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# Effect of microwave radiation pre-treatment of rice flour on gluten-free breadmaking and molecular size of $\beta$ -glucans in the fortified breads

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## Abstract

Cereal  $\beta$ -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  $\beta$ -glucanases in rice flour, in combination with prolonged mixing, fermentation and proofing time, can cause a substantial reduction in  $\beta$ -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  $\beta$ -glucanase activity. Gluten-free breads made from the MIWA treated rice flours were fortified with oat  $\beta$ -glucan concentrate to enhance their nutritional profile. The molecular weight of added  $\beta$ -glucan in the final products increased with increasing both flour water content and time of MIWA treatment, reflecting the magnitude of residual  $\beta$ -glucanase activity in the flour. Pretreatment with MIWA radiation for 4 min of the rice flour tempered at 25% moisture resulted in negligible residual  $\beta$ -glucanase activity and preserved to a great extent the molecular weight of  $\beta$ -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  $\beta$ -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:**  $\beta$ -glucan molecular weight;  $\beta$ -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  $\beta$ -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  $\beta$ -glucan ingestion, with a daily consumption of 3g of  $\beta$ -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  $\beta$ -glucans were also approved by EFSA concerning the reduction in post-prandial glycaemic responses, at recommended doses of about 4g of  $\beta$ -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  $\beta$ -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  $\beta$ -glucans in formulated products, since is related to the viscosity of  $\beta$ -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  $\beta$ -glucanases, in combination with the long contact time during mixing, fermentation and proofing, can cause a substantial reduction in  $\beta$ -glucan molecular weight during production of  $\beta$ -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  $\beta$ -glucan degradation (Andersson et al. 2004; Lazaridou et al. 2014; Moriartey et al. 2010; Vatandoust et al. 2012). Some methods previously used for the  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -glucan concentrate were made from rice flours pretreated by microwave (MIWA) radiation at different times and moisture levels aiming at the retention of  $\beta$ -glucan molecular size in breads and hence at maximizing their physiological functionality. Further to the amount and molecular weight of  $\beta$ -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  $\mu$ m, 150  $\mu$ m > 63.2% > 100  $\mu$ m and 30.8% < 100  $\mu$ m 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  $\beta$ -glucanase

activities, corresponding to about 0, 25, 50, 75 and 100%  $\beta$ -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 $\rightarrow$ 3) (1 $\rightarrow$ 4)- $\beta$ -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;  $\beta$ -glucan content 33-36%. The purification protocol involved aqueous extraction of the polysaccharide (50°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°C x 3 h) by porcine pancreas  $\alpha$ -amylase (100.000U/g flour, Megazyme International Ltd, Bray, Ireland), concentrated (90°C x 2 h to ½ of the volume) and the  $\beta$ -glucan was precipitated with two volumes of ethanol (4°C x 24 h). Finally, the polysaccharide was re-suspended in 2-propanol (4°C x 24 h), filtered and dried (50°C x 18 h) to obtain a high molecular weight  $\beta$ -glucan preparation (HMW-BG). As a result of this purification scheme, the Promoat™ was concentrated from 33 to 72 %  $\beta$ -glucan content (HMW-BG) as assessed by the mixed-linkage (1 $\rightarrow$ 3), (1 $\rightarrow$ 4)  $\beta$ -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 $\beta$ -Glucanase activity determination**

$\beta$ -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  $\beta$ -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°C x 25 min) and they were mixed with an aqueous solution (0.1% w/v) of a high molecular weight ( $2 \times 10^6$ )  $\beta$ -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  $\eta$  and  $\eta_0$  is the viscosity of above mixture and water, respectively) was measured over 1h period at  $20 \pm 0.1$  °C every 5 min intervals. The  $\eta_{sp}$  data versus time were fitted to a linear regression model and the  $\beta$ -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  $\beta$ -glucan solution upon addition of the flour extracts. Residual  $\beta$ -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  $20 \pm 2$  °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  $\beta$ -glucan fortification level following the same breadmaking process.

#### **2.2.5 Content and molecular weight determination of $\beta$ -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  $\beta$ -glucanase activity of rice flour. The concentration of BG in bread was determined using the mixed-linkage (1 $\rightarrow$ 3)(1 $\rightarrow$ 4) $\beta$ -D-glucan assay kit of Megazyme. For molecular weight evaluation, the  $\beta$ -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  $\beta$ -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  $20 \pm 3$  °C 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 2° 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 $\beta$ -glucanase activity of rice flours**

The  $\beta$ -glucanase activity in rice flour estimated from the rate of decrease in  $\eta_{sp}$  of a  $\beta$ -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 84°C (treatment 2), 93°C (treatment 3) and 96°C (treatments 4 and 5). The five microwave treatments (including no treatment) led to rice flours with  $\beta$ -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  $\beta$ -glucanase activity of rice flour on the molecular weight of the resultant BG in the enriched breads.

**Table 1.** Experimental design and residual  $\beta$ -glucanase activities of rice flours treated by microwave energy.

Microwave (MIWA) treatment	Flour water content (%fb) <sup>b</sup>	Treatment time (min)	$\beta$ -glucanase activity <sup>c</sup> ( $\text{h}^{-1}$ ) <sup>d</sup>
1	13	0	0.109 ( $\pm 0.005$ ) e
2 <sup>a</sup>	16	1	0.076 ( $\pm 0.001$ ) d
3 <sup>a</sup>	16	2	0.052 ( $\pm 0.010$ ) c
4 <sup>a</sup>	16	4	0.022 ( $\pm 0.009$ ) b
5 <sup>a</sup>	25	4	0.000 ( $\pm 0.001$ ) a

<sup>a</sup> Flour tempered before the microwave treatment to increase its initial moisture level (13%) to the specified level.

<sup>b</sup> Moisture content of flours after the microwave treatment.

<sup>c</sup> 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.

<sup>d</sup> Expressed as decrease in specific viscosity per hour of a purified  $\beta$ -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  $\beta$ -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).

<b>Microwave (MIWA) treatment</b>	<b>Peak viscosity (mPa·s)</b>	<b>Trough viscosity (mPa·s)</b>	<b>Breakdown viscosity (mPa·s)</b>	<b>Final viscosity (mPa·s)</b>	<b>Setback viscosity (mPa·s)</b>	<b>Peak temperature (°C)</b>
<b>1</b>	2336 a	1406 a	862 b	3271 a	1866 a	78.8 ab
<b>2</b>	2362 a	1448 a	925 b	3305 a	1879 a	78.4 a
<b>3</b>	2364 a	1462 ab	908 b	3332 ab	1870 a	79.2 ab
<b>4</b>	2478 a	1563 b	877 b	3458 b	1904 a	79.9 bc
<b>5</b>	2416 a	1681 c	745 a	3466 b	1885 a	80.9 c
<b>SE</b>	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  $\beta$ -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 (84°C and 93°C, respectively) that can denature other enzymes than  $\beta$ -glucanases, including the  $\alpha$ -amylases necessary for bread development (Caballero et al. 2007; Gujral et al. 2003); the reduction of  $\alpha$ -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  $\alpha$ -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 ( $\alpha$ -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 (Pylar 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 $\beta$ -glucanase inactivation of flour on molecular weight of $\beta$ -glucan isolated from in fortified gluten-free breads

The  $\beta$ -glucan content in the final products fortified with the  $\beta$ -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  $\beta$ -glucanase activity (MIWA treatment 1 and 2) (Tables 1 and 4). The level (3.9% of pure  $\beta$ -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  $\beta$ -glucan breads.

**Table 4.**  $\beta$ -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 ( $\pm$ 0.12)a	49.3 ( $\pm$ 1,08)a	1.4	214
2	3.2	3.0 ( $\pm$ 0.16)ab	49.2 ( $\pm$ 0,92)a	1.5	200
3	3.2	3.2 ( $\pm$ 0.16)ab	48.7 ( $\pm$ 1,07)a	1.7	181
4	3.2	3.4 ( $\pm$ 0.22)b	48.0 ( $\pm$ 0,90)a	1.8	169
5	3.2	3.1 ( $\pm$ 0.09)ab	50.4 ( $\pm$ 1,10)a	1.5	197

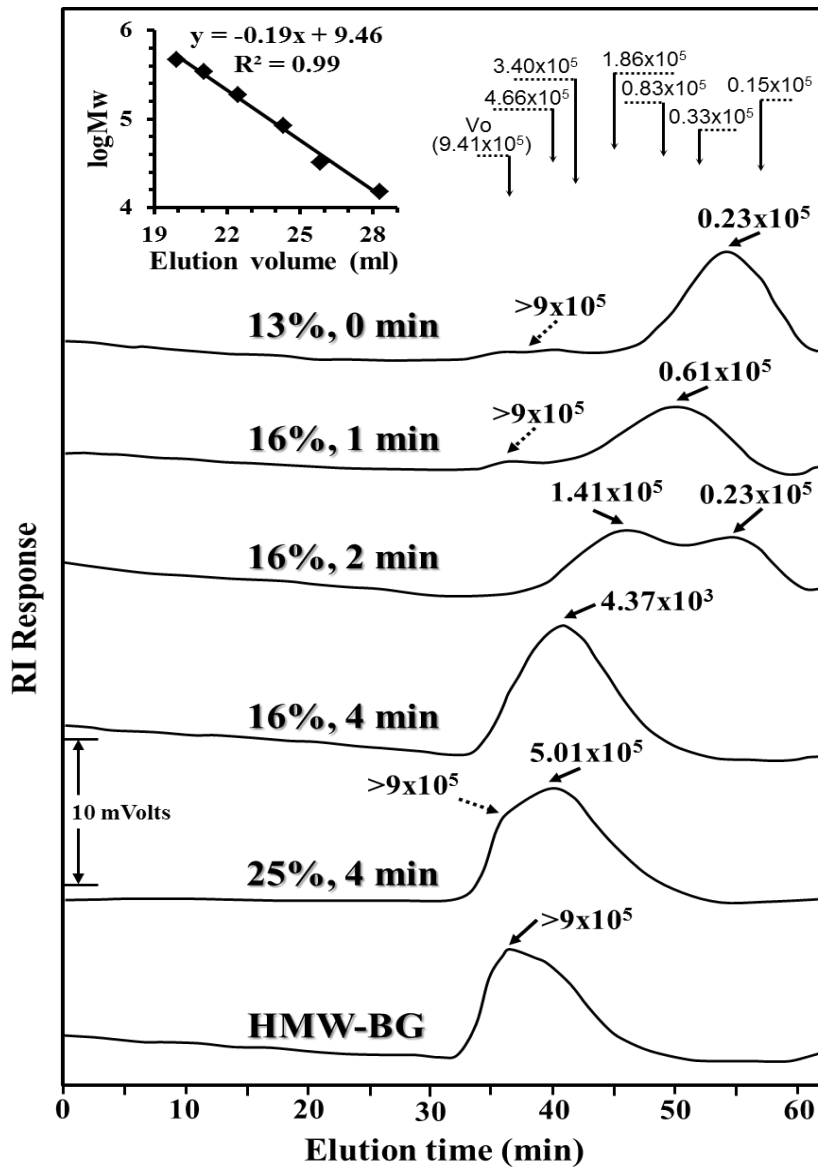
Mean values with different letters for the same parameter imply significant differences between means at  $p < 0.05$ .

The molecular weight distributions of the HMW-BG preparation added to the gluten free formulations as well as the  $\beta$ -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  $\beta$ -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  $\beta$ -glucan added to the gluten-free doughs had a  $M_p > 9 \times 10^5$  Da. However, for the  $\beta$ -glucans isolated from the control bread made from the untreated rice flour there was a large reduction in  $M_p$ , as the main eluting peak was  $\sim 0.23 \times 10^5$  Da, presumably due to polysaccharide degradation by endogenous  $\beta$ -glucanases of the rice flour during the breadmaking process (Table 1). Similarly, several researchers have previously noted a considerable reduction of  $\beta$ -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  $\beta$ -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  $\beta$ -glucans in the final products decreases with increasing mixing and dough fermentation time. Recently, an endogenous  $\beta$ -glucanase activity was also found in rice flour (Perez-Quirce et al. 2016) which can cause severe reduction of oat and barley  $\beta$ -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  $M_p$  ( $0.61 \times 10^5$  Da) compared to that made from the untreated flour (Figure 2), presumably due to a partial decrease in  $\beta$ -glucanase activity as evidenced from the data in Table 1. A minor fraction of  $\beta$ -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  $M_p > 9 \times 10^5$  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  $\beta$ -glucan molecular weight as shown by the shifting of polysaccharide eluting peaks to lower retention times and increase of  $M_p$  up to  $4.37 \times 10^5$  Da (Figure 2); this observation is consistent with the decline in  $\beta$ -glucanase activity of the respective treated flours (Table 1). The eluting profile of  $\beta$ -glucans in bread prepared from the flour heated with MIWA for 2 min showed two main peaks with  $M_p$  values of 1.41 and  $0.23 \times 10^5$  Da which represent 61 and 39% of the total chromatograph area, respectively (Figure 2). Similar bimodal molecular weight distributions of  $\beta$ -glucans isolated from oat, rye and barley breads have been previously

reported and attributed also to the endogenous  $\beta$ -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,  $M_p$ , (slanted solid arrows) of the eluting peaks of the enriched in high molecular weight oat  $\beta$ -glucan concentrate (HMW-BG) added to the gluten-free formulations and of the  $\beta$ -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  $M_p$  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 $\rightarrow$ 3) (1 $\rightarrow$ 4)  $\beta$ -D-glucan standards ( $M_p$ : 15, 33, 83, 186, 340 and 466  $\times 10^3$  Da) used for plotting of the standard curve (inset); (1 $\rightarrow$ 3) (1 $\rightarrow$ 4)  $\beta$ -D-glucan standard with  $M_p$  941  $\times 10^3$  Da is eluted at the void volume of the columns. All  $M_p$  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  $\beta$ -glucan in bread, as indicated by the higher

Mp value,  $5.01 \times 10^5$  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  $\beta$ -glucans maintained to a considerable extent their initial molecular size, since a large portion of the eluted polysaccharides were of high molecular weight ( $>9 \times 10^5$  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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -glucanase activity using the same viscometric method. In the present study, the slight drop of  $\beta$ -glucan molecular weight during production of breads made by the MIWA treatment 5 flour (with no apparent  $\beta$ -glucanase activity) could be attributed either to minor residual enzyme activity, non-detectable by the viscometric method or by oxidative degradation reactions involving of  $\beta$ -glucans; apparently, there is evidence for hydroxyl radical mediated depolymerisation of  $\beta$ -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  $\beta$ -glucans, do not cause reduction in the molecular weight of the bioactive polysaccharides upon baking, presumably due to inactivation of the endogeneous rice flour  $\beta$ -glucanases. The  $\beta$ -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  $\beta$ -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  $\beta$ -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.

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## 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  $\beta$ -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)- $\beta$ -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  $\beta$ -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.
- Horndok, 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., Hoehstoetter, 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  $\beta$ -glucan in thermal treatments. *Carbohydrate Polymers*, 85, 645-652.
- Lazaridou, A., Biliaderis, C. G., Micha-Screttas, M., & Steele, B. R. (2004). A comparative study on structure-function relations of mixed linkage (1 $\rightarrow$ 3), (1 $\rightarrow$ 4) linear  $\beta$ -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  $\beta$ -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 amylo maize V starches. *Starch-Starke*, 58, 468-474.
- Moriarty, 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., Adebawale, 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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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)  $\beta$ -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., Ragae, S.M., Wood, P.J., Tosh, S.M., and 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., Lamarche, B., Thomson, B. A., Duss, R., & Wood, P. J. (2010). Physicochemical properties of oat  $\beta$ -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.