ARTICLE / INVESTIGACIÓN

Effect of rice flour ultrafine particle size on β-glucanase inactivation by microwave treatments and pasting properties in treated flours

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Abstract: The main problem in developing gluten-free products enriched with β-glucans (BG) is the hydrolysis they suffer due to the endogenous β-glucanase content of raw materials. The depolymerization causes a decrease in the molecular weight and viscosity, which is the most important parameter related to the health claims of BG. Hence, it is necessary to inactivate the β-glucanase. Therefore, this study aimed to inactivate the β-glucanase activity present in rice flour (Indica variety) with fine particle size using the microwave (MW) hydrothermal treatment method. The rice flour was conditioned at three different humidities 13, 16, 19 and 25%. Samples of 50 g were subjected to different microwave treatments 1, 2, 4 and 8 min, at 900 W power at 20 seconds intervals of treatment and 1-minute rest. The effect of particle size on inactivating the β-glucanase activity and the effect of MW treatment on the flour pasting property were also studied. The inactivation process followed a first-order kinetic response, and the apparent rate constant of thermal inactivation increased exponentially with the moisture content (M) of the flour, according to the equation $-0.02t \cdot \exp(0.19 \cdot M)$ ($R2 = 0.987$). Among the treatments employed 4 min treatment at 25% initial M was found effective for complete β-glucanase inactivation. The effect of flour particle size did not show a significant ($p > 0.05$) difference on the efficiency of β-glucanase inactivation.

Key words: Particle size, β-glucans, rice flour, microwave treatment, β-glucanase activity.

Introduction

Rice (*Oryza sativa L.*) is one of the world's most important cereals, a staple food for more than half of the world's population. Rice is harvested with husk, which accounts for approximately 20% of the weight[6]. The husk is removed mechanically to obtain white rice grain and rice bran[2]. This brand is a natural source of protein (14-16%), fat (12-23%), crude fiber (8-10%), carbohydrates (CHO's), vitamins, minerals, and essential unsaturated fatty acids, phenolic compounds. In addition to natural antioxidants such as tocopherols, and tocotrienols and a high enzyme load, they mainly consist of lipases, lipoxygenases and peroxidases[16]. These endogenous enzymes in rice, others can also be found, such as: polyphenol oxidases, amylases, catalases, β-glucanases, etc. Most endogenous rice enzymes are found in both the bran and the germ. The (1-3) (1-4) β-Glucans (BG) are essential components in the cell walls in the starchy endosperm and aleurone layer of cereals such as oats and barley and in low amounts in wheat and rye[10] and they are termed as non-starch polysaccharides and are classified as soluble dietary fiber with thickening properties. BG is claimed to have health benefits such as contributing in maintaining normal blood cholesterol levels (recommended intake of 3 g oat BG daily), reduction of the increase in blood glucose or post-prandial glycemia (intake of 4 g oat or barley BG /30g available CHO's per meal), prolongation to the feeling of satiety and with it a reduction in energy intake, improvement of digestive function[7]. The potential beneficial physiological activities of β-glucans are attributed to their ability to increase intestinal contents' viscosity and thereby modify the absorption rates of nutrients and bile acids[31].

Celiac disease is an immune-mediated enteropathy of the digestive tract that causes damage to the small intestine and interferes with the absorption of nutrients from food, characterized by permanent intolerance to gluten[5]. The ingestion of these gluten proteins causes a loss of intestinal villi leading to a reduction in the absorption of nutrients. Today the disease has become one of the most common disorders in the population affecting approximately 0.3-1.0% of the world's population. It affects more females than the male population in a 2:1 ratio. The only efficient treatment for this intolerance is strict lifelong adherence to a gluten-free (GF) diet[20].

Many gluten-free cereals like rice, corn sorghum, etc. They are used for the elaboration of GF foods. In addition, gluten-free products are mainly starchy, which leads to a nutritionally unbalanced diet due to their lack of fiber, vitamins and minerals[28]. Therefore, one of the challenges of the agri-food industry is an improvement in the quality of GF bakery products[4,18,21]. With a mild flavor, white color, better digestibility, hypoallergenic properties and the presence of easily digestible carbohydrates[22]. GF products usually have low amounts of fiber, both soluble and insoluble. Their dietary fiber enrichment seems necessary for their nutritional improvement[21]. Recent studies have opted to enrich breads made with rice flour (RF) with BG whose beneficial health effects have been mentioned above. The effects of BG in baking depend on its molecular weight, concentration and purity. In principle, higher concentration and molecular weight worsen bread quality but improve nutritional proper-

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ties. However, previous studies have shown that the effect of molecular weight depends on the presence of β-glucanases[18-21]. Due to this, it is very important to consider all the factors that could influence the molecular weight of BG. One aspect to consider is the enzymatic activity that can considerably hydrolyse this type of soluble fiber reducing its molecular weight in such a way that its beneficial aspects for health are lost.

The enzymes responsible for this hydrolysis are (1-3) (1-4) β-glucanases. To avoid the degradation of beta-glucans, several solutions have been investigated and proposed the inactivation of (1-3) (1-4) β-glucanases enzymes[11,18]. One method of enzyme inactivation studied is by applying microwave energy[8,9,13,17,18,33]. Microwave heating is based on transforming the alternating energy of the magnetic field into thermal energy because it affects polar and ionizable molecules of matter[3,15,27]. Is generated by the friction and collision of molecules that try to orient themselves within the oscillating field. It is generally assumed that microwave effects contribute to microbial destruction or enzyme inactivation due to thermal effects[9,17]. Mechanical rice flour grinding, characterized by the reduction of particle size, significantly influences the structure and several properties of rice flour[19]. It could therefore be a factor influencing enzyme inactivation. This study aims to experimentally test the inactivation of β-glucanase using microwave energy (MW) by checking the effect of flour particle size to try to preserve both the content and molecular weight of β-glucans avoiding their hydrolysis, from which gluten-free rice flour-based products would be enriched.

Materials and methods

Materials

Ultra-fine white Indica variety rice flour (microcer) supplied by Herba Ricemills S.L.U (Valencia, Spain) and without any preservation process was used for the study. The flour contained the following properties: Moisture <15.0%, acidity (ml of NaOH 0.1N/100g) <3.0mL, density 0. 50-0.70 g/cc, gluten <10ppm, protein >6.5%, ash <0.9, fat <1.0% and particle size: >150 µm; <1%, 150-90 µm; 5-15%, <90µm; >84%) (data provided by the manufacturer). To determine the effect of flour particle size on enzyme inactivation data on NPU flour, Pérez-Quirce et al. (2016). The β-glucan from oats of high purity (95%) was also used.

Sample preparation

The initial moisture content (MC) of the microcer rice flour was measured by the official method 44-19[1], The MC of the flour for the study were adjusted to 13, 16, 19 and 25% with an error of ± 0.3%. To adjust the moisture content of the flours, distilled water was sprayed while it was mixed using a mixer (Model 5KPM50, Kitchen Aid, St. Joseph, MI, USA) for an estimated 10 minutes until the indicated moisture contents were obtained. Subsequently, the hydrated flours were allowed to stand for 24 hours under refrigeration 4°C to equilibrate the moisture in the flour, then stored in heat-sealed polyethylene plastic bags. Each flour with its respective moisture content was subjected to 3 different microwave (MW) treatments times: 1, 2, 4 and 8 minutes at 900 W power. The control was untreated native microcer rice flour.

Microwave treatment

50 grams of hydrated flour were weighed and introduced in 20 x 30 cm bags (polyamide+polypropylene) (NOP101, Cryovac, Sealed air, NY, USA) hermetically sealed by a thermosealing machine (Magneta 300 MG model, Audio Elektro, Holland), each sample with an approximate thickness of 1 mm inside the bag was introduced into the microwave, with the objective that the distribution of the energy inside the MW is uniform on the sample. The test conditions were established as a power of 900W with cycles of 20 seconds of treatment alternating with 1 minute as stop time, successively until the desired treatment time was reached (1, 2, 4 and 8 min). Between each minute of stop time, the bag's temperature was measured by introducing an infrared probe (model Testo 826-T2, Lenzkirch, Germany) sweeping over the entire bag to obtain the maximum temperature reached by the flour during the treatment. In addition to the temperature measurement, the samples were shaken and mixed manually and rotated for homogenization of the treatment. The treated samples were then cooled in the microwave oven to room temperature. They were then stored in a freezer at a temperature of -20°C until their respective analysis. The moisture content of each flour sample was quantified before and after treatment to confirm the hermetic seal of the bags.

Sample	Moisture content (%)	Treatment time (min)
Control	13	-
RF-13-1	13	1
RF-13-2	13	2
RF-13-4	13	4
RF-13-8	13	8
RF-16-1	16	1
RF-16-2	16	2
RF-16-4	16	4
RF-16-8	16	8
RF-19-1	19	1
RF-19-2	19	2
RF-19-4	19	4
RF-19-8	19	8
RF-25-1	25	1
RF-25-2	25	2
RF-25-4	25	4
RF-25-8	25	8

Table 1. Microwave conditions and ID of treated samples used in the study.

β-glucanase activity

The β-glucanase activity of each MW-treated sample was evaluated by employing a viscosity decrease index. Endogenous β-Glucanases in rice flour acted on a BG solution by hydrolyzing and breaking bonds (depolymerization), thereby resulting in a decrease in the molecular weight of the extract-solution mixture by reducing the viscosity, which is governed by the following formula $\eta sp = (\eta\text{-}\eta o)/\eta o$, where ηsp is the specific viscosity, and ηo is the viscosity of water, according to the method used in previous work[11-18]. The method was based on mixing a flour extract added to a dilute

solution of β-Glucan (purity 95%) of concentration 0.1% w/v. The flour was extracted by shaking the flour-water mixture (flour/water 1:10) at 25°C for 30 minutes and then centrifuging it at 2500 rpm for 20 minutes. The supernatant of the mixture was then filtered. Next, a 6-mL aliquot of the flour extract was mixed with 36 mL of the β-glucan solution (0.1% w/v) of high molecular weight (2x106) that was at a temperature of 20°C; this mixture was stirred very slightly with a glass rod and then transferred to a Ubbelohde glass capillary viscometer (UBBEL04NC, K 0. 01, range 2-10 cSt, Paragon Scientific Ltd, Wirral, UK). Measurements of the dissolution time were recorded in the viscometer between the two marks for 1 hour at intervals of every 5 minutes, maintained at a temperature of 20 °C with an error of ± 0.1 °C. Prior to the dissolution measurement, distilled water was measured in the viscometer, and the β-glucanase activity values were adjusted. The values of specific viscosity/time were represented in a linear regression model, the adjusted slope of the equation representing the β-glucanase activity. This activity was expressed as the decrease in specific viscosity per hour of the pure β-glucan solution after adding the flour extracts; after each assay, chromic mixture was added, with a high concentration of sulfuric acid, thus ensuring the complete removal of any residual β-Glucan traces. The β-glucanase activities of each sample were performed in duplicate.

Enzyme inactivation kinetics

Enzyme inactivation was highly dependent on the exposure time to microwave energy as well as the moisture content of the treated sample. Flours with the highest degrees of enzymatic inactivation contained higher moisture percentages and were subjected to longer treatment times. The effect of MW treatment on each % moisture content was described in a kinetic model according to the following equation: $A=A_0 \exp(-k.t)$, where A is the β-glucanase activity, t is the microwave treatment time, A_0 is a constant representing 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. From the MW treatments and the enzyme activity measurements carried out in previous sections, an estimation of the equations for each sample treatment moisture (13, 16, 19 and 25%) was performed.

Pasting properties

Rapid Visco Analyzer studied the pasting properties of MW-treated samples (RVA-4, Newport Scientific Pvt. Ltd. Warriewood, Australia) using the standard method ICC 162. each sample was prepared by weighing 3.0± 0.01 g of rice flour (14% moisture basis) and mixing it with 25± 0.1 ml of distilled water in a test canister. Pasting parameters such as pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown (BV = PV-TV) and setback (SV=FV-TV) were calculated by the thermocline v 2.2 software. each treatment was analyzed in duplicate.

Statistical analysis

Results were statistically analyzed using Statgraphics Centurion XVIII software (Bitstream, Cambridge, MN, USA). Analysis of variance (ANOVA) by Least Significant Difference (LSD) test at p-value ≤ 0.05 was performed.

Results

β-glucanase activity of flours subjected to the different microwave treatments

Specific viscosity decreases in the treated samples and β-glucanase activity are presented in Figure 1 and Table 2. The β-glucanase is the enzyme responsible for producing the hydrolysis of β-glucan molecules, reducing their molecular weight and thus their viscosity. the β-glucanase activity was estimated by measuring the rate of viscosity decrease. The rate of decrease of the equation of the straight line, of the 0.1% w/v β-glucan solution (purity >95%) mixed to a rice flour extract. The 13% moisture control flour without any hydrothermal treatment was observed to have a higher β-glucanase activity than all other samples, with a decrease of 0.109.

At higher moisture contents and MW treatment times, the decrease in BG degradation activity was more significant, resulting in the maintenance of molecular weight and, therefore, nutritional claims. Total inactivation of the β-glucanase activity occurred on the samples with 16% MC in treatment times of 8 min. and samples with 19% MC in treatment times of 4 and 8 min. And in samples with 25% MC at 2 and 4 min. treatment times. It was observed that the treatment time required for total b-glucanase inactivation decreased with increasing moisture content.

When compared to the same treatment intensity at different moisture contents, the importance of this variable in enzymatic inactivation was observed (Figure 1). Compared to the control, the percentages of β-glucanase activity reduction observed in the 2-min treatments at moisture contents of 13, 16, 19 and 25% water content were 18, 52, 82 and 99%, respectively.

Comparison of enzyme inactivation by particle size

The decrease in β-glucanase activity at different particle sizes is presented in Figure 2. The decrease in β-glucanase activity was not affected by the particle size of the flours; this could be due to the fact that moisture level and treatment time are the most significant factors. Except for samples with a moisture content of 13%, b-glucanase inactivation is slower, as shown in Fig. 2 (a).

Kinetics of β-glucanase inactivation in microwave-treated rice flours

To predict the behavior of β-glucanase activity, the statistical results were fitted to the regression Equation 1:

A= Ao.e-K.t; (Equation 1)

with k being dependent on moisture content (M) as shown by Equation 2:

k= ko.e-bM (Equation 2)

Generating the general fitting Equation 3:

A= Ao.exp [-ko.t.exp (-b.M)] (Equation 3)

Where A represents the β-glucanase activity, which depends on the MW treatment time, t (min), and the flour moisture, M (%). A_o, k_o and b are constants estimated for this equation after treated 34 data sets obtained from the study in the general equation.

The estimated value of the constant Ao was (0.109 ± 0.002)/h, representing the initial β-glucanase activity of the untreated flour (t = 0). K_o turned out to be (-0.02 ± 0.01)/min, representing the enzyme inactivation rate when the flour moisture content is 0%; and b was (0.19 ± 0.02), representing a constant quantifying the influence of flour water con-

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Sample	PT (°C)	PV (mPa.s)	TV (mPa.s)	BV (mPa.s)	FV (mPa.s)	SV (mPa.s)	β-glucanase activity [a]
Control	80.3ab	2680g	2036abc	645efg	5048i	3012i	0.109h
RF13-1	80.3a	2679g	2011abc	668g	4988hi	2977hi	0.101gh
RF13-2	80.8ab	2597defg	1941a	657fg	4776g	2836ghi	0.089fg
RF13-4	81.2abc	2581def	1980abc	601ef	4617efg	2637efg	0.075e
RF13-8	81.1ab	2576def	1968ab	608efg	4522de	2554de	0.041d
RF16-1	80.7ab	2648fg	2059bc	589e	4759g	2700efg	0.087ef
RF16-2	80.3a	2637efg	2004abc	633efg	4755fg	2751efg	0.053d
RF16-4	80.3a	2614defg	2024abc	590e	4645efg	2621ef	0.026c
RF16-8	81.6bc	2533cd	2013abc	521d	4364cd	2351c	0.002a
RF19-1	80.3a	2683g	2027abc	656fg	4804fgh	2778fgh	0.046d
RF19-2	80.7ab	2553cde	1967ab	587e	4568ef	2601ef	0.020bc
RF19-4	81.1ab	2532cd	2041abc	491cd	4337cd	2296c	0.009ab
RF19-8	81.6bc	2550cde	2067bc	483cd	4319c	2252c	0.001a
RF25-1	80.0a	2460bc	1956a	504d	4336cd	2380cd	0.012ab
RF25-2	81.2ab	2375ab	1940a	436bc	4125b	2185bc	0.001a
RF25-4	82.4c	2357a	1946a	411b	3980ab	2034b	0.000a
RF25-8	84.1d	2384ab	2075c	309a	3826a	1751a	0.000a
SE	0.4	31	34	21	63	67	0.004
Analysis of variance and significance (p-values)							
F1: Moisture content	ns	***	ns	***	***	***	***
F2: Time	**	ns	ns	ns	*	**	*
F1xF2	*	ns	ns	*	ns	ns	***

Table 2. Pasting properties and β-glucanase activities of the studied flours.
PT = Pasting Temperature. PV = Peak Viscosity. TV = Trough Viscosity. BV = Breakdown Viscosity. FV = Final Viscosity. SV = Setback. The β-glucanase activity was calculated from the slope of the straight line according to the viscosity decrease per hour of the β-glucan solution (0.1 % w/v) following the addition of rice flour extracts. SE: Pooled standard error from ANOVA. The different letters in the corresponding column within each studied factor indicate statistically significant differences between means at $p < 0.05$. Analysis of variance and significance: ***$p < 0.001$. **$p < 0.01$. *$p < 0.05$. ns: not significant.

tent on the inactivation rate. The R^2 of this regression was 0.9877, whereby the equation predicted with high accuracy the variation of β-glucanase activity during microwave treatment between 1-8 min and within the range of moisture content between 13-25% while maintaining the power of 900W.

Pasting properties

The pasting properties of the samples analyzed are shown in Table. 2 and the pasting profiles are depicted in Figure 3. Microwave treatment has a significant influence on the pasting properties of the flours. Studies have shown that starch (the main component of cereal flour) presents
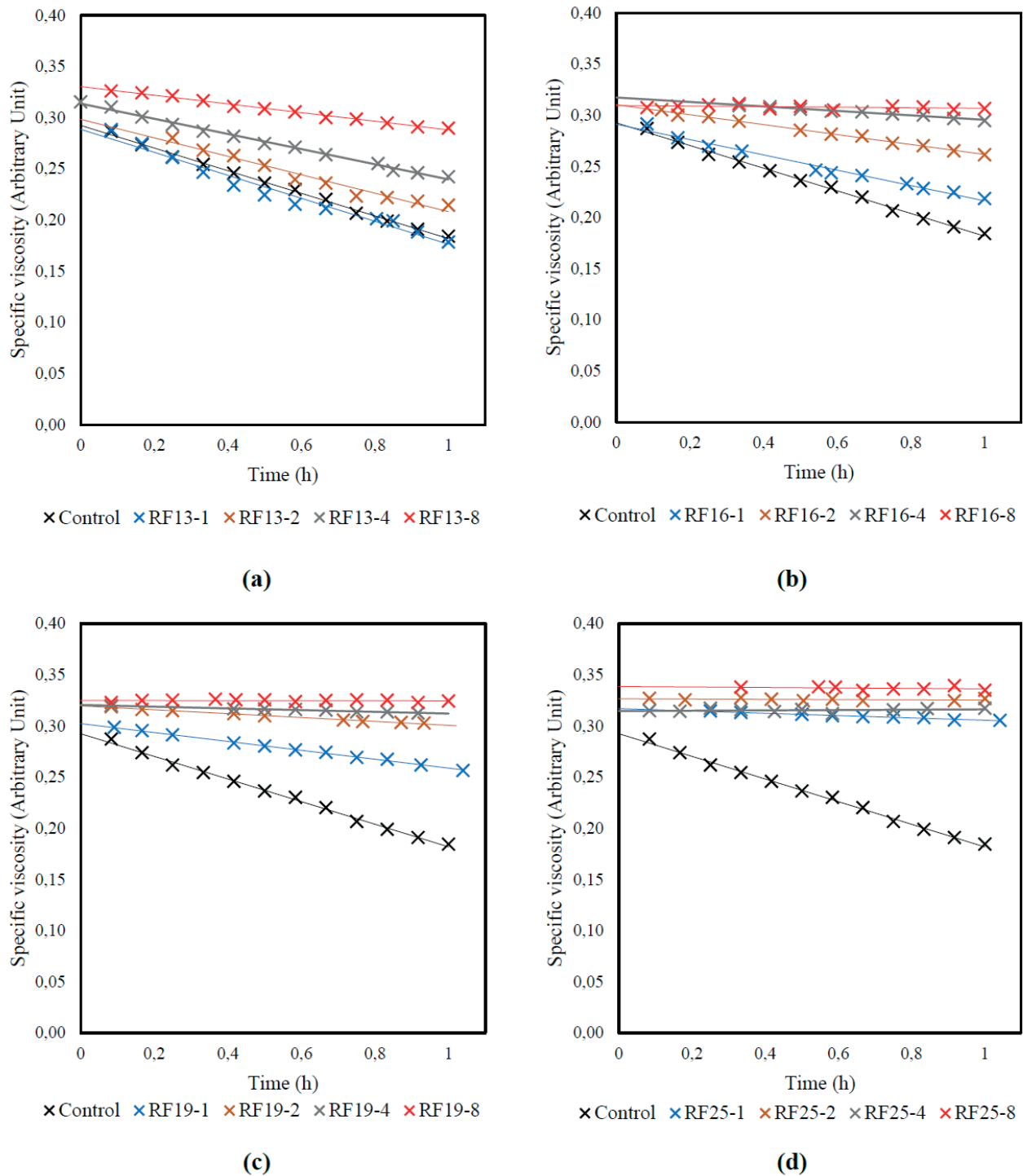
(a)

(b)

(c)

(d)

Figure 1. Plots of specific viscosity decrease versus time at different moisture contents. (13% (a), 16% (b), 19% (c) and 25% (d)).

structural changes when subjected to microwave energy[23]. Starch heated in excess water beyond a certain temperature suffers an irreversible change known as gelatinization, which is the result of the collapse of the molecular order including loss of starch granule crystallinity, water absorption and leaching of amylose into the medium[31], following starch gelatinization there is a point at which the granules cannot support such water absorption and lose its structure known as peak viscosity (PV).

Figure 3 shows the relationship between the decrease in peak viscosity (PV) and the increase in moisture content. Lower PV was observed in flours at 25% moisture level in relation to the control samples and samples treated at 13 and 16% MC, and statistically significant (p<0.05) differences were also found between the different treatment times, with lower PV at longer treatment times. TV showed no differences between the treated flours and the control. BV obtained lower values in the samples treated with 25% MC, and the samples treated with higher MC were the most stable. FV and SV values showed similar tendencies, obtaining significantly lower values than the control flour, and the effect was more pronounced at longer treatment times and higher moisture contents.
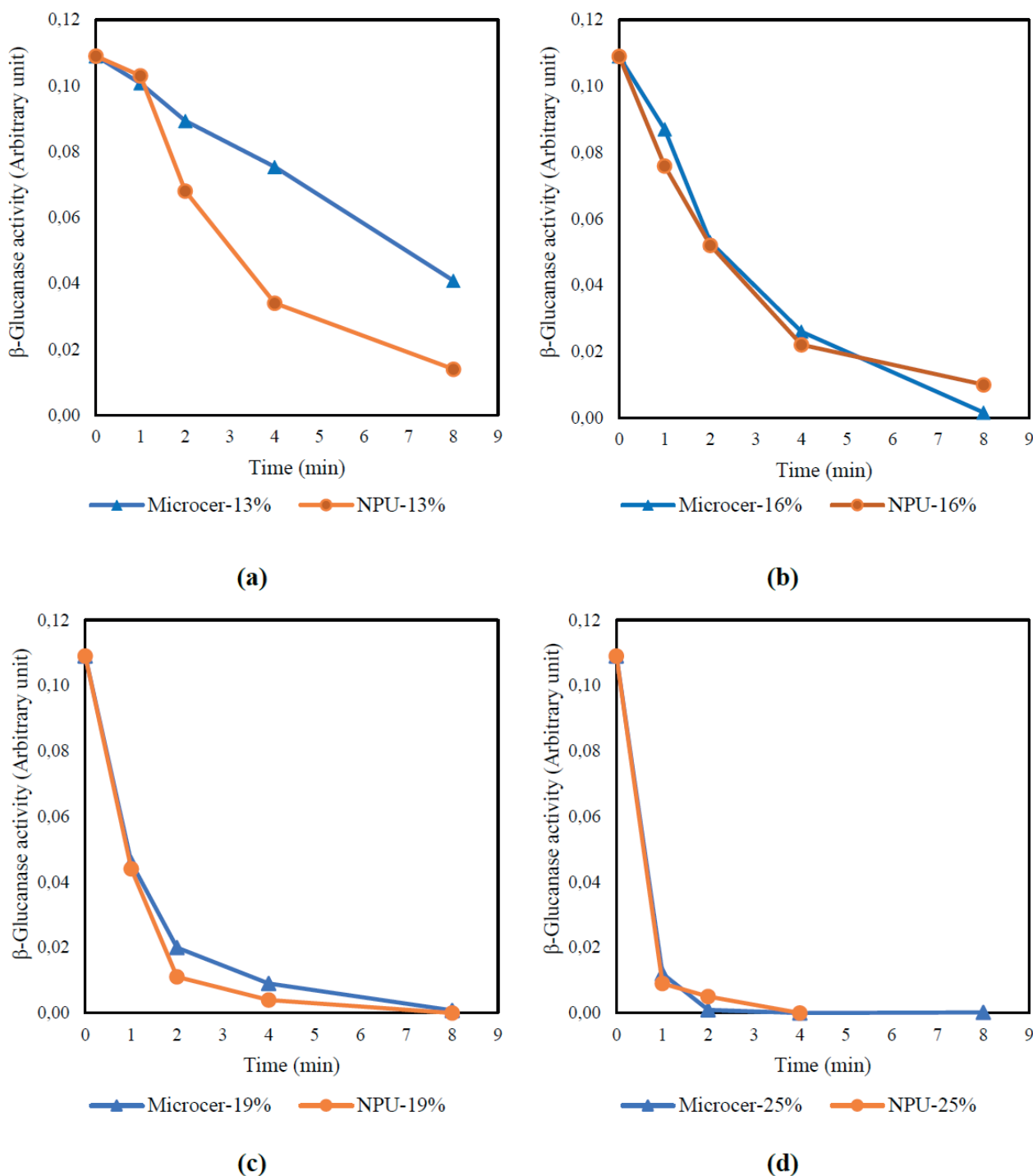
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(a)

(b)

(c)

(d)

Figure 2. Comparison of β-glucanase activity inactivation profiles of flours with different particle size: NPU (larger) and Microcer (ultrafine) at different moisture content (13% (a), 16% (b), 19% (c) and 25% (d)). The data for NPU flour were extracted from Pérez-Quirce et al. (2016).

Discussion

β-glucanase activity of flours subjected to the different microwave treatments

As observed in section 3.1, the control flour obtained a higher β-glucanase activity than all the other samples, with a rate of decrease of 0.109, a value very similar to that obtained in the study of the inactivation of this same enzyme by MW, performed with rice flour with larger particle size Pé-

rez-Quirce *et al.*(2016). However, this result differs from the study by Lazaridou *et al.*(2014) who concluded that β-glucanase activity in barley flour decreases as the particle size decreases without MW treatment.

The primary role of water in MW treatments, and consequently in enzyme inactivation, is because its dipolar nature is the main source of microwave interactions with food, where water molecules absorb microwave energy and orient themselves with the applied electric field, the rapid change in field orientation generates molecular friction that
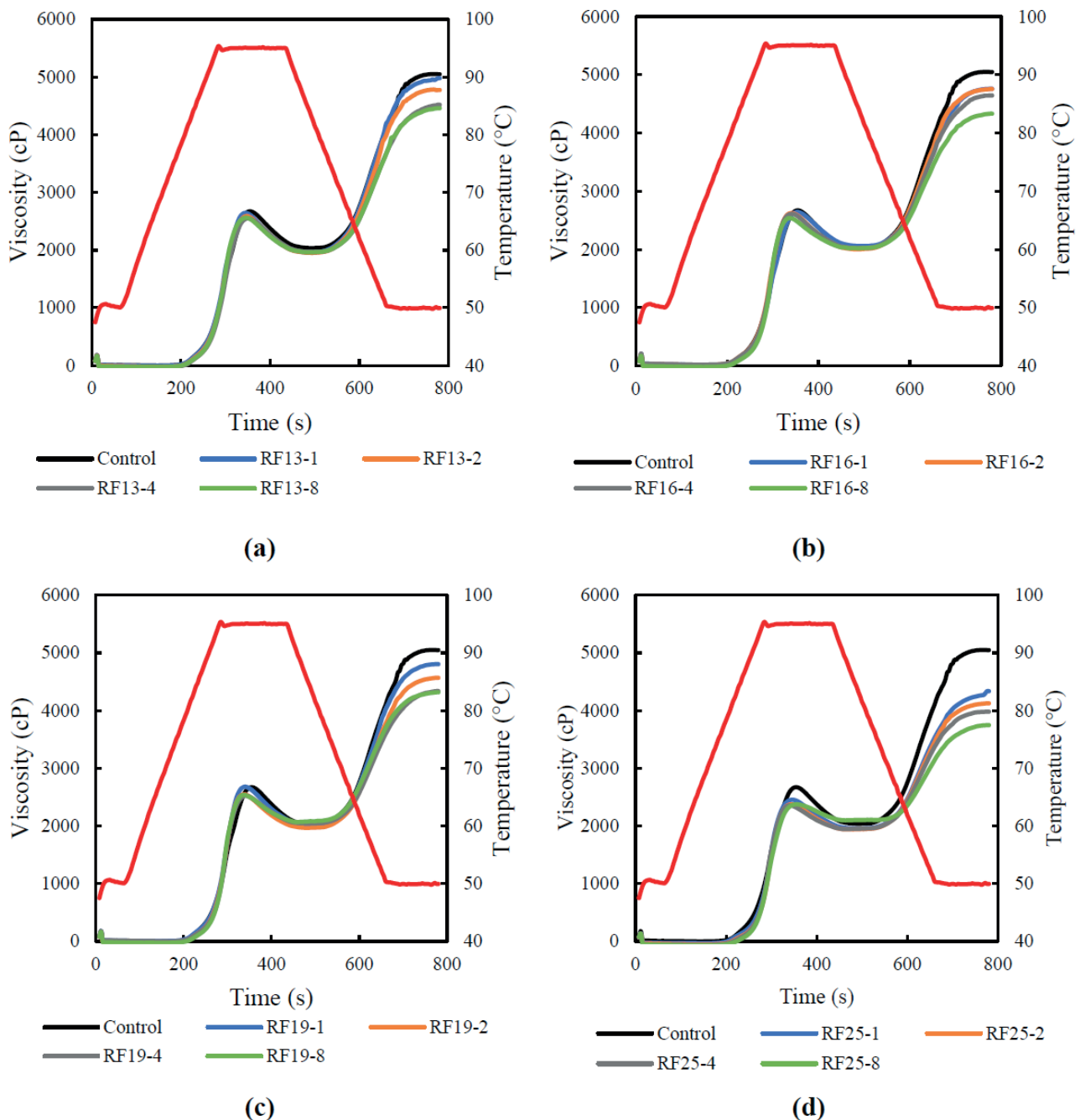
(a)

(b)

(c)

(d)

Figure 3. Pasting profiles of control and MW-treated rice flours at 13% moisture content (a), control and MW-treated rice flours at 16% moisture content (b), control and MW-treated rice flour at 19% moisture content (c) and control and MW-treated rice flour at 25% moisture content (d). rice flour native (control) is represented by microwave treated rice flour at 1 min. by , microwave treated rice flour at 2 min. by , microwave treated rice flour at 4 min by and microwave treated rice flour at 8 min by . The temperature profile is represented by in the second axis.

translates into heat generation[25]. This means that it is most likely that the increase in flour moisture allows faster absorption of the microwave energy during the treatment.

Comparison of enzyme inactivation by particle size

The previous study about the inactivation of β-glucanase activity of rice flours by microwave treatments (Pérez-Quirce et al., 2016) used a flour with more extensive particle size distribution (6%>150µm, 150 µm>63.2%>100µm, 30.8%< 100µm) of the commercial denomination "NPU", concerning the one used in the current investigation with fine particle distribution (1%<150 µm, 150 µm >5-15%>90

µm, 90µm >84%>0µm) of the commercial denomination "Microcer" of the same variety and batch. Both were analyzed with the same moisture and treatment conditions, differentiating only the particle size of the flours between the studies. The behavior of both at the level of enzymatic inactivation concerning time is shown in Figure 2, where it is observed that the profiles of the graphs are very similar in all the samples with moisture content of 16, 19 and 25% without notable differences due to the different size particle. However, a large fluctuation emerged in the flour with 13% moisture. The flour Microcer didn't generate such a pronounced decrease in b-glucanase activity, showing an

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apparently linear reduction in the graph, different from the logarithmic character profile presented by the NPU flour in the viscosity decrease.

Kinetics of β-glucanase inactivation in microwave-treated rice flours

With the values of the equation obtained in section 3.3 it was possible to optimize the enzymatic inactivation results obtained by varying the conditions of treatment time and moisture content the data obtained was used to optimize nonlinear regression in the β-glucanase inactivation equation (Equation 3) and Equation 4 is derived for the such specific condition:

$$A=0.109.\exp[-0.02.t.\exp(0.19*M)] \qquad \text{(Equation 4)}$$

is obtained by replacing the data obtained comparing with Equation 4 with the equation drived by Pérez-Quirce et al.(2016) A= 0.109.exp [-0.0146.t.exp (0.212.M)] of, a slight increase in Ko values and a decrease in b values were observed, indicating that the value of the inactivation kinetic constant is not affected by particle size in the ranges of humidity and treatment times determined in the studies.

Pasting properties of analyzed samples

The decrease in viscosity peaks observed in Figure 3. coincides with the report by Watcharatewinkul et al.(2009), which explained that hydrothermal treatments cause intense changes in starch granules, which translates into increases in pasting temperatures, in addition to decreases in peak and final viscosities. These changes are due to the associations of the chains of the amorphous regions, and changes in the crystallinity that occur during hydrothermal treatment making the moisture content of the samples to be treated is important[14]. The pasting temperature (PT) showed no significant changes in shorter treatment times and lower moisture levels and vice versa for higher moisture levels and treatment times. PT of the sample MW-treated sample at 25% at 4 and 8 min of treatment was delayed by 2 and 4°C, respectively, concerning the control, similar report was given by Stevenson et al.(2005) and Lewandowicz et al.(2000) that heated starches denoted an increase in gelatinization temperature. Sun et al.(2014) explained that flour treated by MW could have greater difficulty in absorbing water and initiating swelling, higher crystallinity and rigidity of starch granules impede water absorption; in fact, the increase in crystallinity promotes a delay in the onset of gelatinization. This is Because water is absorbed mainly by the amorphous regions and thus increases the pasting temperature. The reduction of BV in all treated samples indicates high stability of granule swelling versus shear and heating. The lower SV values suggest that MW treatments could decrease short-term retrogradation (of amylose) under the conditions set and similar trends were obtained by Solaesa et al.(2021).

Conclusions

The enrichment of gluten-free bakery products with β-glucans because of improving the nutritional quality is projected as a necessity to supply many nutritional deficiencies of the celiac population. This research proved the efficacy of microwave heat treatment for the inactivation of β-glucanase, the enzyme responsible for the degradation of BGs, leading to the reduction of molecular weight and consequently showing the potential contribution to health. The degree of inactivation is a value that depends on flour moisture and treatment time, obtaining the relationship that as these factors increase, the enzyme activity is significantly reduced. It is concluded that the MW energy treatment method is very effective in prolonged times (4 min) and high moisture contents (25%) for the inactivation of the endogenous β-glucanase enzyme in rice flour. So, by varying these independent values total inactivation of the enzyme can be achieved and avoid hydrolysis of the BG that reducing its health benefits.

Concerning particle size, the degree of inactivation of small-sized rice flour (Microcer) was compared with that of the study by Perez-Quirce et al.(2016), who worked with coarse-sized rice flour (NPU) at humidities of 13, 16, 19 and 25%, where very similar profiles were observed in the graphs of the decrease in β-glucanase activity with respect to the MW treatment corresponding to each moisture level, without finding notable differences in this factor, with a slight exception for samples with a MC of 13% where a slower inactivation was observed. Rice flour pasting properties analyzed by RVA plots were affected by changes in moisture level and MW treatment time , showing significant effects on peak, breakdown, retrogradation and final viscosities and this indicates higher damage to starch crystallinity in fine particle size flours. This change tends to increase as flour moisture values and treatment time also increase, giving results significantly different from those corresponding to the initial flour with moisture content of 13% and without treatment.

Author Contributions

Caleb S. Calix-Rivera: Writing – original draft, Writing – review & editing, Formal analysis, conceived and designed the experiments; Analyzed and interpreted the data; Contributed analysis tools or data; Wrote the paper, performed the experiments. Sandra Perez-Quirce: Writing – review & editing, Formal analysis, performed the experiments, analyzed and interpreted the data and reviewed the paper. Felicidad Ronda: Funding acquisition, Conceptualization, Methodology, Re- sources, Investigation, Visualization, Supervision, Writing – review & editing, Project administration, Formal analysis, conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Informed Consent Statement

Not applicable.

Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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