	-0.085	-0.011	0.75	0.37	-0.038	-0.032	0.68	2.0	0.66		0.77
BG x WATER	0.022		-0.16	-0.088	0.0016	0.0026	-0.19	-0.40	-0.32	-0.18	-0.16
$WATER^2$	-0.0013		0.0087	0.0047	-0.00011	-0.00015	0.011	0.026	0.019	0.012	0.010
R-SQ	85.1	82.5	92.1	91.7	91.7	92.4	83.7	80.7	79.2	76.0	75.9

Independent variables: BG: β-Glucan level expressed in terms of the actual polysaccharide content on a rice flour basis; WATER: additional water added to the minimum dough hydration of 78%. Blanks correspond to non-significant effects (significance level 5%). R-SQ adjusted square coefficient of the fitting model.

The linear and quadratic significant coefficients for bread hardness (Table 3) showed the reverse trend compared to those found for bread specific volume. In fact, these bread quality parameters displayed a significant ($P<0.001$) negative correlation (see the following section). The individual addition of any of both types of BG increased bread hardness and this effect was more pronounced when barley β-glucan concentrate was added to the dough, as indicated by the higher linear and quadratic coefficients for BBG, compared to those found for OBG. The negative coefficients of the interaction BGxWater explain that the simultaneous addition of BG and water counteracted the individual effect of BG; also, a more pronounced effect of the BBG than OBG was noted. Crumb hardness decreased markedly with the increase of dough hydration until a minimum value that was dependent on the BG dose and coincided with the maximum in bread specific volume (Figure 1C, D). These two bread properties, volume and firmness, were found highly correlated negatively in other works (Perez-Quirce et al., 2014). A higher bread volume usually corresponds to higher amounts of air retained in the dough structure during proofing and baking that justifies a lower crumb firmness. Similar effect was observed for chewiness, probably because this parameter is mainly affected by hardness. Resilience and cohesiveness, which relate to the bread crumb instant and retarded recovery capacity after a compression cycle, are also desirable properties, and they had all coefficients with opposite sign compared to those of hardness and chewiness, implying a negative correlation between these properties (Table 3). The crumb hardening after one and seven days of storage, indicators of bread staling, also increased with the addition of BG and decreased by increasing the water content (plasticizing effect of water on the composite polymeric matrix) in the dough, until a minimum, following identical evolution with the fresh bread hardness. Biliaderis et al. (1997) found that when β-glucans were incorporated in maize starch gels retrogradation was retarded possibly as a result of interference of the intermolecular associations amongst amylopectin molecules by the β-glucans. In this case, the lower extent of amylopectin retrogradation and its lower impact on bread staling may be masked by the counteracting effect of the lower initial bread volume. It must be also noted the lower coefficient values obtained for OBG than BBG, meaning a lower hardening effect of the BG derived from oat than from barley. Again, the coefficient of the interaction BGxWater, corresponding to the models predicting both crumb hardening parameters, were notably lower for OBG than BBG. Further studies would be needed to discriminate the contribution to this quality attribute of the dextrins present in the OBG preparation and the higher MW of OBG compared to BBG.

The hue, h, and luminosity, L^*, of bread crusts were significantly affected by water and BG, and by their interaction. From the multivariate regression equation (Table 3), a 20% increase in bread crust lightness and hue is predicted for the maximum BG content (3.9%) and dough hydration (141%) compared to the lowest levels of both factors (no BG addition and 78% water content, respectively). A higher dough water content and the addition of an ingredient that retains water, such as BG, would decrease the Maillard reactions rate, resulting in a lighter crust (Perez-Quirce et al., 2014). It is remarkable the significance of the interaction BGxWater, having a negative sign in the regression equations of crust L* and h, in particular for OBG breads. This means that the simultaneous increase in BG and water leads to darker and more reddish crusts than expected from the separate action of BG or water. It was only for the colour variables where OBG had more intense interaction with water than its BBG counterpart; this could be possibly attributed to the presence of maltodextrins (reducing carbohydrates) in the former preparation which may contribute to the Maillard reaction products. Particularly remarkable was the non-significant interaction BG x Water on the loss of weight when the commercial isolate from oat was employed, compared to the significant negative interaction when the barley preparation was instead used. This different behaviour of oat BG could be probably due to the plasticizing effect of maltodextrins present in the commercial OBG. These starch hydrolysis products probably alter the distribution of water absorbed among the dough ingredients. This behaviour is also reflected on the lower water requirements mentioned above for the OBG preparation compared to BBG.

3.1.3 Relation between dough and bread properties

Figures 1 illustrates the dependence of dough rheological and bread quality properties on dough hydration, showing the relationships between dough and bread properties. Strong correlations between fundamental and empirical rheological properties of the doughs were also noted. In the current study, only the significant correlations ($p<0.01$), with Pearson coefficient (r) values above 0.7, are highlighted. Thus, the elastic modulus at 1 Hz, G_1', was positively correlated with the viscous modulus, G_1'', ($p<0.001$; $r= 0.88$), with the viscosity, η_o, ($p<0.001$; $r= 0.94$) and the consistency estimated by extrusion tests (force and the energy, $p<0.001$, $r=0.81$ and 0.83, respectively) and was negatively correlated with the loss tangent ($p<0.001$; $r= -0.76$) as well as with the instant and retarded elastic compliances, J_o and J_1, from the creep ($p<0.001$; $r= -0.75$ and -0.74) and the recovery phase ($p<0.001$; $r= -0.77$ and -0.78). In general, the elastic and viscous

moduli increased simultaneously, but the former to a higher extent; therefore, the increase in the elastic modulus coincided with a decrease in the loss tangent. This increase also followed the decrease in the

"a" and "b" exponents ($p<0.001$; r= -0.73 and -0.84). This means that the more consistent were the doughs the least frequency dependent were the moduli values. Notably, a strong correlation was found between the exponent value "a" and the tan δ ($p<0.001$; r=0.973). Such a high Pearson coefficient would allow prediction of the loss tangent from the exponent "a" and vice versa. It means that the more frequency-dependent are the doughs, the less solid-like behaviour they have. The same correlation was previously observed in similar and different GF dough matrices (Ronda et al., 2013; Ronda et al., 2014). The bread specific volume was also negatively correlated with G_1' (r= -0.73) and η_0 (r= -0.78) and positively with the "a" exponent (r=0.80) and tan δ (r=0.83) of the dough. This strong correlation between the dough loss tangent and the bread volume was previously observed in similar GF matrices (Pérez-Quirce et al., 2014). The increase of bread volume, concomitant to the decrease of dough consistency, as predicted from the significant correlation between these properties, should be examined in the light of Figure 1; i.e. the bread volume increases with the decrease in dough consistency, but only up to a certain dough water content. At very low consistency of the dough there was an inversion of the loaf volume values, leading to a decrease in bread volume because the network is incapable of retaining the gas during its expansion, particularly in the oven. The bread volume was also negatively correlated with crumb hardness (r= -0.86), which in turn positively correlated ($p<0.001$) with G_1', G_1'', extrusion energy, and η_o, (r= 0.86, 0.74, 0.88 and 0.93, respectively) and negatively with the exponents *a* and *b* and tan δ (r= -0.69, -0.68 and -0.72, respectively). The minimum bread hardness was observed for the optimal dough hydration that led to a maximum volume. Bread colour parameters correlated significantly with each other, although the Pearson coefficients were lower than those of the previously mentioned relationships; the crust hue correlated positively with crust lightness ($p<0.001$, r= 0.82) and crust chroma ($p<0.05$; r=0.43). This means that lighter crusts were at the same time more yellowish and had more vivid colours.

Table 4. Rheological properties of doughs and physical properties of breads made with different BG contents at optimum dough hydration level.

Dough/Bread Properties	Control	BBG (%) 1.3	BBG (%) 2.6	BBG (%) 3.9	OBG (%) 1.3	OBG (%) 2.6	OBG (%) 3.9	SD
Dough								
G_1' (Pa)	4042 b	2466 a	4141 b	7996 c	2870 a	2661 a	2739 a	246
a (10^{-3})	237 de	271 f	200 b	121 a	264 ef	217 bc	233 cd	8
G_1'' (Pa)	1480 c	1021 ab	1193 b	1474 c	1127 ab	1019 ab	960 a	68
b (10^{-3})	304 c	325 d	306 cd	274 ab	295 bc	271 a	308 cd	7
tan δ_1 (10^{-3})	0.379 d	0.414 e	0.289 b	0.184 a	0.393 de	0.383 d	0.351 c	0.0073
c (10^{-3})	67 b	54 ab	106 c	153 d	31 a	54 ab	75 b	7.3
$J_{0\text{-creep}}$ (10^{-5} Pa^{-1})	37 b	57 c	44 b	27 a	56 c	59 c	80 d	3.6
$J_{1\text{-creep}}$ (10^{-5} Pa^{-1})	49 b	84 cd	40 b	22 a	71 c	72 c	91 d	4.6
λ_{creep} (s)	17 a	16 a	20 a	21 a	18 a	15 a	15 a	2.1
η_0 (10^5 Pa·s)	3.1 c	1.2 a	2.6 bc	6.6 d	1.6 a	1.8 ab	1.5 a	0.31
$J_{0\text{-recov}}$ (10^{-5} Pa^{-1})	50 b	85d	58 b	32 a	70 c	82 cd	110 e	4
$J_{1\text{-recov}}$ (10^{-5} Pa^{-1})	63 cd	78 ef	47 b	22 a	59 bc	66 cd	88 f	6.2
λ_{recov} (s)	92 b	70 ab	81 ab	71 ab	59 a	74 ab	87 ab	9.5
Consistency Force (N)	6.8 c	4.7 ab	4.8 ab	7.6 c	5.6 b	4.5 ab	3.8 a	0.50
Consistency Area (N·s)	503 b	404 ab	420 ab	639 c	437 ab	374 a	321 a	47
Bread								
Loss of weight (%)	16.9a	17.0ab	17.6bc	17.5abc	18.8e	18.0cd	18.5de	0.25
Specific volumen (mL/g)	2.82g	2.75f	2.28b	1.93a	2.50d	2.56e	2.40c	0.017
Hardness (N)	1.25b	0.93a	2.64e	4.15f	2.04d	1.30b	1.63c	0.097
Chewiness (N)	0.617a	0.507a	1.380d	2.160e	1.218c	0.767b	0.891b	0.059
Resilience	0.283a	0.312bc	0.300b	0.320c	0.402d	0.397d	0.298b	0.0058
Cohesiveness	0.546a	0.583b	0.560a	0.554a	0.644d	0.655d	0.611c	0.0071
Springiness	0.902ab	0.936c	0.936c	0.924abc	0.925bc	0.904ab	0.893a	0.016
Cell Area (mm2)	1.27b	1.19b	1.42bc	1.27b	0.63a	0.78a	0.75a	0.13
Wall Thickness (mm)	0.659a	0.681a	0.946b	0.874b	0.681a	0.946b	0.874b	0.035
Crust L*	54.4a	53.3a	58.1b	62.1c	58.3a	57.9b	57.2b	0.60
h	55.6a	62.1b	66.9c	73.2d	63.5b	63.3b	64.4bc	0.90
C*	27.5a	31.6bc	31.4b	32.1bcd	33.4cde	33.5de	34.9e	0.60
Crumb L*	64.4a	67.5bc	69.0cd	69.7d	67.9cd	65.5ab	72.0e	0.76
h	89.4a	94.5b	91.8a	90.9a	92.0a	91.5a	90.6a	0.73
C*	6.5a	7.5b	8.8c	10.4d	8.6c	9.1c	11.4e	0.28
ΔHardness 1 day (N)	2.4b	1.2a	3.1c	3.6c	3.4c	2.9bc	3.0bc	0.25
ΔHardness 7 days (N)	6.5b	3.9a	10.1c	12.8d	9.5c	6.3b	5.9b	0.46

BBG and OBG correspond to barley and oat β-glucan content; they both refer to the actual concentration of β-glucans in dough formulations. Values with a common letter in the same row are not significant different ($p < 0.05$). SD: Standard deviation obtained from ANOVA.

3.2 Effect of β-glucan addition at optimized dough hydration on GF dough and bread quality attributes

To explore the effect of BG addition on GF-bread and compare the two BG sources, doughs and breads prepared with the optimum hydration were made by using identical contents of β-glucans, although different amounts of the commercial products employed; the characteristics of these doughs and breads are summarized in Table 4. The dough consistency varied depending on BG content and source. The G_1' modulus and the loss tangent of the OBG-fortified doughs with optimal hydration hardly varied over the range of BG contents examined, varying from 2700 – 2900 Pa for G_1'and 0.39 – 0.35 for *tan* δ. However, the BBG-enriched doughs varied markedly with the BG content, between 2500 - 8000 Pa for G_1'and 0.41 - 0.18, for *tan* δ (Table 4). Large differences were also noted among the rest of the rheological properties of optimized doughs depending on the BG preparation, particularly, for the viscosity (η_o) and compliance parameters derived from the creep tests. Some authors optimized the water level in oat β-glucan-enriched GF doughs by the amount needed to reach the same viscoelastic complex modulus, G^*, as the control formulation (Hager et al., 2011). This approach would be effective for the OBG-enriched doughs but not for those supplemented with BBG. It is established that high molecular weight β-glucans can form highly viscous pseudoplastic solutions at high polysaccharide concentrations, while the gelation capacity of cereal β-glucans increases with decreasing molecular weight and increasing polysaccharide concentration as well as with increasing amount of cellotriose (DP3) units along the polysaccharide chain (Lazaridou et al., 2004; Lazaridou & Biliaderis, 2007; Tosh et al., 2004). The BBG preparation indeed exhibits such molecular features that could promote interchain (segmental) associations leading to gel structure formation due to possibly its higher DP3 content compared to OBG. It has been reported that the barley β-glucans have a higher frequency of DP3 in the polysaccharide chains compared to those from oat (Lazaridou et al., 2004; Lazaridou & Biliaderis, 2007; Tosh et al., 2004), as well as the β-glucan from the BBG preparation had much lower molecular weight than that of the OBG preparation, which further decreases during breadmaking (see next section, Figure 2). The formation of a gel network structure by the barley β-glucans during the dough proofing stage, could explain the initial

higher consistencies and strength of the BBG-enriched doughs at optimized hydration level, particularly those with the highest β-glucan content (3.9%). Similarly, Symons and Brennan (2004) attributed the increased elasticity of wheat dough with addition of 5% of a β-glucan rich fraction compared with the control formulation to the weak gel-forming ability of these hydrocolloids.

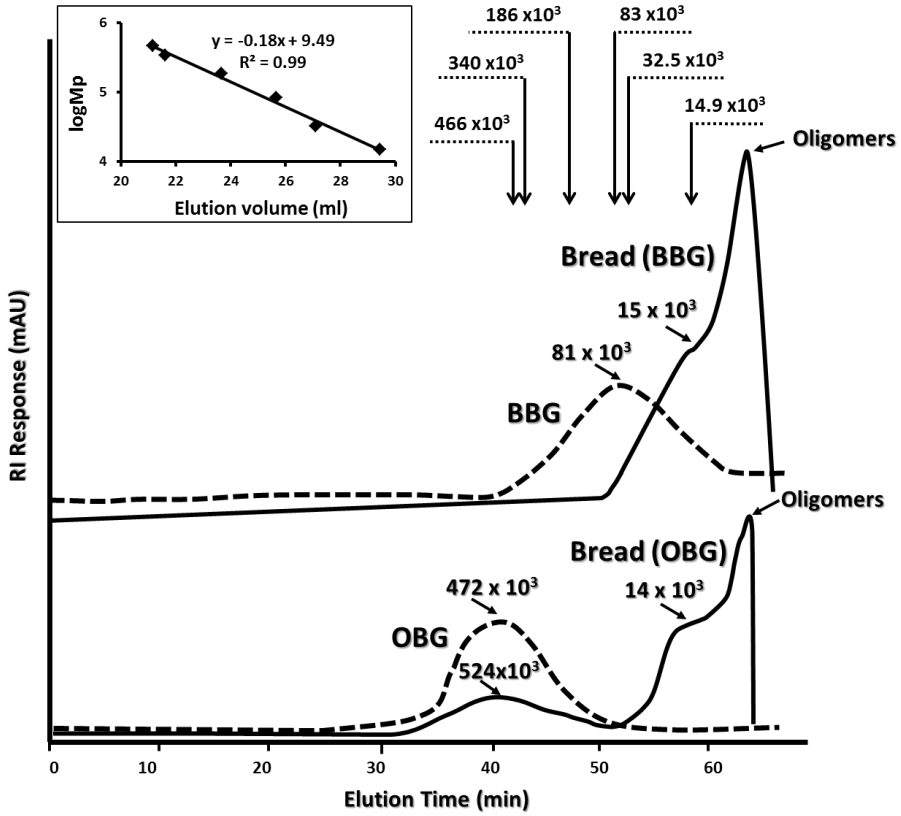


Figure 2. HPSEC elution profiles and Mp of the eluting peaks of barley (BBG) and oat β-glucan (OBG) commercial concentrates and β-glucan isolates derived from bread crumbs, as detected by RI; the commercial oat β-glucan concentrate was further concentrated from 33 to 72 % β-glucan content, whereas the content of β-glucan isolates from the bread crumbs were 75%. The vertical arrows indicate the elution time of the peak fraction of six (1→3, 1→4) β-D-glucan standards (Mp: 14.9, 32.5, 83, 186, 340, and 466 x 10^3) used for plotting of the molecular weight-elution volume standard curve (inset).

Breads with 1.3 % BBG had the highest specific volume among all fortified breads; only 2% lower than that of the control, and significantly higher than the volume of breads with 1.3% OBG (Table 4). However, with additional amount of BBG there was a decline in bread volume, reaching values significantly lower than the respective OBG-enriched breads of equivalent BG content, probably due to gel network formation by the β-glucan chains themselves that increases the rigidity of

the doughs and thereby resulting in lower expansion and hence, a lower loaf volume. The highest volume of OBG-enriched bread volume was observed for the intermediate dose, 2.6%, whereas at the highest dose tested, 3.9% (corresponding to 11.8% of the commercial product) a 15% volume reduction was noted compared to the control bread formulation (no β-glucan addition). Other researchers reported an up to 50% loaf volume reduction with inclusion of barley β-glucans into wheat-based breads at levels ranging from 1.7 to 6.7% in the flour mixtures (Brennan & Cleary, 2007; Cavallero et al., 2002; Cleary et al. 2007; Symons & Brennan, 2004). Similarly to our findings, Skendi et al. (2010) found that with increasing concentration of β-glucans added to yeast-leavened wheat doughs the loaf volumes increased up to a certain polysaccharide concentration, above which a reduction in bread volume was noted; these authors also reported that the optimum concentration of added β-glucan in the dough formulation for maximum loaf volume depends on its molecular structure (size).

In general, all breads had soft crumbs with increasing dough hydration, since the latter was adapted to the actual BG content. However, while the smallest BBG dose (1.3 %) slightly affected bread hardness, higher doses doubled and quadrupled the hardness of the control bread. The hardness of OBG-fortified breads increased more than 60% with the addition of 1.3%, although for products with higher OBG the hardness values were comparable to that of the control bread. Similar results showing crumb hardening with inclusion of oat β-glucans into breads from both wheat (Hager et al., 2011) and gluten-free (Lazaridou et al., 2007) flours have been previously reported. The BG-enriched breads had always higher resilience than the control bread, while only breads with OBG had significantly higher cohesiveness than the control. Springiness of the BG-fortified bread crumbs was similar to that of the control sample, in particular when OBG was used (Table 4).

The fibre enriched breads showed crumbs and crusts with significantly higher L*, h, and C* parameters than those of the control samples. This means that BG-fortified breads were more yellowish, lighter and with more vivid colours than the control bread as has been previously noted by Hager et al. (2011). The weight loss during baking of OBG-enriched doughs was significantly higher than that of BBG-supplemented doughs and higher than the control as well. In BBG-supplemented breads, the weight loss was similar to the control formulation in spite of the notably

higher dough hydration of these fortified products. This might be due to the high water binding capacity of this low molecular weight soluble fibre.

The mean cell area of crumb (Table 4) was not significantly affected by BBG supplementation, while OBG decreased this parameter to about half of the control bread. At the same time, the cell density showed a concomitant increase from 28 cells/cm^2 in the control bread to 40 cells/cm^2 in OBG-enriched breads, while BBG-fortified breads exhibited an average value of 21 cells/cm^2 (no significantly different from the control value). Higher crumb porosities were previously reported for GF-breads when oat β-glucans were added to the dough formulation (Lazaridou, et al., 2007). These changes in crumb characteristics denote a finer structure of the OBG-enriched breads. Wang et al. (1998) found that incorporation of β-glucan into wheat bread improved crumb grain by stabilizing air cells in the dough and preventing coalescence of the cells during proofing and baking. As can be seen in Table 4, the wall thickness of the cells increased with the two highest BG additions, independently of the BG source.

The bread shelf-life, as assessed by the extent of crumb hardening between a short (1 day) and a long (7 days) storage period in hermetically sealed plastic bags at 4ºC, is also presented in Table 4. The hardness changes after 1 and 7 days followed a very similar tendency to that observed in the initial hardness of fresh breads. The addition of 1.3% BBG led to breads with the least crumb hardening, even lower than the control bread, but the extent of hardening of the enriched breads, both with OBG and BBG, was significantly higher than the control bread at short storage period (around 25% higher); after 7 days of storage the staling of 2.6% and 3.9% OBG-enriched breads was the same as for the control bread.

3.3 Nutritional implications of gluten-free β-glucan-fortified breads

Table 5. β-Glucan content in flour mix and bread crumbs.

		BG added to the dough (% dry matter)	BG content measured in the final product (% dry matter)	Bread Moisture content (%)	BG content (% in bread)	Bread intake to fulfil the EFSA claim (g)
Control BBG (% flour basis)		-	0.05 (±0.01)a	48.8 (±0.15)a	-	-
	1.3	1.09	1.19 (±0.03)b	52.3(±0.18)c	0.57	529
	2.6	2.14	2.32 (±0.06)c	54.4(±0.06)e	1.06	284
	3.9	3.17	3.29 (±0.03)e	56.3(±0.06)g	1.44	209
OBG (% flour basis)						
	1.3	1.07	1.21 (±0.04)b	51.1(±0.24)b	0.59	507
	2.6	2.07	2.23 (±0.15)c	53.0(±0.17)d	1.05	286
	3.9	3.01	3.00 (±0.01)d	54.8(±0.06)f	1.36	221

Table 5 presents the β-glucan content obtained for bread crumbs. The intermediate BG dose (2.6% flour basis) can meet the health claim requirements of FDA (US Food and Drug Administration (USFDA, 2005) and EFSA (EFSA, 2011) for reduction of serum cholesterol and can be accomplished by a daily intake of ~ 280 g of the GF-fortified products. The maximum BG addition tested (3.9% f.b.) would allow reduction of the daily intake of bread to ~ 210 – 220 g which corresponds to four portions of 50 g.

The MW of the β-glucans present in the commercial concentrate and in the 3.9% BG-enriched bread crumbs was determined to evaluate any change in MW during the bread making process. In the case of OBG preparation (low purity preparation) and for the bread crumb samples, a purification step was needed prior to the HPLC-SEC determination. The purity reached in the analysed OBG concentrate was 72.4%, whereas the BG isolated from the bread crumbs attained a BG concentration of 75 %. In the case of OBG purification, a previous reflux to inactivate any endogenous β-

glucanases was carried out. The apparent peak molecular weight (Mp) values were estimated from the peak fraction of the eluting polysaccharides using a calibration curve made with six β-glucan standards of known molecular weight (inset, Fig. 2). The β-glucans present in the commercial preparations showed Mp of $81 \cdot 10^3$ and $472 \cdot 10^3$ for BBG and OBG, respectively. However, the isolated BGs from the bread crumbs denoted a significant degradation of the β-glucans during bread making as evidenced by the higher elution volume and a lower estimate of the Mp. Both BBG- and OBG-fortified breads showed an oligomer's peak besides the β-glucans fraction of low molecular size, ~ $15 \cdot 10^3$. In the case of OBG-added breads an additional small fraction of high molecular weight peak ($524 \cdot 10^3$), similar to the initial OBG preparation, was also noted. In agreement with these findings, previous studies have shown that during production of yeast-leavened breads from oat bran and barley flour, and rye crisp breads, the β-glucans are partially degraded by endogenous β-glucanases in the flour mixtures and that the MW of β-glucans in the final products decrease with increasing time of mixing and dough fermentation (Aman et al., 2004; Andersson et al., 2004; Lazaridou et al., 2014; Trogh et al., 2004). The decrease in molecular weight of β-glucans in GF breads might be also attributed to the β-glucanase activity of either the rice flour and/or the yeast preparation used in this study in addition to the barley and oat flour raw materials utilized for production of the BGG and OBG concentrates.

4. Conclusions

Fortification of GF dough formulations with commercial BG concentrates requires optimization of the dough hydration level depending on the β-glucan content, to maximize the bread quality attributes; both dough and bread properties are dependent on the molecular weight and the structure of β-glucans. Adopting a constant dough consistency as a sole criterion to compare different GF formulations containing β-glucans might not be relevant to the final bread properties, particularly when low MW β-glucans are used in breadmaking. BG-enriched GF breads that fulfil the EFSA claim of improved quality can be obtained. A marked impact of bread making process on the final MW of β-glucans was noted, pointing to the presence of endogenous β-glucanase activity, responsible for the polysaccharide degradation.

The physiological implications of the remaining β-glucan of reduced MW in the final product are not known and need to be further explored. Nevertheless, in view of the well-known relationships between the physiological function of cereal β-glucans and their viscosity enhancement properties, efforts can be made to eliminate endogenous β-glucanase activity and to maintain the original molecular size of β-glucans in order to deliver the desired health benefits for these bioactive polysaccharides.

Acknowledgement

The authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad and the European Regional Development Fund (FEDER) (Projects AGL2012-35088) and Comunidad de Castilla y León (Project VA252A12-2). Special thanks to Ana M. Moraleja for her help in baking and other laboratory tasks.

References

Aman, P., Rimsten, L., & Andersson, R. (2004). Molecular weight distribution of β-glucan in oat-based foods. *Cereal Chemistry*, 81, 356-360.

Andersson, A. A. M., Armo, E., Grangeon, E., Fredriksson, H., Andersson, R., & Aman, P. (2004). Molecular weight and structure units of (1-3, 1-4)-β-glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science*, 40, 195-204.

Angioloni, A. & Collar, C. (2011). Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. *LWT - Food Science and Technology*, 44(3), 747-758.

Biliaderis, C.G., Arvanitoyannis, I., Izydorczyk, M.S. & Prokopowich, D.J. (1997). Effect of hydrocolloids on gelatinization and structure formation in concentrated waxy maize and wheat starch gels. *Starch-Starke* 49: 278-283.

Brennan, C. S. & Cleary, L. J. (2007). Utilisation of Glucagel in the beta-glucan enrichment of breads: A physicochemical and nutritional evaluation. *Food Research International*, 40, 291-296.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A. M., 2002. High (1→3, 1→4)-β-glucan barley fractions in breadmaking and their effects on human glycemic response. Journal of Cereal Science, 36, 59-66.

Cleary, L. J., Andersson, R., & Brennan, C. S. (2007). The behaviour and susceptibility to degradation of high and low molecular weight barley b-glucan in wheat bread during baking and in vitro digestion. *Food Chemistry*, 102, 889-897.

Doublier, J. L. & Wood, P. J. (1995). Rheological properties of aqueous solutions of (1-3) (1-4)-B-glucano from oats (Avena sativa L.). *Cereal Chemistry,* 72(4), 335-340.

EFSA. (2011). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal,* 9(6), 2207.

Gill, S., Vasanthan, T., Ooraikul, B., & Rossnagel, B. (2002). Wheat bread quality as influenced by the substitution of waxy and regular barley flours in their native and extruded forms. *Journal of Cereal Science,* 36(2), 219-237.

Hager, A. S., Ryan, L. A. M., Schwab, C., Ganzle, M. G., O'Doherty, J. V., & Arendt, E. K. (2011). Influence of the soluble fibres inulin and oat beta-glucan on quality of dough and bread. *European Food Research and Technology*, 232(3), 405-413.

Houben, A., Hoechstoetter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology*, 235(2), 195-208.

Kinner, M., Nitschko, S., Sommeregger, J., Petrasch, A., Linsberger-Martin, G., Grausgruber, H., Berghofer, E., & Siebenhandl-Ehn, S. (2011). Naked barley-Optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. *Journal of Cereal Science*, 53(2), 225-230.

Lazaridou, A. & Biliaderis, C. (2007). Molecular aspects of cereal b-glucan functionality: Physical properties,technological applications and physiological effects. *Journal of Cereal Science,* 46, 101-118.

Lazaridou, A., Biliaderis, C. G., Micha-Screttas, M., & Steele, B. R. (2004). Acomparative study on structure-function relations of mixed linkage (1→3), (1→4) linear β-D-glucans. *Food Hydrocolloids,* 18, 837-855.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering,* 79(3), 1033-1047.

Lazaridou, A., Marinopoulou, A., Matsoukas, N. P., & Biliaderis, C. G. (2014). Impact of flour particle size and autoclaving on β-glucan physicochemical properties and starch digestibility of barley rusks as assessed by in vitro assays. *Bioactive Carbohydrates and Dietary Fiber,* 4, 58-73.

Nunes, M. H., Ryan, L. A. M., & Arendt, E. K. (2009). Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. *European Food Research and Technology,* 229, 31-41.

Pérez-Quirce, S., Collar, C., & Ronda, F. (2014). Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads. *International Journal of Food Science and Technology,* 49, 1375-1382.

Ronda, F., Perez-Quirce, S., Angioloni, A., & Collar, C. (2013). Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: A fundamental and empirical rheological approach. *Food Hydrocolloids,* 32, 252-262.

Ronda, F., Villanueva, M., & Collar, C. (2014). Influence of acidification on dough viscoelasticity of gluten-free rice starch-based dough matrices enriched with exogenous protein. *LWT - Food Science and Technology,* 59, 12-20.

Sabanis, D., Lebesi, D., & Tzia, C. (2009). Effect of dietary fibre enrichment on selected properties of gluten-free bread. *LWT-Food Science and Technology*, 42(8), 1380-1389.

Skendi, A., Biliaderis, C. G., Papageorgiou, M., & Izydorczyk, M. S. (2010). Effects of two barley b-glucan isolates on wheat flour dough and bread properties. *Food Chemistry,* 119, 1159-1167.

Steffe, J. F. (1996). *Rheological methods in food process engineering*. (Second ed.). East Lansing MI: Freeman Press.

Symons, L. J., & Brennan, C. S. (2004). The influence of (1→3)(1→4) β-D-glucan-rich fractions from barley on the physicochemical properties and *in vitro* reducing sugar release of white wheat breads. *Journal of Food Science*, 69, C463-C467.

Thomson, T. (2009). The nutritional quality of gluten-free foods.In E. Gallagher (Ed.), *Gluten-free food science and technology* (45-51). New Delhi: Wilet-Blackwell.

Tosh, S.M. (2013). Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. *European Journal of Clinical Nutrition,* 67, 310-317.

Tosh, S.M., Brummer, Y., Wood, P.J., Wang, Q., & Weisz, J. (2004). Evaluation of structure in the formation of gels by structurally diverse (1→3)(1→4)-β-D-

glucans from four cereal and one lichen species. *Carbohydrate Polymers*, 57, 249-259.

Trogh, I., Courtin, C. M., Andersson, A. A. M., Aman, P., Sorensen, J. F., & Delcour, J. A. (2004). The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley breads with increased arabinoxylan and (1-3, 1-4) β- D-glucan levels. *Journal of Cereal Science,* 40, 257-267.

Wang, L., Miller, R. A., & Hoseney, R. C. (1998). Effects of (1-3)(1-4)-b-D-glucans of wheat flour on breadmaking. *Cereal Chemistry*, 75, 629-633.

Whitehead, A., Eleanor J Beck, E. J., Tosh, S., & Wolever, T. M. S. (2014). Cholesterol-lowering effects of oat b-glucan: a meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition*, (in press), doi: 10.3945/ajcn.114.086108.

Witczak, M., Korus, J., Ziobro, R., & Juszczak, L. (2010). The effects of maltodextrins on gluten-free dough and quality of bread. *Journal of Food Engineering*, 96, 258-265.

Wood, P. J. (2010). Oat and Rye beta-Glucan: Properties and Function. *Cereal Chemistry*, 87(4), 315-330.

CAPÍTULO IV

Effect of β-glucan molecular weight on rice flour dough rheology, quality parameters of breads and *in vitro* starch digestibility [*]

*Perez-Quirce, S., Lazaridou, A., Biliaderis, C.G., Ronda, F., (2017) "Effect of β-glucan molecular weight on rice flour dough rheology, quality parameters of breads and in vitro starch digestibility". LWT - Journal of Food Science and Technology. DOI: 10.1016/j.lwt.2017.04.065.

Effect of β-glucan molecular weight on rice flour dough rheology, quality parameters of breads and in vitro starch digestibility

Sandra Perez-Quirce[1], Athina Lazaridou[2], Costas G. Biliaderis[2], Felicidad Ronda[1*]

[1]Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

[2]Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 235, Thessaloniki, Greece, 541 24.

Abstract

The study aimed at investigating the effects of molecular weight(peak molecular weight, Mp, 83, 192 and 650 kDa) and level (1.3, 2.6 and 3.9 g/100 g flour basis) of enriched in β-glucan (BG) concentrates (from oat and barley) added into rice flour gluten-free (GF) doughs on their viscoelastic and pasting properties, as well as the quality parameters of bread and the in vitro starch digestibility. A purification process of a commercial BG concentrate, followed by an acid hydrolysis step were employed to reduce the content of interfering excipients (e.g. maltodextrins) and obtain preparations with a range of molecular weights. BG-enriched GF breads of improved quality, that can fulfil the EFSA claims (ingest of 3 g of BG per day with a daily bread intake of ~200 g of bread), were obtained, exhibiting slower starch digestibility (*in vitro* assay) dependent on the molecular weight and concentration of BG. With the higher Mp BG used, showing the largest impact on dough rheology characteristics and having a greater potential for health benefits, higher specific volume and lower bread crumb hardness were noted among the GF breads. The medium and lowest Mp BG also had an influence on dough rheological behavior and bread quality attributes. The rapidly available glucose of the bread decreased from 81g/100 g to 72g/100 g as result of the 3.9g/100 g addition of the highest Mp BG in the GF formulations.

Keywords: Gluten free bread; pasting properties; oscillatory test, creep-recovery test; digestible starch.

1. Introduction

In recent years β-glucans (BG) have received significant consumer and research attention because their consumption has been linked with certain health benefits recognized by EFSA (EFSA 2011). These health claims have largely contributed to increased consumption of cereal based foods, such as breads, muffins, pasta and breakfast cereals, mostly whole flour products (Wolever et al., 2010). However, celiacs cannot ingest wheat, barley or even oat, the main sources of this dietary fiber, since they are not gluten-free grains. The BG enrichment of gluten-free breads to fulfil the requirements for the above EFSA claims can be achieved by addition of BG concentrates obtained from these cereals by means of water extraction processes that cannot co-solubilize the allergenic storage proteins of the prolamin fraction (Perez-Quirce, Collar & Ronda, 2014; Ronda, Perez-Quirce, Angioloni & Collar, 2013; Ronda, Perez-Quirce, Lazaridou & Biliaderis, 2015).

The demand for gluten-free (GF) products steadily increases as a result of enhanced consumer education on diet related pathogenic conditions. Despite the current demand for development of food products with improved nutritional quality, the GF products often receive only marginal attention from a nutrient-rich formulation point of view. The enrichment of GF breads with BG, does hold a special interest among the vulnerable population of celiac patients which also encounters a significant incidence rate of other associated chronic diseases, such as obesity-metabolic syndrome and diabetes due to their higher fat and calorie denser diets compared to the general population (Cronin & Shanahan, 1997).

The functionality and physiological properties of cereal BG are strongly related to their viscosity, which depends on the molecular weight (Mw), fine structure, concentration and physical state of the polysaccharide (Lazaridou & Biliaderis, 2007; Wolever et al., 2010). Generally, high Mw BG are reported to enhance the viscosity of the liquid in the upper digestive tract (digesta) and, thereby, it is beneficial for exerting many physiological activities (Wood, 1994); it reduces the peak blood glucose response (Regand, 2009) and attenuates the glycemic response and the *in vitro* starch digestion (Thondre, Monro, Mishra & Henry, 2010). Overall, the Mw and amount of BG solubilised in the gut might be critical in its capacity to reduce glucose release and transport as a function of the increased viscosity of the digesta and reduced motility (Regand, Chowdhury, Tosh, Wolever & Wood, 2011).

Previous studies have shown a great variability in the effect of BG on dough viscoelastic behaviour and bread quality characteristics of wheat flour bakery items (Brennan & Cleary, 2007; Cleary, Andersson & Brennan, 2007; Skendi, Biliaderis, Papageorgiou & Izydorczyk, 2010) and GF products (Hager et al., 2011; Lazaridou, Duta, Papageorgiou, Belc & Biliaderis, 2007; Perez-Quirce, 2014; Ronda et al., 2013), depending on dough water and BG contents. A loss of quality when GF breads are fortified with BG is partially related to their Mw, as a result of enhanced water-binding and viscosity (Perez-Quirce et al., 2014; Ronda et al., 2015). Thus, although the Mw is a key determinant of the health promoting potential of BG, addition of high levels of high Mw BG preparations can be a challenge for food producers because of their low solubility and high viscosity, affecting detrimentally the dough handling properties and the sensory attributes of the final product. These negative changes could be avoided by incorporating lower BG concentrations and/or BG preparations of lower Mw (Kennan et al., 2007). However, with lower Mw BG, a reduction in physiological responses is anticipated (Cleary et al., 2007).

The effects of Mw on GF dough and bread quality have mostly been studied by means of commercial BG concentrates, often of low purity. In this context, the BG-enrichment is accompanied by addition of high amounts of other substances, such as maltodextrins, present in the commercial product (~72g/100 g). Maltodextrins could have adverse effects, such as weakening of the dough network structure (Witczak, Korus, Ziobro & Juszczak, 2010) or dough stickiness, and thereby modify the true effects caused by BG itself (Ronda et al., 2015). Moreover, maltodextrins, being starch hydrolysis products, can aggravate the glycemic responses of the final products since they are a form of readily digestible carbohydrates. Thus, a comprehensive study of the effects of Mw and amount of highly concentrated BG on GF dough and bread properties is required.

This study reports on the enrichment in BG of a commercial BG concentrate by means of a purification protocol and a reduction of their Mw by acid hydrolysis of the polysaccharides in a paste form of the enriched BG preparation. Under properly selected hydrolysis conditions the production of a tailor-made lower Mw BG preparation was feasible. Moreover, this study aimed at investigating the effects of Mw and level of the BG preparations introduced into rice flour GF doughs on their viscoelastic and pasting properties and the quality parameters of the resultant breads,

bread volume and firmness. The impact of BG enrichment on the *in vitro* starch digestibility of breads was also assessed.

2. Materials and methods

2.1. Materials

Rice flour (12.5 g/100 g moisture, 0.46 g/100 g ash, 7.5 g/100 g protein, 0.49 g/100 g fat and 79.1 g/100 g starch) was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar, and sunflower oil were purchased from the local market. Hydroxypropyl-methyl-cellulose (HPMC) 4KM was a gift from Dow Chemical (Midland, USA).

Barley (1→3)(1→4)-β-D-glucan (BBG)(Glucagel™), a low Mw BG, was given as a free sample from DKSH (Hamburg, Germany). The oat (1→3)(1→4)-β-D-glucan concentrate (OBG) (Promoat™) which was a high Mw BG preparation, was supplied by Biovelop AB (Kimstad, Sweden). The proximate composition of these materials as given by the suppliers was: for BBG, 2.52 g/100 g moisture, 4.75 g/100 g soluble protein, 1.43 g/100 g ash, 1.32 g/100 g fat, >85 g/100 g total carbohydrates, and >72 g/100 g BG; for OBG, 6 g/100 g moisture, 54-56 g/100 g carbohydrates (dextrin), <4.5 g/100 g protein, 1-3 g/100 g ash and 0.5-1 g/100 g fat, and 33-36 g/100 g BG. The gluten content of the commercial BBG and OBG samples was analyzed by the ELISA test based on the R5 antibody; for OBG, the gluten content was under the detection limit (<6.2 mg/kg), while for BBG 1.76 g/kg, which rendered the latter BG concentrate as not gluten-free. Nevertheless, the BBG was included in this study as a raw material for evaluation of the effect of BG Mw on the rice flour-based gluten-free dough formulations since it is technically feasible to obtain a gluten-free barley BG concentrate (Ronda et al., 2013).

2.2. β-glucan isolation and characterization

The OBG was of relatively low purity in BG (33-36 g/100 g). Hence, for this study further purification of this preparation was carried out, by a modification of the method of Lazaridou, Biliaderis, Micha-Screttas & Steele (2004) to obtain a preparation with much higher BG concentration (~70 g/100 g) as shown in Figure 1; this concentrate was designated as HWB. Following the method of Sibakov et al.

(2013) with some modifications, an amount of this enriched HWB concentrate was subsequently hydrolyzed by a 8 g/100 mL phosphoric acid solution at 82 ^{o}C acting for 25 min to obtain a second BG preparation with a desired medium Mw (MWB). The hydrolysis step was carried out at high solids level (paste like mixture) to more effectively control the degradation rate, yielding the preferred Mw of the polysaccharide. The entire purification/isolation protocol is outlined in detail in Fig 1. The β-glucan content and the apparent peak molecular weight (Mp) of the isolated BG preparations were determined using the mixed-linkage (1→3)(1→4)β-D-glucan assay kit purchased from Megazyme (Megazyme International Ireland Ltd., Co., Bray, Ireland) as well as a high performance size exclusion chromatography system with a refractive index detector, respectively. The running conditions of the chromatography and the sample preparation are described in detail elsewhere (Lazaridou et al., 2004; Lazaridou, Marinopoulou, Matsoukas & Biliaderis, 2014). The low Mw BG (LWB) preparation used in this study for bread enrichment was the commercial product Glucagel® (BBG), having an adequate BG content (72 g/100 g) for the requirements of the present work.

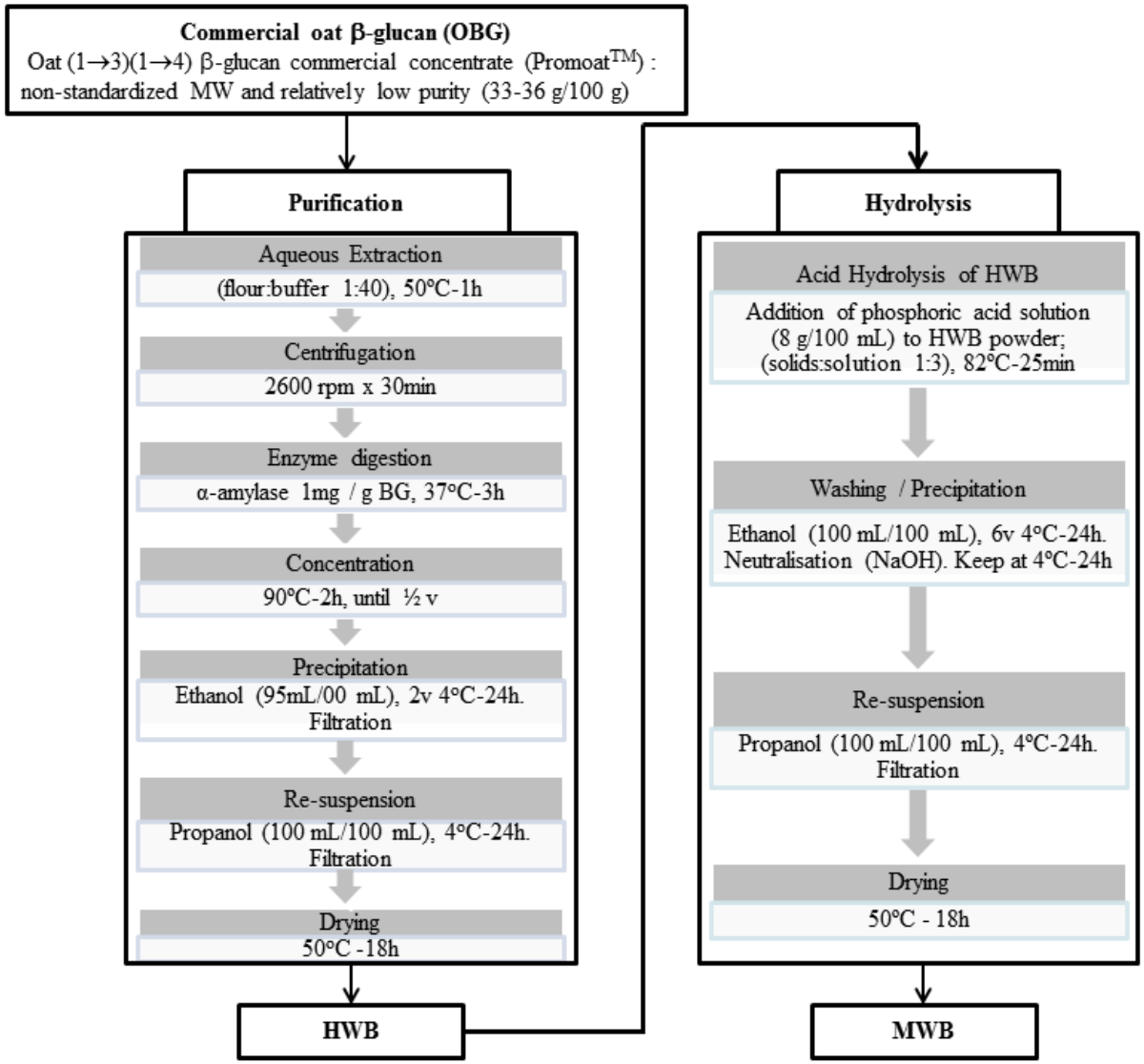


Figure 1: Flowchart of purification of the commercial β-glucan and hydrolysis process. MW: Molecular Weight; HWB: β-glucan of high molecular weight; MWB: β-glucan of medium molecular weight.

2.3. Dough preparation and breadmaking

A straight dough process was performed for the breadmaking using the following ingredient formulation expressed on a 100 g rice flour basis: 6 g oil, 5 g sucrose, 2 g HPMC, 1.8 g salt and 3 g dried yeast. The levels of BG incorporated into this formulation were 0 (control), 1.3, 2.6 and 3.9 g/100 g (rice flour basis) of pure BG by adding different amounts of BG preparations according to their BG contents and added on top of the other ingredients. The amounts of added water to the dough were adapted according to the BG level based on the findings of our previous work (Ronda et al., 2015) and were 92, 105, 120 and 130 g/100 g (rice flour basis) with increasing BG content from 0 to 3.9 g/100 g. Dough rheological tests were performed without inclusion of yeast in the formulated doughs in order to obtain stable readings (avoiding CO_2 evolution). BG samples were hydrated in hot water (40-45 ºC) prior to their mixing with the remaining dough ingredients. The GF dough and breadmaking procedures are described in detail elsewhere (Ronda et al., 2015). After baking, breads were removed from the pan and stored for one hour at room temperature before any analysis.

2.4. Evaluation of dough rheology

2.4.1. Fundamental rheology

Oscillatory and creep-recovery tests were carried out at least in duplicate and triplicate, respectively with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) following the same procedure described in detail elsewhere (Ronda et al., 2013; 2015). A stress sweep from 0.1 to 200 Pa at 1 Hz and 25 ^{o}C was performed to establish the linear viscoelastic region (LVR). Frequency sweep data were fitted to a power law model as previously described by Ronda et al. (2013). The recorded viscoelastic parameters, G_1' and G_1'', and tan δ_1, represent the elastic and viscous moduli and the loss tangent, respectively, at a frequency of 1 Hz. The a, b and c exponents quantify the dependence of the dynamic moduli and the loss tangent on the oscillation frequency.

Creep tests were performed by imposing a step of shear stress of 50 Pa, exceeding the LVR, for 60 s. In the recovery phase, the stress was suddenly removed and the sample was allowed for 180 s to recover the elastic (instantaneous and retarded) part

of the deformation. Creep data are described in terms of creep compliance, J, which is defined as the strain divided by the stress applied. The data from creep and recovery tests were modelled to the 4- and 3-parameter Burger's models, respectively (Lazaridou et al., 2007; Ronda et al., 2013). Concerning the calculated parameters, J_0 is the instantaneous compliance, J_1 is the retarded elastic or viscoelastic compliance, λ_1 is the retardation time and η_0 is the steady viscosity (estimated from the creep step). Additionally, J_{max} is the maximum creep compliance obtained at the end of the creep step and J_{steady}, the steady-state compliance in the recovery step, calculated by subtracting the compliance value at the terminal region of curve (where dough recovery reached equilibrium) from the J_{max}. Recovery (%) was calculated as $100 \cdot (J_{steady}/J_{max})$.

2.4.2. Pasting properties

Viscometric profiles of formulated and then lyophilized rice flour doughs (without yeast) were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using the ICC Standard 162 protocol. The re-hydrated freeze-dried samples were transferred into RVA canisters and processed as previously described by Ronda et al. (2013). These tests were carried out in triplicate.

2.5. Evaluation of bread quality

Bread volume was determined in duplicate by a Volscan profiler 300 analyser (Stable Microsystems, Godalming, UK). Breads were weighed immediately after removal from the pan once cooled down. Crumb hardness was determined in duplicate with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) using the software "Texture Expert". An aluminum 20 mm diameter cylindrical probe was employed in a compression test at 1 mm/s speed test and deformation level up to 50 %. Crumb hardness (N) was calculated from the maximum force of the curves. Analysis was carried out at 20 ± 3 ºC on bread slices, with 20 mm thickness, taken from the center of each loaf.

2.6. *In vitro* starch digestibility of breads

In vitro starch enzymatic digestibility of breads was measured according to the modified method of Englyst, Hudson & Englyst (2000). The hydrolyzed glucose

released by digestive enzymes at 20 min (G_{20}) and 120 min (G_{120}) and the total glucose (TG) content were determined by the glucose oxidase/peroxidase colorimetric method. The free sugar glucose (FGS) content was also determined through a separate test following the procedure proposed by Englyst et al. (2000). From these tests, rapidly digested starch (RDS), slowly digestible starch (SDS), resistant starch (RS), total starch (TS) and rapidly available glucose (RAG) were calculated. Starch digestibility rate index (SDRI) was computed from the percentage of RDS in TS of the breads. This test was carried out in quadruplicate.

2.7. Statistical analyses

Statgraphics Centurion v.16 (Bitstream, Cambridge, MN, USA) software was used for the non-linear regressions and for Pearson correlation analysis. STATISTICA package (Tulsa, OK, EEUU) v.6 was used for ANOVA analysis. Fisher's least significant difference (LSD) test was adopted to evaluate significant differences ($p<0.05$) among samples. Homogeneity of variance was checked for each studied variable.

3. Results and discussion

The three BG preparations (LWB, MWB and HWB) employed in this study largely differed in molecular size, having peak molecular weight values of 83 kDa, 192 kDa and 650 kDa (Fig. 2) and BG contents of 72 g/100 g, 68 g/100 g and 73 g/100 g, respectively.

3.1. Fundamental rheology of bread doughs

The effect of addition of enriched BG concentrates on rheological properties of GF doughs prepared with the optimum hydration level (to maximize the bread making performance of the composite doughs) was investigated in this study. The elastic modulus values ($1439\ Pa<G'_1<11187\ Pa$) were higher than those of viscous modulus ($690\ Pa<G''_1<3120\ Pa$), and the values for tan δ_1 were <1 for the dough formulations (Table 1). Both moduli slightly increased with frequency; this dependence, which is quantified by the 'a' and 'b' exponents, calculated from G' and G" fittings to the power law model, became less pronounced with BG addition (Table 1). The visco-

elastic behaviour of all doughs corresponded to solid-like materials, in agreement with earlier findings on GF doughs enriched with BG concentrates (Lazaridou et al., 2007; Ronda et al., 2015).

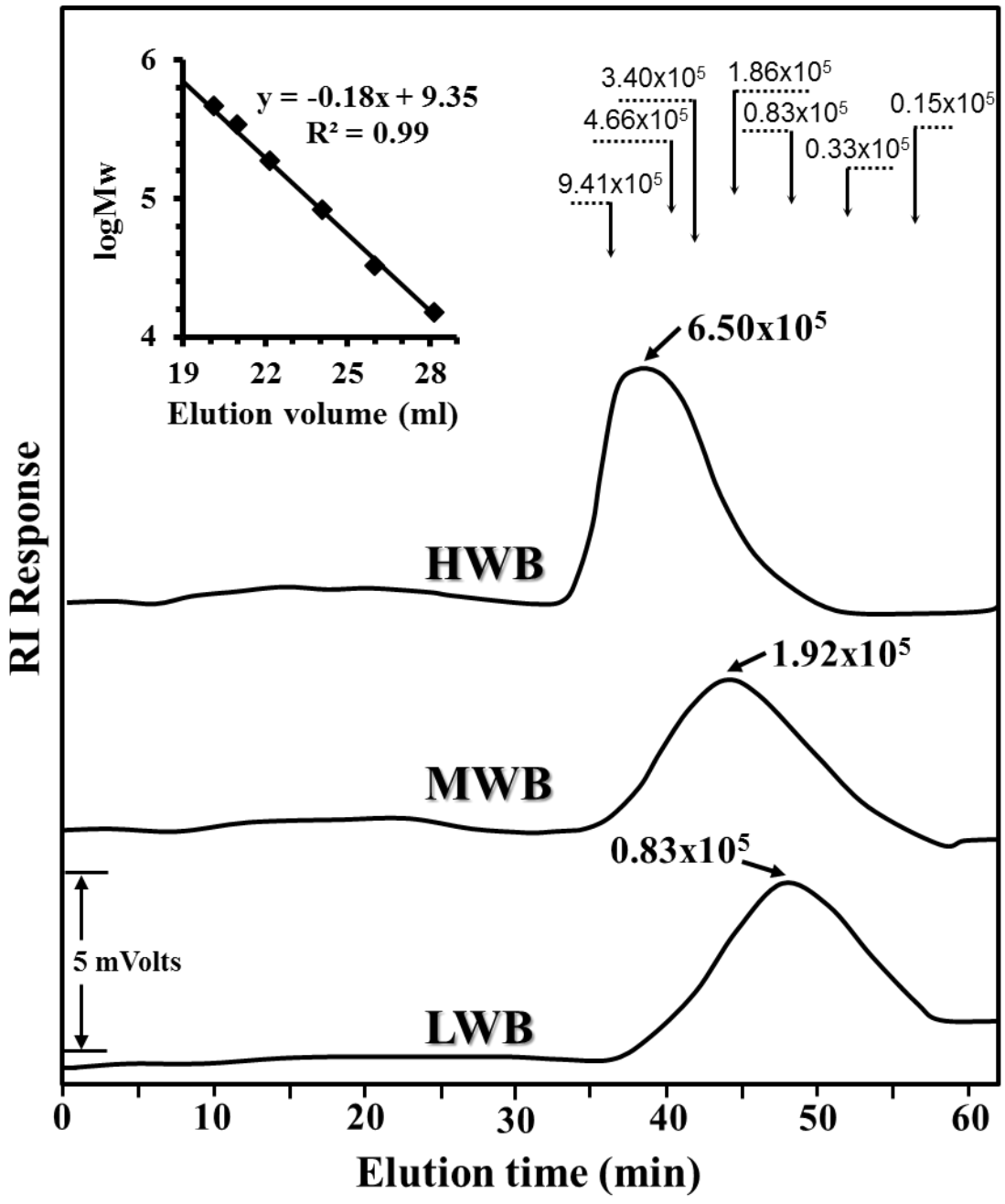


Figure 2. HPSEC elution profiles and peak molecular weight (Mp) (slanted arrows) of the eluting peaks of enriched in β-glucan concentrates added to the GF bread formulations, as detected by RI. The vertical arrows indicate the elution time of the peak fraction of seven (1→3)(1→4)-β-D-glucan standards (Mp: 0.15, 0.33, 0.83, 1.86, 3.40, 4.66 and 9.41 x 10^5) used for plotting of the molecular weight-elution volume standard curve (inset). LWB: β-glucan of low molecular weight; MWB: β-glucan of medium molecular weight; HWB: β-glucan of high molecular weight.

Table 1. Effects of molecular weight and level of added β-glucan on viscoelastic parameters of β-glucan-enriched rice flour-based (gluten-free) doughs and specific volume and crumb hardness of breads.

BG Mp		CONTROL	LWB			MWB			HWB			SE
BG level (g/100 g flour basis)		0	1.3	2.6	3.9	1.3	2.6	3.9	1.3	2.6	3.9	
Oscillatory test	G'_1(Pa)	3361 c	3243 c	2465 b	9745 e	1439 a	1729 a	3228 c	3128 bc	5936 d	11187 f	250
	a	0.283 e	0.279 de	0.282 de	0.167 a	0.276 de	0.245 cd	0.219 bc	0.308 e	0.227 bc	0.199 ab	0.016
	G''_1(Pa)	1512 d	1418 d	1025 c	2436 f	702 ab	690 a	956 bc	1536 d	2002 e	3120 g	106
	b	0.337 f	0.306 def	0.333 ef	0.256 ab	0.293 bcde	0.278 abcd	0.251 a	0.322 ef	0.300 cde	0.268 abc	0.014
	tan δ_1=G''/G'	0.449 e	0.437 de	0.417 de	0.250 a	0.488 f	0.403 d	0.297 bc	0.492 f	0.337 c	0.279 ab	0.016
	c	0.054 cd	0.027 abc	0.051 bcd	0.089 e	0.017 ab	0.033 abc	0.032 abc	0.014 a	0.073 de	0.069 de	0.012
Creep-recovery tests – Creep phase	J_0 (10^{-4} Pa^{-1})	2.2 ab	3.0 c	3.0 c	2.2 ab	4.0 d	6.9 f	6.0 e	1.7 a	2.7 bc	1.9 a	0.2
	J_1 (10^{-4} Pa^{-1})	40.5 e	37.0 de	30.7 c	4.2 a	20.4 b	40.1 e	18.8 b	34.5 cd	6.2 a	2.6 a	1.6
	λ	11.3 e	11.8 e	11.6 e	8.1 bcd	5.2 a	6.6 abc	9.1 d	10.8 e	8.2 bcd	6.6 abc	0.6
	η_0 (10^3 Pa·s)	2.4 a	2.7 a	2.4 a	162.1 d	1.0 a	1.4 a	37.3 b	2.4 a	124.3 c	376.3 e	11
	J_{max} (10^{-4} Pa^{-1})	309 bc	254 b	337 c	10 a	640 e	461 d	39 a	310 bc	14 a	6.2 a	23
Creep-recovery tests – Recovery phase	J_0 (10^{-4} Pa^{-1})	4.7 bc	6.2 c	5.7 c	3.7 ab	4.2 abc	13.9 e	10.1 d	4.9 bc	4.6 abc	2.8 a	0.7
	J_1 (10^{-4} Pa^{-1})	19.3 c	21.3 cd	18.2 c	1.9 a	37.3 e	37.7 e	10.1 b	22.6 d	3.7 a	1.6 a	1.6
	λ	14.7 a	19.0 d	16.8 c	28.38 f	16.6 c	18.26 d	15.4 ab	18.3 d	15.8 bc	20.8 e	0.5
	Recovery (%)	7.6 ab	10.3 b	7.1 ab	56.0 d	5.3 a	9.73 b	50.9 c	7.9 ab	60.7 e	73.0 f	0.8
Pasting properties	Peak viscosity (cp)	998 de	800 b	665 a	602 a	986 de	951 cd	789 b	1080 f	912 c	1032 ef	21
	Trough viscosity (cp)	833 c	659 b	555 a	504 a	881 c	859 c	706 b	992 d	831 c	965 d	19
	Breakdown (cp)	165 f	142 e	110 d	98 bcd	105 cd	92 bcd	83 ab	88 abc	81 ab	67 a	7
	Final Viscosity(cp)	1630 c	1182 b	967 a	887 a	1799 d	1610 c	1257 b	2107 e	1908 d	1874 d	40
	Setback (cp)	798 c	524 b	413 a	383 a	919 d	751 c	552 b	1115 e	1076 e	909 d	33
	Peak Time (min)	6.103 a	6.035 a	6.035 a	6.035 a	6.335 b	6.365 b	6.430 bc	6.535 bc	6.333 b	6.665 c	0.085
	Pasting Temp.(ºC)	88.0 a	90.5 b	92.2 bc	92.7 c	91.8 bc	91.9 bc	92.7 c	92.3 bc	91.0 bc	93.1 c	0.67
Bread properties	Specific volume (g/100mL)	2.60 c	3.08 e	2.69 d	2.04 a	3.03 e	2.70 d	2.46 b	3.17 f	2.63 c	2.60 c	0.019
	Hardness (N)	2.38 d	1.21 a	1.90 bc	6.53 f	1.37 a	1.67 ab	3.71 e	1.63 ab	2.63 d	2.22 cd	0.16

BG: β-glucan; Mp: peak molecular weight; LWB: β-glucan of low molecular weight (83 kDa); MWB: β-glucan of medium molecular weight (192 kDa); HWB: β-glucan of high molecular weight (650 kDa). The parameters from oscillatory tests correspond to the fitting of experimental measurements to power law model. The parameters from creep-recovery tests correspond to the fittings to Burger's model. Values in the same row with a letter in common are not significantly different ($p > 0.05$). SE: Pooled standard error obtained from ANOVA analysis. Oscillatory tests and bread measurements were made at least in duplicate; creep-recovery tests were carried out at least in triplicate.

Both viscoelastic moduli, G' and G", increased with BG addition to the doughs, with the extent of these changes being dependent on the Mp of BG, in accordance with the observations made by Cleary et al. (2007) and Ronda et al. (2015) for wheat-based, and GF rice flour-based doughs, respectively. The increments of moduli with respect to the control dough were larger for HWB, (averaged increasesof 201%- and 147% for G'_1 and G''_1 respectively), than MWB increases of 63.4%, and 51.8% for G'_1 and G''_1) and LWB (increases of 153%-, and 108% for G'_1 and G''_1 -). This can be probably due to the large increase of water absorption capacity of the dough when high levels of high Mw BG preparations are included in the GF formulation (Ronda et al., 2015). Cleary et al. (2007) observed the same behavior in wheat doughs fortified with high Mw barley BG. According to the data, the G_1' and G_1'' moduli of the MWB fortified doughs exhibited the lowest G_1' and G_1'' values among the three different BG samples. The barley LWB preparation indeed exhibits molecular features that could promote interchain (segmental) associations leading to gel formation due to its higher DP3 content (Lazaridou et al., 2004) and the much lower molecular weight compared to the oat BG preparations (MWB, HWB); formation of a gel network structure by barley BG could explain the higher consistency and strength of the respective doughs at an optimized hydration level compared to their MWB counterparts (Table 1). The enhancement of solid-like behavior of doughs with increased levels of oat BG concentrates is opposed to previous findings (Ronda et al., 2015) where similar tan δ_1 values were reported for all levels of BG added to the GF doughs; this was attributed to a weakening effect of the maltodextrins present in the oat commercial BG concentrate (Witczak et al., 2010). The reduction of such excipients in the oat BG preparations of the present study could be responsible for the strengthening effect when the MWB and HWB were added to the GF doughs.

The obtained creep-recovery curves were similar to those previously found for rice flour and gluten-free doughs fortified with BG into the LVR (Ronda et al., 2013; 2015) or outside the LVR (Lazaridou & Biliaderis, 2007). Both, the level and the Mp of the incorporated BG affected the creep–recovery parameters (Table 1). However, the effect on creep data was not proportional to the Mp of the added BG. High levels of LWB led to lower instantaneous (J_0) and retarded (J_1) elastic compliances ($p<0.05$) than those of MWB fortified doughs, associated to a lower deformation of the dough when submitted to a constant stress, probably due to the higher gelation

capacity of the LWB preparation. These elastic compliances values were similar to those obtained for the HWB, which is probably attributed to its ability to form highly viscous (pseudoplastic) solutions (Lazaridou et al., 2004; Lazaridou & Biliaderis, 2007).

Dough formulations with high elasticity, as manifested by low J_0 and J_1 compliances, could restrict dough expansion and lead to lower volume breads (Perez-Quirce et al., 2014). The elastic compliances of BG-formulated doughs although did not follow a clear trend with BG level, however, showed the lowest values for the highest levels of added BG preparations (Table 1). For the dough maximum compliance values, J_{max}, there was a decrease with increasing levels of added BG. A clear opposite trend was noted for the evolution of the steady state viscosity, η_o, which is measured from the reciprocal of flowability of the material at the end of the applied load in the creep test; it increased from 2.4 kPa·s for the control dough to the 376 kPa·s for the highest addition level of HWB, explaining the higher resistance to deformation under a constant stress of the latter sample; the former is in agreement with findings reported elsewhere, where high Mw β-glucans had significant higher viscosities in aqueous systems compared to their lower molar mass counterparts (Lazaridou et al., 2004; Mikkelsen et al., 2010). The recovery of the dough, when the stress was released, increased from <10% for the control dough to >70% for the HWB-fortified dough (Table 1). Taken into account that only the elastic part of the viscoelastic behavior can be recovered after application of a stress, this means that the 3.9g/100 g BG-fortified doughs exhibited the most pronounced elastic behavior among all dough formulations. These data are consistent with the much lower tan δ values observed by the oscillatory testing for all the 3.9g/100 g BG fortified doughs (Table 1).

3.2. Pasting properties

The impact of Mp and BG level on the RVA primary parameters is evidenced by the significant changes on the pasting and gelling behaviour of BG-enriched rice flour-based doughs (Table 1). Major single effects on cooking and cooling parameters were noted for the LWB GF formulation. With addition of LWB there was a significant ($p<0.05$) decrease of the peak (from 20 % to 40 %), breakdown (from 14 % to 41 %), final (from 27 % to 46 %) and setback (from 34 % to 52 %) viscosities, compared to control, in accordance with findings reported for wheat flour substituted

with 2.5 and 5 g/100 g BG (Brennan & Cleary, 2007). A similar tendency was noted with incorporation of the MWB although this effect was significant at the highest level of substitution. A slight and opposite trend was observed in the case of HWB addition. Brennan & Cleary (2007) proposed that barley BG could limit the available water within the paste mixture and hence limit the swelling of the starch granules, leading to greater retention of granular integrity and a reduction in gelatinization. A synergistic effect for the peak viscosity of rice starch and BG composite aqueous dispersions was reported by Banchathanakij & Suphantharika (2009) and interpreted by assuming that the system is biphasic, with the BG located entirely in the continuous phase; as a result, its concentration increases as the volume of the phase accessible to the soluble hydrocolloid is reduced, due to swelling of the starch granules during pasting. The changes observed in the present study could be explained by a combination of these two phenomena, the water binding and the thickening of the continuous phase caused by BG addition; such opposing contributions to viscosity changes vary with the molecular weight and level of the soluble BG. It is also important to note that in the present work, compared to aforementioned studies, real dough systems were examined by RVA which consist of many flour ingredients, all contributing to the rheological responses.

The pasting temperature (PT) increased significantly with BG addition, regardless its Mp. The effect of fibres on the PT could be interpreted on the basis of the changes induced on granule swellingand amylose leaching processes responsible for commencement of starch pasting (Mira, Eliasson & Persson, 2005). The observed decrease of breakdown with the inclusion of fibres denoted an increase in the paste resistance to cooking and a less intense shear thinning behavior; the latter implies less starch granule rupturing during cooking in the RVA. It was also noted that, the breakdown viscosity decreased with increasing Mp and BG fortification level.

The final viscosity (FV) decreased significantly, from 27 % to 46 % with LWB addition. Similarly, the setback viscosity decreased, up to 52 % reduction (at 3.9 g/100 g added BG); such behavior can be attributed to a restriction of amylose retrogradation in the presence of LWB. However, the MWB and HWB oat BG showed different behavior; i.e., at the lowest level of added BG there was an increase in FV (10 % and 30 % for MWB and HWB, respectively), while with further addition of BG lower FV values of the gelled BG-containing doughs were recorded.

Similar responses were noted for the setback viscosities. In general, it is clear that with inclusion of high amounts of soluble dietary fibres in the composite mixtures, the intermolecular associations within the starch network upon cooling are weakened.

3.3. Evaluation of bread quality

The specific volume of breads was positively affected by the addition of BG at low levels; at 1.3 g/100 g bread specific volume increased (17–22 %) compared to the control bread, regardless the molecular weight of the BG preparation. However, fortification of the GF doughs with higher amounts of BG (up 3.9 g/100 g) reduced successively the loaf volume (Table 1). The lowest specific volume was presented for breads enriched with 3.9 g/100 g LWB (Table 1 and Figure 3). Therefore, it seems that the structuring ability of the BG depends on its molecular weight and primarily on its concentration. The possible cause of the lowest specific volume of LWB could be the increased gelation potential of the BG that increases the rigidity of the doughs and thereby results in a lower dough expansion and loaf volume. The less pronounced loaf volume reduction with increasing contents of MWB and HWB preparations derived from the OBG concentrate could be attributed to the reduction and/or elimination of the maltodextrins in these samples; apparently, the addition of maltodextrins has a strong weakening effect on structure of GF dough and increases its susceptibility to deformation (Witczak et al., 2010). Other researchers have reported a significant decrease up to 50 % in loaf volume and height with the inclusion of BG into wheat-based bread formulations (Brennan & Cleary, 2007; Cleary et al., 2007; Hager et al., 2011); such a decline was more pronounced at higher levels of fortification. Skendi et al. (2010) demonstrated a dependence of loaf volume on the molecular size and concentration of barley BG added to the dough, as well as on the quality of wheat flour base used in the formulations. Other authors obtained the greatest reduction in loaf volume when a high Mw BG was added to wheat flour doughs (Cleary et al., 2007). Brennan & Cleary (2007) observed that enrichment of dough with soluble BG preparations imparts reduced extensibility, which would limit oven spring. BG added to wheat doughs can interrupt the continuity of the gluten network (Skendi et al., 2010). In our case, the water amount added to the GF doughs was adjusted in such a way that BG enriched doughs showed neither the highest consistency nor the lowest compliances. The specific volume was

correlated positively ($p<0.001$) to tan δ_1 values ($r=0.91$) and to the exponents a ($r=0.91$) and b ($p<0.05$, $r=0.69$) as well as negatively to c ($p<0.001$; $r=-0.81$) and the percentage of compliance recovery ($p<0.05$; $r=-0.70$). The above relations mean that the higher the viscous-like behavior of the GF dough, with a structure more dependent on frequency, and the lower the ratio of elastic to total deformation under an applied stress, the higher the specific volume of the bread, at least within the range of values obtained for these variables in the current work.

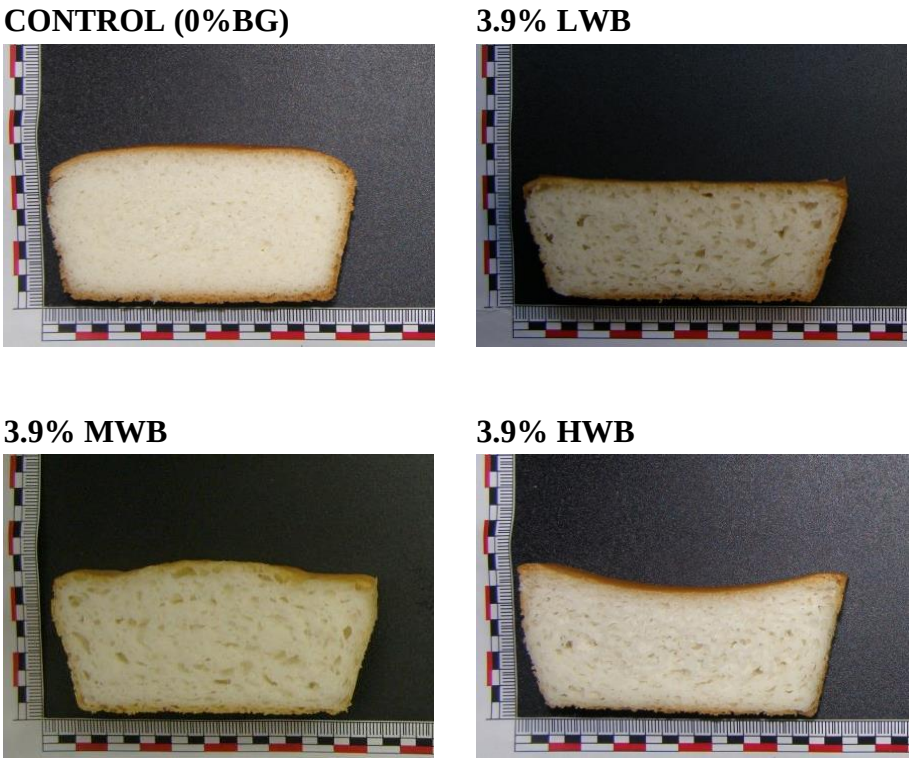


Figure 3. Cross-sections of gluten-free breads formulated with β-glucan preparations at the maximum level tested (3.9 g/100 g flour). BG: β-glucan; Mw: Molecular Weight; LWB: β-glucan of low molecular weight (83 kDa); MWB: β-glucan of medium molecular weight (192 kDa); HWB: β-glucan of high molecular weight (650 kDa).

Enriched breads at the highest BG content showed the highest crumb hardness (Table 1); the highest value among the three different BG preparations was noted for the LWB samples, probably due to the lower loaf volume and the greater gelling potential of the LWB; correlation analysis showed that crumb hardness was negatively correlated with specific volume ($p<0.001$; $r=-0.89$). A higher bread

volume usually corresponds to higher amounts of air retained in the dough structure during proofing and baking, and this generally yields lower crumb hardness. Hager et al. (2011) also found a softening effect on the crumb with the incorporation of an oat BG preparation. Crumb hardness was further correlated (negatively) with the exponent a ($p<0.01$; $r=-0.80$) and the tan δ_1 ($p<0.01$; $r=-0.77$) values of the dough, and positively correlated with the c ($p<0.05$; $r=0.69$) parameter of the dough

3.4. In vitro starch digestibility of breads

Table 2 shows the effect of inclusion of the three BG preparations at the lowest and highest concentration studied on the *in vitro* starch digestibility of the GF rice breads. Free sugar glucose (FSG) contents (g/100 g dry basis) of breads enriched with the lowest Mp BG were significantly lower ($p<0.05$) among all breads analyzed. Taken into account that sugar was added to the dough formulation at 5 g/100 g level (rice flour basis) the low final concentration (<1 g/100 g) found in breads means that sucrose was largely used by yeast during fermentation. Rapidly available glucose (RAG) and rapidly digestible starch (RDS) values for breads with the lowest concentration of BG (1.3 g/100 g) were not different from the control regardless of the BG molecular weight. However, with increase of BG concentration there was a significant decrease of rapidly released glucose from starch by the digestive enzymes for MWB and HWB fortified breads, in agreement with previous *in vivo* studies (Stamataki et al., 2016). For RAG and RDS, the lowest values were achieved at the highest concentration of HWB, 72 g/100 g and 64 g/100 g versus 81 g/100 g and 72 g/100 g in the control bread, respectively (Table 2), in agreement with a previous study (Hager et al., 2011). The digestible starch (DS) showed a small decrease in the presence of BG, yet large in cases of LWB and HWB inclusion at 3.9 g/100 g level. As for the resistant starch (RS), at the highest fortification levels of BG, regardless of Mp, there was a significant increase. The starch digestibility rate index (SDRI), which quantifies the starch digestion rate irrespective of the bread total starch content, showed a significant decrease in breads fortified with MWB and HMW preparations when the highest BG levels were used; this effect was enhanced with the increase of molecular weight of the BG preparation; at 3.9 g/100 g addition of HWB the SDRI decreased from 93 % (control) to 85 %. These observations related to the reduced rate and extent of starch digestion in the *in vitro* starch digestibility assays can be attributed to viscosity effects, as modulated by the concentration and

molecular weight of the BG preparation (C x Mw); such trends have been also reported by Regand et al. (2011) for similar food products.

Table 2. Starch fractions FSG and RAG expressed as g/100 g of total solids of the gluten-free breads (*in vitro* digestion).

BG Mp	**CONTROL**	**LWB**		**MWB**		**HWB**		
BG level (g/100 g flour basis)	**0**	**1.3**	**3.9**	**1.3**	**3.9**	**1.3**	**3.9**	**SE**
FSG	0.84 b	0.32 a	0.38 a	0.96 b	0.60 ab	0.93 b	0.96 b	0.2
RAG	80.5 c	79.5 c	78.0 bc	80.0 c	75.5 b	77.7 bc	71.5 a	1.4
RDS	71.7 c	71.3 c	69.8 bc	71.2 c	67.4 b	69.1 bc	63.5 a	1.3
SDS	4.9 b	2.4 a	1.8 a	3.8 ab	7.6 c	4.9 b	8.6 c	1.2
DS	76.5 c	73.7 ab	71.6 a	75.0 bc	75.0 bc	74.4 b	72.0 a	1.3
RS	0.0 a	1.4 abc	4.6 d	0.6 ab	1.6 bc	2.0 bc	2.9 cd	1.0
TS	76.3 ab	75.1 ab	76.2 ab	75.6 ab	76.6 b	76.1 ab	74.9 a	1.0
SDRI	93.3 c	94.8 c	91.6 bc	95.5 c	88.0 ab	91.4 bc	85.0 a	0.02

BG: β-glucan; Mp: peak molecular weight; LWB: β-glucan of low molecular weight (83 kDa); MWB: β-glucan of medium molecular weight (192 kDa); HWB: β-glucan of high molecular weight (650 kDa). FSG: Free glucose and sucrose; RAG = rapidly available glucose; RDS = rapidly digestible starch; SDS = slowly digestible starch; DS = digestible starch; RS = resistant starch; TS = total starch; and SDRI = starch digestion rate index (100· RDS/TS). SE: Pooled standard error from ANOVA analysis. Values with a letter in common in the same column are not significantly different ($p > 0.05$). The tests were made at least in quadruplicate.

4. Conclusions

This study examined the potential impact of three different BG samples varying in their molecular weight and origin (oat and barley), but of similar purity, on physical and nutritional quality of rice flour based doughs and breads (gluten-free). A purification process of a commercial BG concentrate was adopted for the fortification of dough formulations to avoid the interference of other excipients present in this preparation and make clear the true impact of the BG addition in the composite formulation. Furthermore, it allowed the production of BG samples of reduced molecular weight by controlled acid hydrolysis of BG in a concentrated aqueous paste form. This study demonstrates the feasibility for production of BG-enriched GF breads with acceptable quality attributes that can fulfill the EFSA claim

(cholesterol lowering effect for daily consumption of 3 g BG with ~ 200 g of fortified bread). It also indicated that formulation optimization (molecular weight and concentration of BG, as well as dough water content) is required to properly target for specific quality attributes of the end-product, while ensuring the full health potential of this physiologically active polysaccharide. Moreover, the use of high molecular weight BG preparations in bakery items must be preferred as it is well documented the relation between viscosity enhancement and hypoglycemic and hypocholesterolemic effects. Apparently, in the present study, at maximum fortification level (to justify the health claim requirements) acceptable quality characteristics (crumb texture) were noted with the highest molecular weight preparation. Finally, the results of this work can find special interest among the celiac patients who usually encounter great difficulty to access functional products, especially gluten free bakery items, with enhanced health benefits and acceptable sensory properties.

Acknowledgement

The authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad and the European Regional Development Fund (FEDER) (Projects AGL2012-35088 and AGL2015-63849-C2-2-R).

References

Banchathanakij, R. & Suphantharika, M. (2009). Effect of different β-glucans on the gelatinisation and retrogradation of rice starch. Food Chemistry, 114 (1), 5-14.

Brennan, C. S. & Cleary, L. J. (2007). Utilisation of glucagel in the beta-glucan enrichment of breads: a physicochemical and nutritional evaluation. Food Research International, 40, 291-296.

Cleary, L. J., Andersson, R. & Brennan, C. S. (2007). The behaviour and susceptibility to degradation of high and low molecular weight barley b-glucan in wheat bread during baking and in vitro digestion. Food Chemistry, 102, 889-897.

Cronin, C. & Shanahan, F. (1997). Insulin-dependent diabetes mellitus and celiac disease. Lancet, 349(9058), 1096-1097.

EFSA. (2011). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, 9 (2207).

Englyst, K.N., Hudson, G.J., & Englyst, H.N. (2000). Starch analysis in food. In: Meyers, R.A. (Ed.), Encyclopedia of Analytical Chemistry. John Wiley & Sons, Chichester, pp. 4246-4262.

Hager, A. S., Ryan, L. A. M., Schwab, C., Ganzle, M. G., O'Doherty, J. V., & Arendt, E. K. (2011). Influence of the soluble fibres inulin and oat beta-glucan on quality of dough and bread. European Food Research and Technology, 232(3), 405-413.

Keenan, J.M., Goulson, M., Shamliyan, T., Knutson, N., Kolberg, L., & Curry, L., (2007). The effects of concentrated barley b-glucan on blood lipids in a population of hypercholesterolaemic men and women. British Journal of Nutrition, 97, 1162–1168.

Lazaridou, A. & Biliaderis, C. G. (2007). Molecular aspects of cereal b-glucan functionality: physical properties, technological applications and physiological effects. Journal of Cereal Science, 46, 101-118.

Lazaridou, A., Biliaderis, C. G., Micha-Screttas, M., & Steele, B. R. (2004). A comparative study on structure-function relations of mixed linkage (1/3), (1/4) linear b-D-glucans. Food Hydrocolloids, 18, 837-855.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79(3), 1033-1047.

Lazaridou, A., Marinopoulou, A., Matsoukas, N. P., & Biliaderis, C. G. (2014). Impact of flour particle size and autoclaving on b-glucan physicochemical properties and starch digestibility of barley rusks as assessed by in vitro assays. Bioactive Carbohydrates and Dietary Fiber, 4, 58-73.

Mikkelsen, M.S., Jespersen, B.M., Moller, B.L., Laerke, H.N., Larsen, F.H., & Engelsen, S.B. (2010). Comparative spectroscopic and rheological studies on crude and purified soluble barley and oat beta-glucan preparations. Food Research International, 43 (10), 2417–2424.

Mira, I., Eliasson, A., & Persson, K. (2005). Effect of surfactant structure on the pasting properties of wheat flour and starch suspensions. Cereal Chemistry, 82(1), 44-52

Perez-Quirce, S., Collar, C., & Ronda, F. (2014). Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads. International Journal of Food Science and Technology, 49, 1375-1382.

Regand, A. T. (2009). Physicochemical properties of beta-glucan in differently processed oat foods influence glycemic response. Journal of Agricultural and Food Chemistry, 57(19), 8831–8838.

Regand, A., Chowdhury, Z., Tosh, S.M., Wolever, T.M.S., & Wood, P. (2011).The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. Food Chemistry, 129, 297–304.

Ronda, F., Perez-Quirce, S., Angioloni, A., & Collar, C. (2013). Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: a fundamental and empirical rheological approach. Food Hydrocolloids, 32, 252-262.

Ronda, F., Perez-Quirce, S., Lazaridou, A., & Biliaderis C.G. (2015). Effect of barley and oat b-glucan concentrates on gluten-free rice based doughs and bread characteristics. Food Hydrocolloids, 48, 197-207.

Sibakov, J., Myllymäki, O., Suortti, T., Kaukovirta-Norja, A., Lehtinen, P., & Poutanen K. (2013). Comparison of acid and enzymatic hydrolyses of oat bran β-glucan at low water content. Food Research International, 52, 99–108.

Skendi, A., Biliaderis, C. G., Papageorgiou, M., & Izydorczyk, M. S. (2010). Effects of two barley b-glucan isolates on wheat flour dough and bread properties. Food Chemistry, 119, 1159-1167.

Stamataki, N. S., Yanni, A. E., Stoupaki, M. G., Tsigkas, A., Konstantopoulos, P., Pateras, I., Tentolouris, N., Perrea, D. N., & Karathanos, V. T. (2016). Incorporation of beta-glucans into bread: postprandial glucose, insulin and ghrelin response of healthy subjects. Clinical Nutrition ESPEN. 13, e72, 2016.

Thondre, P.S., Monro, J.A., Mishra, S., & Henry, C.J.K. (2010). High molecular weight barley b-glucan decreases particle breakdown in chapattis (Indian flat breads) during in vitro digestion. Food Research International, 43, 1476–1481.

Witczak, M., Korus, J., Ziobro, R., & Juszczak, L. (2010). The effects of maltodextrins on gluten-free dough and quality of bread. Journal of Food Engineering, 96, 258-265.

Wolever, T.M.S., Tosh, S.M., Gibbs, A.L., Brand-Miller, J., Duncan, A.M., Hart, V., Lamarche, , Thomson, B.A. Duss, R., & Wood, P.J. (2010). Physicochemical properties of oat β-glucan influence its ability to reduce serum LDL cholesterol in humans: a randomized clinical trial. American Journal of Clinical. Nutrition. 92, 723–32.

Wood, P. B. (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. The British Journal of Nutrition, 72(5), 731-743.

CAPÍTULO V

Inactivation of endogenous rice flour β-glucanase by microwave radiation and impact on physico-chemical properties of the treated flour *

* Sandra Pérez-Quirce[a], Felicidad Ronda[a]*, Carmen Melendre[a], Athina Lazaridou[b], Costas G. Biliaderis[b]. (2016). "Inactivation of Endogenous Rice Flour β-Glucanase by Microwave Radiation and Impact on Physico-chemical Properties of the Treated Flour". Food Bioprocess Technology. 9 (9): 1562–1573.

Inactivation of endogenous rice flour β-glucanase by microwave radiation and impact on physico-chemical properties of the treated flour

Sandra Perez-Quirce[1], Felicidad Ronda[1*], Carmen Melendre[1], Athina Lazaridou[2], Costas G. Biliaderis[2]

[1]Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

[2]Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 235, Thessaloniki, Greece, 541 24.

Abstract

The apparent reduction of β-glucan (BG) molecular weight in rice based gluten-free (GF) breads fortified with cereal BG concentrates reveals the presence of β-glucanase activity in rice flour. Inactivation of endogenous β-glucanase in rice flour thus seems to be necessary step when developing GF breads enriched with BG of high molecular-weight. The aim of this work was to study the thermal inactivation of endogenous β-glucanase in rice flour by means of microwave (MW) processing; rice flours preconditioned at four different moisture levels (13%, 16%, 19%, 25%) were treated by MW radiation at 900 W and five MW treatment times (ranging from 40s to 8 min, applied stepwise at 20s intervals). The effects of microwaves on starch crystallinity, pasting and thermal properties of MW-treated rice flours were also explored. The β-glucanase activity in rice flours was assessed by the rate of decrease in specific viscosity of a dilute solution of a purified β-glucan preparation, upon addition of flour extracts. MW proved to be a useful alternative for thermal inactivation of endogenous β-glucanase in rice flours when applied to moistened samples. The inactivation process followed a first order kinetic response and the apparent rate constant of thermal inactivation increased exponentially with the moisture content of the flour, M, according to the equation $0.0146 \cdot \exp(0.212 \cdot M)$ ($R^2 = 0.97$). The MW time required for complete β-glucanase inactivation was only 4 min when the initial flour moisture increased to 25%. Following MW treatment, the

starch crystallinity was unaffected ($p>0.05$) and the side effects of the treatment on flour pasting and thermal properties were rather negligible.

Keywords: β-Glucanase inactivation; microwave treatment, pasting properties, rice flour; thermal properties; X-Ray diffractometry

1. Introduction

The (1-3) (1-4) β-D-glucans (BG) are major components of cell walls in the starchy endosperm and the aleurone layer of commercially important cereals, mostly oat and barley and to a lesser extent rye and wheat (Lazaridou and Biliaderis 2007). These polysaccharides are classified as soluble dietary fibre with well recognized nutrtitional implications as specified in several health claims authorized by many regulatory authorities around the globe. The US Food and Drug Administration (USFDA) (2005) has approved a health claim for the reduction of coronary heart disease risk with a daily consumption of 3g of β-glucan soluble fiber from whole grain barley or oat and dry milled barley/oat grain products as part of a low saturated fat and low cholesterol diet. Recently, the European Food Safety Authority (EFSA) has also authorized a health claim, according to which barley β-glucan ingestion leads to the reduction of blood plasma cholesterol levels, which is a major risk factor for the development of coronary heart disease (EFSA 2011a); the recommended daily intake is 3g of oat β-glucan as part of a balanced diet. Other health claims for oat and barley β-glucans were also approved by EFSA concerning the reduction in post-prandial glycemic responses, at doses of about 4g of β-glucans per 30g of available carbohydrates in bread and pasta products (EFSA 2011b), and the increase of faecal bulk (EFSA 2011c); the latter claim can be used for foods containing barley or oat grain fiber at least 6g/100g product or 3g/100 kcal.

On the other hand, the demand for gluten-free products steadily increases. Although several gluten free (GF) products are nowadays available on the market, baked products with gluten-free ingredients are generally of poor nutritional and sensorial quality and exhibit undesirable physicochemical properties; i.e. they contain low amounts of fibre, vitamins and other essential nutrients, which exert a worsening

effect on the already nutritionally unbalanced diet of celiac disease (CD) sufferers (Thomson 2009). Despite the current trend for development of food products with improved nutritional quality, the GF products often receive only marginal attention from a formulation point of view. The enrichment of GF breads and other bakery items (cookies, pasta products, etc.) with BG, holds a special interest among the vulnerable population of celiac patients which encounters a significant incidence rate of other associated chronic diseases, such as obesity and diabetes due to higher fat and caloric intensity diets (Cronin and Shanahan 1997).

Rice flour is the most suitable ingredient for GF bakery formulations due to its bland taste, white colour, digestibility and hypoallergenic properties. Other attributes such as the low content of protein and sodium as well as the presence of easily digested carbohydrates are additional benefits (Rosell et al. 2014). Furthermore, in rice flour, the ratio of albumin-globulin-prolamin-glutelin is rather unique among the cereals, revealing a high concentration in glutelins and low in prolamins. As with other cereals, rice proteins are deficient in the essential amino acid lysine, but as a consequence of the respective ratio of protein fractions, rice has higher content of lysine than other cereals, and this is shared by oats (Rosell et al. 2014).

Previous works have demonstrated the potential of baking rice-based GF breads enriched with commercial BG concentrates to fulfill the EFSA health claim requirements as well as to provide products with acceptable quality (Perez-Quirce et al. 2014; Ronda et al. 2015). In these studies, although the final content of BG in the bread was not affected, the molecular weight of BG was notably reduced compared with the initial concentrate used as ingredient in the formulation mixture (Hager et al. 2011; Ronda et al. 2015).

The ability of β-glucans to decrease serum cholesterol levels and moderate the glycemic responses is often linked to the potential of these polysaccharides to enhance the viscosity of the intestinal contents (Tosh et al. 2008; Wolever et al. 2010). Since the viscosity of a β-glucan solution is a function of its molecular weight and polysaccharide concentration, the intensity of MW treatment and the amount of water-extractable β-glucans in a given food product can influence the extent of their physiological effect (Brummer et al. 2012; Lazaridou and Biliaderis 2007; Tosh 2013; Tosh et al. 2008; Wolever et al. 2010). In order to retain the full physiological

impact of β-glucan in formulated products it is therefore crucial to minimize its depolymerization (hydrolysis) during food processing and storage. This is a challenging problem during production of β-glucan enriched bread, since the activity of endogenous flour β-glucanases, in combination with the long contact time during mixing of ingredients, fermentation and proofing, can cause a substantial reduction in β-glucan molecular weight (Aman et al. 2004; Andersson et al. 2004; Trogh et al. 2004).

The role of enzymes from wheat flour in β-glucan degradation during dough handling has been demonstrated by Moriartey et al (2010), who showed that ethanol refluxed wheat flour (resulting in enzyme inactivation) gave lower β-glucan degradation and higher extract viscosities from doughs prepared with added barley β-glucan concentrate. The addition of yeast, on the other hand, did not seem to affect β-glucan degradation (Andersson et al. 2004; Moriartey et al. 2010) nor did differences in fermentation temperature or the water addition (Andersson et al. 2004). Andersson et al. (2004) also suggested that the baking process itself does not result in further degradation of the polysaccharide, whereas the endogenous β-glucanase activity in flour (wheat or barley) and reaction time in the dough system (during mixing, fermentation and proofing) are the most important determinants of β-glucan degradation during production of β-glucan enriched breads (Andersson et al. 2004; Moriartey et al. 2010). Inactivation of flour enzymes and the use of relatively short processing times have been proposed as effective means to minimize β-glucan degradation (Andersson et al. 2004; Moriartey et al. 2010; Vatandoust et al. 2012). However, adopting long mixing and fermentation-proofing regimes is often crucial for quality parameters of the final products, i.e. improved loaf volume, crumb porosity and texture. Thus the reduction of fermentation time to avoid β-glucan degradation does not appear as a feasible-practical approach. Consequently, the destruction of β-glucanase activity in raw flour materials seems to be a necessary and convenient step to develop breads enriched with BG of high molecular weight. Endogenous β-glucanase activity has not been demonstrated in rice flour so far. In spite of the low BG content of rice flour, that would make someone to anticipate a rather low β-glucanase activity, an apparent reduction of the BG molecular weight in rice based GF breads, fortified with barley and oat BG concentrates, has pointed

towards the presence of β-glucanase activity in rice flour (Ronda et al. 2015; Hager et al. 2011).

The aim of this work was to study the inactivation of β-glucanase enzymes in rice flour by means of microwave treatments (MW). Some of the methods previously used to inactivate β-glucanase activity are autoclaving, scalding, oven heating and ethanol refluxing (Lazaridou et al. 2014; Rieder et al. 2015; Moriartey et al. 2010). However, there is no literature information on the use of microwave heating for inactivation of β-glucanase enzymes. The high efficiency and the relatively short treatments adopted in MW processing, in comparison to other conventional heating procedures, led us to explore this alternative method for thermal inactivation of the endogenous rice flour β-glucanase. Only few works, with promising results, have been reported till now on flours or whole cereal grain stabilization by MW (Jiaxun-Tao et al. 1993). In rice processing MW has been applied to control the growth of pests and mildew (Zhao et al. 2007) as well as to inactivate lipases and lipoxygenase enzymes in order to increase its stability during storage (Chang and El-Dash 1998; Zhong et al. 2013).

In other heat treatments of flours (e.g. autoclaving), hydration has been identified as a critical parameter for successful β-glucanase inactivation in barley flours (Lazaridou et al. 2014); only in flours with the highest moisture content there was a full destruction of this enzyme. Taken this into account and the fact that the principle of microwave heating is mainly based on water molecules polarization, a study of the effect of rice flour moisture content on β-glucanase inactivation kinetics by MW seemed to be necessary. Moreover, to limit starch gelatinization during the thermal processing stage the moisture content of the flour was kept below 30% (Biliaderis et al. 1980; Maache-Rezzoug et al. 2008; Biliaderis 2009). The effect of MW heating under these conditions on starch crystallinity, pasting and thermal properties of microwaves-treated rice flours was also tested.

2. Materials and methods

2.1. Materials

Sixteen different rice flour samples, varying in moisture content and hydrothermal treatment using MW, were examined in this work as specified in Table 1. Rice flour from an Indica variety was supplied by Herba Ricemills SLU (Tarragona, Spain), having 13.12% moisture, 79.1% starch, 0.46% ash, 7.5% protein and 0.49% fat. The particle size distribution of the flour was 6% > 150 μm, 150 μm > 63.2% > 100 μm and 30.8% < 100 μm) (data provided by the manufacturer).

2.2. Methods

Several preliminary tests were conducted in order to establish the working conditions for microwave treatment. A particular attention was paid to achieve a uniform temperature and constant water content during the process. The initial moisture content of the rice flour was measured following the AACC 49.19 method, and the water needed to adjust it to the selected value was calculated. Flours were sprayed with the appropriate amount of water while they were mixed in a Kitchen-Aid (Model 5KPM50, Kitchen Aid, St. Joseph, MI, USA) mixer for 10 min. The samples were then allowed to stand 24 h at 4±2 ºC in order to equilibrate the moisture. Four different water contents (13%, 16%, 19%, 25%) and five microwave treatment times, (1, 2, 4 and 8 min for moistures of 13 to 19%, and 40 s, 1, 2, 4 min for 25% moisture) were tested. The β-glucanase activity of untreated flour, was taken as the value corresponding to zero time conditions.

2.2.1. Microwave Treatment of Flour Samples

Rice flours were heated in a Panasonic Inverter NN-GD566M (Osaka, Japan) microwave oven. The frequency of microwave radiation was 2450 MHz. Samples of hydrated flours (50.0 g) were introduced into (polyamide + polypropylene) bags of 20 x 30 cm (NOP101, Cryovac, Sealed air, NY, USA) and hermetically closed by heat-sealing (Magneta 300 MG model, brand Audio Elektro, Holland) in order to maintain the moisture content constant during the MW treatment. All samples were spread out to form a thin layer inside the bag (layer thickness ~ 1 mm) to ensure a uniform moisture and temperature distribution in the flour during treatment. The microwaves dish, where the sample was placed, rotated during the treatment to

assure a good distribution of MW energy in the sample. The microwaves power, 900W, was applied in cycles of 20 seconds intervals combined with downtimes of 1 min. Under these conditions the sample bags withstood the water vapor pressure without breaking during the treatment. Between stops the packed flours were manually mixed by moving and turning the bags to facilitate a common temperature within the sample. Samples were subsequently left to cool for 30 min and then were stored at 4 °C until further analyses. The moisture contents of the flour before and after the microwave treatment were determined following the AACC 49.19 method . Water losses did not exceed 1%, which implies a good hermetic sealing of the bags. Previous tests have showed a marked loss of water in samples processed in non-hermetically closed containers; i.e. samples in open containers treated for 6 min in the microwave oven exhibited moisture losses from 13% to 5%. Some experiments described in literature also used open or non-hermetically closed containers (glass beakers sealed with a perforated polyethylene film), but the moisture retention in the samples was not reported. As moisture shows a marked effect on heat-induced inactivation of enzymes in plant materials, its control seems of extreme importance in order to obtain conclusive results; therefore, sealing of the bags was adopted in all experimental trials of the present study. The temperature evolution over time of the microwave treatment was monitored with an infrared thermometer Testo 826-T2 (Lenzkirch, Germany) in the shutdown periods of the treatment cycles. All microwave treatments were carried out at least in duplicate.

2.2.2. β-Glucanase Activity Determination

The β-glucanase activity in control and hydrothermally treated rice flours was assessed by measuring the rate of decrease in specific viscosity of a dilute solution of a pure β-glucan preparation, following addition of flour extracts, according to the method reported in a previous work (Lazaridou et al. 2014). The flour extracts were obtained by aqueous extraction (flour: water 1:10) under stirring at 25 ºC for 30 min and subsequent centrifugation at 2500 g for 20 min. An aliquot (6 ml) of the resultant supernatant was added to 36 ml of the aqueous solution (0.1% w/v) of a high molecular weight ($2x10^6$) β-glucan preparation (purity 95%). The mixture of flour extract with the β-glucan solution was then transferred into an Ubbelohde glass capillary viscometer (UBBEL04NC, K 0.01, range 2-10 cSt, brand Paragon Scientific Ltd, Wirral, UK) and the specific viscosity ($\eta_{sp} = (\eta-\eta_o)/\eta_o$, where η_o is the

viscosity of water) was measured over 1h period at 20±0.1 ºC every 5 min intervals. The η_{sp} data versus time were fitted to a linear regression model and the β-glucanase activity in rice flours was calculated from the slope of the fitted line and expressed as the decrease in specific viscosity per hour of the pure β-glucan solution upon addition of the flour extracts. The principle of this procedure is based on the linear relationship between the intrinsic viscosity and the molecular weight of some polymers in solution which, in turn, follows an inverse linear response (depolymerization) with the reaction time when the polymer, i.e. β-glucan, is subjected to a random depolymerization by the action of β-glucanase (Rieder et al. 2015). At low polymer concentrations specific viscosity can be used instead of intrinsic viscosity (Hjerde et al. 1994). Residual β-glucanase activity of each treated flour sample was analyzed at least in triplicate.

2.2.3 β-Glucanase Inactivation Kinetics

The β-glucanase activity values obtained in duplicate from heat treated flours under the sixteen treatment conditions, plus the untreated flour activity value, were fitted against the flour moisture and the time of microwave treatment using a nonlinear multivariate regression model. The individual effect of the time of microwaves application on β-glucanase inactivation kinetics at each level of moisture content of the flour was described by a first order kinetic model, according to the equation, $A = A_0 \exp(-k \cdot t)$, where A is the β-glucanase activity, t is the time of the microwave treatment, A_0 is a constant that represents the initial activity of the untreated flour (t = 0); and k (min^{-1}) is the apparent rate of enzyme inactivation, representing the reduction of enzyme activity per unit time. The flour moisture content also had a marked effect on the rate constant k which varied exponentially with it.

2.2.5. Pasting Properties of Flours

Pasting properties were studied by using the Rapid Visco Analyzer (RVA-4, Newport Scientific Pvt. Ltd., Australia) using ICC Standard method 162. The pasting temperature (PT), peak time (when peak viscosity occurred) (VT), peak viscosity (PV), holding strength or trough viscosity (TV), breakdown (BD), final viscosity (FV) and setback (final viscosity minus peak viscosity) (SB) were calculated from the pasting curve using Thermocline v.2.2 software. Viscoamylography of aqueous

flour dispersions (3 g of flour 14% moisture basis to 28 g total weight with distilled water) was carried out in triplicate.

2.2.6. X-ray Diffraction

Samples were analyzed using a Bruker D8 Discover A25 diffractometer (Bruker AXS, Rheinfelden, Germany) equipped with a copper tube operating at 40 kV and 40mA, using the CuKa radiation of 0.154-nm wavelength. Diffractograms were obtained by scanning from 5° to 40° (2θ) at a rate of 1.2°/min, a step size of 0.02°, a divergence slit width variable (DS) of 5mm and a scatter slit width (SS) of 2.92° and a nickel filter 0.02 to exclude the Kβ radiation.

2.2.7. Differential Scanning Calorimetry

Thermal characteristics of flours were determined using a differential scanning calorimeter (DSC-822e, Mettler Toledo, SAE). Flour samples were weighed into aluminum pans (40 μL) and distilled water was added using a micropipette to make 70% moisture content of the aqueous flour dispersions to avoid the effects of water scarcity on the thermal profile (non-equilibrium melting) of granular starch (Biliaderis et al. 1980; Biliaderis, 2009). Flour weights were about 8 mg. The samples were scanned from 0ºC to 110 ºC at 5 ºC/min using an empty pan as reference. Starch retrogradation was evaluated in the samples which have been previously gelatinized in the DSC oven and stored in the pans at (4 ± 2) ºC for 7 days; the staled samples were re-scanned using the same heating protocol as for gelatinization. The enthalpy (△H) values, expressed in J/g, based on dry-flour basis, the onset and endset temperatures (T_o and T_e) and the peak temperature (T_p) were established in both scans, of gelatinization and retrogradation. Samples were run in duplicate.

2.2.8. Statistical Analysis

Statgraphics Centurion v.6 (Bitstream, Cambridge, MN, USA) was used for multivariate non-linear regression. STATISTICA package (Tulsa, OK, EEUU) v.6, allowed performance of MANOVA analysis, and LSD (Least Significant Difference) test was used to evaluate significant differences ($p<0.05$) between samples.

3. Results and discussion

3.1. β-Glucanase Activity Rice Flours Treated by Microwaves

Endogenous β-glucanase activity of rice flours was estimated by measuring the rate of decrease in specific viscosity of a purified BG solution after addition of the rice flour extract at constant temperature of 20 ± 0.2 °C (Table 1). A pronounced decrease in specific viscosity over time was observed with the untreated rice flours, corresponding to an average activity value of (0.109 ± 0.005) h^{-1}, which denotes a high β-glucanase activity in untreated rice flour, only slightly smaller than that reported previously for barley flours, i.e. 0.143 or 0.117 for coarse or fine barley flours, respectively (Lazaridou et al. 2014). However, for the microwaves-treated flours the decline in specific viscosity of the mixed standard BG solution-flour extracts over time was significantly smaller ($p<0.05$), implying that the hydro-microwave treatment brought about a large reduction in β-glucanase activity of the rice flours; the effect being more pronounced when the time of the treatment and the moisture content of flours increased (see Table 1).

The total inactivation of β-glucanase activity was effected after 8 min and 4 min of MW treatment for flours tempered to moisture contents of 19% and 25%, respectively. For the same periods of MW treatment residual β-glucanase activities were still measured in flours with 13% and 16% moisture contents, even though they were reduced by 87% and 91% with respect to the initial flour enzymatic activity. Lazaridou et al. (2014) also found that increasing the moisture content of barley flours before hydrothermal treatment by autoclaving, resulted in complete inactivation of the endogenous barley β-glucan hydrolysing enzymes. This behavior could be explained by the large reduction of the denaturation temperature of proteins even with a slight increase of moisture content in low moisture protein systems (Arntfield et al.1990), such as hydrated flours. In other studies, hydrothermal treatments such as steaming and autoclaving of barley grain and oat groats also led to no detectable β-glucanase activity (Izydorczyk et al. 2000; Zhang et al. 1998). The water content is particularly important when the heating energy is generated by microwave radiation. Microwaves are electromagnetic waves in the frequency range of 300–300,000 MHz. In a MW field, where the polar molecules absorb microwave energy and orient themselves with respect to the applied electric field, the rapid

change in their orientation generates heat by molecular friction (Sumnu 2001). This results in bulk heating throughout the sample and a faster heating rate compared with other forms of conventional heating. Because of its dipolar nature, water, is the main source of microwave interactions with food materials. Most likely the increase in moisture content of the flour above its monolayer value allows a faster absorption of energy from microwaves during the treatment. As a result, a pronounced reduction in the time needed to inactivate the β-glucanase with microwave energy in comparison to other heating systems is effected; i.e. the time is shortened from 4 h heating the flour in an oven at 130ºC (Rieder et al. 2015) to 20 min, when autoclaving flours at 120 ºC (Lazaridou et al. 2014), to only 4 min with microwave heating. These findings imply significant savings of time and energy.

Table 1. Residual β-glucanase activities of rice flours treated by microwave energy.

Initial moisture content (% fb)	Treatment time (min)	β-glucanase Activity [a, b]	Final moisture content (% fb)[c]
Control	0	0.109 h	13.00
13	1	0.103 h	13.02
13	2	0.068 fg	13.10
13	4	0.034 d	12.57
13	8	0.014 b	12.83
16	1	0.076 g	15.93
16	2	0.052 ef	15.42
16	4	0.022 c	15.44
16	8	0.010 ab	15.10
19	1	0.044 de	18.61
19	2	0.011 b	17.81
19	4	0.004 ab	18.15
19	8	0.000 a	17.66
25	0.67	0.012 b	24.44
25	1	0.009 ab	24.20
25	2	0.005 ab	24.21
25	4	0.000 a	23.87

[a] Calculated as decrease in specific viscosity per hour of a purified β-glucan solution (0.1%w/v) following the addition of rice flour extracts.

[b] Values are means of duplicate treatments and duplicate measurements. Values with the same letter for the same parameter are not significantly different (p>0.05); means were compared using the LSD test.

[c] Moisture content of flours after the microwave treatment.

The evolution of temperature versus microwave treatment time is shown in Figure 1 for flours with 13% to 25% moisture contents. In the first 20 s of treatment the flours had got 59ºC and 72ºC, respectively. After 2 ½ min and 2 min (for 13 and 25% moisture) both flours attained a constant temperature around 95ºC and 97ºC, respectively, leading to a plateau value as found by other authors (Lewandowicz et al. 1997). Lewandowicz et al. (1997) reported that the plateau interval length increased with the rise in moisture content and also when the sample was introduced in sealed containers instead of open ones; the sealed beakers used were covered by these authors with a perforated polyethylene foil that probably allowed some water loss during heat treatment. As can be concluded from the temperature-time responses, all the attained plateau temperature values were always below 100ºC (presumably due to the 'colling effect' from water evaporation), with water acting as 'protector' of the flour constituents. This relatively low temperature probably explains the smaller changes in the physico-chemical properties of the heat-treated flours.

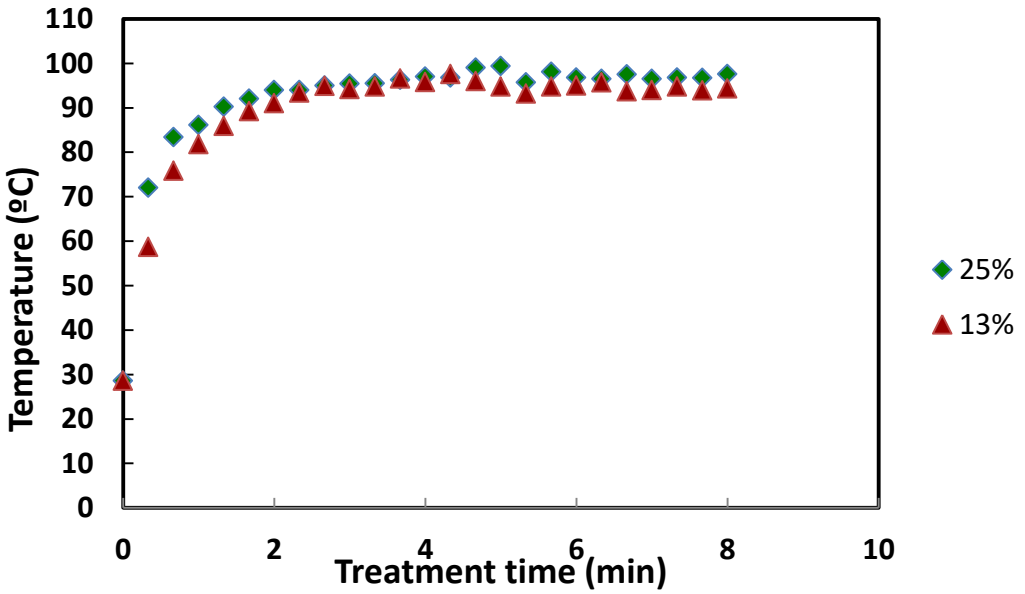


Figure 1. Flour temperature evolution versus time upon microwave treatment of two rice flour samples at 13 and 25% water contents, respectively.

3.2 β-Glucanase Inactivation Kinetics in Hydrated Rice Flours by Microwave Heating

Figure 2 shows the regression surface response of the residual β-glucanase activity versus the microwave treatment time and the moisture content of the flour treated. The regression equation obtained was:

$$A = A_o \cdot e^{-k \cdot t} \qquad \text{(eq. 1)}$$

with k being dependent on moisture content (M) as follows:

$$k = k_o \cdot e^{b \cdot M} \qquad \text{(eq. 2)}$$

leading to the general equation:

$$A = A_o \cdot \exp[-k_o \cdot \mathrm{t} \cdot \exp(b \cdot M)] \qquad \text{(eq. 3)}$$

Where A is the time dependent β-glucanase activity (t); t is the time of microwave treatment (min) and M is the moisture content of the hydrated flour (% in wet basis), k (min^{-1}) is the apparent rate constant of enzyme inactivation that is dependent exponentially on moisture flour. A_0, k_o and b are constants estimated for this specific system after fitting the 33 data sets to the model (equation 3). The estimated value of A_o constant was (0.109 ± 0.0027) h^{-1}, representing the initial β-glucanase activity of the untreated flour (t = 0); k_o (0.0146 ± 0.0033) min^{-1} represents the rate of enzyme inactivation when the moisture content of the flour is 0%; and b (0.212 ±0.013) is a constant that quantifies the influence of water content of the flour to the inactivation rate. The correlation coefficient of this regression, R^2, was 0.9667 which means that the model explains successfully the variation of β-glucanase activity during the microwave treatment within the range of moistures (13-25%) and treatment times (0.67 – 8 min) applied in the present study. The standard error of the estimate was 0.0070 h^{-1}. The fitting value (estimated) of A_o, was very near to the experimental initial β-glucanase activity of the flour, before any treatment, 0.109 h^{-1} (see Table 1), implying the good agreement of the model to the experimental data. Regarding the correlation equation (eq. 3) it must be noted the marked effect of flour moisture, M, on β-glucanase inactivation kinetics, as the rate constant, k, depends exponentially on it. The kinetics constant, k, for the completely dry flour, 0.0146 min^{-1}, increased to 0.231, 0.438, 0.828 and 2.969 min^{-1} when the moisture content of the microwave-

treated flour increased to 13, 16, 19 and 25%, respectively. The model can also predict the time of MW treatment needed to completely inactivate the endogenous β-glucanase (to reduce its original activity value in rice flour from ~0.1 h^{-1} to 0.0001 h^{-1}); this is reduced from more than 30 min at 13% hydration level to less than 3 min for 25% moisture.

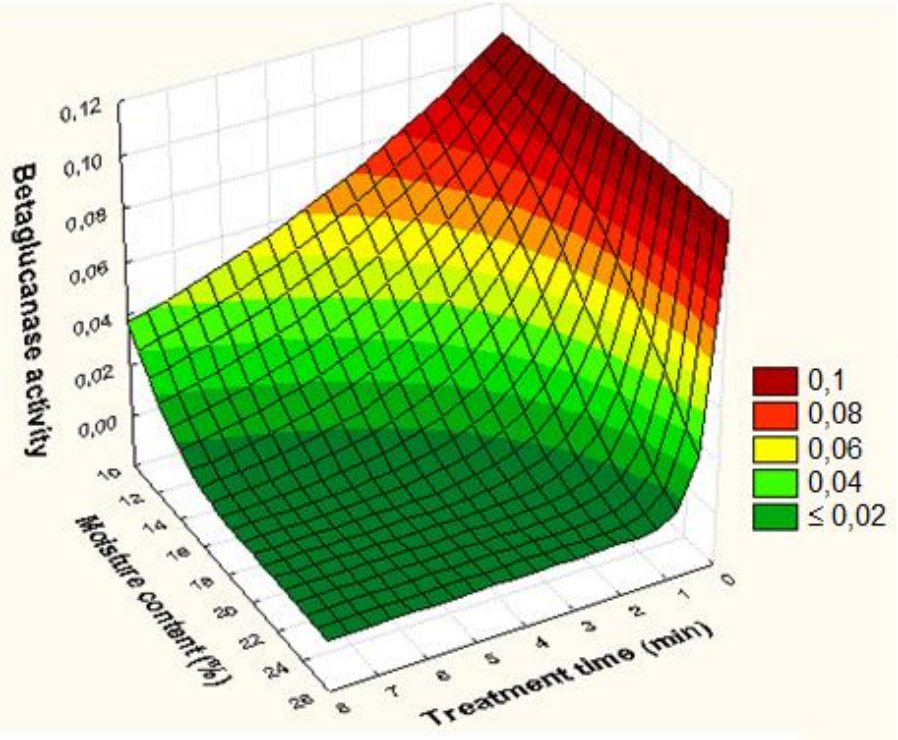


Figure 2: Kinetics of β-glucanase inactivation by microwave treatment depending on flour moisture content.

3.3. Pasting Properties of Flours

The impact of microwave treatments on rheological responses of aqueous flour dispersions, i.e. upon cooking (gelatinization and pasting) and cooling (gelling) of the starch component of the rice flours, was studied to evaluate any side effects that microwave β-glucanase inactivation treatment might have on the flours. As can be seen in Table 2, although some significant differences were found between the pasting properties of the native rice flour and those of the microwave treated samples, particularly for the highest moisture flours and the longest treatment times, none of them were of great quantitative importance. In general, microwave treated flours did not show significant differences in peak viscosity compared to the control (untreated) flour. However, the microwave treatment increased trough viscosity (TV)

when the two longest treatment times were applied in all the flours, independently of the moisture content. The most important increase in TV, 14%, was noted for the flour with the highest moisture content, 25%, and the longest treatment time, 4 min. This increase in TV, concomitant with the decrease in the Breakdown, demonstrates that microwave treated flours are more stable during continuous heating and agitation, which concurs with the findings of Adebowale et al. (2005), Hormdok and Noomhorm (2007), Olayinka et al. (2008) and Watcharatewinkul et al. (2009). The final viscosity (FV) of the gelled flours after the heating/cooling cycle, hardly varied in any of the MW treated flours with respect to the untreated flour; only flours with 25% moisture, following a 2 - 4 min MW treatment, showed a significant, although moderate (<10%) increase in FV. The setback viscosity (SB) was neither affected by MW treatment, as also happened with the pasting temperature (PT). Only the flour with 25% moisture and treated for 4 min showed a feeble significant increase in the PT.

Table 2. Effects of microwaves treatment on the viscometric parameters of rice flours.

Moisture content (%fb)	Treatment time (min)	Peak Viscosity (mPa·s)	Trough Viscosity (mPa·s)	Breakdown Viscosity (mPa·s)	Final viscosity (mPa·s)	Setback Viscosity (mPa·s)	Pasting Temperature (ºC)
Control	0	2352 ab	1467 ab	885 fg	3336 abc	1869 ab	78.5 a
13	1	2414 abc	1488 abc	926 gh	3338 abc	1850 ab	78.6 a
13	2	2422 abc	1504 abcd	918 gh	3353 abc	1850 ab	78.6 a
13	4	2357 abc	1577 ef	780 cde	3392 abcd	1852 ab	79.6 ab
13	8	2419 abc	1538 cde	881 efg	3404 abcd	1866 ab	80.0 ab
16	1	2363 abc	1446 a	917 gh	3315 abc	1869 ab	78.6 a
16	2	2365 abc	1465 ab	900 fgh	3335 abc	1870 ab	78.9 a
16	4	2450 bc	1564 cde	887 cde	3493 bcde	1900 ab	79.9 ab
16	8	2344 abc	1561 cde	783 c	3438 abcd	1877 ab	80.2 ab
19	1	2375 abc	1451 a	924 gh	3340 abc	1888 ab	79.7 ab
19	2	2277 a	1452 a	825 cd	3262 a	1810 a	79.8 ab
19	4	2341 abc	1611 fg	730 b	3477 bcde	1866 ab	79.7 ab
19	8	2285 a	1617 fgh	668 a	3417 abcd	1800 a	79.2 ab
25	0.67	2396 abc	1563 cde	833 def	3333 abc	1766 a	79.6 ab
25	1	2437 bc	1585 ef	852 de	3463 bcd	1878 ab	79.7 ab
25	2	2456 c	1647 gh	809 cd	3635 e	1989 b	79.8 ab
25	4	2439 bc	1675 h	764 bc	3551 de	1875 ab	81.0 b

Within the data set of each parameter, different letters in the mean values of therespective columns imply significant differences between means at $p < 0.05$.

Some studies have been conducted on the effect of MW on cereals, mainly starches. Lewandowicz et al. (2000) found an increase in gelatinization temperature and a decrease in solubility of microwaved maize and wheat starches. Stevenson et al. (2005) also reported an increase of gelatinization temperature and a decrease of paste viscosity of microwaved maize starch. Anderson and Guraya (2006) and Luo et al. (2006) investigated, respectively, the effect of microwave on rice and maize starches with different proportions of amylose/amylopectin. These authors reported rearrangements of the molecular structure upon microwave heating that could explain the significant changes in viscosity properties of both waxy and non-waxy starches. Pinkrová et al. (2003) have observed that the peak viscosity of rice flour decreased as temperature and microwave power level applied to rice grain increased. On the other hand, Fan et al. (2012) reported that microwave irradiation had no effect on the optical and thermal properties of rice starch during gelatinization compared to conventional heating. Many studies have been carried out on the structural and physical properties of starches after heat-moisture (HMT) treatment (Zavareze and Dias 2011). HMT promotes intense changes in starches, thus significantly altering their pasting profile as evidenced by increased pasting temperature and decreased peak viscosity, final viscosity, and breakdown (Watcharatewinkul et al. 2009). According to these researchers, the changes in heat-treated starch's pasting properties are due to associations between the chains in the amorphous regions of the granule as well to changes in crystallinity during the hydrothermal treatment. Such structural modifications are intensified as the moisture content in the HMT increases (Olayinka et al. 2008). As the forces of the intra-granular chain interactions are strengthened, the starch requires more heat for structural disintegration and paste formation (Olayinka et al. 2008). A high pasting temperature thus indicates that more forces and cross-links exist within the starch granules (Olayinka et al. 2008). Lan et al. (2008) have shown that the retrogradation process is influenced by the amount of leached amylose, granule size, and the presence of rigid, non-fragmented swollen granules. Chung et al. (2009) also found that HMT reduces amylose leaching from starch granules and that this reduction is more significant in starches containing high levels of amylose. They have reported that HMT promotes additional amylose–amylose and/or amylopectin–amylopectin chain interactions, which restrict amylose leaching and decrease retrogradation events. A recent study by Roman et al. (2015) reports on the effects of short time (0.5 to 4 min) MW treatments of corn flours at

30% moisture. They found opposite effects on pasting properties depending on the MW treatment time. Treated samples showed increased viscosity during the heating-cooling cycle when a very short time of microwave treatment was applied (0.5 and 1 min). Instead, longer treatments (4 min) induced the opposite effect, and a decrease in the viscosity was noted. A decrease of the maximum (peak) viscosity was also observed by Pinkrová et al. (2003) by increasing temperature of microwave treatment of the rice grain and increasing power output at a moisture content of 30 %. Roman et al. (2015) have examined the effect of MW on flour structure. They found a more disaggregated structure and a less compact matrix, with starch granules being more naked and slightly swollen. Luo et al. (2006) observed even more marked changes on starch granule structure when moistened (30 %) maize starch was treated for 20 min with microwaves at 1 W/g. They have noted some breakage, cracks and pores on the surface of the starch granules. These observations can be attributed to chain segmental transfers and internal rearrangements in the granular material, facilitated by water plasticization. The absence of such effects in our MW treated flours could be due to the shorter treatment times employed (lower power) as well as the use of completely airtight bags, where large water transfers within the sample were probably hindered by the vapor pressure raised in the bag headspace. Figure 3 shows the different pasting properties of two aliquots of 30% moistened-rice flour treated with the same MW heating procedure used in all the experiments carried out in this study, and in an opened beaker. Although further studies should be carried out to fully unravel the different effects heat-moisture treatments have on the starch granular material using MW, the notable differences in pasting properties shown in Fig. 3 clearly indicate that water plays an important role in starch modifications during MW treatments. Moreover, it is important to note that the flour treated in the close bag maintained its original white color compared to that treated in the open beaker for the same time which showed a visible darker-brown color.

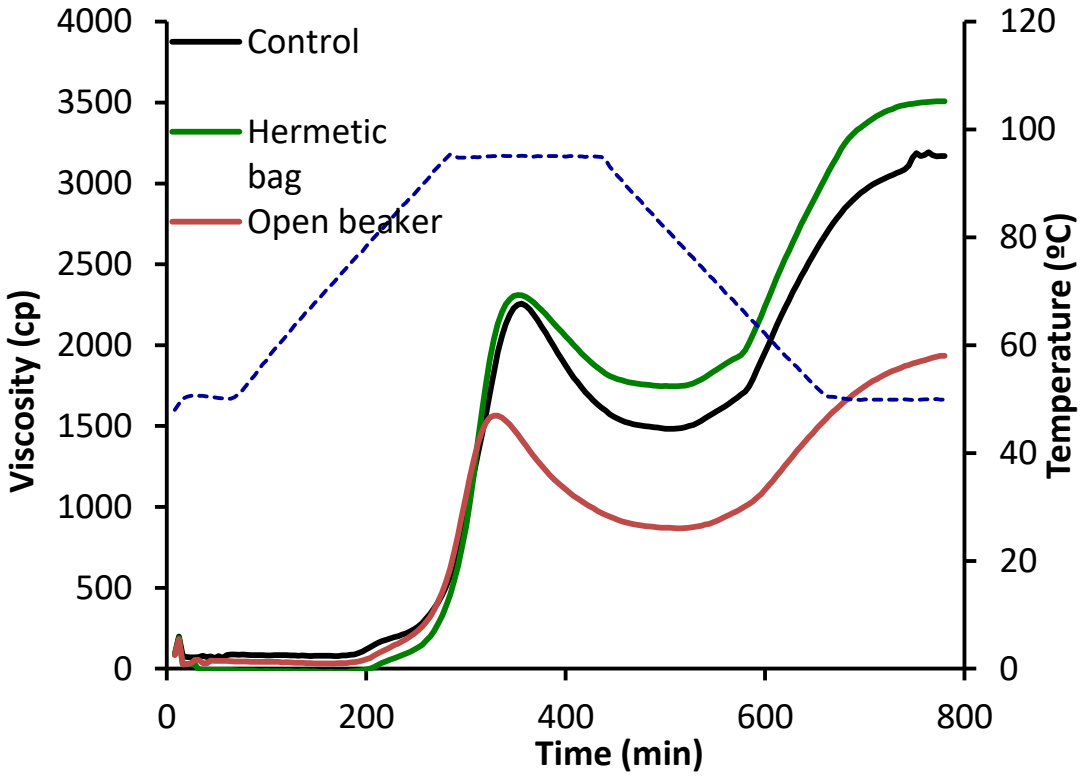


Figure 3: Pasting characteristics of (9.2 g dry flour/ 100 g) aqueous dispersions of rice flours; control and microwave heat-treated at 30% moisture content for 4 min. Viscosity profile for native (—), and microwave-treated samples in hermetic bags (—) and open beaker (—).

3.4. X-ray Diffraction

The diffractograms obtained from the microwave-treated and the untreated 25% moistened-rice flours (Fig. 4) showed that all samples maintained the A-type crystallinity, typical of many cereal starches (Imberty, et al. 1991; Zobel 1964) with peaks centered at approximately 15°, 17.1°, 18° and 23° (2θ), for both native sample and all microwave treated rice flours. Indeed, the A-type crystalline pattern was conserved in the MW treated flours with 25%, for any processing time (0.67, 1, 2 and 4 min), and no significant differences in the relative crystallinity value compared to the native flour (starch) were observed. Luo et al. (2006) and Roman et al. (2015) reported an increase in X-ray intensities for microwave treated maize starch and flour, respectively. It has been postulated that microwave radiation can lead to formation of additional double helical structures within the starch crystallites, leading to a higher molecular order than that in native starch (Luo et al. 2006). The movement of double helices might be related to the vaporization of water molecules that release positions originally occupied by water molecules, allowing for more

compact ordered crystalline arrays (Luo et al. 2006). The use of hermetically sealed bags in our study provided a small positive pressure inside the bags and prevented the loss of water, and this in turn might have resulted in limited molecular reorganization within the starch granules. Different effects of HMT on starch crystallinity have been noted by other authors, however, for more prolonged thermal teatments. Jyothi et al. (2010) observed an increase in crystallinity of sweet potato and arrowroot starches and a slight decrease in the crystallinity of cassava starch, after the three starches were heat-moisture treated at 120ºC for 14 h at 20% moisture. Similar results to our findings were obtained by Maache-Rezzoug et al. (2008). These authors did not find any significant difference in the relative crystallinity when applied a controlled pressure drop (DIC) hydrothermal treatment at 1 bar of pressure (100 ºC, 14-15% moisture) for any processing time (10 – 60 min) to maize starch, whereas they have noted a significant reduction in relative crystallinity when applied treatments at 2 and 3 bar of pressure (122 and 135ºC, respectively); in the latter case, the A-type crystalline pattern was progressively changing to a V-type crystalline pattern, implying the formation of amylose-lipid complexes in the heat-treated starch granules. Overall, the findings of the present work clearly indicate that it is possible to apply MW treatments for complete inactivation of β-glucanase without modifying starch structure, even at the highest moisture content of 25%. Maintaining a constant water content of the flour and the flour temperature below 100 °C, throughout the microwave treatment are probably the most important parameters in this respect.

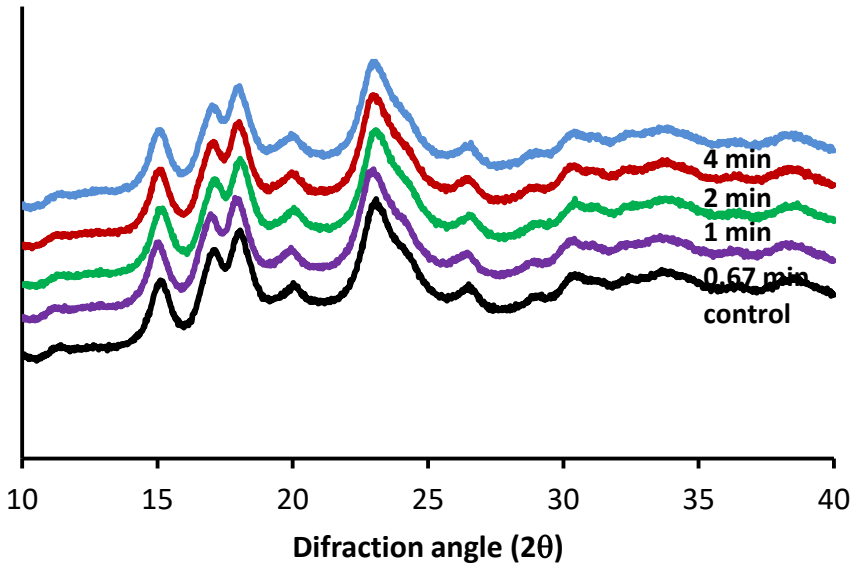


Figure 4: X-ray diffraction patterns of untreated rice flour (control) and microwaved (different treatment times) rice flours at moisture content 25%. .

3.5. Impact of MW on Thermal Properties

The results of X-ray diffractometry were further confirmed by differential scanning calorimetry. The thermal properties of untreated (control) and microwave-treated 25% moistened-rice flours are summarized in Table 3. In the range of temperatures tested, the aqueous dispersions of flours exhibited two endothermic peaks, corresponding to the gelatinization of starch and the dissociation of amylose-lipid complexes (Biliaderis 2009). After seven-day storage (in the DSC pans) at 4ºC of the gelatinized samples a second scan also showed two endothermic transitions. The peak corresponding to the melting of the recrystallized amylopectin that appeared at lower temperatures than the gelatinization peak of native starch, and the amylose-lipid complex transition found in the first scan that appeared at the same temperature and had the same enthalpy value. As can be seen, the effect of microwaves on starch gelatinization, amylopectin retrogradation and amylose-lipid complex temperatures and enthalpies was practically negligible, even though the samples tested by DSC were those of the highly moistened-flours. The only significant effect ($p<0.01$) was obtained in the gelatinization peak temperature (weak annealing, Biliaderis 2009), although the quantitative importance of this change was rather minor. Other authors have also reported an increase in the gelatinization peak temperatures of microwave treated 30% moistened-maize flours (Roman et al. 2015) when MW energy was applied for 2, 4 min; the increases observed by these authors were ~3°C, while that of the present work was less than 1°C. Lewandowicz et al. (2000) also observed rises in the gelatinization peak temperatures of 30% moistened-starches treated by MW for 60 min at 0.5 W/g, that varied from 13ºC for wheat to 6ºC for waxy corn starch, in comparison to their untreated counterparts. Moreover, these authors have reported an important decrease, ~72%, in the apparent gelatinization enthalpy value of wheat starch that was only ~ 50% for corn, and insignificant for waxy corn compared with the untreated starches. Other authors also found a reduction in starch gelatinization enthalpy for MW treated corn starches (Luo et al. 2006; Stevenson et al. 2005). In contrast, Roman et al. (2015) have reported an almost double gelatinization enthalpy of all MW-treated corn flour samples with respect to the native one. Consequently, the extent of MW heating effects on thermal behavior of granular starches depends on the starch source, the amylose and moisture content, as well as the intensity and time of the applied microwave treatment (Zavareze and Dias 2011).

Table 3. Thermal properties of aqueous dispersions (70 % water) of flour treated by microwaves (at 25% water content).

Treatment time (min)	ΔH_{gel} (J/g db)	$T_{o\text{-}gel}$ (ºC)	$T_{p\text{-}gel}$ (ºC)	$T_{e\text{-}gel}$ (ºC)	R (ºC)	$\Delta H_{am\text{-}lip}$ (J/g db)	$T_{p\text{-}am\text{-}lip}$ (º C)	ΔH_{ret} (J/g db)	$T_{p\text{-}ret}$ (ºC)
0	7.7 a	68.0 a	74.16 a	89.6 a	12.4 a	1.22 bc	100.7 a	4.7 bc	52.7 a
0.67	7.5 a	68.3 ab	74.30 b	90.2 a	12.0 a	1.09 ab	100.0 a	4.2 a	54.5 ab
1	8.0 a	68.1 a	74.25 ab	91.2 a	12.2 a	1.32 c	100.2 a	4.4 ab	55.2 b
2	7.7 a	68.4 ab	74.47 c	91.5 a	12.2 a	1.06 ab	101.3 a	4.7 bc	53.3 ab
4	7.9 a	68.9 b	74.71 d	91.5 a	11.6 a	0.97 a	101.7 a	4.8 c	52.8 a

ΔH_{gel}: Enthalpy value associated with gelatinization; $T_{o\text{-}gel}$ and $T_{e\text{-}gel}$: onset and endset temperatures of the gelatinization peak; $T_{p\text{-}gel}$, $T_{p\text{-}ret}$, $T_{p\text{-}amil}$: T_{peak} of *gelatinization*, *retrogradation* and *amylose-lipid complex* dissociation peaks, respectively; $R= 2\cdot(T_p\text{-}T_o)$; $\Delta H_{amyl\text{-}lipid}$: Enthalpy value of the dissociation of the amylose-lipid complex; ΔH_{ret}: Melting enthalpy of the recrystalized amylopectin after storage of the gelatinized sample at 4ºC for 7 days. Each value is the average of duplicate measurements.

Within the data set of each parameter, different letters in the mean values of the respective columns imply significant differences between means at $p < 0.05$.

4. Conclusions

Microwave treatment is a useful alternative for inactivation of the endogenous β-glucanase in rice flours when applied to moistened flour samples. The enzyme inactivation process follows a first order kinetic response (R^2 = 0.97). The constant rate of the thermal inactivation by MW increased exponentially with the moisture content of the flour, so that, the microwave treatment time required for complete β-glucanase inactivation was only 4 min when the initial flour moisture wasraised to 25%. Following the MW treatment, the crystallinity of the starch was unaffected and the side effects of the treatment on flour pasting and thermal properties were rather negligible.

Acknowledgments

The authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad and the European Regional Development Fund (FEDER) (Projects AGL2012-35088).

References

AACC, American Association of Cereal Chemists, 2000. Method 44-19. In: Anonymous Approved Methods of the AACC, (10th ed.). The Association, St. Paul, MN.

Adebowale, K. O., Afolabi, T. A.,&Olu-Owolabi, B. I. (2005). Hydrothermal treatments of Finger millet (Eleusine coracana) starch. *Food Hydrocolloids*, 19, 974–983.

Åman, P., Rimsten, L., Andersson, R., 2004. Molecular weight distribution of beta glucan in oat-based foods. *Cereal Chemistry*, 81, 356–360.

Andersson, A. A. M., Armo, E., Grangeon, E., Fredriksson, H., Andersson, R., & Aman, P. (2004). Molecular weight and structure units of (1-3, 1-4)-β-glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science*, 40, 195–204.

Anderson, A. K., & Guraya, H. S. (2006). Effects of microwave heat moisture treatment on properties of waxy and non-waxy rice starches. *Food Chemistry*, 97, 318–323.

Arntfield, S.D., Ismond, M.A.H., Murray, E.D. (1990). Thermal analysis of food proteins in relation to processing effects. In V. R. Harwalkar, & C. –Y. Ma (Eds.), *Thermal analysis of foods* (pp. 51–91). London & NewYork: Elsevier Applied Science.

Biliaderis, C. G., Maurice, T. J., & Vose, J. R. (1980). Starch gelatinization phenomena studied by differential scanning calorimetry. *Journal of Food Science*, 45, 1669–1674.

Biliaderis, C.G. (2009). Structural transitions and related physical properties of starch. In R. L. Whistler, & J. N. BeMiller (Eds.), *Starch: Chemistry and Technology*, 3rd Ed. (pp. 293-372), Academic Press.

Brummer, Y., Duss, R., Wolever, T. M. S., & Tosh, S. M. (2012). Glycemic response to extruded oat bran cereals processed to vary in molecular weight. *Cereal Chemistry*, 89, 255–261.

Chang, Y. K. & El-Dash, A. A. (1998). Effect of microwave energy on lipase inactivation and storage stability of brown rice. *Acta Alimentaria*, Vol. 27, No.2, 193–202.

Chung, H. J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers*, 75, 436–447.

Cronin, C., & Shanahan, F. (1997). Insulin-dependent diabetes mellitus and coeliac disease. *Lancet*, 349 (9058), 1096–1097.

EFSA. (2011a). Scientific opinion on the substantiation of a health claim related to barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to article 14 of regulation (EC) No. 1924/2006. *EFSA Journal*, 9 (2470), 14.

EFSA. (2011b). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13 (1) of Regulation (EC) No. 1924/2006. *EFSA Journal*, 9 (2207), 21.

EFSA. (2011c). Scientific Opinion on the substantiation of health claims related to oat and barley grain fibre and increase in faecal bulk (ID 819, 822) pursuant to Article 13 (1) of Regulation (EC) No. 1924/2006. *EFSA Journal*, 9 (2249), 13.

Fan, D., Ma., S., Wang, L., Zhao, J., Zhang, H., Chen, W. (2012). Effect of microwave heating on optical and thermal properties of rice starch. *Starch*, 64 (9), 740-744.

Hager, A. S., Ryan, L. A. M., Schwab, C., Ganzle, M. G., O'Doherty, J. V., & Arendt, E. K. (2011). Influence of the soluble fibres inulin and oat beta-glucan on quality of dough and bread. *European Food Research and Technology*, 232 (3), 405-413.

Hjerde, T., Kristiansen, T. S., Stokke, B. T., Smidsrod, O., & Christensen, B. E. (1994). Conformation-dependent depolymerization kinetics of polysaccharides studied by viscosity measurements. *Carbohydrate Polymers*, 24 (4), 265–275.

Hormdok, R., & Noomhorm, A. (2007). Hydrothermal treatments of rice starch for improvement of rice noodle quality. *LWT-Food Science and Technology*, 40, 1723–1731.

Imberty, A., Buléon, A., Tran, V., Perez S. (1991). Recent advances in knowledge of starch structure. *Starch*, 43 (10), 375–384.

Izydorczyk, M.S., Storsley, J., Labossiere, D., Mac. Gregor, A.W., & Rossnagel, B.G. (2000). Variation in total and soluble β-glucan content in hulless barley: Effects of thermal, physical and enzymatic treatments. *Journal of Agricultural and Food Chemistry*, 48, 982–989.

Jiaxun-Tao; Rao, R. & Liuuzo, J. (1993). Microwave heating for rice bran stabilization. *Journal of Microwave, Power and Electromagnetic Energy*, 28 (3), 156-164, ISSN-0832-7823.

Jyothi, A. N., Sajeev, M. S., & Sreekumar, J. N. (2010). Hydrothermal modifications of tropical tuber starches. 1. Effect of heat-moisture treatment on the physicochemical, rheological and gelatinization characteristics. *Starch*, 62 (1), 28-40.

Lan, H., Hoover, R., Jayakody, L., Liu, Q., Donner, E., Baga, M., et al. (2008). Impact of annealing on the molecular structure and physicochemical properties of normal, waxy and high amylose bread wheat starches. *Food Chemistry*, 111, 663–675.

Lazaridou, A., & Biliaderis, C.G. (2007). Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science*, 46, 101–118.

Lazaridou, A., Marinopoulou, A., Matsoukas, N. P., & Biliaderis, C. G. (2014). Impact of flour particle size and autoclaving on β-glucan physicochemical properties and starch digestibility of barley rusks as assessed by in vitro assays. *Bioactive Carbohydrates and Dietary Fibre*, 4 (1), 58-73.

Lewandowicz, G., Fornal, J., & Walkowski, A. (1997). Effect of microwave radiation on potato and tapioca starches. *Carbohydrate Polymers*, 34, 213-220.

Lewandowicz, G., Jankowskib, T., & Fornal, J. (2000). Effect of microwave radiation on physicochemical properties and structure of cereal starches. *Carbohydrate Polymers*, 42, 193–199.

Luo, Z., He, X., Fu, X., Luo, F., & Gao, Q. (2006). Effect of microwave radiation on the physicochemical properties of normal corn, waxy corn and amylomaize V starches. *Starch-Starke*, 58, 468–474.

Maache-Rezzoug, Z., Zarguili, I., Loisel, C., Queveau, D., & Buléon, A. (2008). Structural modifications and thermal transitions of standard maize starch after DIC hydrothermal treatment. *Carbohydrate Polymers*, 74, 802–812.

Moriartey, S., Temelli, F., & Vasanthan, T. (2010). Effect of formulation and processing treatments on viscosity and solubility of extractable barley beta-glucan in bread dough evaluated under in vitro conditions. *Cereal Chemistry*, 87(1), 65-72.

Olayinka, O. O., Adebowale, K. O., & Olu-Owolabi, B. I. (2008). Effect of heat-moisture treatment on physicochemical properties of white sorghum starch. *Food Hydrocolloids*, 22, 225–230.

Perez-Quirce, S., Collar, C., & Ronda, F. (2014). Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads. *International Journal of Food Science and Technology*, 49, 1375-1382.

Pinkrová, J., Hubáčková, B., Kadlec, P., Příhoda, J.,& Bubník, Z. (2003). Changes of starch during microwave treatment of rice. *Czech Journal of Food Sciences*, 21, 176–184.

Rieder, A., Ballance, S., Knutsen, S.H. (2015). Viscosity based quantification of endogenous β-glucanase activity in flour. *Carbohydrate Polymers*, 115, 104–111.

Roman, L., Martinez, M. M., Rosell, C. M., & Gomez, M. (2015). Effect of microwave treatment on physicochemical properties of maize flour. *Food and Bioprocess Technology*, 8(6), 1330-1335.

Ronda, F., Perez-Quirce, S., Lazaridou, A., & Biliaderis, C. G. (2015). Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics. *Food Hydrocolloids, 48*, 197-207.

Rosell C.M., Barro, F.; Sousa C., Mena, M.C. (2014), Cereals for developing gluten-free products and analytical tools for gluten detection. *Journal of Cereal Science*, 59, 354-364.

Scientific opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to article 13(1) of Regulation (EC) No 1924/2006, 2207. (2011).

Stevenson, D. G., Biswas, A., Inglett, G. E. (2005). Thermal and pasting properties of microwaved corn starch. *Starch*, 57 (8), 347-353.

Sumnu, G. (2001). A review on microwave baking of foods. *International Journal of Food Science and Technology*, 36, 117–127.

Thomson, T. (2009). The nutritional quality of gluten-free foods. In: Gluten-Free Food Science and Technology (edited by E. Gallagher). pp. 45–51. New Delhi: Wilet-Blackwell.

Tosh, S. M., Brummer, Y., Wolever, T. M. S., & Wood, P. J. (2008). Glycemic response to oat bran muffins treated to vary molecular weight of β-glucan. *Cereal Chemistry*, 85, 211–217.

Tosh, S. M. (2013). Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products. *European Journal of Clinical Nutrition*, 67, 310–317.

Trogh, I., Courtin, C. M., Andersson, A. A. M., Aman, P., Sorensen, J. F., & Delcour, J. A., (2004). The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1/3, 1/4)- β-D-glucan levels. *Journal of Cereal Science*, 40, 257-267.

US Food and Drug Administration (USFDA) (2005). Food labeling: Soluble dietary fibre from certain foods and coronary heart disease. Federal Register, 70, 76150–76162.

Vatandoust, A., Ragaee, S., Wood, P. J., Tosh, S. M., & Seetharaman, K. (2012). Detection, localization, and variability of endogenous beta-glucanase in wheat kernels. *Cereal Chemistry*, 89 (1), 59-64.

Watcharatewinkul, Y., Puttanlek, C., Rungsardthong, V., & Uttapap, D. (2009). Pasting properties of a heat-moisture treated canna starch in relation to its structural characteristics. *Carbohydrate Polymers*, 75, 505–511.

Wolever, T. M. S., Tosh, S. M., Gibbs, A. L., Brand-Miller, J., Duncan, A. M., Hart, V., *et al.* (2010). Physicochemical properties of oat β-glucan influence its ability to reduce serum LDL cholesterol in humans: A randomized clinical trial. *The American Journal of Clinical Nutrition*, 92, 723–732.

Zavareze, E. R. & Dias, A. R. G. (2011). Impact of heat–moisture treatment and annealing in starches: A review. *Carbohydrate Polymers*, 83, 317–328.

Zhang, D., Doehlert, D. C., & Moore, W. R. (1998). Rheological properties of (1-3), (1-4) β-D-glucans from raw, roasted and steamed oat groats. *Cereal Chemistry*, 75, 433–438.

Zhao, S., Xiong, S., Qiu, C., Xu, Y. (2007). Effect of microwaves on rice quality. *Journal of Stored Products Research,* 43, 496–502.

Zhong, Y., Tu, Z., Liu, C., Liu, W., Xu, X., Ai, Y., et al. (2013). Effect of microwave irradiation on composition, structure and properties of rice (*oryza sativa* L.) with different milling degrees. *Journal of Cereal Science*, 58 (2), 228-233.

Zobel, H. F. (1964). X-ray analysis of starch granules. Page 109 in: Methods in Carbohydrate Chemistry. Vol. 4. R. L. Whistler, ed. Academic Press: Orlando, FL.

CAPÍTULO VI

Effect of microwave radiation pre-treatment of rice flour on gluten-free breadmaking and molecular size of β-glucans in the fortified breads *

* Sandra Pérez-Quirce[a], Felicidad Ronda[a]*, Athina Lazaridou[b], Costas G. Biliaderis[b]. (2016). "Effect of microwave radiation pre-treatment of rice flour on gluten-free breadmaking and molecular size of β-glucans in the fortified breads". Food Bioprocess Technology. DOI: 10.1007/s11947-017-1910-7.

Effect of microwave radiation pre-treatment of rice flour on gluten-free breadmaking and molecular size of β-glucans in the fortified breads

Sandra Perez-Quirce[1], Felicidad Ronda[1*], Athina Lazaridou[2], Costas G. Biliaderis[2]

[1]Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

[2]Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 235, Thessaloniki, Greece, 541 24.

Abstract

Cereal β-glucan concentrates can be used in gluten-free breads to improve dough handling properties and quality of final products as well as to enhance their nutritional value; however, the presence of endogenous β-glucanases in rice flour, in combination with prolonged mixing, fermentation and proofing time, can cause a substantial reduction in β-glucan molecular weight, affecting detrimentally their efficacy for bioactivity. In this study, microwave (MIWA) heating was applied to the rice flours before breadmaking at different flour water contents (13-25%) and treatment times (0- 4min) to reduce β-glucanase activity. Gluten-free breads made from the MIWA treated rice flours were fortified with oat β-glucan concentrate to enhance their nutritional profile. The molecular weight of added β-glucan in the final products increased with increasing both flour water content and time of MIWA treatment, reflecting the magnitude of residual β-glucanase activity in the flour. Pretreatment with MIWA radiation for 4 min of the rice flour tempered at 25% moisture resulted in negligible residual β-glucanase activity and preserved to a great extent the molecular weight of β-glucans in the enriched breads. End-product quality was not affected by flour MIWA pretreatment, and even a slightly higher loaf specific volume was noted for breads made from the MIWA -treated flours (4min MIWA at 25% moisture content) compared to that of untreated flour. These findings

can contribute to the improvement of nutritional value of rice-based gluten-free breads for celiac consumers as well as of any β-glucan containing yeast-leavened bakery product without altering its sensorial attributes. Additional studies are still required for further evaluation of the effect of more intense microwave treatment on rice flour and its application on breadmaking.

Keywords: β-glucan molecular weight; β-Glucanase inactivation; gluten-free bread; microwave treatment; rice flour

1. Introduction

Celiac disease (CD) is a genetically linked autoimmune disorder affecting the gastrointestinal system and characterized by life-long intolerance to the ingestion of gluten. CD has a diverse clinical picture ranging from tangible symptoms such as nutrients malabsorption, diarrhea, weight loss, and abdominal discomfort to vaguer symptoms such as iron and folic acid deficiency, arthralgia, fatigue, and osteoporosis (Sollid & Lundin, 2009). The demand of gluten-free (GF) products is increasing as a result of the increased numbers of CD diagnosed patients. Market trends have encouraged extensive research for the development of gluten-free products, especially breads and other bakery items (Houben et al. 2012; Foschia et al. 2016). Generally, dough and bread development without gluten involves the use of diverse ingredients and additives with the aim of imitating the viscoelastic properties of gluten and thereby obtaining high quality products (Demirkesen et al. 2014, Hager and Arendt, 2013, Sciarini et al. 2010). Hydrocolloids are one group of polysaccharides which can fulfil this need. They are used in gluten-free breads to improve the dough handling properties and enhance the quality attributes (volume, texture, moisture retention, etc.) and shelf-life of bread (Ahmad et al. 2016). Moreover, some hydrocolloids, belonging to the dietary fibers group, are used in dough formulations to enhance the nutritional value of gluten-free breads. Nowadays, a wide range of non-starch polysaccharides, including cereal β-glucan (BG), are thought to exert several nutritive and physiological effects in the human digestive track.

The US Food and Drug Administration (USFDA) (2005) and recently, the European Food Safety Authority (EFSA) have approved health claims according to which barley and oat β-glucan ingestion, with a daily consumption of 3g of β-glucan soluble fiber, leads to the reduction of blood plasma cholesterol levels, which is a major risk factor for the development of coronary heart disease (EFSA 2011a). Other health claims for oat and barley β-glucans were also approved by EFSA concerning the reduction in post-prandial glycemic responses, at recommended doses of about 4g of β-glucans per 30g of available carbohydrates in bread and pasta products (EFSA 2011b), and the increase of faecal bulk (EFSA 2011c); the latter claim can be used for foods containing barley or oat grain fiber of at least 6g/100g product or 3g/100 kcal.

Our previous works have demonstrated the potential of baking rice-based gluten-free (GF) breads enriched with commercial BG concentrates to fulfill the EFSA health claim requirements as well as to provide products with acceptable quality (Perez-Quirce et al. 2014; Ronda et al. 2015). However, a reduction of the BG molecular weight in rice-based GF breads compared with that of the initial concentrates used as ingredients in the formulation mixture has been noted probably implying the presence of endogenous β-glucanase activity in rice flour (Hager et al. 2011; Ronda et al. 2015). It seems to be necessary to minimize BG depolymerization during food processing in order to retain the full physiological impact of β-glucans in formulated products, since is related to the viscosity of β-glucan aqueous dispersions; the latter is a function of molecular weight and concentration of the polysaccharide (Tosh, 2008; Wood, 2007; Wolever et al. 2010).

The activity of endogenous flour β-glucanases, in combination with the long contact time during mixing, fermentation and proofing, can cause a substantial reduction in β-glucan molecular weight during production of β-glucan containing yeast-leavened baked products (Aman et al. 2004; Andersson et al. 2004; 2009; Lazaridou et al. 2014; Ronda et. al. 2015; Trogh et al. 2004). The inactivation of flour enzymes and the use of relatively short processing time have been proposed as effective means to minimize β-glucan degradation (Andersson et al. 2004; Lazaridou et al. 2014; Moriartey et al. 2010; Vatandoust et al. 2012). Some methods previously used for the β-glucanase inactivation were autoclaving, scalding, oven heating, microwave

heating and ethanol refluxing (Lazaridou et al. 2014; Rieder et al. 2015; Moriartey et al. 2010; Perez-Quirce et al. 2016). Among these options, the process with the greatest potential on the inactivation of endogenous β-glucanase rice flour seemed to be microwave heating since it has been previously found that a treatment for just 4 min after tempering of the flour to 25% moisture content resulted in enzyme inactivation (Perez-Quirce et al. 2016); moreover, the degree of crystallinity of the starch was unaffected and the side effects of such treatment on flour pasting and thermal properties were rather negligible. Lazaridou et al. (2014) and Perez-Quirce et al. (2016) have recently evaluated the β-glucanase inactivation including the flour tempering up to certain moisture level before the thermal treatments as a critical parameter for the sufficient enzyme inactivation. However, maintaining the molecular weight of β-glucans in rice-based breads when they are fortified with these polysaccharides has not been yet verified. Furthermore, the effect of the microwave heating on quality attributes of the gluten-free breads made with the microwave-treated rice flours is still unknown.

Therefore, in the present study gluten-free breads fortified with a high molecular weight β-glucan concentrate were made from rice flours pretreated by microwave (MIWA) radiation at different times and moisture levels aiming at the retention of β-glucan molecular size in breads and hence at maximizing their physiological functionality. Further to the amount and molecular weight of β-glucans found in the end-products, the effects of rice flour heat treatment on physical properties of flours and gluten-free breads were also explored.

2. Materials and methods

2.1. Materials

Five samples of rice flour, varying in water content and time of microwave heating were examined in this work. Rice flour from an Indica variety was supplied by Herba Ricemills SLU (Tarragona, Spain), having 13.12% moisture, 79.1% starch, 0.46% ash, 7.5% protein and 0.49% fat. The particle size distribution of the flour was 6% > 150 μm, 150 μm > 63.2% > 100 μm and 30.8% < 100 μm according to data provided by the manufacturer. Different combinations of flour water content and time of

MIWA treatment that could lead to five almost equally-spaced residual β-glucanase activities, corresponding to about 0, 25, 50, 75 and 100% β-glucanase inactivation, have been tested. These treatment conditions are shown in Table 1 and were adopted according to the findings from a previous work (Perez-Quirce et al. 2016).

A high molecular weight oat (1→3) (1→4)-β-D-glucan concentrate available on the market (Promoat™) was supplied by Biovelop AB (Kimstad, Sweden) and was further purified. The proximate composition of this commercial concentrate as provided by the supplier was: 6% moisture, 54-56% carbohydrates (dextrins), <4.5% protein, 1-3 % ash and 0.5-1 % fat; β-glucan content 33-36%. The purification protocol involved aqueous extraction of the polysaccharide (50^{o}C x 1 h) using an aqueous slurry of the Promoat™ flour (1:40 solids:liquid) followed by centrifugation (2400 g x 30 min). The supernatant was digested (37^{o}C x 3 h) by porcine pancreas α-amylase (100.000U/g flour, Megazyme International Ltd, Bray, Ireland), concentrated (90^{o}C x 2 h to ½ of the volume) and the β-glucan was precipitated with two volumes of ethanol (4^{o}C x 24 h). Finally, the polysaccharide was re-suspended in 2-propanol (4^{o}C x 24 h), filtered and dried (50^{o}C x 18 h) to obtain a high molecular weight β-glucan preparation (HMW-BG). As a result of this purification scheme, the Promoat™ was concentrated from 33 to 72 % β -glucan content (HMW-BG) as assessed by the mixed-linkage (1→3), (1→4) β-D-glucan assay kit purchased from Megazyme.

The ingredients used in the breadmaking process, like salt, sugar and sunflower oil were obtained from the local market, whereas the hydroxy-propyl-methyl-cellulose (HPMC) 4 KM preparation was a gift from Dow Chemical (Midland, EEUU).

2.2 Methods

2.2.1 Microwave treatment of rice flour samples

Rice flours were heated in a Panasonic Inverter NN-GD566M (Osaka, Japan) microwave oven following the method previously developed by Perez-Quirce et al. (2016), according to which samples of hydrated flours (50.0 g) were introduced and hermetically closed into polyamide and polypropylene bags and subsequently heat-

treated with microwave power (900 W) applied in cycles of 20 seconds intervals combined with downtimes of 1 min. Several batches from each treatment were processed and then mixed in order to obtain the required amount of flour required for the breadmaking.

A particular attention was paid to achieve a uniform temperature and constant humidity during the MIWA process. The moisture contents of the flour before and after the microwave treatment were determined following the AACC 44-19 method, and the water required to adjust it to a certain value was calculated as in a previous work (Perez-Quirce et al. 2016); the tempering procedure of flours before MIWA treatment was also described in the latter study.

2.2.2 β-Glucanase activity determination

β-Glucanase activity in control (untreated) and heat treated rice flours was assessed by measuring the rate of decrease in specific viscosity of a dilute solution of a pure β-glucan preparation, following addition of flour extracts, according to a method described in our previous works (Lazaridou et al. 2014; Perez-Quirce et al. 2016); flour extracts (1:10 w/w rice flour:water) were obtained by aqueous extraction (25^{o}C x 25 min) and they were mixed with an aqueous solution (0.1% w/v) of a high molecular weight ($2x10^{6}$) β-glucan preparation (purity 95%). The mixture was then transferred into an Ubbelohde glass capillary viscometer (UBBEL04NC, K 0.01, range 2-10 cSt, brand Paragon Scientific Ltd, Wirral, UK) and the specific viscosity ($\eta_{sp} = (\eta-\eta_0)/\eta_0$, where η and η_0 is the viscosity of above mixture and water, respectively) was measured over 1h period at 20±0.1 ºC every 5 min intervals. The η_{sp} data versus time were fitted to a linear regression model and the β-glucanase activity in rice flours was calculated from the slope of the fitted line and expressed as the decrease in specific viscosity per hour of the pure β-glucan solution upon addition of the flour extracts. Residual β-glucanase activity of each treated flour sample as well as enzyme activity of the untreated flour were analyzed at least in triplicate.

2.2.3 Pasting properties of rice flour aqueous dispersions

Pasting properties were studied in the microwave-treated and control flours by the Rapid Visco Analyzer (RVA-4, Newport Scientific Pvt. Ltd., Australia) using the ICC Standard method 162. The pasting temperature, peak viscosity, holding strength or trough viscosity, as well as breakdown, final and setback viscosity were calculated from the pasting curve using the Thermocline v.2.2 software. Viscoamylography of aqueous flour dispersions (3 g of 14% moisture basis flour in 28 g of total weight) was carried out in triplicate.

2.2.4 Breadmaking process

A straight dough process was performed using the following formula on a 100 g rice flour basis: 92% water, 6% oil, 5% sucrose, 2% HPMC, 1.8% salt and 3% dried yeast. GF dough-making was achieved by blending the solid ingredients first in a kitchen-aid professional mixer (Model 5KPM50, Kitchen Aid, St. Joseph, MI, USA) for 2 min at speed 2. Then, the liquid ingredients (oil and water at 20 ± 2 °C) were added and mixed for 8 min at speed 4. The dough (200 g) was placed into an aluminum pan and proofed at 30 °C and 90% relative humidity for 50 min. Subsequently, baking was carried out in a Sveba Dahlen oven (Fristad, Sweden) at 170°C for 20 min with steam injection for 7s at the beginning of the process. After baking, breads were removed from the pan and stored for one hour at room temperature before further analysis. Breads from MIWA treated rice flours enriched with the HMW-BG preparation were also made at 3.9 % of pure β-glucan fortification level following the same breadmaking process.

2.2.5 Content and molecular weight determination of β-glucan in breads

The content and molecular weight of the added BG in enriched breads were determined to evaluate any change during the breadmaking process due to endogenous β-glucanase activity of rice flour. The concentration of BG in bread was determined using the mixed-linkage (1→3)(1→4)β-D-glucan assay kit of Megazyme. For molecular weight evaluation, the β-glucans were firstly extracted from the fortified breads according to an isolation protocol described in details elsewhere (Lazaridou et al. 2014); this includes an aqueous digestion with Termamyl 120 L (1% v/v, Novozymes), followed by a suspension with Fuller's earth for protein

removal, hydrolysis with xylanase (100U/100g bread, Megazyme), exhaustive dialysis, concentration and repeated precipitations with ethanol. The content of β-glucan concentrates derived from the bread crumbs were 55-80% as assessed by the respective assay kit of Megazyme.

The apparent peak molecular weight (Mp) of the extracted-BG from breads was estimated following the method described in detail by Lazaridou et al. (2004; 2014) using a high performance size exclusion chromatography system (HPSEC) system, which consisted of a single pump (Marathon IV, Rigas Labs, Thessaloniki, Greece), a guard TSKPWH column and two SEC columns in series, 7.5x300 mm TSK G6000 PW and 7.5x600 mm TSK G5000 PW (Tosoh Bioscience GmbH, Stuttgart, Germany), and a refractive index (RI) detector (ERC-7515A, ERC- Inc. Nishiaoki, Kawaguchi-City, Japan).

2.2.6 Evaluation of bread quality

For loaf specific volume determination, breads were weighed after removal from the pan and cooling down and the loaf volume was measured in four replicates using a Volscan profiler 300 analyser (Stable Microsystems, Surrey, UK). Texture parameters of the bread crumb were evaluated in quadruplicate samples with a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) using the software "Texture Expert"; for this analysis an aluminum 20 mm diameter cylindrical probe was employed to submit crumb specimens to a two-cycle compression test (Texture Profile Analysis, TPA) at 1 mm/s speed test, with a 30 s delay between first and second compression, and at a deformation level up to 50%. This test was carried out at 20 ± 3 ºC on bread slices, with 20 mm thickness, taken from the center of each loaf. Hardness (N), chewiness (N), cohesiveness, springiness and resilience of the bread crumb were calculated from the TPA curves.

Bread crust colour was measured with a Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Japan); results were obtained in the CIE L*a*b* and CIE L*C*h coordinates using the D65 standard illuminant, and the 2º standard observer (International Commission on Illumination, CIE). Colour determinations were made 4 x 4 times; i.e. colour parameters were measured in four different bread loaves at four different points of their crust.

2.2.7 Statistical analysis

The Statgraphics Centurion v.6 (Bitstream, Cambridge, MN, USA) was used for ANOVA analysis, and significant differences ($p<0.05$) between samples were identified by the LSD (Least Significant Difference) test.

3 Results and discussion

3.1 Effect of microwave treatment on β-glucanase activity of rice flours

The β-glucanase activity in rice flour estimated from the rate of decrease in η_{sp} of a β-glucan solution-flour extract mixture significantly ($p<0.05$) decreased with increase of MIWA treatment time and flour moisture content before heating (Table 1). The enzyme activity seemed to be eliminated when 4 min heating by microwave radiation applied to rice flour tempered at 25% moisture content before the treatment; these findings are in agreement with our previous study (Perez-Quirce et al. 2016). The flour temperatures at the end of the MIWA treatment were 84ºC (treatment 2), 93ºC (treatment 3) and 96ºC (treatments 4 and 5). The five microwave treatments (including no treatment) led to rice flours with β-glucanase activities 100%, ~75%, ~50%, ~25% and 0% of the value of the native flour (Table 1). This range allowed us to study the effect of β-glucanase activity of rice flour on the molecular weight of the resultant BG in the enriched breads.

Table 1. Experimental design and residual β-glucanase activities of rice flours treated by microwave energy.

Microwave (MIWA) treatment	Flour water content (%fb) [b]	Treatment time (min)	β-glucanase activity [c] (h^{-1}) [d]
1	13	0	0.109 (±0.005) e
2 [a]	16	1	0.076 (±0.001)d
3 [a]	16	2	0.052 (±0.010)c
4 [a]	16	4	0.022 (±0.009)b
5 [a]	25	4	0.000 (±0.001)a

[a] Flour tempered before the microwave treatment to increase its initial moisture level (13%) to the specified level.
[b] Moisture content of flours after the microwave treatment.
[c] Values are means of duplicate treatments and duplicate measurements. Values with the same letter are not significantly different ($p > 0.05$); means were compared using the LSD test.
[d] Expressed as decrease in specific viscosity per hour of a purified β-glucan solution (0.1 % w/v) following addition of rice flour extracts (1:10 w/w rice flour:water).

3.2 Pasting properties of rice flours

The pasting properties of aqueous dispersions of the treated rice flours were studied to evaluate the impact of microwave heat treatment on starch functionality. Minor differences were noted between the pasting properties of the native rice flour and those of the microwave treated samples (Table 2) in agreement with our previous study (Perez-Quirce et al. 2016). Significant, although small, differences ($p < 0.05$) were only obtained between the control and the most intense microwave treated flours, i.e., the sample tempered at the highest moisture level (25%) and submitted to the longest treatment time (4 min). The slight increase in trough viscosity in combination with the decrease in breakdown viscosity of the latter flour implies that microwave treated flours are more stable during continuous heating and agitation as have been reported by several researchers for other hydrothermally treated flours (Adebowale et al. 2005; Hormdok & Noomhorm, 2007; Olayinka et al. 2008; Watcharatewinkul et al. 2009). Such changes in pasting properties of heat treated starches have been attributed to associations between the polymeric chains in the

amorphous regions of the starch granule as well to changes in crystallinity caused by the hydrothermal treatment. The structural modifications were found more pronounced as the flour moisture content before the hydrothermal treatment increased (Olayinka et al. 2008). As the intra-granular chain interactions strengthen (annealing effects), the reorganized starch structures require more heat energy for structural disintegration and paste formation; i.e., a higher pasting temperature, as found in the current study (Table 2), indicates a more dense cross-linking within the starch granules. A decrease of the peak viscosity was observed by Pinkrová et al. (2003) by increasing temperature and power output upon microwave treatment applied on rice grain at a moisture level of 30 %. Luo et al. (2006) have reported even more marked changes in the starch granule structure when maize starch tempered at 30% moisture content and treated for 20 min with microwaves at 1 W/g. The absence of such effects in our MIWA treated flours could be due to the shorter treatment times and the lower MIWA power applied as well as the use of completely airtight bags, in which water loss from the sample was probably hindered by the vapor pressure raised within the bag headspace (Perez-Quirce et al. 2016). Overall, the proposed MIWA treatment of rice flour, besides the efficient elimination of β-glucanase activity that was the main goal of the hydrothermal process, only slightly affected starch functionality.

Table 2. Effects of microwave treatment on the viscometric parameters of rice flours (RVA viscoamylography).

Microwave (MIWA) treatment	Peak viscosity (mPa·s)	Trough viscosity (mPa·s)	Breakdown viscosity (mPa·s)	Final viscosity (mPa·s)	Setback viscosity (mPa·s)	Peak tempearture (ºC)
1	2336 a	1406 a	862 b	3271 a	1866 a	78.8 ab
2	2362 a	1448 a	925 b	3305 a	1879 a	78.4 a
3	2364 a	1462 ab	908 b	3332 ab	1870 a	79.2 ab
4	2478 a	1563 b	877 b	3458 b	1904 a	79.9 bc
5	2416 a	1681 c	745 a	3466 b	1885 a	80.9 c
SE	52.1	31.6	23.1	39.1	21.5	0.4

Mean values with different letters for the same parameter imply significant differences between means at $p<0.05$. SE: Pooled Standard Error obtained from ANOVA.

3.3 Effect of microwave treatments on gluten-free bread quality attributes

To explore the effect of the MIWA treatment on breadmaking properties of flours, breads were prepared using the microwave treated rice flour under different conditions that result in various flour β-glucanase activities. The quality characteristics of the resultant breads are summarized in Table 3. Breads made from the most intensively treated flour (MIWA treatment 5) reached the highest specific volume among all breads, ~ 7% higher than that of the control flour (MIWA treatment 1). On the other hand, the intermediate treated flours (MIWA treatments 2 and 3) led to lower specific volumes, with a maximum decrease of 14% compared with the bread loaves which had the highest specific volume. This could be attributed to the high temperature reached during these MIWA treatments (84ºC and 93ºC, respectively) that can denature other enzymes than β-glucanases, including the α-amylases necessary for bread development (Caballero et al. 2007; Gujral et al. 2003); the reduction of α-amylase activity would explain the lower loaf volume. However, when the MIWA treatment became more intense (treatments 4 and 5), possibly the effect derived from the reduction of α-amylase activity is masked by the differences in the pasting properties, e.g. higher pasting temperature, peak viscosity and final viscosity, which led to higher bread volume during the baking step compared to the control product. The higher viscosity of the dough matrix might restrict the coalescence phenomenon and allow a better retention of the gas produced during fermentation. At the same time the higher pasting temperature would allow a greater development of the dough during baking before the fixation of the crumb structure upon baking (Ronda et al., 2016). Nevertheless, there were no large differences either in volume and appearance of bread loaves or crumb structure with the use of MIWA pre-treated rice flours (Table 3 and Figure 1).

The crumb hardness values varied between 0.67 and 1.02 N (Table 3). Breads with the lowest specific volume showed the higher hardness, in agreement with the negative correlation between these parameters reported in earlier works (Perez-Quirce et al. 2014; Ronda et al. 2015); i.e., the lower the specific volume, the smaller crumb air fraction and thus the more compact the structure. However, there was no apparent trend between the intensity of MIWA treatment and crumb hardness (Table 3). The treated flour breads showed lower resilience than the control bread, whereas

breads made with untreated rice flours exhibited the highest crumb chewiness among all samples. Springiness of the control bread and those of the less treated flours were significantly lower than breads from flours heated for longer time. Instead, crumb cohesiveness was not affected by rice flour pre-treatment. However, the differences in texture parameters noted among the tested samples did not seem to be large. Furthermore, the flour tempered at the highest moisture level (25%) gave loaves with the highest water loss during the baking process; i.e. ~ 7.4% higher weight loss than that of the control bread (Table 3). However, as can be seen in Table 4 the bread moisture content was not significantly different among all breads studied.

Regarding the crust colour, breads made from flour with the greatest extent of enzyme inactivation (MIWA treatment 5) showed the highest L* value (Table 3). In addition, h and C* values increased as the MIWA treatment time of the flour increased. This means that bread made with treated flours were more yellowish, lighter and with more vivid colours than the control bread. It is possible that some of the enzymes responsible for colour development (α-amylases) have been partially inactivated by the heat treatment and thus the colour is lighter since the Maillard reactions proceed at a lower rate and extent than in breads from the untreated flour (control); i.e. a lower concentration of reducing sugars in the fermented dough (Pyler et al., 2000).

Table 3. Effects microwave treatment of rice flour on physical properties of the resultant breads.

Microwave (MIWA) treatment	Specific volume (mL/g)	Loss of weight (%)	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience	Crust L *	Crust a	Crust b	Crust h	Crust C *
1	3.384 c	15.73 ab	0.900 c	0.8851 b	0.6424 b	0.511 c	0.3888 d	60.20 b	14.43 ab	22.51 a	57.32 a	26.74 a
2	3.231 b	15.83 b	0.669 a	0.8088 a	0.6439 b	0.294 a	0.3688 c	61.54 b	14.21 ab	25.98 b	61.34 b	29.62 b
3	3.122 a	14.88 a	1.019 d	0.9226 c	0.5847 a	0.337 ab	0.3484 b	58.31 a	15.41 c	29.33 c	62.27 b	33.14 c
4	3.435 d	15.18 ab	0.796 b	0.9219 c	0.6283 b	0.460 c	0.3485 b	60.59 b	14.47 b	29.81 c	64.08 c	33.15 c
5	3.649 e	16.98 c	0.967 cd	0.9050 bc	0.6419 b	0.356 b	0.2952 a	64.55 c	13.77 a	29.44 c	64.94 c	32.50 c
SE	0.015	0.25	0.032	0.0085	0.0053	0.017	0.0042	0.59	0.24	0.28	0.49	0.26

Mean values with different letters for the same parameter imply significant differences between means at $p<0.05$. SE: Pooled Standard Error obtained from ANOVA.

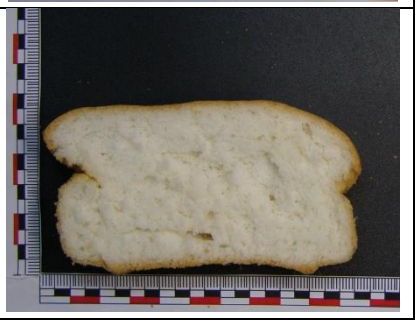
Microwave (MIWA) treatment	External appearance	Crumb structure
1		
2		
3		
4		
5		

Figure 1. Effect of flour microwave treatment on the external appearance and internal crumb structures of gluten-free rice-based breads.

3.4 Effect of β-glucanase inactivation of flour on molecular weight of β-glucan isolated from in fortified gluten-free breads

The β-glucan content in the final products fortified with the β-glucan isolate was similar to that expected from the amount of polysaccharide added to the dough formulation (Table 4); a slight decline in the BG content in breads was noted only in products made by flours with high β-glucanase activity (MIWA treatment 1 and 2) (Tables 1 and 4). The level (3.9% of pure β-glucan on rice flour basis) of HMW-BG added to the gluten-free formulations can meet the health claim requirements of US Food and Drug Administration (FDA, 2005) and EFSA (EFSA, 2011) for the reduction of serum cholesterol and can be accomplished by a daily intake of ~ 170 – 214 g product which on average, corresponds to four servings of 50 g of the GF fortified with β-glucan breads.

Table 4. β-Glucan (BG) content added to dough and measured in bread and bread intake to fulfil the EFSA claim (cholesterol reduction).

Microwave (MIWA) treatment	BG added to the dough (% dry matter)	BG content measured in bread (% dry matter)	Bread moisture content (%)	BG content (% in bread)	Bread intake to fulfil the EFSA claim (g)
1	3.2	2.8 (± 0.12)a	49.3 (± 1,08)a	1.4	214
2	3.2	3.0 (± 0.16)ab	49.2 (± 0,92)a	1.5	200
3	3.2	3.2 (± 0.16)ab	48.7 (± 1,07)a	1.7	181
4	3.2	3.4 (± 0.22)b	48.0 (± 0,90)a	1.8	169
5	3.2	3.1 (± 0.09)ab	50.4 (± 1,10)a	1.5	197

Mean values with different letters for the same parameter imply significant differences between means at $p<0.05$.

The molecular weight distributions of the HMW-BG preparation added to the gluten free formulations as well as the β-glucan concentrates derived from breads made from rice flours submitted to different microwave pre-treatments were analysed by a HPLC-SEC-RI system to evaluate any changes in molecular weight of the polysaccharide during breadmaking (Figure 2); the Mp values of these preparations were estimated from the peak fraction of the eluting peaks of the polysaccharides

using a calibration curve made with β-glucan standards. A large portion of the eluting peak and the peak fraction of the HMW-BG sample were eluted in the void volume of the size exclusion columns, i.e. the β-glucan added to the gluten-free doughs had a Mp > 9 x 10^5 Da. However, for the β-glucans isolated from the control bread made from the untreated rice flour there was a large reduction in Mp, as the main eluting peak was ~ 0.23 x 10^5 Da, presumably due to polysaccharide degradation by endogenous β-glucanases of the rice flour during the breadmaking process (Table 1). Similarly, several researchers have previously noted a considerable reduction of β-glucan molecular weight during production of yeast-leavened bakery products from oat bran as well as rye and barley flour which was attributed to endogenous β-glucanase activity (Aman et al. 2004; Andersson et al. 2004; 2008; 2009; Lazaridou et al. 2014; Trogh et al. 2004); apparently, the molecular weight of β-glucans in the final products decreases with increasing mixing and dough fermentation time. Recently, an endogenous β-glucanase activity was also found in rice flour (Perez-Quirce et al. 2016) which can cause severe reduction of oat and barley β-glucans during breadmaking when these polysaccharides are added as concentrates to rice-based gluten-free bread formulations (Ronda et al. 2015).

Tempering of rice flour up to 16% moisture content followed by 1 min microwave heating resulted in bread with higher Mp (0.61 x 10^5 Da) compared to that made from the untreated flour (Figure 2), presumably due to a partial decrease in β-glucanase activity as evidenced from the data in Table 1. A minor fraction of β-glucans in both breads from the control and this treated (MIWA treatment 1) flour preserved their initial molecular size, since small peaks representing 3% and 6% of the chromatograph area, respectively, had Mp>9 x 10^5 Da (Figure 2, slanted dotted arrow). With increasing time of MIWA treatment up to 4 min for the flour tempered at 16% moisture there was a gradual increase of β-glucan molecular weight as shown by the shifting of polysaccharide eluting peaks to lower retention times and increase of Mp up to 4.37 x 10^5 Da (Figure 2); this observation is consistent with the decline in β-glucanase activity of the respective treated flours (Table 1). The eluting profile of β-glucans in bread prepared from the flour heated with MIWA for 2 min showed two main peaks with Mp values of 1.41 and 0.23 x 10^5 Da which represent 61 and 39% of the total chromatograph area, respectively (Figure 2). Similar bimodal

molecular weight distributions of β-glucans isolated from oat, rye and barley breads have been previously reported and attributed also to the endogenous β-glucanase action in the flours (Aman et al. 2004; Andersson et al. 2004; 2009).

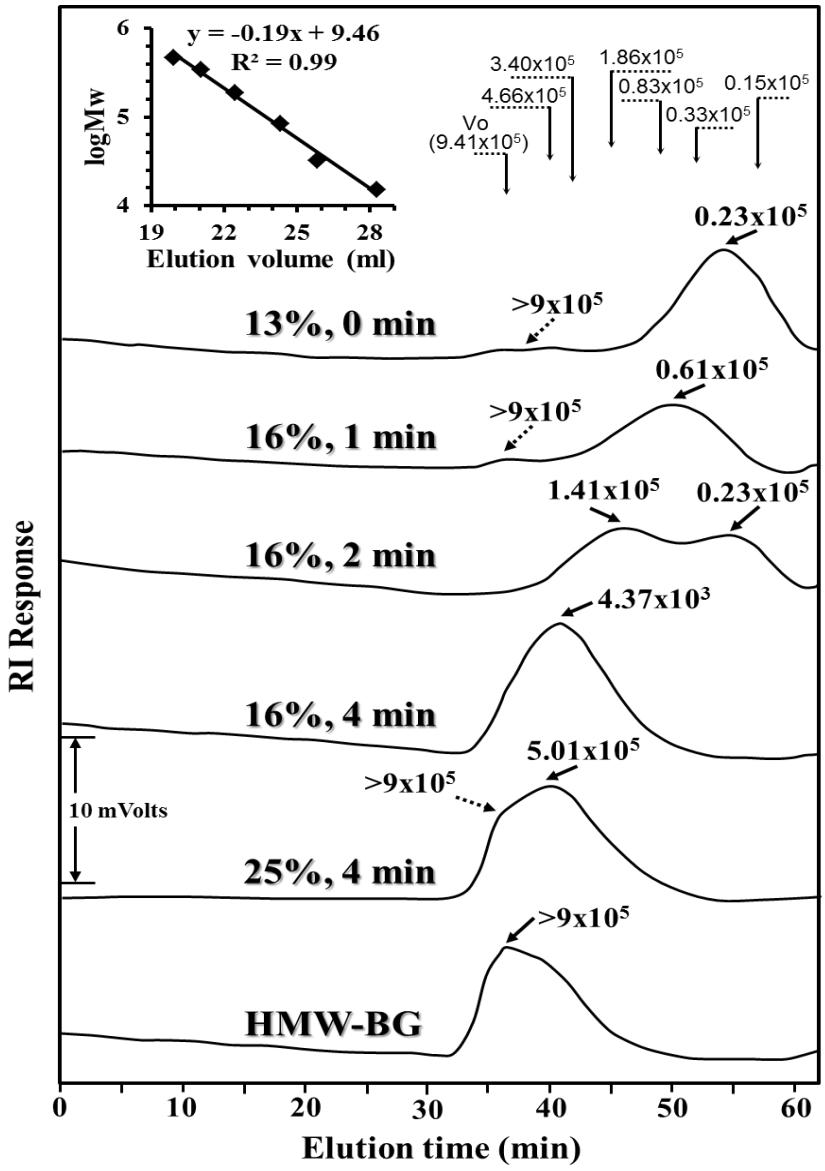


Figure 2. HPSEC elution profiles detected by RI and apparent peak molecular weight, Mp, (slanted solid arrows) of the eluting peaks of the enriched in high molecular weight oat β-glucan concentrate (HMW-BG) added to the gluten-free formulations and of the β-glucan extracted from the crumbs of the fortified breads made by the microwave (MIWA) treated flours; MIWA treatment conditions (moisture content and heating time) of the rice flours are also given on the respective elution curves. The slanted dotted arrows show the Mp of minor eluting peaks or the molecular weight of the fraction of shoulders. The vertical arrows indicate the elution time of the peak fraction of six (1→3) (1→4) β-D-glucan standards (Mp: 15, 33, 83, 186, 340 and 466 x 10^3 Da) used for plotting of the standard curve (inset); (1→3) (1→4) β-D-glucan standard with Mp 941 x 10^3 Da is eluted at the void volume of the columns. All Mp values showed on figure is expressed in Daltons (Da).

With increase of flour moisture level to 25% before MIWA treatment there was a further increase of the molecular weight of β-glucan in bread, as indicated by the higher Mp value, 5.01 x 10^5 Da, compared to that from flour with 16% moisture level treated with MIWA for the same time, 4 min (Figure 2). In contrast, for the MIWA treatment 5, it seemed that the β-glucans maintained to a considerable extent their initial molecular size, since a large portion of the eluted polysaccharides were of high molecular weight (>9 x 10^5 Da) and appeared as a shoulder of the main peak eluted in the void volume of the chromatograph (slanted dotted arrow). This fact is in accordance with the β-glucanase activity of the respective treated flour used for breadmaking which appeared to be negligible, i.e. apparently, non-detectable activity by the employed viscometric method (Table 1). As found previously, increased levels of flour moisture are crucial for adequate β-glucanase inactivation, most likely due to the drop of denaturation temperature of the enzyme with increasing water content (Lazaridou et al. 2014; Perez-Quirce et al. 2016). Perez-Quirce et al. (2016) have recently reported that residual activity of endogenous β-glucanase in rice flour decreased with increasing time of microwave heating and moisture level of the flour; for instance, an increase of initial water content up to 19 and 25% in rice flour following by microwave treatment for 8 and 4 min, respectively, resulted in non-measurable β-glucanase activity using the same viscometric method. In the present study, the slight drop of β-glucan molecular weight during production of breads made by the MIWA treatment 5 flour (with no apparent β-glucanase activity) could be attributed either to minor residual enzyme activity, non-detectable by the viscometric method or by oxidative degradation reactions involving of β-glucans; apparently, there is evidence for hydroxyl radical mediated depolymerisation of β-glucans that could occur in cereal baked products (Kivela et al., 2011).

4. Conclusions

Microwave pretreatment of hydrated rice flours used as a base material to produce gluten-free breads, fortified with β-glucans, do not cause reduction in the molecular weight of the bioactive polysaccharides upon baking, presumably due to inactivation of the endogeneous rice flour β-glucanases. The β-glucan molecular weight in the final product increased with time of microwave heating and initial moisture content

of the flour in accordance with the magnitude of the residual endogenous β-glucanase activity found in the treated flours. A slight increase in loaf volume was also observed for the breads made from the rice flour treated for the longest time, while no practically important changes in the pasting properties of the flour as well as in texture and color of the final products were noted as a result of flour microwave treatment. The findings of the present study could contribute to improving the quality and bioactivity of rice-based gluten-free baked products, containing cereal β-glucan concentrates, to broaden the food item choices for celiac consumers. Additional studies are still required to extensively evaluate the effect of more intense microwave treatments on rice flour functionality and its applicability on the breadmaking process.

Acknowledgments

The authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad and the European Regional Development Fund (FEDER) (Projects AGL2012-35088 and AGL2015-63849-C2-2-R).

References

AACC, American Association of Cereal Chemists, (2000). Method 44-19. In: Anonymous Approved Methods of the AACC, (10th ed.). The Association, St. Paul, MN.

Adebowale, K. O., Afolabi, T. A., & Olu-Owolabi, B. I. (2005). Hydrothermal treatments of Finger millet (Eleusine coracana) starch. *Food Hydrocolloids*, 19, 974–983.

Ahmad Mir, S., Ahmad Shah, M., Rashid Naik, H., Ahmad Zargar, I. (2016). Influence of hydrocolloids on dough handling and technological properties of gluten-free breads. *Trends in Food Science & Technology*, 51, 49-57.

Aman, P., Rimsten, L., Andersson, R., (2004). Molecular weight distribution of β-glucan in oat-based foods. *Cereal Chemistry*, 81, 356–360.

Andersson, A. A. M., Armo, E., Grangeon, E., Fredriksson, H., Andersson, R., & Aman, P. (2004). Molecular weight and structure units of (1-3, 1-4)-β-glucans in

dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science*, 40, 195–204.

Andersson, R., Fransson, G., Tietjen, M., & Aman, P. (2009). Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. *Journal of Agricultural and Food Chemistry*, *57*, 2004-2008.

Andersson, A. A. M., Ruegg, N., & Aman, P. (2008). Molecular weight distribution and content of water-extractable β-glucan in rye crisp bread. *Journal of Cereal Science*, 47, 399-406.

Caballero, P.A., Gomez, M., Rosell, C.M. (2007). Bread quality and dough rheology of enzyme-supplemented wheat flour. *European Food Research Technology*, 224, 525–534.

Chung, H. J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers*, 75, 436–447.

Demirkesen, I., Kelkar, S., Campanella, O.H., Sumnu, G., Sahin, S., Okos, M. (2014). Characterization of structure of gluten-free breads by using X-ray Microtomography. *Food Hydrocolloids*, 36, 37–44.

EFSA (2011a). Scientific opinion on the substantiation of a health claim related to barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to article 14 of regulation (EC) No. 1924/2006. EFSA Journal, 9 (2470), 14.

EFSA (2011b). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and "digestive function" (ID 850) pursuant to Article 13 (1) of Regulation (EC) No. 1924/2006. EFSA Journal, 9 (2207), 21.

EFSA (2011c). Scientific Opinion on the substantiation of health claims related to oat and barley grain fibre and increase in faecal bulk (ID 819, 822) pursuant to Article 13 (1) of Regulation (EC) No. 1924/2006. EFSA Journal, 9 (2249), 13.

Foschia, M., Horstmann, S., Arendt, E.K., Zannini, E. (2016). Nutritional therapy – Facing the gap between coeliac disease and gluten-free food. *International Journal of Food Microbiology*, 239, 113–124.

Gujral, H. S., Haros, M., & Rosell, C. M. (2003). Starch hydrolyzing enzymes for retarding the staling of rice bread. *Cereal Chemistry*, 80(6), 750–754.

Hager, A.S., Arendt, E.K. (2013). Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free breads based on rice, maize, teff and buckwheat. *Food Hydrocolloids*, 32, 195–203.

Hager, A. S., Ryan, L. A. M., Schwab, C., Ganzle, M. G., O'Doherty, J. V., & Arendt, E. K. (2011). Influence of the soluble fibres inulin and oat beta-glucan on quality of dough and bread. European *Food Research and Technology*, 232(3), 405-413.

Hormdok, R., & Noomhorm, A. (2007). Hydrothermal treatments of rice starch for improvement of rice noodle quality. *LWT–Food Science and Technology*, 40, 1723–1731.

Houben, A., Hoechstoetter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European Food Research and Technology*, 235(2), 195-208.

Kivela, R., Sontag-Strohm, T., Loponen, J., Tuomainen, P., & Nystrom L. (2011). Oxidative and radical mediated cleavage of β-glucan in thermal treatments. *Carbohydrate Polymers*, 85, 645-652.

Lazaridou, A., Biliaderis, C. G., Micha-Screttas, M., & Steele, B. R. (2004). Acomparative study on structure-function relations of mixed linkage (1→3), (1→4) linear β-D-glucans. *Food Hydrocolloids*, 18, 837-855.

Lazaridou, A., Marinopoulou, A., Matsoukas, N. P., & Biliaderis, C. G. (2014). Impact of flour particle size and autoclaving on β-glucan physicochemical properties and starch digestibility of barley rusks as assessed by in vitro assays. *Bioactive Carbohydrates and Dietary Fibre*, 4 (1), 58-73.

Luo, Z., He, X., Fu, X., Luo, F., & Gao, Q. (2006). Effect of microwave radiation on the physicochemical properties of normal corn, waxy corn and amylomaize V starches. *Starch-Starke*, 58, 468–474.

Moriartey, S., Temelli, F., & Vasanthan, T. (2010). Effect of formulation and processing treatments on viscosity and solubility of extractable barley beta-glucan in bread dough evaluated under in vitro conditions. *Cereal Chemistry*, 87(1), 65-72.

Olayinka, O. O., Adebowale, K. O., & Olu-Owolabi, B. I. (2008). Effect of heat-moisture treatment on physicochemical properties of white sorghum starch. *Food Hydrocolloids*, 22, 225–230.

Perez-Quirce, S., Collar, C., & Ronda, F. (2014). Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads. *International Journal of Food Science and Technology*, 49, 1375-1382.

Perez-Quirce, S., Ronda, F., Melendre, C., Lazaridou, A., Biliaderis, C.G. (2016). Inactivation of endogenous rice flour β-glucanase by microwave radiation and impact on physico-chemical properties of the treated flour. *Food and Bioprocess Technology*, 9 (9), 1562-1573.

Pinkrová, J., Hubácková, B., Kadlec, P., Příhoda, J.,& Bubník, Z. (2003). Changes of starch during microwave treatment of rice. *Czech Journal of Food Sciences*, 21, 176–184.

Pyler, E.J., & Gorton L.A. (2000). Fundamental Bakery Dough Processes. In Baking Science & Technology, Volume II: Formulation and Production. Sosland Publishing Company, Kansas City, MO, US.

Rieder, A., Ballance, S., Knutsen, S.H. (2015). Viscosity based quantification of endogenous β-glucanase activity in flour. *Carbohydrate Polymers*, 115, 104–111.

Roman, L., Martinez, M.M., Rosell, C. M., & Gomez, M. (2015). Effect of microwave treatment on physicochemical properties of maize flour. *Food and Bioprocess Technology*, 8(6), 1330–1335.

Ronda, F., Perez-Quirce, S., Lazaridou, A., & Biliaderis, C. G. (2015). Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics. *Food Hydrocolloids*, 48, 197-207.

Ronda, F.; Pérez-Quirce, S.; Villanueva, M. (2016) Rheological properties of Gluten-free bread doughs. Relationship with bread quality, in Advances in Food Rheology and Its Applications. Eds: Ahmed, J,; Ptaszek, P. and Basu, S. Elsevier, WP., Cap 12, pp 297-334

Sciarini, L.S., Ribotta, P.D., Leon, A.E., Perez, G.T. (2010). Effect of hydrocolloids on gluten-free batter properties and bread quality. *International Journal of Food Science and Technology*, 45, 2306–2313.

Sollid, L. M., & Lundin, K. E. A. (2009). Diagnosis and treatment of celiac disease. Mucosal Immunology, 2, 3-7.

Tosh, S. M., Brummer, Y., Wolever, T. M. S., & Wood, P. J. (2008). Glycemic response to oat bran muffins treated to vary molecular weight of β-glucan. *Cereal Chemistry, 85*, 211-217.

Trogh, I., Courtin, C. M., Andersson, A. A. M., Aman, P., Sorensen, J. F., & Delcour, J. A. (2004). The combined use of hull-less barley flour and xylanase

as a strategy for wheat/hull-less barley breads with increased arabinoxylan and (1-3, 1-4) β- D-glucan levels. *Journal of Cereal Science*, 40, 257-267.

US Food and Drug Administration (USFDA) (2005). Food labeling: Soluble dietary fibre from certain foods and coronary heart disease. *Federal Register*, 70, 76150–76162.

Vatandoust, A., Ragaee, S.M., Wood, P.J., Tosh, S.M., and Seetharaman, K. (2012). "Detection, Localization, and Variability of Endogenous β-Glucanase in Wheat Kernels". *Cereal Chemistry*, 89(1), 59-64.

Watcharatewinkul, Y., Puttanlek, C., Rungsardthong, V., & Uttapap, D. (2009). Pasting properties of a heat-moisture treated canna starch in relation to its structural characteristics. *Carbohydrate Polymers*, 75, 505–511.

Wolever, T. M. S., Tosh, S. M., Gibbs, A. L., Brand-Miller, J., Duncan, A. M., Hart, V., Lamarche, B., Thomson, B. A., Duss, R., & Wood, P. J. (2010). Physicochemical properties of oat β-glucan influence its ability to reduce serum LDL cholesterol in humans: a randomized clinical trial. *The American Journal of Clinical Nutrition*, *92*, 723-732.

Wood, P. J. (2007). Cereal beta-glucans in diet and health. *Journal of Cereal Science*, *46*, 230-238.

CAPÍTULO VII

Impact of yeast and fungi (1 → 3)(1 → 6)-β-glucan concentrates on viscoelastic behavior and breadmaking performance of gluten-free rice-based doughs*

* Sandra Pérez-Quirce, Pedro A. Caballero, Marina Villanueva, Felicidad Ronda*. (2017). "Impact of yeast and fungi (1 → 3)(1 → 6)-β-glucan concentrates on viscoelastic behavior and breadmaking performance of gluten-free rice-based doughs". Submitted to Food Hydrocolloids.

Impact of yeast and fungi (1 → 3)(1 → 6)-β-glucan concentrates on viscoelastic behavior and breadmaking performance of gluten-free rice-based doughs

Sandra Perez-Quirce, Pedro A. Caballero, Marina Villanueva, Felicidad Ronda

Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain.

Abstract

The application of β-glucan for gluten-free breads enrichment has a special interest of people suffering from celiac disease with significant cases associated with auto-immune diseases and unbalanced diet. Most of the studies found in literature are referred to cereal (1 → 3)(1 → 4)β-glucan, while those from yeast and fungi are still nearly unexplored. This work has studied the effect of fortification with (1 → 3)(1 → 6)-β-glucan extracts derived from yeasts –two types of products soluble (SBG) and insoluble (IBG)– and fungi (Pleurotus Ostreatus) (FBG), to gluten-free rice based doughs and breads. A fundamental rheological approach showed the lowest values of G' and G'' and the highest compliances for SBG-enriched samples, while doughs with FBG or IBG showed the opposite trend, increasing their resistance to deformation as the concentration increased. Higher consistent doughs (FBG and IBG enriched doughs) corresponded to those with larger dynamic moduli and poorer frequency dependence, lower elastic deformation and higher viscosity at steady state. The physical quality of breads was significantly improved by all β-glucan addition at optimized dough hydration. They caused an increase in the specific volume of the breads, a reduction on their hardness and led to delayed crumb hardening during storage. Sensory evaluation also demonstrated an improvement in bread organoleptic attributes when SBG was added.

Keywords: (1 → 3)(1 → 6)-β-glucan, rheology, gluten-free, dough, bread

1. Introduction

β-glucan (BG) is a homoglucose polymer widely distributed in the cell walls of microorganisms - particularly of the baker's and brewer's yeast *Saccharomyces cerevisiae*, mushrooms and cereals (mainly oats and barley) (Kittisuban et al., 2014; Liu et al., 2008). BG from baker's yeast and fungi is characterized by β-(1 → 3)(1 → 6) linkages, while those derived from cereals are polysaccharides of glucopiranosyl residues linked with β-(1 → 3)(1 → 4) linkages. BG from cereals is classified as dietary fiber which is not hydrolyzed in the human digestive tract, being a non-calorific ingredient (Borchani et al., 2016). Simultaneously BG obtained from yeast and mushrooms presents positive effects on human health such as immune-stimulation, anti-inflammatory, anti-microbial, anti-tumoral (Kim et al., 2011; Santipanichwong & Suphantharika, 2009; Worrasinchai et al., 2006). Certain preparations of yeast-derived β-glucan have been recently approved as novel food ingredients by the European Food Safety Authority (EFSA) and given "Generally Recognized as Safe" status by US Food and Drug Administration. So far, EFSA has not yet approved a health claim on immune function for yeast β-glucan preparations (EFSA, 2011; Samuelsen et al., 2014).

The cell wall of yeast (*Saccharomyces cerevisiae*) contains about 50–65% of β-glucan (Kogan & Kocher, 2007). The difference in molecular and structural features according to the origin of BG lead to differences in their physical properties and thereby different effect on the functionality of food systems (Banchathanakij & Suphantharika, 2009). The potential applications of β-(1 → 3)(1 → 6) glucan in food stuffs as a thickening, water holding, oil binding agent and emulsion stabilizer as well as a fat replacer in food emulsions (Santipanichwong & Suphantharika, 2009; Worrasinchai et al., 2006) and a texture modifier in starch gels (Satrapai & Suphantharika, 2007) have been previously reported. However, its application on gluten-free products enrichment are mostly unexplored although holds a special interest of people suffering from celiac disease which encounters a significant occurrence rate of other associated chronic diseases, such as gastritis, vitamin B deficiency, cardiomyopathy, skin problems as well as obesity-metabolic syndrome and diabetes due to their higher fat and calorie denser diets compared to that of general population (Lerner & Matthias, 2015; Lebwohl et al., 2014; Helert et

al.,2009; Cronin & Shanahan 1997). Only a few studies have focused on β-(1 → 3)(1 → 6) glucan enrichment of cakes (Kim et al., 2011), gluten-free rice noodles (Heo et al., 2014) and starch breads (Kittisuban et al., 2014). Kim et al. (2011) used BG enriched product (51,4 % of BG) from *Lentinus edodes* (insoluble fiber) as a high-fiber and low-calorie substitute for wheat flour cakes, concluding an increase in batter viscosity with more shear-thinning behavior and elastic properties improved. Overall, the cakes containing more BG showed decreased volume and increased hardness although no significant differences were observed between the control and cakes containing 1 g of yeast β-glucan per serving. Heo et al. (2014) added also *Lentinus edodes* powdered extract (40,1 % of BG) to improve the functional properties of gluten-free rice noodles, showing that the use of such fibrous material increased the thermo-mechanical properties of rice flour in a dough system, leading to rice noodles with greater extensibility and firmness in all three worked concentrations (4, 8 and 12%). Kittisuban et al. (2014), used response surface methodology to analyze effects of hydroxypropylmethylcellulose (HPMC), yeast β-glucan extract (insoluble, 72,2 % of BG), and whey protein isolate (WPI) on physical properties of gluten-free bread baked from formulas based on rice starch. β-glucan affected the specific volume differently depending on the WPI levels, i.e. slightly increased with β-glucan levels at low WPI levels. This was explained by the ability of β-glucan to increase dough consistency that would improve gas retention and dough development. However, they found a decrease on the loaf volume of gluten-free bread assisted by crumb hardness with further increase of the BG level from 1 g/100 g to 2 g/100 g, indicating that there is an optimum value for dough consistency. The constant dough hydration applied by these authors could be, at least in part, responsible for results obtained since a limiting dough hydration as result of the addition of ingredients with specific water absorption behavior as insoluble BG enriched materials can mask the impact due to its presence further affecting on dough consistency that it is well known affects dramatically on dough development ability (Ronda et al., 2015).

The great impact of the solubility of fibers on gluten-free dough rheology and bread-making has been concluded from previous works (Martinez et al., 2014). Soluble fibers, mainly being pure carbohydrate polymers as inulin and polydextrose,

decreased dough consistency, favoured the volume increase during fermentation and produced breads with higher specific volumes, lower hardness, darker loaves and greater cell density than control breads. In contrast, insoluble fibers from oat, bamboo, potato and pea, particularly those of coarse particle size, decreased specific volume and increased markedly bread firmness. As enrichment of gluten-free bread with health promoting ingredients which additionally can positively affect the sensorial quality of this product is worth to study the aim of this work was to investigate the microstructural features of different (1 → 3)(1 → 6)-β-glucan commercial concentrates of high purity and different declared water solubility, derived from various sources: yeasts – soluble powder (SBG) and insoluble powder (IBG)- and fungi – from *Pleurotus Ostreatus* insoluble powder (FBG)– and to study the effect of these materials on rice based dough rheology at constant water content and physical and sensorial properties of gluten-free breads formulated at adapted dough hydration to the same consistency than the control non-enriched dough.

2. Materials and methods

2.1. Materials

Rice flour (12.5% moisture, 0.46% ash, 7.5% protein, 0.49% fat and 79.1% starch, particle size distribution: 6%>150 mm,150 mm>63.2%>100 mm, 30.8% < 100 mm) was supplied by Herba Ricemills S.L.U (Tarragona, Spain). Salt, sugar, and sunflower oil were purchased from the local market. HPMC 4 KM was a gift from Dow Chemical (Midland, EEUU). β-glucans (BG) used in GF formulations were declared as insoluble (1-3)(1-6) β-glucan (IBG) (Wellmune WGP® Dispersible Powder) obtained from the yeast *Saccharomyces cerevisiae* . The proximate composition given by the supplier was: 79.25% purity (dry basis), 84.49% carbohydrates, 7.50% fat, 4.51% moisture, 2.86% protein and 0.77% ash. Declared as soluble (1 → 3)(1 → 6)-β-glucan (SBG) (Wellmune Soluble Powder®) derived from the yeast *Saccharomyces cerevisiae* of 91.35% purity (dry basis), 92.11% carbohydrates, 6.79% moisture, 0.89% ash, 0.68% protein, <0.01% fat. Both were provided as free samples by Biothera, (Eagan, Minnesota, USA). Insoluble Fungal (1 → 3)(1 → 6)-β-glucan (FBG) (Pleuran®) (Bratislava, Slovak Republic) derived from the fungus *Pleurotus ostreatus*, given as a gift by Merck (Barcelona, Spain).

According to producer it contains 90.62% purity (dry basis), 2.56% moisture, and absence of lipids and proteins.

2.2. Dough preparation

A straight dough process was performed following the formula on a 100 g rice flour basis: 92% water, 6% oil, 5% sucrose, 1.8% salt, 2% HPMC, and BG. The levels of pure BG incorporated into this formulation were 0 (control), 0.5, 1 and 2% in flour basis. Different amounts of BG commercial concentrates were added on top of the other ingredients according to BG purity of each ingredient. The dough preparation procedure is described in detail elsewhere (Pérez-Quirce et al., 2014).

2.3. Rheological characterization of dough

2.3.1. Small deformation mechanical test. Oscillatory and creep recovery tests

Oscillatory and creep-recovery tests were carried out at 25ºC with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) using 60 mm serrated parallel plate geometry. The GF dough was placed on the rheometer plate using a 3mm gap, trimmed and then vaseline oil was applied on the exposed to air surfaces to prevent sample drying during testing. Before the measurement, the dough was allowed to rest for 500 s. A stress sweep from 0.1 to 200 Pa at 1Hz was performed to establish the linear viscoelastic region (LVR). Frequency sweeps were carried out from 10 to 0.1 Hz in the LVR and data were fitted to a power law model as in previous works (Ronda et al., 2013). The recorded viscoelastic parameters, G_1' and G_1'', and $(\tan \delta)_1$, represent the elastic and viscous moduli and the loss tangent, respectively, at a frequency of 1Hz. The *a*, *b* and *c* exponents quantify the dependence of the dynamic moduli and the loss tangent on the oscillation frequency. Each test was carried out at least in duplicate.

Creep tests were performed by imposing a sudden step of shear stress at 1.5 Pa in the LVR for 150 s. In the recovery phase, the stress was suddenly removed and the sample was allowed for 300 s to recover the elastic (instantaneous and retarded) part of the deformation. Each test was done in triplicate.. Burger's model was fitted to creep and recovery tests data, described in terms of compliance J (strain divided by the stress) (Lazaridou et al., 2007; Ronda et al., 2013). J_0 is the instantaneous compliance, J_1 is the retarded elastic compliance or viscoelastic compliances, λ_1 is

the retardation time obtained from both, the creep and the recovery phases. η_0 is the steady viscosity estimated from the creep phase. J_{max} is the maximum creep compliance obtained at the end of the creep step. Similar equations were used for the recovery compliance J_r (t). As there is no viscous flow in the recovery phase, equations consist only of parameters describing the elastic response after removal of the shear stress.

2.3.2. Large deformation mechanical test: forward extrusion test

Forward extrusion assays of formulated rice doughs were done in a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell and operating at 10 mm/s head speed following the method described in detail elsewhere (Ronda et al., 2015). Compression force–time curve allowed evaluating maximum force determined as the force at which the slope changed. The curve plateau representing the force necessary to continue with the extrusion process and the area under the curve were both used to define the sample consistency. All measurements were performed in triplicate.

2.4. Bread preparation

Breads were prepared according the formulation described above (see 2.2), including 3 g of dried yeast per 100 g of rice flour and a water amount optimized individually for each type and level of BG to obtain equal force during the extrusion test (see 2.3.2) than the control dough formulated with 92 g water per 100 g of rice flour. The optimized dough hydration increased with rising amounts of IBG and FBG and decreased with SBG, becoming between 90% and 104% depending on the formulation (Table 1). Baking process, described in detail elsewhere (Pérez-Quirce et al., 2014), was carried out in a Sveba Dahlen oven (Fristad, Sweden) at 170 °C for 20 min with 7s steam at the beginning of the baking. After baking, breads were left for one hour at room temperature before analysis. To study the effect on staling, breads were stored at 4±2°C in polyethylene bags. An experimental design of 12 elaborations (see Table 1) was carried out.

Table 1. Experimental design: amounts of pure β-glucan from commercial concentrates obtained from yeast (IBG and SBG) and fungal (FBG) and dough hydration in each gluten-free bread elaboration.

BG Type	BG (% rfb)	WATER (% rfb)
Control	0	92
SBG	0.5	91.5
SBG	1	91
SBG	2	90
IBG	0.5	95
IBG	1	98
IBG	2	104
FBG	0.5	94
FBG	1	96
FBG	2	100

Rfb: rice flour basis. Design factors are: Yeast soluble (1-3)(1-6) β-glucan (SBG). Yeast insoluble (1-3)(1-6) β-glucan (IBG) and fungal (1-3)(1-6) β-glucan (FBG).

2.5. Electron microscope photomicrographs of fibers and crumb breads

Fibers and bread crumbs photomicrographs were taken with a Quanta 200FEI (Hillsboro, Oregon, USA) environmental scanning electron microscope (ESEM). Fiber photomicrographs were taken in beam deceleration mode (BDM) at 2 keV in low vacuum mode with a backscattered electron detector (BSED). Crumb samples were directly mounted on stubs. Observations were made with an accelerating voltage of 10 keV.

2.6. Evaluation of bread quality

Bread volume was determined in four replicates by a Volscan profiler analyzer (Stable Microsystems, Surrey, UK). Breads were weighed immediately after removal from the pan once cooled. Crumb texture was determined in quadruplicate samples with a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) using the software "Texture Expert". An aluminum 20 mm diameter cylindrical probe was employed in a double compression test (TPA) to penetrate to 50 % depth at 1 mm/s

speed test, with a 30 s delay between first and second compression. Hardness (N), chewiness (N), cohesiveness, springiness and resilience were calculated from the TPA graph. Analyzes were made at 20 ± 3 ºC on two central slices (20 mm thickness) from two breads of each dough. Breads were analyzed on fresh and one day (stored at 4 ±2ºC) after their elaboration. Moreover, a staling kinetics study was carried out on breads enriched with the maximum addition of BG (2%) by measuring texture at 0, 1, 2, 4, 7 and 9 days after baking and storage at 4 ± 2ºC. Avrami equation was used for fitting the evolution of crumb firmness with time (Ronda and Roos, 2011). Crumb and crust color was measured with a Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Japan) in the CIE L*C*h coordinates using the D65 illuminant, and the 2º standard observer as reported elsewhere (Ronda et al., 2015). Crumb grain characteristics of bread were assessed by using a digital image analysis system using the ImageJ software. The analysis was performed on 30 x 50 mm squares taken from the center of the loaf. The crumb grain characteristics studied were the mean cell area (mm^2) and the cell density ($cells/cm^2$). Crumb grain parameters were measured in duplicate.

2.7. Sensory analysis

Sensory analysis was conducted on BG-supplemented bread samples using a Multisample Difference Test following the guidelines suggested by Meilgaard et al. (2007). A trained panel of eight panelists rated the intensity of nine attributes on a numerical intensity scale of nine points ranging from 1 (not perceived or very low) to 9 (extremely intense or very high). The control sample was used as a reference and was positioned on the middle of the scale (Ronda et al., 2005). The samples were evaluated in terms of crust uniformity, crumb grain uniformity, odor and flavor intensity, aftertaste persistency, crumb humidity, crumb adhesiveness, crumb softness and crumb cohesiveness, being presented separately for each attribute.

2.8. Statistical analyses

Statgraphics Centurion v.16 (Bitstream, Cambridge, MN, USA) was used for non-linear regressions to fit creep-recovery data to Burger's model. ANOVA analysis, LSD (Least Significant Difference) test ($p < 0.05$) and Pearson correlation analysis were performed by the v.6. Statistica package (Tulsa, OK, EEUU).

3. Results and discussion

3.1. BG ingredients microstructure

Fig. 1 shows the photomicrographs of the different BG containing ingredients used in this study. BG from yeast exhibited spherical or slightly lenticular morphologies responding to its origin as yeast cells (Pat 1, Pat 2, Pat3), showing SBG a smooth surface and IBG a grainy surface. The particles of FBG reflects the crushed matrix of mushroom tissue showing irregular surface, polyhedral shape and seemed to be more disintegrable. All particles of BG ingerdient show an average particle size bellow 50 μm, which means ingredients offine structure.

3.2. Viscoelastic properties of fiber-enriched doughs at constant dough hydration

Dough prepared with either constant consistency or constant water addition is used for testing the effect of added substances. The latter, previously used by Krupa-Kozak et al. (2012) and Nunes, Ryan, and Arendt (2009), was selected in this study for dough rheology characterization because recorded differences in dough behavior may be directly related to the added ingredients. Moreover, this approach allows better objectivity in the comparison of the availability of water for starch gelatinization.

The viscoelasticity of gluten-free doughs was examined by oscillatory and creep measurements. Table 2 shows the parameters obtained from fitting frequency sweeps and creep tests data to power law and Burger's model respectively. All rheological properties of doughs were significantly ($p<0.05$) affected all the types of ingredients and the level of addition; and, except for the exponents a, b and the phase shift tangent, also by their double interactions, denoting a different effect of the level depending on the type of ingredient in most viscoelastic properties. In all cases the storage modulus (G') was much larger than the loss one (G'') implying the prevalence of elastic features over viscous, suggesting a typical weak gel structure, in agreement with previous studies on GF dough enrichment with cereal BG (Hager et al, 2011; Lazaridou et al., 2007; Ronda et al., 2013, 2015). Both moduli showed slight increases with angular frequency as denotes the low a and b exponents. The elastic and viscous moduli at 1 Hz, G_1' and G_1'', decreased with soluble product

SBG addition, until 30% at the highest addition level, denoting a lower consistency of the dough as observed by Ziobro et al., (2013) and Peressini et al. (2015) with the addition of inulin to gluten-free or wheat doughs respectively. This effect is probably because soluble ingredient dissolved in the aqueous solution, envelops the starch granules lubricating the dough and decreasing the capacity of the starch to absorb water, which leads to a reduction of dough consistency and elasticity (Martinez et al., 2014). Insoluble products, IBG or FBG, showed the opposite trend, increasing G_1' and G_1'' moduli until 280% and 200% for IBG and 140% and 85% for FBG, respectively. The more marked effect of IBG from yeast than from FBG from fungi on dough consistency confirms the more regular the insoluble fiber is (see the shape of IBG versus FBG in Fig.1) the greater impact has on dough structure as demonstrated Martinez et al., (2014) in agreement with Sabanis et al. (2009) who reported that insoluble fibers increased dough consistency, requiring an increase of the water content in their formulations. Insoluble fiber remains almost unaltered in the dough leading to larger, more irregular structures than in the control dough (Martinez et al., 2014). The loss tangent (tan δ_1) was unaffected by SBG and decreased by IBG and FBG indicating doughs enriched with insoluble ingredients had a higher elastic behavior besides a higher consistency. The degree of dependence of G 'and G'' with the frequency, quantified from a and b exponents that ranged 0.25-0.35, decreased with the addition of insoluble BGs denoting a less dependence of viscoelastic moduli on frequency.

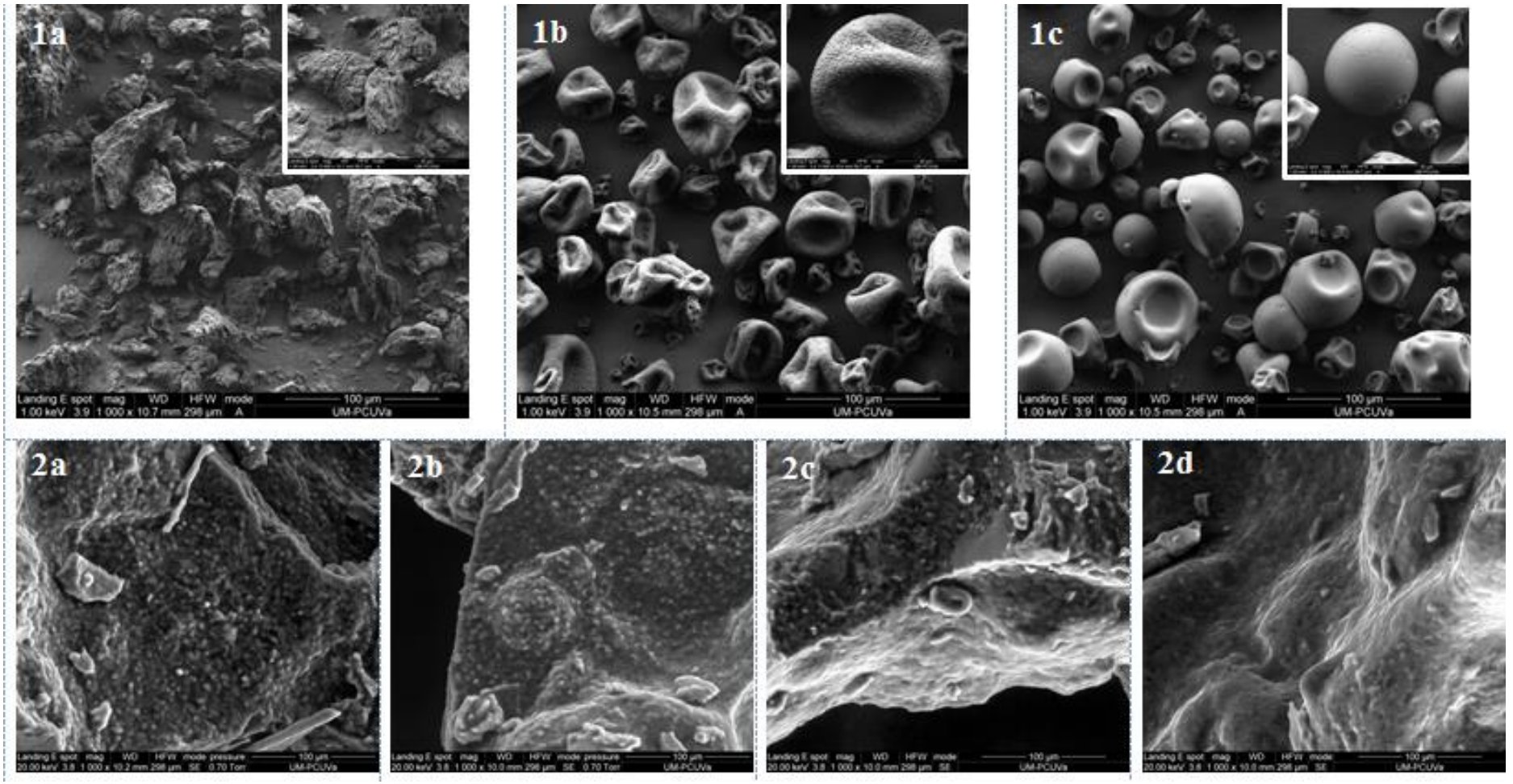


Fig. 1. Scanning electron microscope photomicrographs of the beta-glucan containing products: (1a) FBG. (1b) IBG. (1c) SBG; and of the enriched gluten-free breads with 2% BG content (flour basis): (2a) Control bread (without BG). (2b) FBG. (2c) IBG. (2d) SBG

Table 2
Effects of design factors on the dynamic oscillatory and creep parameters of gluten-free doughs at constant water content of 92%.

Sample	Dose	Oscillatory tests- Parameters of power law model					Creep tests- Parameters of Burger model				
		G'_1	a	G''_1	b	$(\tan\delta)_1$	J_0	J_1	λ_1	η_0	J_{max}
	(%)	(Pa)		(Pa)			(10^{-4} Pa^{-1})	(10^{-4} Pa^{-1})	(s)	(10^{-4} Pa·s)	(10^{-4} Pa^{-1})
Control	0	3209 bc	0.328 f	1654 bcd	0.324 e	0.518 e	6.2 e	8.9 e	18.6 a	9.6 ab	30.8 e
SBG	0.5	2587 ab	0.326 def	1385 abc	0.318 de	0.535 e	6.3 e	13.1 f	22.4 a	7.9 ab	38.3 f
SBG	1	2478 ab	0.330 ef	1321 ab	0.304 cde	0.534 e	6.9 e	12.2 f	23.9 a	8.5 ab	36.4 f
SBG	2	2260 a	0.298 cde	1158 a	0.295 bcd	0.512 de	5.7 e	12.5 f	16.8 a	6.7 a	40.2 f
IBG	0.5	4033 d	0.281 abc	1778 cde	0.301 cd	0.442 abc	4.5 d	7.4 de	19.3 a	15.9 c	21.7 cd
IBG	1	5169 e	0.255 a	2079 ef	0.276 ab	0.402 ab	2.9 bc	4.4 bc	23.2 a	25.3 d	13.2 b
IBG	2	12200 g	0.264 ab	4817 h	0.264 a	0.395 a	1.4 a	2.2 a	22.7 a	46.8 e	6.7 a
FBG	0.5	3886 cd	0.295 bcde	1913 def	0.281 abc	0.492 cde	3.6 cd	9.0 e	23.0 a	13.9 bc	23.1 d
FBG	1	4772 de	0.293 bcd	2187 f	0.297 bcd	0.459 bcd	3.1 c	5.8 cd	21.2 a	18.9 cd	16.8 bc
FBG	2	7623 f	0.254 a	3054 g	0.266 a	0.401 ab	1.8 ab	2.6 ab	21.3 a	41.6 e	8.2 a
SE		332	0.012	136	0.0080	0.023	0.44	0.73	2.7	2.5	0.2
Factor I (type)		***	***	***	**	***	*	***	ns	***	***
Factor II (level)		***	*	***	**	*	ns	***	ns	***	***
Factor I x Factor II		***	ns	***	ns	ns	ns	**	ns	***	***

Values with a common letter in the same column are not significantly different ($p > 0.05$). SE Standard error obtained from ANOVA analysis. *. **. *** and ns indicate the level of significance in the effects of type of BG and the level of addition and their interaction. * $p<0.05$. ** $p<0.01$. ***$p<0.001$ and ns= not significant ($p>0.05$).

Parameters of Burger model from creep tests are collected in Table 2. Creep/recovery curves of gluten-free doughs (not shown) exhibited a typical viscoelastic behavior combining both viscous fluid and elastic components, similar to the corresponding curves obtained previously for rice flour (Ronda et al., 2013; 2015) and other gluten-free doughs (Lazaridou et al., 2007). Because the tests were performed in the LVR, all creep compliance parameters and the equivalents for the recovery phase followed a strong parallelism and setting parameters of both segments were equivalent, because of that recovery phase data are not shown. The addition of SBG did not affect the instantaneous elastic compliance (J_0) of the dough although increased significantly the retarded (J_1) and the maximum (J_{max}) ones, regardless the addition level. On the opposite, insoluble products, FBG or IBG, decreased J_0, J_1 and J_{max}, with changes being magnified with the addition level but unaffected by the source of the fiber. Maximum depletion in all compliance values, at 2 % addition, were around -75% denoting stiffer doughs with lower dough deformation submitted to a constant stress. Factors providing a decrease in compliance parameters increased the viscosity at the steady state, (η_0), agreeing with previous studies on GF doughs enriched with cereal BG (Ronda et al., 2013; 2015; Lazaridou et al., 2007) in tests performed both in and outside the LVR. Any significant effect was detected on dough steady viscosity as result of SBG presence meanwhile the addition of IBG or FBG increased it markedly until +350% at 2% level. Dough viscosity increase might be attributed to the high water retention capacity of insoluble ingredients, as observed by Rosell et al. (2009) for insoluble fibers.

3.3. Optimization of dough hydration to constant consistency by forward extrusion test

The knowledge of the important differences on rheological behavior of doughs enriched with different ingredients depending on their solubility and source led us to optimize the hydration of doughs used for bread elaboration to similar consistency to remove the important and well known effect of this factor on breadmaking. The water content of enriched doughs was adapted to obtain the same consistency than the control dough formulated with 92% water per 100g of rice flour, evaluated from the forward extrusion test. The force and energy required by the control dough during the extrusion test were 7.2 g and 598 g·s respectively. The dough hydration

necessities increased with rising amounts of IBG and FBG and decreased with SBG until 104% and 100% for insoluble ones and 90% for the soluble one at the highest additions (Table 1). The level of dough hydration to adapted consistency obtained with this procedure was coherent with the results obtained from fundamental rheological tests: The lower G', G'' and η_0 and the larger J_o, J_1 and J_{max} the lower the water necessities for constant dough consistency.

3.4. Bread properties

Enriched breads characteristics are shown in Table 3 and Fig 2. All BGs increased the specific volume of breads, effect being lower at the highest addition level - 2%. The FBG addition led to breads with the highest specific volume. There were no significant differences among breads enriched with SBG or IBG. Other authors found the highest bread specific volumes with soluble fibers (Martinez et al., 2014) when compared with those made with insoluble fibers. These authors concluded that, in general, higher specific volumes were found to be greater in doughs with a lower consistency which can be attributed to the fact that a high consistency can limit dough expansion during proofing and baking. The optimization of the water content to constant dough consistency can explain our different result and allows confirming that these ingredients had a beneficial effect on bread volume beyond its effect on dough consistency. Besides, the fine structure of the used (1 → 3)(1 → 6)-β-glucan containing insoluble products can also justify the higher bread volumes obtained as it was reported fine fibers lead to higher bread volumes than the coarser ones (Martinez et al., 2014). At 2% fiber addition the specific volume of breads started to decrease although they still surpass the control bread volume. Lazaridou et al (2007), working at adapted dough hydration, also found an increase in the specific volume of gluten-free breads as result of oat BG (90% purity) enrichment that was higher at 1% than at 2% addition level. Kim et al. (2011) observed that the replacement of flour by 1 g BG from *Lentinus edodes*/100g flour led to cakes of similar volume than the control meanwhile the replacement of 3g BG decreased the volume significantly.

Table 3
Textural and morphogeometrical properties of bread

Sample	Dose (%)	Specific volume (mL/g)	Loss of weight (%)	Hardness (N)	Chewiness (N)	Resilience (N)	Mean cell area (mm^2)	Cell density ($cells/cm^2$)	Crust Colour			Crumb Colour			Δ Hardness 1 day (N)
									L*	C*	h	L*	C*	h	
Control	0	3.336 a	16.57 a	0.804 d	0.412 d	0.343 de	0.192 a	47.9 d	58.3 a	61.3 a	31.1 c	75.3 e	94.4 de	97.8 c	1.25 c
SBG	0.5	3.831 d	17.31 b	0.531 bc	0.258 bc	0.342 cde	0.260 bc	48.5 d	62.6 de	67.7 e	33.5 d	72.0 bcd	96.3 ef	96.6 bc	0.80 ab
SBG	1	3.825 d	17.80 bc	0.597 c	0.282 c	0.342 bcde	0.232 ab	48.3 d	59.0 ab	65.1 d	32.9 d	71.3 bc	93.6 cde	97.9 c	0.69 ab
SBG	2	3.574 b	16.22 a	0.588 c	0.285 c	0.359 e	0.187 a	46.8 d	61.3 cd	63.2 bc	30.5 c	76.7 e	92.5 bcd	98.3 c	1.25 bc
IBG	0.5	3.896 d	18.42 cd	0.549 bc	0.266 bc	0.313 a	0.238 ab	46.1 cd	60.5 c	61.7 ab	25.9 b	71.0 bc	90.3 b	97.4 bc	0.39 a
IBG	1	3.766 cd	18.74 de	0.507 bc	0.246 ac	0.325 abc	0.308 cd	41.4 b	58.5 a	65.3 d	25.5 b	72.7 cd	92.0 bc	97.8 c	0.67 a
IBG	2	3.682 bc	19.33 e	0.483 abc	0.220 abc	0.328 abc	0.490 e	35.2 a	60.3 bc	64.1 cd	22.6 a	67.6 a	85.7 a	97.5 bc	1.01 ab
FBG	0.5	4.427 f	19.29 e	0.374 a	0.167 a	0.314 ab	0.247 b	46.5 cd	63.7 e	67.4 e	29.9 c	74.5 de	92.7 cd	95.6 ab	0.66 a
FBG	1	3.881 d	19.08 de	0.455 ab	0.204 abc	0.320 abc	0.227 ab	48.1 d	60.2 bc	68.4 e	31.3 c	69.6 ab	93.9 cde	96.8 b	0.73 a
FBG	2	4.074 e	21.46 f	0.371 a	0.181 ab	0.308 a	0.336 d	42.6 bc	61.9 d	63.0 bc	25.5 b	67.6 a	95.4 ef	95.6 a	0.86 a
SE		0.046	0.27	0.043	0.003	0.010	0.016	1.3	1.0	1.5	1.2	1.4	1.4	1.2	0.20
Factor I (type)		***	***	***	**	**	***	***	***	***	***	***	***	***	ns
Factor II (level)		***	**	ns	ns	ns	***	**	***	***	***	*	**	**	*
Factor I x Factor II		***	***	ns	ns	ns	***	*	ns	***	*	***	***	ns	ns

Values with a common letter in the same column are not significantly different ($p > 0.05$). ΔHardness 1 day: Firmness increase in 1 storage day.SE Standard error obtained from ANOVA analysis. *. **. *** and ns indicate the level of significance in the effects of type of BG and the level of addition and their interaction. * $p<0.05$. ** $p<0.01$. ***$p<0.001$ and ns= not significant ($p>0.05$)

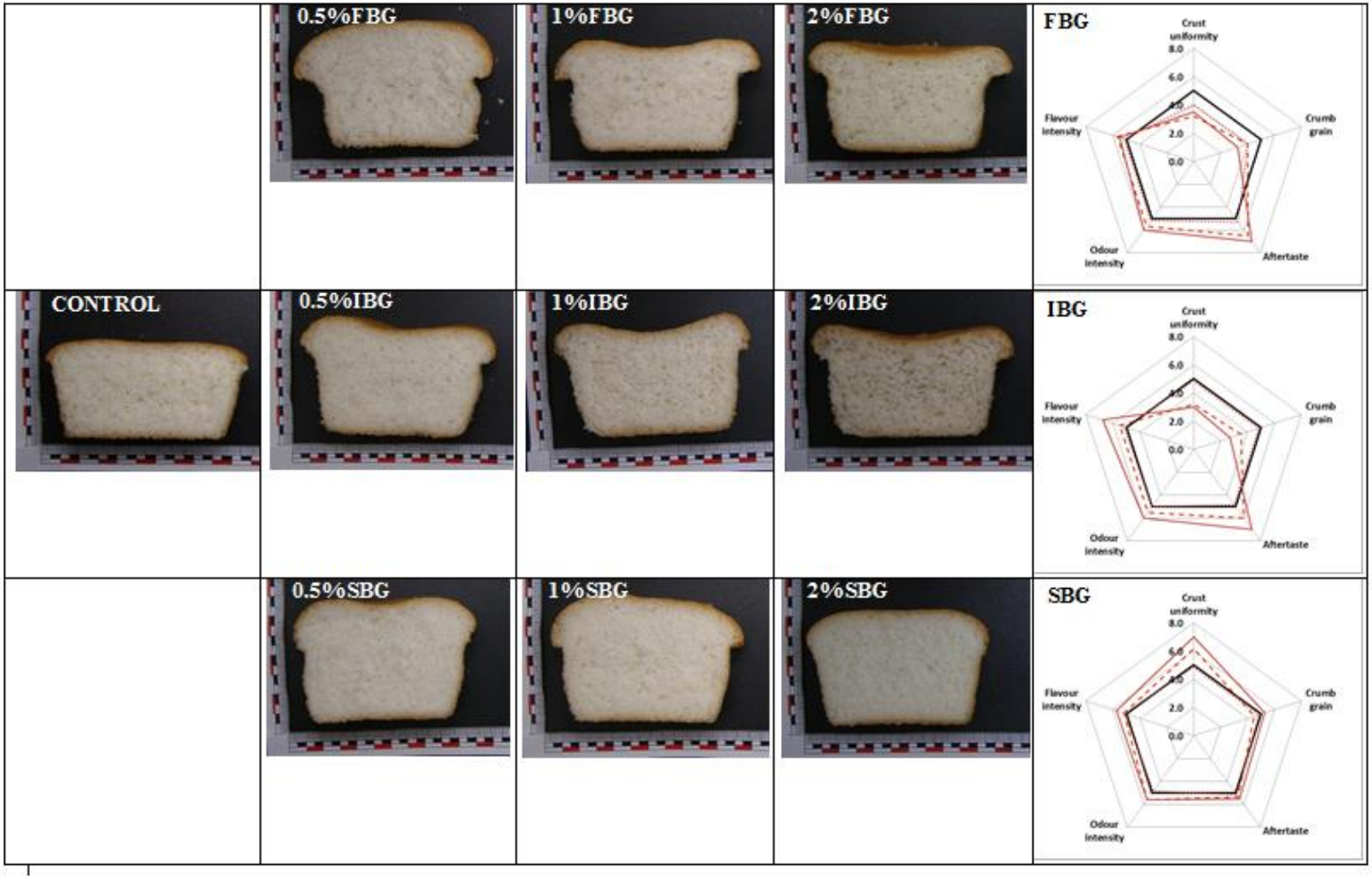


Figure 2. Effect of BG addition on crumb structure and sensory properties of gluten-free breads depending on the addition level and type of BG.
····· 0.5%BG - - - 1% BG; —— 2% BG; —— Control

Regarding bread texture it was found that all the BG tested products reduced bread crumb hardness and chewiness significantly ($p<0.05$) with respect to the control bread with no significant effect of the addition level. SBG and IBG showed similar effect on these textural properties while FBG led to the lowest values. It was found a reciprocal relationship between the specific volume of breads and their hardness. This significant correlation ($p <0.01$; $r=0.85$) has been reported in previous studies on gluten-free bread, including studies on the effects of adding fiber (Sabanis et al., 2009; Kittisuban et al., 2014; Martinez et al., 2014) and it is probably due to the lower resistance of crumb to deformation with a higher percentage air content in breads of higher volume. Kim et al. (2011) reported an increase in the hardness of wheat cakes as the dose of BG from *Lentinus edodes* increased. Kittisuban et al. (2014) also found that the hardness of the crumb increased with the concentration of BG, and attributed that effect to the water binding capacity of the BG when they worked at constant hydration. Our better results are surely due to the optimization of dough hydration in function of ingredient presence. Springiness and Cohesiveness, whose average values in crumb breads were 0.812 and 0.582, were not significantly affected by ingredient presence which can be related to the very small size of them all. However, as can be seen in Table 3, resilience decreased significantly with insoluble BG although SBG did not affect it. Resilience is a more sensitive parameter than springiness and cohesiveness. It quantifies the instant recovery capacity after a compression of the crumb meanwhile the later evaluate the recovery capacity after a waiting (recovery) time. It should be noted that breads made with soluble ingredient were more capable of recovering, with less friable crumbs, what improved their quality with respect to those enriched with insoluble fibers (see section 3.5).

Mean cell area of bread crumb increased by the addition of insoluble BGs until more than double of the control bread value in the case of 2% IBG (see Table 3 and Fig. 2). This change took place with simultaneous decrease in cell density being a significant correlation between them ($p<0.001$; $r=-0.93$). SBG-enriched breads showed lower mean cell area and higher cell density, similar to the control bread. Different effects of ingredients on crumb grain depending on its solubility can be explained in view of the microphotographs that show the structure of 2% BG-enriched bread crumbs (Fig. 2). Soluble BG product help to create a uniform network which help to envelope the bubbles and avoid coalescence phenomena. Fig 2d shows

the crumb surface of bread with SBG was smoother than control and insoluble ones enrichedbreads, indicating that the addition of SBG improves the homogeneity of the crumb structure, leading to a fine structure. Insoluble ingredient, particularly the more rounded one (as IBG in Fig 2c) as demonstrated Martinez et al (2014), creates points of rupture in the structure, thus making it easier for bubbles to associate leading to coarser crumb grain structure. An improved crumb was observed previously with the addition of oat BG both to GF breads (Lazaridou et al., 2007; Ronda et al., 2015) and to wheat breads (Wang, Miller, and Hoseney, 1998) which was also attributed to its capacity of preventing coalescence of the cells during proofing and baking.

The BG products enriched breads showed crusts with significantly higher L* and C* coordinates than those of the control sample. This means that BG-fortified breads were lighter and with more vivid colors than the control bread. The higher dough water content (in the case of insoluble ingredient addition) and the addition of an ingredient that retains water, such as BG, decrease the Maillard reactions rate, resulting in a lighter crust (Perez-Quirce et al., 2014) as has been previously noted by Hager et al. (2011) and Ronda et al. (2015) for cereal BG. BG moderately affected the crust hue, h, depending on BG solubility: IBG decreased it, denoting reddish crust color, meanwhile SBG increased it leading to yellowish ones. Enriched breads showed slightly darker crumbs (lower L*) and in the case of insoluble ingredients, significantly lower Chroma (IBG) and hue (FBG) denoting less vivid and reddish crumbs respectively. The slight effect of fiber on bread crumb color could be related to the original –although very slight– color of these ingredients.

Figure 3 presents the crumb firmness evolution during the storage of BG-added GF breads at 4±2ºC. The correlation coefficients of the fitting of Avrami model to experimental data, R^2, ranged 0.96 (for FBG) and >0.99 (for the rest). The values of the Avrami model parameters (F_o, F_∞, k, n) were (0.59N, 2.5N, 0.59 d^{-n}, 0.37), (0.48N, 2.2 N, 0.93 d^{-n}, 0.19), (0.38N, 2.3 N, 0.35 d^{-n}, 0.50) and (0.80N, 3.4 N, 0.39 d^{-n}, 0.69) for SBG-, IBG-, FBG-added breads and the control bread respectively. The half-life time, $t_{1/2}$, that reports the time needed to change from the initial firmness value, F_o, to the half of the maximum change observed at infinite time, $(F_\infty-F_o)/2$, was 1.5, 0.2, 3.9 and 2.3 days respectively, meaning a faster hardening during

storage of yeast BGs-added crumbs. The rate constants of crumb firming found in this study, was of the same order of magnitude than that found in other studies for gluten-free breads, 0.44 d^{-n} (Ronda and Roos, 2011) and inulin enriched wheat breads, 0.35 d^{-n} (Ronda et al., 2014) stored at 4±2ºC. Since the first days, FBG-added breads showed the lowest crumb firmness meanwhile breads with yeast BG fiber (IBG and SBG) showed similar firmness after one day of storage to that of the control bread. However, the lower levelling-off firmness values, F_∞, of fiber added breads, that ranged 2.2 - 2.5N versus 3.5N for the control bread, and Fig 3 show the beneficial effect of BG fiber, in particular FBG, in reducing bread hardening after long-term storages. The parameter F_o resulted to be similar to values of firmness measured in fresh breads (Table 3) which confirm the good fit of the Avrami model to experimental data. The higher rate constant, k, and the lowest half-life time, $t_{1/2}$, obtained for IBG-added breads denote a faster firmness change, from the initial fresh bread value to the levelling-off one in comparison with the remaining breads.

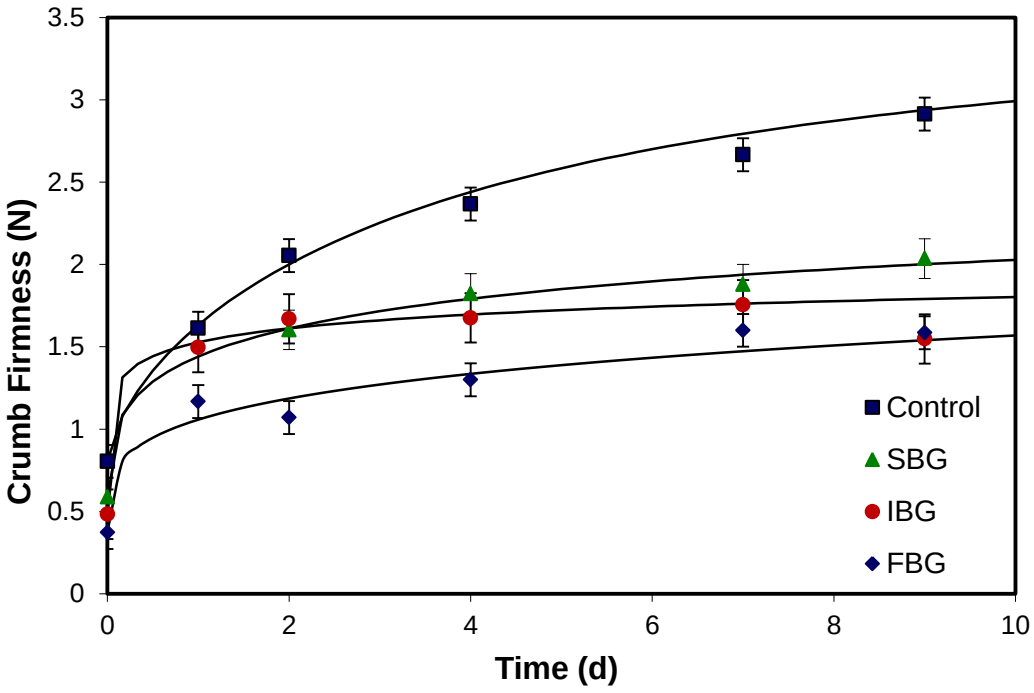


Figure 3: Hardening kinetics in BG-enriched breads stored at 4 ± 2 ºC. The continuous lines resulted from fitting the Avrami equation to experimental data. The error bars represent the standard deviation. ■ Control; ▲ SBG ● IBG ◆ FBG

3.5. Sensory evaluation

Panelists were unable to find significant ($p<0.05$) differences between BG enriched - and control samples in terms of crumb humidity, crumb adhesiveness, crumb softness and crumb cohesiveness (data not shown). Similar results were obtained previously by Martins et al. (2015) who studied BG-bread fortification with dry spent yeast and reported no significant differences in sensorial attributes of the final product. An analogous behavior was also stated in fiber-enriched products such as inulin-fortified snacks (Peressini et al., 2015). Figure 2 shows the sensory evaluation results of BG-supplemented gluten-free breads in terms of crust uniformity, crumb grain uniformity, odor and flavor intensity and aftertaste persistency. SBG-supplemented bread showed a sensorial profile very similar to the control bread except for crust uniformity which significantly ($p<0.05$) increased with SBG addition, probably due to the higher volumes obtained in the fortified breads. Conversely, the addition of IBG and FBG resulted in lower scores than the control sample, denoting a worse aspect of the loaves and lower crumb grain uniformity. Insoluble BGs fortification led to higher flavor and odor intensities (mainly for IBG) and aftertaste (for both IBG and FBG) scores than control bread. Panelists recognized these differences coming from foreign and a bit undesirable flavor and odor.

4. Conclusions

This study demonstrated the availability of the gluten-free bread enrichment with $(1 \rightarrow 3)(1 \rightarrow 6)$-β-glucan enriched products obtained from yeast or fungi at 0,5%-2% level. The effect on dough rheological properties was very dependent on BG source and solubility. In general, soluble one decreased G', G'' and steady viscosity and increased compliances, while doughs with insoluble fibers showed the opposite trend, increasing their resistance to deformation as the concentration increased. The physical quality of breads was significantly improved by β-glucan addition at optimized dough hydration. The addition of β-glucan products caused an increase in the specific volume of the breads, a reduction on their hardness and led to delayed crumb hardening during storage. Sensory evaluation also demonstrated an improvement in bread organoleptic attributes when soluble BG was added. Insoluble

BG extracts, both from yeast or fungi, at 1 and 2% level, gave to breads a foreign and slightly undesirable flavor and odor not found in the control rice flour bread. The use of more complex and optimized formulation could help to overcome this slight undesirable taste/odour. Additional studies are still pending in this sense

Acknowledgements

The authors gratefully acknowledge the financial support of the Spanish Institutions Ministerio de Economía y Competitividad and the European Regional Development Fund (FEDER) (Projects AGL2012-35088 and AGL2015-63849-C2-2-R). Authors thank Dr. Joanna Harasym for the revision of the paper and her comments about fungi and yeast β-glucans extraction and properties.

References

Banchathanakij R.; Suphantharika M. (2009). Effect of different β-glucans on the gelatinisation and retrogradation of rice starch. Food Chemistry 114, 5–14.

Borchani, C.; Fonteyn, F.; Jamin, G.; Paquot, M.; Thonart, P.; Blecker, C. (2016). Physical, functional and structural characterization of the cell wall fractions from baker's yeast *Saccharomyces cerevisiae*. Food Chemistry, 194, 1149–1155.

Cronin, C.C.; Shanahan, F. (1997). Insulin-dependent diabetes mellitus and coeliac disease. The Lancet, 349, Issue 9058, 1096-1097.

EFSA (2011). Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on the safety of "yeast beta-glucans" as a novel food ingredient. EFSA J. (2011), 9, 2137.

Hager, A.; Ryam, L.; Schwab, C.; Gänzle, M.; O'Doherty, J. & Arendt, E. (2011). Influence of the soluble Fibers inulin and oat β-glucan on quality of dough and bread. European Food Research and Technology. 232, 405 – 413.

Hellert, C., Svensson, M., Tholstrup, J., Hultberg, B. (2009). Clinical trial: B vitamins improve health in patients with coeliac disease living on a gluten-free diet. Aliment Pharmacol Ther; 29(8):811-816.

Heo, S.; Jeon, S. & Lee, S. (2014). Utilization of *Lentinus edodes* mushroom β-glucan to enhance the functional properties of gluten-free rice noodles. Food Science and Technology 55, 627 – 631

Kim, J.; Lee, S.; Bae, I.; Park, H.; Lee, H. & Lee, S. (2011). (1 – 3)(1 – 6)-β-Glucan-enriched materials from Lentinus edodes mushroom as a high-Fiber and low-calorie flow substitute for baked foods. Journal of Science of Food and Agriculture 91 (10), 1915 – 1919.

Kittisuban, P.; Ritthiruangdej, P. & Suphantharika, M. (2014). Optimization of hydroxypropylmethylcellulose, yeast β-glucan, and whey protein levels based on physical properties of gluten-free rice bread using response surface methodology. Food Science and Technology 57, 738 – 748.

Kogan, G.; Kocher, A. (2007). Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livestock Science, 109, Issues 1–3, 161-165.

Krupa-Kozak, U., Altamirano-Fortoul, R., Wronkowska, M., & Rosell, C. M. (2012). Breadmaking performance and technological characteristic of gluten-free bread with inulin supplemented with calcium salts. European Food Research and Technology, 235, 545-554.

Lazaridou, A.; Duta, D.; Papageorgiou, M.; Belc, N. & Biliaderis, C. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79 (3), 1033-1047.

Lebwohl, B., Green, P.H.R, Genta, R.M. (2015). The coeliac stomach: Gastritis in patients with coeliac disease. Aliment Pharmacol Ther; 42(2):180-187.

Lerner, A., Matthias, T. (2015). Celiac disease: Intestinal, heart and skin interconnections. Intl J Celiac Dis; 3(1):28-30.

Liu, X.; Wang, Q.; Cui, S. & Liu, H. (2008). A new isolation method of β-D-glucans from spent yeast Saccharomyces cerevisiae. Food Hydrocolloids 22, 239 – 247.

Martinez, M. M.; Díaz, A.; Gómez, M. (2014). Effect of different microstructural features of soluble and insoluble Fibers on gluten-free dough rheology and bread-making. Journal of Food Engineering 142, 49–56.

Martins, Z.E.; Erben, M.; Gallardo, A.E.; Silva, R.; Barbosa, I.; Pinho, O. & Ferreira, I. M. P. L. V. O. (2015). Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread. International Journal of Food Science and Technology, 50, 1855-1863.

Meilgaard, M.; Civille, G.V.; Carr, B.T. (2006). Sensory Evaluation Techniques. Crc Press Inc., 4th Ed., 448p.

Nunes, M. H. B., Ryan, L. A. M., & Arendt, E. K. (2009). Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. European Food Research and Technology, 229, 31-41.

Peressini, D.; Foschia, M.; Tubaro, F.; Sensidoni, A. (2015). Impact of soluble dietary Fiber on the characteristics of extruded snacks. Food Hydrocolloids 43, 73-81

Pérez-Quirce, S.; Collar, C. & Ronda, F. (2014). Significance of healthy viscous dietary Fibers on the performance of gluten-free rice-based formulated breads. International Journal of Food Science and Technology, 49, 1375-1382.

Ronda, F.; Gómez, M.; Blanco, C.A. & Caballero, P.A. (2005). Effects of polyols and nondigestible oligosaccharides on the quality of sugar-free sponge cakes. Food Chemistry 90, 549-555.

Ronda, F.; Pérez-Quirce, S.; Angiolini, A. & Collar, C. (2013). Impact of viscous dietary Fibers on the viscoelastic behaviour of gluten-free formulated rice doughs: A fundamental and empirical rheological approach. Food Hydrocolloids 32, 252-262.

Ronda, F.; Pérez-Quirce, S.; Lazaridou, A. & Billiaderis, C. (2015). Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics. Food Hydrocolloids 48, 197 – 207.

Ronda, F.; Roos, Y.H. (2011). Staling of fresh and frozen gluten-free bread. Journal of Cereal Science, 53, (3), 340-346.

Ronda, F., Quilez, J., Pando, V., Roos, Y. (2014). Fermentation time and fiber effects on recrystallization of starch components and staling of bread from frozen part-baked bread. Journal of Food Engineering, 131, 116-123.

Rosell, C. M., Santos, E., & Collar, C. (2009). Physico-chemical properties of commercial 431 Fibers from different sources: A comparative approach. Food Research International, 42, 432 176-184.

Sabanis, D., Lebesi, D., Tzia, C., (2009). Effect of dietary Fiber enrichment on selected properties of gluten-free bread. LWT-Food Sci. Technol. 42, 1380–1389.

Samuelsen, A.; Schrezenmeir, J. & Knutsen, H. (2014). Effects of orally administered yeast-derived β-glucans: A review. Molecular Nutrition & Food Research 58 (1) 183 – 193.

Santipanichwong, R., & Suphantharika, M. (2009). Influence of different β-glucans on the physical and rheological properties of egg yolk stabilized oil-in-water emulsions. Food Hydrocolloids, 23, 1279–1287.

Satrapai, S., & Suphantharika, M. (2007). Influence of spent brewer's yeast β-glucan on gelatinization and retrogradation of rice starch. Carbohydrate Polymers, 67, 500-510.

Wang, L., Miller, R.A., Hoseney, R.C. (1998). Effects of (1→3), (1 →4)-β-D-glucans of wheat flour on breadmaking. Cereal Chemistry. 75(5):629–633.

Worrasinchai, S., Suphantharika, M., Pinjai, S., & Jamnong, P. (2006). β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. Food Hydrocolloids, 20, 68–78.

Ziobro, R.; Witczak, T.; Juszczak, L.; Korus, J. (2013). Supplementation of gluten-free bread with non-gluten proteins. Effect on dough rheological properties and bread characteristic. Food Hydrocolloids, 32, (2), 213-220.

CONCLUSIONES

CONCLUSIONES

La principal conclusión de esta tesis doctoral se resume en que es posible el enriquecimiento de pan sin gluten con β-glucanos en dosis que permiten cumplir con la alegación de salud de EFSA mediante el consumo diario de 170 g de pan (adición del 3.9% en base harina), para la reducción del colesterol sérico y preservando su peso molecular durante el proceso de panificación para asegurar los beneficios para la salud que se le suponen a esta fibra soluble También se ha concluido la viabilidad del enriquecimiento de pan sin gluten con β-glucanos de levadura y hongos, (en dosis hasta el 2% en base harina), manteniendo e incluso mejorando la calidad físico-química y sensorial de los panes, en particular cuando se empleó el ingrediente soluble.

Profundizando en estas conclusiones, se extraen las siguientes conclusiones más relevantes en relación a los capítulos de los que proceden:

1- La adición de β-glucano por sí sólo no logró imitar la viscoelasticidad del gluten. Su incorporación dio lugar a un perfil de viscosidad y una consistencia mayores y una marcada disminución en la altura y volumen de los panes resultantes. Por lo tanto, su adición no impide la necesidad de adicionar algún otro hidrocoloide formador de gel del tipo de los comúnmente empleados en productos sin gluten, como la hidroxipropilmetilcelulosa (HPMC).

   Entre los tipos de HPMC recomendados en panificación el formador de gel débil dio lugar a panes de menor volumen en comparación con el HPMC que forma un gel semi-firme. Sin embargo, el empleo de este último en la formulación, junto con el exceso de agua que requieren las masas en presencia de altos contenidos de β-glucano, favoreció la aparición de grandes defectos alveolares en la miga de pan que no resultaron aceptables. Por lo tanto, se desaconseja el empleo de este HPMC en beneficio del primero.

2- La optimización de la hidratación en las formulaciones sin gluten ha resultado ser un factor clave, afectando de manera determinante en la reología de las masas y la calidad de los panes. La adición de β-glucano a una masa de baja

hidratación disminuyó el volumen del pan, mientras que el aumento del agua en la masa ejerció el efecto opuesto, dando lugar a un aumento en el volumen hasta un valor máximo, correspondiente a la hidratación óptima de la masa. Un exceso de hidratación ocasiona que el volumen específico del pan descienda nuevamente. Se recomienda una hidratación de la masa alrededor del 90-92% (en base harina) para obtener el volumen máximo de panes sin BG añadido. Esta adaptación de las necesidades de agua en función del contenido de β-glucano se ha confirmado en gran parte de los capítulos de esta Tesis Doctoral (II, III, IV, VI y VII).

3- Los factores que han resultado determinantes en el estudio del enriquecimiento de panes con β-glucano fueron el origen del β-glucano, su concentración y su peso molecular. Suponiendo un nivel de hidratación óptimo, el volumen específico de panes se vio afectado positivamente por la adición de BG a niveles bajos (1.3% en base harina) independientemente del peso molecular del BG. Sin embargo, la fortificación de las masas sin gluten con mayores cantidades de BG (hasta el 3.9% base harina) aumentó significativamente la consistencia de las masas y redujo el volumen del pan. Este efecto fue observado desde el primer capítulo de la Tesis y ha quedado ratificado a lo largo de los capítulos II, III y IV.

4- Se obtuvieron grandes diferencias en función del origen del β-glucano. A igual contenido de β-glucano puro y con la hidratación óptima, la reología de las masas y los atributos de calidad de panes enriquecidos con BG de cebada, de bajo peso molecular y pureza elevada, se vieron notablemente afectados, frente a aquellos enriquecidos con BG de avena, de alto peso molecular y baja pureza, que mostraron efectos menos pronunciados comparados con el control. El alto contenido en excipientes, fundamentalmente maltodextrinas, en el concentrado comercial de avena podría ser el causante de un efecto debilitador en estas masas.

5- El peso molecular de los β-glucanos de cereal purificados y aislados resultó ser un factor de estudio decisivo en la elaboración de panes enriquecidos. La incorporación de BG de alto peso molecular mostró el mayor impacto en la reología de las masas, conduciendo al mayor volumen específico y menor dureza de la miga, al tiempo que un mayor potencial para los beneficios sobre la salud, ya que a elevadas concentraciones (3.9% base harina) se observó una disminución significativa de la glucosa rápidamente digerible en el pan. Por su parte, formulaciones con BG de medio o bajo peso molecular tuvieron un impacto significativo, aunque no tan destacado, en los parámetros reológicos y en los atributos de calidad del pan. La reducción y / o eliminación de las maltodextrinas en estas muestras también contribuyó a esos efectos.

6- Se requiere un control de la actividad β-glucanásica en las materias primas, ya que se apreció una hidrólisis de los β-glucanos en el producto final que limitaría los beneficios para salud asociados al consumo de estos polisacáridos bioactivos. El empleo de tratamientos con energía microondas se planteó como método eficaz de inactivación térmica de la β-glucanasa endógena de la harina de arroz. Con tan sólo 4 minutos de tratamiento en harinas al 25% de humedad se alcanzó la inactivación completa de la enzima β-glucanasa. El empleo de estas harinas tratadas en la fortificación de panes sin gluten con β-glucanos permitió mantener el elevado peso molecular de estos polisacáridos tras la panificación. El tratamiento térmico no perjudicó a las propiedades de los panes obtenidos con las harinas de arroz tratadas.

7- Se concluyó el gran efecto de la procedencia, dosis y solubilidad del (1 → 3)(1 → 6)-β-glucano extraído de levaduras u hongos, en la calidad del pan sin gluten enriquecido. La adición del extracto de levadura en su forma soluble disminuyó G', G'' y la consistencia, mientras que las masas enriquecidas con β-glucanos insolubles mostraron la tendencia opuesta, aumentando su resistencia a la deformación a medida que aumentaba la concentración. La calidad física de los panes se mejoró significativamente mediante la adición de β-glucano a la masa con hidratación optimizada, aumentando el volumen específico de los panes, reduciendo su dureza y retrasando el endurecimiento de la miga durante el

almacenamiento. Los panes enriquecidos con BG soluble mostraron, a su vez, una mejora sustancial en los atributos organolépticos del pan.

Los hallazgos del presente estudio podrían contribuir a mejorar la calidad y la bioactividad de los productos horneados sin gluten basados en arroz, al incorporar concentrados de β-glucano de cereales o levaduras y hongos, para ampliar las opciones de alimentos al alcance de los consumidores celíacos.

A partir de los resultados presentados en esta Tesis Doctoral, las perspectivas de trabajos futuros se orientan en dos direcciones. En primer lugar, los destinados a completar algunos aspectos de caracterización nutricional, como la realización de estudios de digestibilidad del almidón *in vivo*, o la realización de estudios más detallados sobre el efecto de las radiaciones microondas sobre diversas harinas entre otros. En segundo lugar, la aplicación industrial de cualquiera de los β-glucanos evaluados en la Tesis para su incorporación en productos aptos para enfermos celiacos. En este sentido ya hay una empresa que se dedica a la extracción de β-glucanos de hongos que está interesada en la aplicación de los resultados que de aquí se deriven. La citada empresa apoyaría esta investigación por el interés que para ella encierra el conocimiento de la viabilidad de aplicar estos beta-glucanos en un alimento de alto consumo, como es el pan.

CONCLUSIONS

The main conclusion of this doctoral thesis is that it is possible the enrichment of gluten-free breads with cereal β-glucans to fulfil EFSA's health claim for the reduction of serum cholesterol and simultaneously preserve polysaccharide molecular weight during the baking process that ensures the health benefits of this soluble fiber; a daily consumption of 170 g of bread that contained 3.9 % β-glucans (flour basis) was required for obtained the claimed effect. The feasibility of gluten-free bread fortification with yeast and fungi β-glucans (at levels up to 2% flour basis) was also concluded, since they maintain or even improve the physicochemical and sensorial quality of the breads, particularly when the soluble ingredient was used.

Further to these conclusions, the following concluding remarks were drawn from each of the 7 chapters of the thesis:

1- The addition of cereal β-glucan by itself to the gluten-free formulations failed to mimic the gluten viscoelastic behavior in dough. Its incorporation led to a higher viscosity and greater consistency of dough and a marked decrease in the height and volume of the resultant breads compared to a control formulation. Therefore, its addition did not eliminate the requirements for some other gel forming hydrocolloid, commonly used in gluten-free products, such as hydroxypropylmethylcellulose (HPMC).

   Among the different types of HPMC recommended for breadmaking, a weak-gel type resulted in lower bread volume compared to HPMC which formed a semi-firm gel network. However, the use of the latter in the gluten-free formulation, in combination with the excess of water required by the doughs when β-glucan at high levels is added, favored the appearance of non-uniform porosity in the bread crumb. Therefore, the use of this HPMC type was discouraged in the best interest of the weak-gel type.

2- The optimization of the water level in gluten-free doughs has proved to be a key factor, affecting in a decisive way the dough rheology and the bread quality. The individual addition of β-glucan to a dough with low hydration decreased the bread volume, while the increase of water content exerted the opposite effect,

resulting in an increase in bread volume up to a maximum value, corresponding to the optimal dough hydration level; a further increase of water caused the decline of specific volume. It is recommended a dough water content of 90-92% (flour basis) to obtain the maximum bread volume in the absence of BG. The need for adaptation of the added water levels according to the β-glucan content of the enriched dough formulations was confirmed in most of the individual studies of this Doctoral Thesis (chapters II, III, IV, VI and VII).

3- Determinant factors for the dough and bread quality attributes were found to be the origin, concentration and molecular weight of cereal β-glucans. Adopting the optimum level of dough hydration, bread specific volume was positively affected by the addition of BG at low levels (1.3% flour basis), regardless the molecular weight of BG. However, fortification of gluten-free doughs with higher amounts of BG (up to 3.9% flour basis) significantly increased the dough consistency and reduced the specific volume. This effect was observed in several studies of the Thesis (chapters I, II, III and IV).

4- Great impact on gluten-free breadmaking had the origin and type of the added β-glucan commercial concentrates. Using the same content of pure β-glucan at optimum hydration levels, the effects of a barley β-glucan concentrate with low molecular weight and high purity on dough rheological behavior and bread quality attributes were more pronounced than those of an oat β-glucan concentrate having high molecular weight and low purity. The high content of other substances present in the commercial oat β-glucan preparation, mainly maltodextrins, could be cause a weakening effect in these doughs.

5- Molecular weight of isolated and purified cereal β-glucan concentrates proved to be a decisive factor in the bread enrichment. Among three β-glucan preparations differing in molecular weight the incorporation of the high molecular weight sample showed the greatest impact on dough rheological parameters leading to the highest loaf specific volume and the lowest crumb hardness, as well as the greatest potential for health benefits, since at high concentrations (3.9% based on flour) a significant decrease of rapidly available glucose in bread was observed. On the other hand, formulations with medium or low molecular weight β-glucans had also a significant impact on dough rheological parameters and bread

quality attributes, but not so great as the high molecular weight preparation. The reduction and / or elimination of maltodextrins in these concentrates also contributed to these effects.

6- The inhibition of β-glucanase activity in raw materials of gluten-free breads was required, as a hydrolysis of β-glucans during breadmaking process was found, which would limit the health benefits associated with the consumption of these bioactive polysaccharides. The use of microwave energy treatment was proposed as an effective method for thermal inactivation of the endogenous β-glucanase in rice flours. Only 4 minutes of heat treatment in flours at 25% water content was enough to result in enzyme inactivation. The use of these treated flours in gluten-free breads fortified with cereal β-glucans allowed to maintain the high molecular weight of these polysaccharides during breadmaking process. The heat treatment did not impair the physical properties of the breads obtained from the treated rice flours.

7- The great effect of the source, concentration and solubility of (1 → 3)(1 → 6)-β-glucan extracts from yeast or fungi on the quality of gluten-free enriched breads was also concluded. The addition of the soluble yeast extract decreased the elastic (G') and loss (G'') moduli and the dough consistency, while enriched doughs with insoluble β-glucans showed the opposite trend, increasing their resistance to deformation with increase of polysaccharide concentration. The bread physical properties were significantly improved by the addition of microbial β-glucans, at optimized dough hydration level as evidenced by the increase of loaf specific volume, reduction of crumb hardness and delay of crumb hardening during bread storage. Breads enriched with soluble yeast β-glucan showed also a significant enhancement of their sensorial attributes.

The findings of the present thesis could contribute to improving the quality and bioactivity of rice-based gluten-free baked products that contain β-glucan concentrates from cereal grains or yeast and fungi and expanding the food choices available to celiac consumers.

Based on the results presented in this doctoral Thesis, future work perspectives are oriented in two directions. Firstly, to go in depth in the nutritional effects of the products studied by means of *in vivo* tests, such as *in vivo* starch digestibility studies, and to carry out more detailed studies on the effect of microwave energy on different types of flours. Secondly, the application at an industrial level of the β-glucans evaluated in the Thesis for the enrichment of products suitable for celiac patients. In this respect, a company that is dedicated to the extraction of β-glucan from fungi is interested in the knowledge of the feasibility of the application of these β-glucans in a staple food such as bread.