Effect of microwave radiation pre-treatment of rice flour on gluten-free breadmaking and molecular size of β-glucans in the fortified breads [*]

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

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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.

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 $(1\rightarrow3)$ $(1\rightarrow4)$-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°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 α-amylase (100.000U/g flour, Megazyme International Ltd, Bray, Ireland), concentrated (90°C x 2 h to ½ of the volume) and the β-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 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 $(1\rightarrow3)$, $(1\rightarrow4)$ 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°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 ℃ 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 $(1\rightarrow3)(1\rightarrow4)$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 ℃ 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).
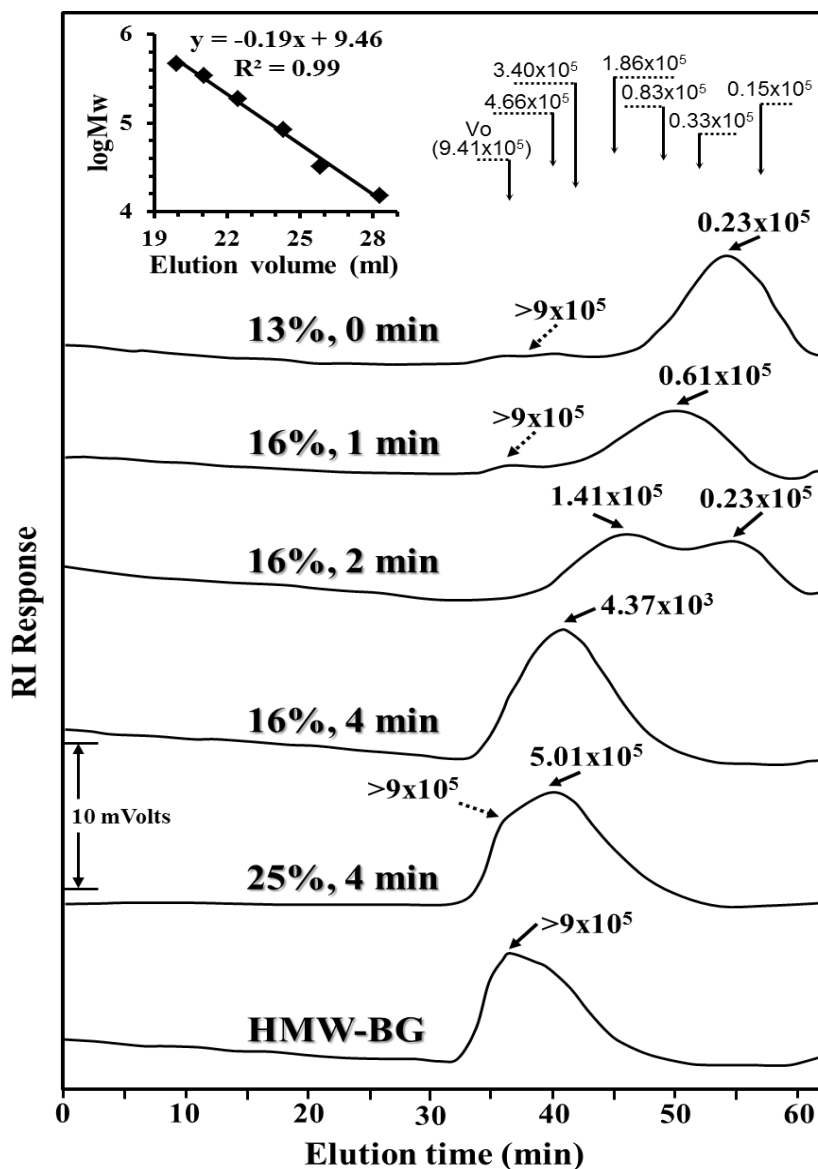

Figure 2. HPSEC elution profiles detected by RI and apparent peak molecular weight, Mp, (slanted solid arrows) of the eluting peaks of the enriched in high molecular weight oat β-glucan concentrate (HMW-BG) added to the gluten-free formulations and of the β-glucan extracted from the crumbs of the fortified 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

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