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ORIGINAL PAPER

The role of cyclodextrinase and glucose oxidase in obtaining gluten-free laminated baked products

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Abstract
Currently, there is a general awareness of the need of having affordable and tasty gluten-free food. However, the food market does not offer baked products of laminated conformation. The aim of this work was to study the effect of enzymes on the technological quality of gluten-free laminated baked product and dough properties. Cyclodextrinase—CGT and glucose oxidase—GOX were evaluated. Enzyme-containing products had a higher specific volume than control and a lower masticability. However, only CGT produced a layered inner structure. The results observed in enzyme-containing products can be related to the formation of non-covalent protein aggregates and the effect on dough behavior during heating. Samples with enzymes showed a delay at the starting temperature of starch gelatinization. Furthermore, CGT dough had a lower viscosity increment than GOX and control, between 70 and 100 °C. Consequently, a marked positive effect in the technological quality of laminated products was observed with CGT.

Keywords Gluten-free · Laminated baked product · Enzymes · Cyclodextrinase · Glucose oxidase

Introduction

Celiac disease is defined as a chronic enteropathy of the small intestine immuno-mediated and promoted by exposure to dietary gluten in genetically predisposed individuals [1]. Currently, a strict gluten-free diet is the only treatment that leads to a recovery of the normal architecture and function of the intestine, as well as to the remission of symptoms [2, 3]. Furthermore, in recent years, a new gluten-free product consumer group has been growing, people with no gluten restriction but who follow a gluten-free diet as part of a healthy eating plan [4]. Concomitantly, there is a general awareness of the need of having safe, affordable and tasty gluten-free food.

However, in products where gluten plays a structural role, as in bakery goods, its absence makes dough manipulation more difficult in the production process and provokes a detrimental effect on technological and sensory quality. Particularly, in products of laminated conformation such as Danish and puff pastry, croissants and yeast-leavened salty products, gluten fulfills a critical function. The extent to which the dough sheets remain discrete from fat and the capacity of the laminated system to expand in a vertical direction are related to the protein network characteristics.

In this sense, with the aim to find answers to the technological problem of gluten-free dough manipulation, many authors have evaluated the use of flour and starch combinations to mimic the viscoelastic gluten properties. However, most of the studies are focused on sponge-like structure products, such as bread and cake [5–7], while no studies about gluten-free products of layered inner conformation, such as Danish or puff pastry, have been found. The use of a wide range of additives, including enzymes, emulsifiers, and hydrocolloids, has also been identified [8–10]. Particularly, enzymatic treatment of gluten-free dough has gained importance due to the structure and functionality modifications of flour proteins, which are expected to lead to a better quality baked product. In this sense, the incorporation of oxidative enzymes, such as glucose oxidase, is of particular interest in the development of this kind of products. Glucose oxidase (EC 1.1.3.4) promotes the cross-linking of gluten proteins through oxidation of glucose to form gluconic acid and

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hydrogen peroxide [11, 12]. The mechanism through which glucose oxidase (GOX) improves the quality of wheat-baked products is still controversial. In gluten-free systems, the effect of the enzyme has been investigated [13, 14], since some gluten-free flours, such as soy and rice, have free sulfhydryl groups that can be potentially oxidized, besides the protein fraction modification.

Cyclodextrin glucanotransferase (CGT), an enzyme produced by species of *Bacillus*, catalyzes four different reactions: cyclization, coupling, disproportionation, and hydrolysis. The CGT (EC 2.4.1.19) uses the starch as substrate and breaks α-1,4 glycosidic linkages. Besides, cyclization reactions lead to the formation of α-, β-, and γ-cyclodextrins, which contain six, seven, or eight glucose units, respectively. Some authors [15] have reported the addition of CGTase produced an increase of specific volume, an improvement in texture, and a decrease of the ability of amylopectin to retrograde during storage. However, the use of this enzyme on gluten-free systems and on products with a laminated conformation has not been extensively reported. The aim of this work was to study the effect of cyclodextrinase and glucose oxidase on the technological quality of a gluten-free laminated baked product and dough properties.

Materials and methods

Materials

Commercial samples of soy (Complementos Proteicos SA, Argentina), rice flours (Cultivos de Avena SRL, Argentina) and cassava starch (LITESOL MB, Argentina) were used. The humidity contents of the samples were 7.1 ± 0.0, 9.2 ± 0.3, and 11.5 ± 0.3%, respectively. The soy flour had an ash and protein percentage of 5.7 ± 0.0 and 41.8 ± 0.1%, respectively. While rice flour and cassava starch presented a 0.8 ± 0.0 and 0.1 ± 0.0% of ash, respectively, and a 6.9 ± 0.1 and a 0.4 ± 0.1% of protein. The evaluated enzymes were cyclodextrin glucanotransferase (CGT, Novozymes, 3 KNU/g) and glucose oxidase (GOX, Novozymes, 10,000 U/g).

Gluten-free laminated product elaboration

Gluten-free laminated products were elaborated with two doses of CGT (0.2 and 0.8 g/100 g flour, CGT 1 and CGT 2) and GOX (0.01 and 0.02 g/100 g flour, GOX 1 and GOX 2) and without additives (control). The CGT and GOX concentrations were selecting according to the previous publications [6, 8]. The dough pieces were prepared with 35 g of rice flour, 45 g of cassava starch, 20 g of soy flour, 12.8 g of commercial shortening (Mkt CALSA, Argentina), 0.5 g of compressed yeast (Red Saf-instant, Lesaffre, Argentina), 2.5 g of refined dry salt (Dos Anclas, Argentina), 1.4 g of sugar (Ledesma, Argentina), and 3.5 g of vanilla essence. The flours mix was according to the previous studies on gluten-free products, where the authors reported a good quality of the sample-related proteins and starch interactions [16–18]. The ingredients were mixed with 61 g of water for 3 min in a mixer (MPZ Pedro Zambom e hijos, Argentina) until the dough was made. Water temperature was such that the dough obtained had a final temperature of 21 ± 1 °C. The bulk dough was covered with film, saved in a plastic container, and let rest at 9 °C for 24 h. After the refrigerated rest period, a 33.3 g shortening sheet was folded envelope style into a dough sheet and gaged to 60 mm thickness in six steps, with a sheeter (MA-AR ACRILIC Tissot, Argentina). The dough was given a twofold turn and allowed to rest for 20 min at 9 °C; then, it was gaged to 50 mm thickness in seven steps and given another twofold turn. The dough was let rest again for 20 min at 9 °C and was gaged to 50 mm thickness. It was laminated with a twofold turn and the final gaging was about to 15 mm of thickness. Round holes (diameter $d = 2$ mm) were cut into the dough 1.6 cm apart from each other to prevent complete separation of layers during baking. Square dough pieces (5 × 5 cm) were cut. Samples were baked in a convector oven Beta 107 IPA (Pauna, Argentina) at 210 °C for 20 min. The dough samples used in the evaluations were made at least twice and six pieces of each sample were analyzed.

Baked product technological quality evaluation

The assessment of physical and textural attributes of gluten-free laminated baked products was done at least twice and six pieces of each sample were analyzed. The conformational evolution, defined as the system behavior during the production process, was evaluated following the methodology proposed by de la Horra et al. [19]. The height of laminated dough pieces before cooking and of baked products was determined at three points on the surface (at 5 mm from the edges and at the center), and an average height was calculated. The width of dough samples and products was registered before and after the baking step and the average was presented. The height (H) and width (W) ratios were determined with the dimensions (height and width) of the baked products (bp) and the laminated dough pieces (dp) (Eqs. 1, 2). The shape factor (SF) of the products was calculated with the baked sample dimensions (Eq. 3). The specific volume was obtained from the baked product weight and the volume determined by rapeseed displacement after cooling for 1 h. The specific volume was expressed as the volume/weight ratio of the final product according method 10–05.01 of AACC [20]:

$$H = \frac{H_{bp}}{H_{dp}} \quad (1)$$

$$W = \frac{W_{bp}}{W_{dp}} \quad (2)$$

$$SF = \frac{\text{Height}}{\frac{\text{Width+Length}}{2}}. \quad (3)$$

The crust color was determined using a CM-700d/600d KONICA MINOLTA spectrophotometer (Ramsey, USA). Measurements were done at three points on the crust (left-upper edge, center, and right-lower edge). The results were expressed as CIE $L^*a^*b^*$ [20].

The textural characterization of the samples was assessed by a texture analyzer INSTRON 3342 (Norwood, MA, USA). A central piece of the baked product was cut and compressed up to 40% of its initial height using a cylindrical probe (diameter $d = 2.5$ cm) [21]. Force deformation curves were determined at a crosshead speed of 1 mm/s. Crumb firmness was defined as the maximum force obtained and it was expressed in Newtons (N).

Image texture analysis was used to evaluate the inner structure of the products. Cross-sectional images of the samples were obtained with a scanner (HP Scanjet G3010, Palo Alto CA, USA) and analyzed with Image J Software (National Institutes of Health, USA). Different fields of view were selected in each image depending on the sample size. The images were pre-processed by turning to grayscale, subtracting the background, and enhancing the contrast. The gray-level co-occurrence matrix algorithm was applied to the images and textural features were obtained [22]. Contrast, homogeneity, and entropy were considered. The Otsu's threshold algorithm was applied to binarize the images. The fractal texture was evaluated by the fractal box counting method and the fractal dimension was established [23]. The image analysis was done in duplicate and three pieces of each sample were considered.

Dough properties evaluation

The dough samples used in the evaluations were prepared as described previously and were evaluated after the refrigerating step without lamination. The dough samples were made at least twice and six pieces of each enzyme were analyzed.

Protein fraction analysis

The influence of enzymes on protein interactions was studied from dough pieces subjected to a rest period of 24 h at 9 °C, covered with film, and saved in plastic containers. Prepared dough samples were freeze dried and defatted with hexane (1:3 w/v) for 24 h at 22 ± 2 °C. Protein sequential extractions of samples (1 g) were carried out with 6.5 ml of 0.05 M phosphate buffer (PB), pH 6.9. Suspensions were stirred for 2 h at room temperature and then centrifuged at 10,000×*g* for 10 min (25 °C). The supernatant (PB fraction) was kept and the precipitate was dissolved in PB containing 2% w/v sodium dodecil sulfate (SDS). The suspensions were stirred under the previous conditions and two fractions were obtained, the supernatant (PB + SDS), and the precipitate. The protein content of each fraction was determined according to the micro-Kjeldahl method modified with boric acid [20]. Dough samples were prepared in duplicate and the extractions and protein determinations were done each time.

SDS-PAGE electrophoresis of protein fractions was performed according to Laemmli [24] using a Mini Protean II Slab Cell (Bio-Rad Laboratories, Richmond, CA, USA). A stacking gel of 2 cm and 4% acrylamide and a running gel of 8 cm and 12% were used. Molecular masses (Broad range, Bio-Rad Laboratories, Hercules, USA) of myosin (200,000), β-galactosidase (116,250), phosphorylase b (97,400), serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), trypsin inhibitor (21,500), and lysozyme (14,400) were used.

Rheological assessment

Dough pieces with lower doses of enzymes and without additives were prepared as described previously. The rheological properties were evaluated by a Physica MCR 301 rheometer (Anton Paar, Germany); a parallel plate geometry (25 mm diameter and 2 mm gap) was used; and the temperature was set by a controlled Peltier system. After loading, the upper plate was lowered until the final gap (2.0 mm), the sample was allowed to rest for 9 min, and the excess dough was trimmed. Dehydration was prevented by adding low-viscosity silicone around the plate edges. The tests performed were:

(a) Creep and recovery test, applying a constant stress of 200 Pa during 300 s inside the linear viscoelastic region and allowing the strain sample recovery for 600 s when the stress was removed [25]. The test took place at 9 °C, after a set of samples were prepared and allowed to rest for 24 h at 9 °C. The data obtained were modeled according to Burgers model [26] and analyzed with the fitted according to Eq. (4):

$$J(t) = J_0 + J_1\left(1 - e^{-t/\lambda \text{ret}} + \left(\frac{t}{\eta_0}\right)\right) \quad (4)$$

$$J(t) = J_{max} - J_0 - J_1(1 - e^{-t/\lambda \text{rec}}) \quad (5)$$

where J_0 is the instantaneous compliance, J_1 is the viscoelastic compliance, λ_{ret} is the Kelvin component retardation time, and η_0 is the zero shear viscosity.

Equation (5) was used to fit the recovery phase, where J_{max} is the maximum creep compliance and λ_{rec} is the Kelvin component recovery time. The elastic compliance (J_e) is evaluated by the sum of J_0 and J_1 corresponding to the recovery phase; the viscous compliance (J_v) is obtained by subtracting J_e from J_{max}. The J_e/J_{max} ratio gives information about the relative elastic part of the maximum creep compliance, while J_v/J_{max} of the relative viscous part of J_{max}.

(b) Temperature sweep, dough samples after a rest period of 24 h at 9 °C were heated from 25 to 100 °C (4 °C/min). The test was performed at strain of 0.1% and frequency of 1 Hz. The storage modulus (G'), loss modulus (G''), and complex viscosity (η^*) were obtained. The first derivate of the storage modulus ($d1$, G') was calculated using the rheometer software, the value, and temperature of the $d1$; G' peak was registered. Each test was performed on different dough samples in triplicate, and every time, three pieces of the dough bulk were analyzed.

Statistical analysis

The results obtained were compared by analysis of variance (ANOVA) using the least significant difference (LSD) multiple comparison test, where the relationship between the measured parameters was assessed by the Pearson's test (significant level at $p \leq 0.05$) (Infostat statistical software, Facultad de Ciencias Agropecuarias, UNC, Argentina).

Results and discussion

Effect of enzymes on the technological quality of gluten-free laminated baked products

The overall effect of enzyme incorporation on the macrostructure of gluten-free laminated baked products is shown by the general appearance and the inner conformation of the samples (Fig. 1). The evolution of the structure during the production process and the quality of baked products were assessed through quality parameters (Table 1). At the end of lamination and folding steps, dough pieces with enzymes were higher than control, although the differences observed were not significant. There were no significant differences in the height and width relationship values when enzymes were added; this indicates that enzymes did not affect the system capacity to grow vertically during baking or its lateral expansion. At the end of baking, products with enzymes were higher than control; there was a greater effect with the higher CGT dose and both doses of GOX. No significant differences were found in the shape factor values. There was a positive effect on products specific volumes; samples with enzymes presented higher values than control. Other authors [15, 27] found a positive effect of CGT over rice bread-specific volumes. The incorporation of enzymes did not cause changes in crusts lightness; only in GOX 1, a slight decrease in the L^* parameter was observed. The a^* parameter values were positive in all the analyzed samples and no significant differences were found between the redness intensity when enzymes were added. The same tendency was observed in the parameter b^*, related to yellowness intensity.

The texture properties of the products were affected by the enzyme incorporation. Non-significant variations were observed in firmness with both CGT doses and GOX 1, while it was significantly different with GOX 2. Other authors have studied the effect of GOX in textural parameters of baked

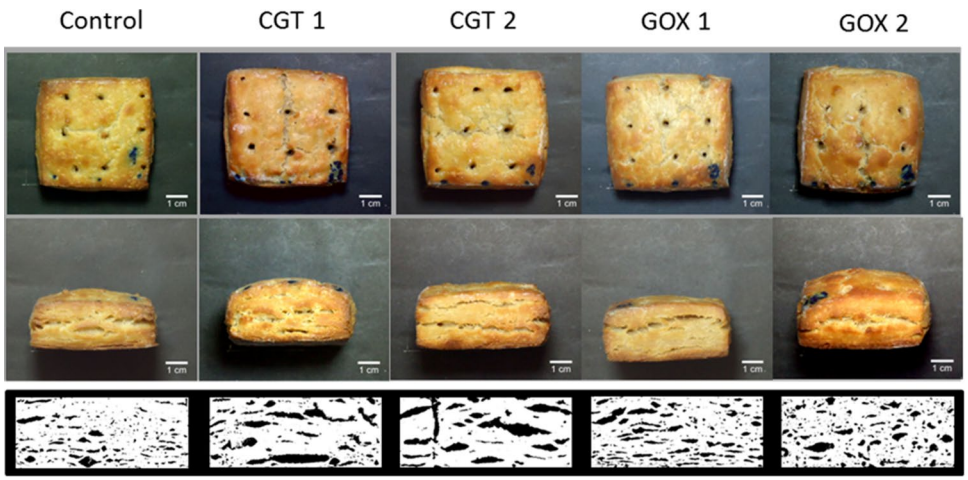

Fig. 1 Gluten-free laminated baked products. CGT 1: 0.2 µl cyclodextrin glucanotransferase/100 g flour, CGT 2: 0.8 µl cyclodextrin glucanotransferase/100 g flour, GOX 1: 0.01 g glucose oxidase/100 g flour, and GOX 2: 0.02 g glucose oxidase/100 g flour. CGT 1: 0.2 µl cyclodextrin glucanotransferase/100 g flour, CGT 2: 0.8 µl cyclodextrin glucanotransferase/100 g flour, GOX 1: 0.01 g glucose oxidase/100 g flour, and GOX 2: 0.02 g glucose oxidase/100 g flour

Table 1 Technological quality parameters of gluten-free laminated products

Sample	Control	CGT 1	CGT 2	GOX 1	GOX 2
Dough height (cm)	$(1.4 \pm 0.0)^{a}$	$(1.9 \pm 0.0)^{a}$	$(1.9 \pm 0.0)^{a}$	$(1.9 \pm 0.0)^{a}$	$(1.8 \pm 0.0)^{a}$
H	$(1.5 \pm 0.1)^{a}$	$(1.4 \pm 0.1)^{a}$	$(1.3 \pm 0.0)^{a}$	$(1.3 \pm 0.1)^{a}$	$(1.4 \pm 0.0)^{a}$
Product height (cm)	$(2.81 \pm 0.0)^{a}$	$(2.2 \pm 0.2)^{b}$	$(2.5 \pm 0.0)^{b}$	$(2.5 \pm 0.0)^{b}$	$(2.5 \pm 0.1)^{b}$
W	$(1.0 \pm 0.0)^{a}$	$(1.0 \pm 0.0)^{ab}$	$(1.1 \pm 0.0)^{b}$	$(1.0 \pm 0.1)^{ab}$	$(1.0 \pm 0.0)^{ab}$
SF	$(0.4 \pm 0.0)^{a}$	$(0.4 \pm 0.0)^{a}$	$(0.5 \pm 0.1)^{a}$	$(0.5 \pm 0.0)^{a}$	$(0.5 \pm 0.0)^{a}$
SV (cm^3)	$(64.4 \pm 4.4)^{a}$	$(76.3 \pm 1.8)^{b}$	$(75.8 \pm 2.4)^{b}$	$(70.0 \pm 1.2)^{ab}$	$(74.2 \pm 1.2)^{b}$
*L**	$(55.8 \pm 1.1)^{ab}$	$(56.4 \pm 1.1)^{b}$	$(56.7 \pm 0.7)^{b}$	$(57.0 \pm 1.7)^{b}$	$(53.2 \pm 0.8)^{ab}$
*a**	$(12.5 \pm 1.1)^{a}$	$(11.2 \pm 0.3)^{a}$	$(9.8 \pm 1.0)^{a}$	$(10.1 \pm 0.8)^{a}$	$(12.6 \pm 0.2)^{a}$
*b**	$(31.4 \pm 0.1)^{a}$	$(30.6 \pm 1.1)^{a}$	$(31.8 \pm 1.9)^{a}$	$(31.5 \pm 1.8)^{a}$	$(31.4 \pm 0.5)^{a}$
Firmness (N)	$(62.2 \pm 4.0)^{b}$	$(58.5 \pm 0.8)^{b}$	$(63.5 \pm 1.5)^{b}$	$(60.1 \pm 3.2)^{b}$	$(49.1 \pm 1.7)^{a}$
Masticability	$(62.7 \pm 3.0)^{c}$	$(29.7 \pm 0.0)^{ab}$	$(18.4 \pm 1.3)^{ab}$	$(60.5 \pm 10.6)^{c}$	$(36.4 \pm 4.9)^{b}$
Homogeneity	$(0.2 \pm 0.1)^{b}$	$(0.2 \pm 0.0)^{a}$	$(0.2 \pm 0.0)^{a}$	$(0.2 \pm 0.0)^{a}$	$(0.2 \pm 0.0)^{a}$
Contrast	$(90.4 \pm 15.5)^{b}$	$(79.9 \pm 3.5)^{a}$	$(79.4 \pm 4.8)^{a}$	$(84.4 \pm 4.9)^{ab}$	$(81.8 \pm 3.4)^{ab}$
Entropy	$(7.1 \pm 0.3)^{a}$	$(7.2 \pm 0.1)^{a}$	$(7.2 \pm 0.4)^{a}$	$(7.2 \pm 0.1)^{a}$	$(7.2 \pm 0.1)^{a}$
FD	$(1.4 \pm 0.0)^{a}$	$(1.6 \pm 0.0)^{c}$	$(1.6 \pm 0.0)^{d}$	$(1.5 \pm 0.0)^{b}$	$(1.5 \pm 0.0)^{b}$

Mean ± standard deviation. Different letters in the same row indicate significant differences ($p \leq 0.05$) between samples

CGT 1 0.2 µl cyclodextrin glucanotransferase/100 g flour, *CGT 2* 0.8 µl cyclodextrin glucanotransferase/100 g flour, *GOX 1* 0.01 g glucose oxidase/100 g flour, *GOX 2* 0.02 g glucose oxidase/100 g flour, *H* height relationship, *W* weight relationship, *SF* shape factor, *SV* specific volume, *FD* fractal dimension

products and observed dependence between the firmness and the type of gluten-free flour and GOX doses used. Gujral and Rosell [8] found a decrease in firmness of rice bread samples with GOX. The firmness tendency was in accordance with a decrease in free sulfhydryl and amino groups, associated with cross-linking of rice proteins and the occurrence of covalent additional bonds. Renzetti and Arendt [13] did not find an effect of GOX in bread crumb firmness made with buckwheat and teff flour. However, they observed an increment in the textural parameter in sorghum bread samples and a reduction when corn flour was used. The product masticability significantly declined with CGT and with the highest dose of GOX. A decrease in this parameter has a positive effect, due to a lower force to disintegrate the sample until it is ready to be swallowed is required [28].

Changes in the inner product structure due to the enzyme addition were assessed through texture image analysis and four parameters were obtained (Table 1). Figure 1 shows binarized images of sample inner structures. Control presented a compact crumb with isolated pores. In CGT samples, there were pores with extended conformation and some layers throughout the structure. GOX 1 presented some extended pores, but they were not present along the structure; concomitantly, no sheets were detected. With the highest GOX dose, a spongier structure similar to the control was observed. Samples with enzymes had a less homogenous distribution of the structural elements, pores, and layers than control, although the values were not significantly different. A decrease in contrast values was observed when enzymes were in the formulation and this tendency was accentuated with CGT. De la Horra et al. [16] observed a diminution in contrast values of gluten-free laminated product images when diacetyl ester of mono and diglycerides (DATEM) was added. The authors associated it with the presence of layers in the product inner structure. Entropy measures the randomness of the intensity distribution in the image. Although CGT incorporation produced changes in the inner conformation and crumb general appearance, the randomness of the intensity distribution was not affected. To quantitatively describe the morphology of objects with complex and irregular structures, such as pores and layers, fractal dimension was used [29]. Higher fractal dimension values were found in samples with enzymes. The most marked effect of CGT over this parameter can be associated with a rougher surface [30], due to structural elements of greater tortuosity than control, related to layer formation and the presence of pores with extended conformation.

Effect of enzymes in dough properties

Dough samples were subjected to a sequential extraction in different solvents to evaluate if the presence of enzymes had affected protein solubility due to the formation of new interactions. Non-reduced protein fractions of dough samples are shown in Fig. 2. Control presented on top of the separating and stacking phosphate buffer (PB) gel (Fig. 2a) large aggregates, which correspond to protein aggregates of high molecular weight that did not enter to the gel, because the size of aggregates was larger than pore size.

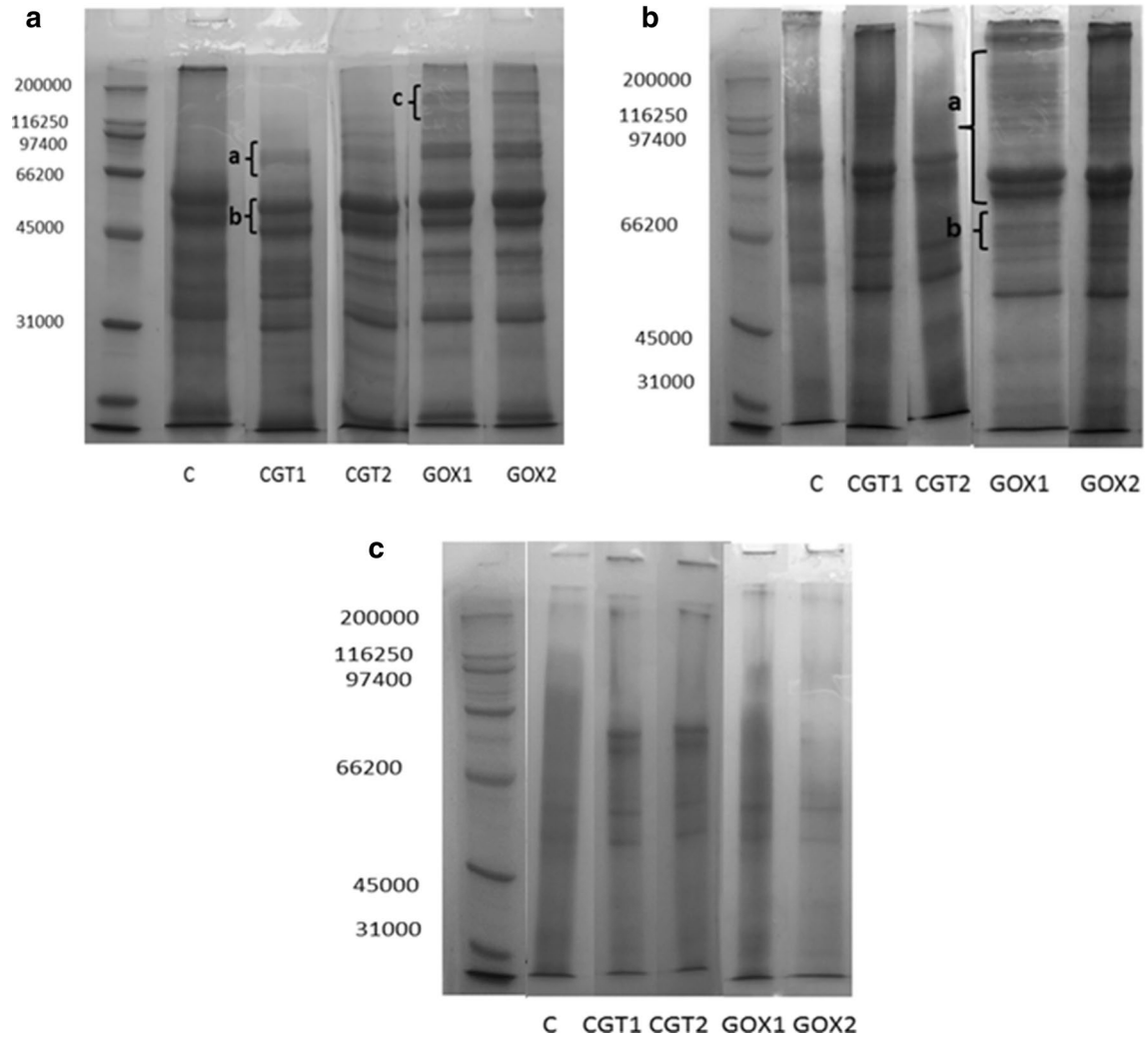

Fig. 2 Electrophoretic patterns of protein fractions from the sequential extraction. **a** Buffer phosphate gel. **b** Buffer phosphate gel containing 2% w/v sodium dodecil sulfate. **c** Final precipitate gel. CGT 1: 0.2 µl cyclodextrin glucanotransferase/100 g flour, CGT 2: 0.8 µl cyclodextrin glucanotransferase/100 g flour, GOX 1: 0.01 g glucose oxidase/100 g flour, GOX 2: 0.02 g glucose oxidase/100 g flour

Samples with CGT had a higher amount of proteins in PB fraction (Table 2) and the gel showed bands (116–66 kDa) which were not present in control (marked with letter a, Fig. 2a); these bands were more intense in CGT 2. Lines with CGT did not show protein aggregates on top of the separating and stacking gel, but presented a pair of bands (66–45 kDa) which were more intense than control (marked with letter b). This revealed that the presence of CGT promotes a higher solubility of proteins in their native states. Gujral et al. [15] observed an increment in the protein solubility of rice bread. They associated it to the formation of complexes between the cyclodextrins (CD) inner cavity of hydrophobic nature and the rice protein (globulins and glutelins) during the bread production process. Lee and Fennema [31] studied the formation of inclusion complexes between CD and globular hydrophobic proteins at 3 and 31 °C. The

Table 2 Protein contents of fractions from sequential extraction

Sample	BP (%)	BP + SDS (%)	Precipitate (%)
Control	$(4.7 \pm 0.1)^{b}$	$(4.7 \pm 0.1)^{a}$	$(1.8 \pm 0.2)^{ab}$
CGT 1	$(5.1 \pm 0.1)^{c}$	$(5.3 \pm 0.2)^{b}$	$(1.9 \pm 0.2)^{ab}$
CGT 2	$(5.3 \pm 0.1)^{c}$	$(5.2 \pm 0.1)^{b}$	$(1.6 \pm 0.1)^{a}$
GOX 1	$(3.9 \pm 0.2)^{a}$	$(5.8 \pm 0.2)^{c}$	$(2.1 \pm 0.2)^{b}$
GOX 2	$(4.a \pm 0.2)^{b}$	$(5.9 \pm 0.1)^{c}$	$(1.8 \pm 0.2)^{ab}$

Percentage of protein considering flour as 100%. Different letters in the same column indicate significant differences ($p \leq 0.05$) between samples for each fraction

CGT 1 0.2 µl cyclodextrin glucanotransferase/100 g flour, *CGT 2* 0.8 µl cyclodextrin glucanotransferase/100 g flour, *GOX 1* 0.01 g glucose oxidase/100 g flour, *GOX 2* 0.02 g glucose oxidase/100 g flour. *BP* buffer phosphate fraction, *BP + SDS* buffer phosphate containing 2% w/v sodium dodecil sulfate fraction

authors reported that the interaction between CD and proteins generated an increment in the solubility at 31 °C and improved its stability during heating. CGT required certain pH and temperature conditions to present its maximum activity. Although the dough sample conditions were not those of maximum enzyme activity when the sequential extractions were done, it was observed that CGT presented some activity affecting the protein fraction. An increment of 11.0 and 9.6% of proteins soluble in PB and SDS was observed in CGT samples compared with control. This revealed a higher amount of protein aggregates held by non-covalent bonds, as well as more intense CGT patterns (Fig. 2b). There was a non-significant decrease in protein percentages retained in the precipitate when CGT was added. However, in CGT samples, bands on top of the separating and stacking gel and bands of 200 and 66–97 kDa proteins were more intense than control (Fig. 2c). These results suggested that CGT does not promote the formation of covalent bonds among soy and rice proteins in dough. Instead, the enzyme has an effect on hydrophobic proteins, which are more soluble due to the formation of CD complexes and enables the formation of protein aggregates through non-covalent bonds.

Samples with GOX had lower PB soluble protein percentages than control. GOX samples had a less intense 200 kDa band, while other samples were more intense (45–66 kDa) (Fig. 2a, marked with letter b). This revealed that high molecular soy and/or rice proteins were interacting through GOX action and that their solubility decreased. Renzetti et al. [32] found a decrease in relative concentration of proteins at 70 kDa when GOX was added in oat dough. GOX samples also showed bands (200–116 kDa) that were not present in control (Fig. 2a; marked with letters c and a, respectively). Sciarini et al. [33] observed a high molecular weight band in a pattern of proteins from a dough made with soy and rice flours and GOX, which was absent in control. PB + SDS protein contents were higher in samples with GOX. This can be associated with the formation of non-covalent aggregates formed by proteins of (200–97) kDa and of approximately 66 kDa (Fig. 2b; marked with letters a and b, respectively). There was no significant increment of proteins retained in the precipitate when GOX was added.

The dough rheological behavior was assessed through a creep-recovery test, where the sample deformation due to the stress imposed and the recovery of the elastic part of the deformation can be related to dough behavior in lamination and rest periods. The creep-recovery test was carried out in dough samples with enzyme lower doses as no significant differences were found in the protein fraction between the evaluated doses; there was no clear improvement in the product technological quality with higher doses. The profiles were according to a typically viscoelastic behavior (Fig. 3). Sivaramakrishan et al. [34] found similar profiles for dough samples made with rice flour.

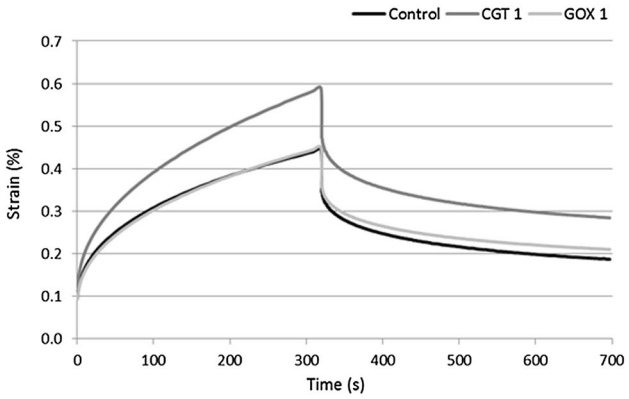

Fig. 3 Creep test curves of gluten-free dough samples. *CGT 1* 0.2 µl cyclodextrin glucanotransferase/100 g flour, *GOX 1* 0.01 g glucose oxidase/100 g flour

Table 3 Creep test parameters of gluten-free dough samples

Sample	Control	CGT 1	GOX 1
Creep phase			
J_0 (1/Pa) × 10^{-6}	$(6.1 \pm 1.0)^{ab}$	$(6.4 \pm 0.8)^{b}$	$(5.5 \pm 0.5)^{a}$
J_1 (1/Pa) × 10^{-6}	$(6.6 \pm 1.0)^{a}$	$(7.5 \pm 1.1)^{b}$	$(6.0 \pm 0.7)^{a}$
λ_{ret} (s)	$(20.8 \pm 2.0)^{a}$	$(20.8 \pm 0.9)^{a}$	$(21.6 \pm 0.7)^{a}$
η_0 (Pa s) × 10^{7}	$(2.5 \pm 0.4)^{b}$	$(1.9 \pm 0.2)^{a}$	$(2.5 \pm 0.3)^{b}$
J_{max} (1/Pa) × 10^{-5}	$(2.5 \pm 0.3)^{a}$	$(2.8 \pm 0.3)^{b}$	$(2.3 \pm 0.3)^{a}$
Recovery phase			
J_0 (1/Pa) × 10^{-6}	$(7.8 \pm 1.5)^{a}$	$(8.1 \pm 0.9)^{a}$	$(7.2 \pm 0.9)^{a}$
J_1 (1/Pa) × 10^{-6}	$(7.4 \pm 1.1)^{ab}$	$(8.2 \pm 1.0)^{b}$	$(7.1 \pm 0.1)^{a}$
λ_{rec} (s)	$(192.9 \pm 13.2)^{b}$	$(171.1 \pm 2.0)^{a}$	$(175.9 \pm 23.0)^{a}$
Je_0 (1/Pa) × 10^{-5}	$(1.5 \pm 0.1)^{a}$	$(1.6 \pm 0.1)^{b}$	$(1.5 \pm 0.1)^{a}$
J_e/J_{max}	$(61.0 \pm 4.2)^{b}$	$(56.2 \pm 4.3)^{a}$	$(60.6 \pm 4.7)^{b}$
J_v/J_{max}	$(39.6 \pm 3.9)^{b}$	$(43.9 \pm 3.6)^{a}$	$(39.0 \pm 3.9)^{b}$

Mean ± standard deviation. Different letters in the same row indicate significant differences ($p \leq 0.05$) between samples

CGT 1 0.2 µl cyclodextrin glucanotransferase/100 g flour, *GOX 1* 0.01 g glucose oxidase/100 g flour, J_0 instantaneous compliance, J_1 viscoelastic compliance, λ_{ret} Kelvin component retardation time, η_0 zero shear viscosity, J_{max} maximum creep compliance, λ_{rec} Kelvin component recovery time, Je_0 steady-state compliance, J_e/J_{max} *and* J_v/J_{max} relative elastic (J_e) and viscous (J_v) portion of maximum creep compliance

During creep phase, dough with CGT presented higher instantaneous and viscoelastic compliances than control (Table 3). An increment in compliance values when stress is being applied is related to a higher capacity of the system to be deformed [35]. CGT sample zero shear viscosity was lower than control and the maximum creep compliance was higher. The enzyme addition caused an increment in the fluidity of the system, associated with a greater behavior of viscoelastic liquid. Accordingly, the dough sample presented a lesser elastic capacity and had a greater deformation.

The CGT dough rheological behavior can be related to the enzyme action over the damage starch fraction. During the test, no heat was applied and water conditions were limited; therefore, the access of the enzyme to the amylose and amylopectin chains was restricted. Hence, the enzyme hydrolyzed the starch, which is more available due to the physical damage of its structure during milling. Gujral et al. [15] reported a decrease in the viscoelastic solid character of the system due to the action of CGT and its hydrolytic effect over starch. Furthermore, the starch modification can lead to the formation of non-covalent protein aggregates and cyclodextrin–protein complexes, which also contribute to the decrease of the dough viscous character due to the inability of these proteins to interact. There were no significant differences between the retardation time values, which reveal that samples need the same time to reach the maximum deformation.

When the stress was removed, samples had an instantaneous response, followed by a retarded one, which ended in a non-recovery deformation. The recovery extent was different between samples (Table 3). CGT dough presented higher instantaneous and viscoelastic compliances and a lower recovery time than control. This reveals that the immediate and retarded compliance changes were higher and faster in CGT sample. The portion of the deformation that remains after removal of stress represents the viscous part of the maximum compliance (J_v/J_{max}), whereas the reformed part is represented by the elastic recovery of the maximum compliance (J_e/J_{max}) [36]. In both samples, control and CGT, the recovery of the maximum compliance had a greater elastic character. However, in control, the elastic portion of the maximum compliance was higher, while CGT showed a more viscous recovery. The CGT dough behavior led to higher laminated dough pieces after the laminating and folding steps than control.

There were no significant differences in creep phase parameters when GOX and control were compared. The dough samples showed similar rheological behaviors when subjected to a constant stress and deformed in the same extent. In the recovery phase, the changes in compliances were similar for both samples, but there was a significant effect of GOX in the time recovery. This can be related to a dough piece that, after the folding steps and during the rest period, recovered faster than control and whose baked product presented a greater height than control (Table 1). The maximum compliance presented a higher elastic character, and CGT value was lower than control and GOX. GOX did not present different J_v/J_{max} or J_e/J_{max} values from control. Renzetti et al. [32] did not find significant differences in creep-recovery parameters when GOX was added in oat dough samples.

To evaluate the structural changes in dough during heating, temperature sweeps were performed in samples without lamination and after a refrigerated rest period. The evolution of complex viscosity during heating is shown in Fig. 4. Initially, the profiles of the samples analyzed decreased to around 50 °C; this was associated to the fat melting contained in dough and, concomitantly, to an increase of the system plasticization. Then, viscosities remained constant to 70 °C. From approximately 70–90 °C, samples presented a marked increment related to the starch gelatinization process and a subsequent peak. Dreese et al. [37] studied wheat dough samples with different starch and gluten contents in a temperature sweep and attributed the G' changes between 55 and 75 °C to the starch gelatinization. Sciarini et al. [38] studied the influence of soy flours on different starches. When the samples were heated, the pasting properties of cassava and corn starches were drastically reduced by soy flours, and this effect was more noticeable in cassava sample; active soy flour had the highest effect. The authors concluded that cassava starch interacts specifically with active soy flour. The interaction governs the dough behavior, and concomitantly, a good technological gluten-free bread was obtained [33]. The addition of CGT did not affect dough viscosity until it reached 70 °C, while GOX contributed to a decrease of dough flowability. Between 70 and 100 °C, viscosity decreased in CGT and it was greater than GOX. Gujral et al. [15] confirmed the hydrolytic and cyclizing activity of CGT during the production process of rice bread. Thus, the starch breakdown promoted by the enzyme affected the system, obtaining a lesser viscous and elastic dough. On the other hand, the cyclodextrins produced can form complexes with the proteins and lipids present in the dough and hinder the swelling of starch granules. Gujral et al. [8] studied the pasting properties of starch pastes with CGT and reported a reduction in the viscosity. They also found a decrease in the viscous and elastic character when the sample was analyzed through a frequency test. In the dough sample with GOX, the reduction of viscosity can be

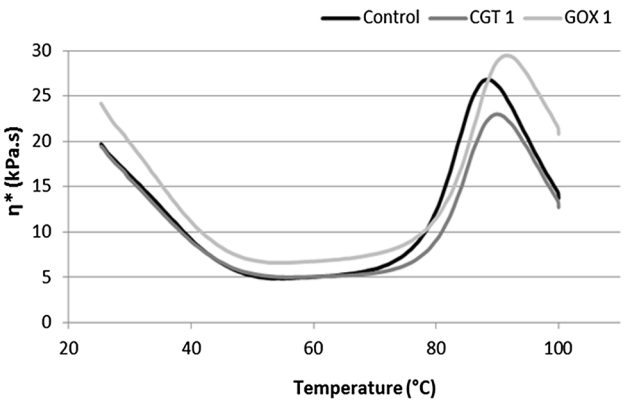

Fig. 4 Evolution of complex viscosity during heating of gluten-free dough samples. CGT 1: 0.2 µl cyclodextrin glucanotransferase/100 g flour, GOX 1: 0.01 g glucose oxidase/100 g flour

related to protein aggregation, which strengthened the protein barrier surrounding the starch granules and reduced its swelling. In agreement with our results, Renzetti et al. [32] observed a decline in paste viscosity of oat flour treated with GOX.

Changes that took place between 70 and 100 °C can also be analyzed in terms of the elastic moduli (G') and their first derivate ($d1$, G') (Table 4). The maximum values of $d1$, G' are associated with the beginning of G' increment. Dough samples with enzymes had $d1$, G' maximum values at higher temperatures than control. Moreira et al. [39] and Mariotti et al. [40] studied the rheological behavior of dough samples made with chestnut and rice flours and corn starch, amaranth flour and pea isolate, respectively. The authors related the temperature sweep parameters with starch behavior during the heating. Moreira et al. [39] associated the temperature of the $d1$, G' with the beginning of starch gelatinization and observed an increment in this value when additives were incorporated. This was related to the additive capacity to delay the beginning of starch gelatinization due to a reduction in water availability. Samples with enzymes were less elastic than control until the maximum was reached. The G' maximum, reached between 70 and 100 °C, was displaced at higher temperatures when the enzymes were added.

During baking, dough piece grows. In this type of products, they are expected to grow vertically more than horizontally. In yeast-laminated dough pieces, the vertical growth of the system results from water vapor generated when, during baking, the water contained in dough sheets evaporates and gets trapped in the melting fat layers [41]. In addition, the action of yeast generates gases, such as CO_2 and ethyl alcohol, which, during heating, exert pressure on dough layers and produce a vertical expansion of the structure. The degree of expansion of the system and the ability of the layers to maintain their integrity under the pressure of gases are related to its rheological properties, which depend on the degree of starch hydration and the development of the protein network. Some authors have reported that during the baking of laminated dough pieces, such as cookies, an expansion of the system and a decrease in viscosity take place. This occurs until a point, where the structure is suddenly fixed. Systems that expand faster and fix their structure later during baking are associated with samples that increase their viscosity more slowly and at higher temperatures [42, 43]. Other authors [44] have found a positive association between dough samples that gelatinize at higher temperatures and, therefore, fix their structure later during baking; as a result, the baked products show greater values for the parameter depending on its desired expansion.

The higher delay in temperature at the beginning of starch gelatinization and the least viscosity increment (70–100 °C) observed in CGT, allowed the system to fix its structure later during baking. On the other hand, CD–protein complexes can exert a protective film effect, entrapping more efficiently the gases generated during baking. Consequently, the baked products with CGT had a greater specific volume and were higher, showed a more laminated inner structure and no significant difference was found in firmness when compared with control. Although CGT mainly has an action over starch, CGT indirectly generates an effect on the protein fraction of the system, which has a positive effect on the technological characteristics of gluten-free laminated products. Jemli et al. [45] determined the CD content of baked products and corroborated the production of these compounds, mainly during baking. They also related the presence of CD–protein complexes with gluten-free breads of greater volume and less firmness.

Table 4 Temperature sweep parameters of gluten-free dough samples

Samples	Control	CGT 1	GOX 1
G'			
Max (kPa)	$(18.4 \pm 2.0)^{b}$	$(14.0 \pm 0.3)^{a}$	$(18.1 \pm 0.4)^{b}$
T (°C)	$(88.6 \pm 0.4)^{a}$	$(90.7 \pm 1.1)^{b}$	$(91.8 \pm 1.1)^{b}$
$d1$, G'			
Max (kPa)	$(15.5 \pm 1.7)^{b}$	$(13.1 \pm 0.2)^{a}$	$(15.6 \pm 1.5)^{ab}$
T (°C)	$(82.6 \pm 1.3)^{a}$	$(85.2 \pm 0.0)^{b}$	$(85.9 \pm 0.8)^{b}$

Mean ± standard deviation. Different letters in the same row indicate significant differences ($p \leq 0.05$) between samples

CGT 1 0.2 µl cyclodextrin glucanotransferase/100 g flour, *GOX 1* 0.01 g glucose oxidase/100 g flour, η^* complex viscosity, G' elastic moduli, *d1, G′* first derivate of G'

Conclusions

The use of enzymes leads to an improvement in the technological quality of gluten-free laminated baked products. Particularly, a marked effect was observed when CGT was added, although samples with both enzymes showed greater specific volume values and a positive effect was registered in the textural parameters. However, only the sample with CGT presented a greater tortuosity related to a layered structure. The differences found in the technological quality of the products can be associated with the effect of enzymes in the components of the system, proteins and starch, and concurrently, in dough behavior during the production steps. Enzymes promoted the formation of protein aggregates held by non-covalent bonds. In CGT sample, an increment in SDS-soluble proteins was observed, possibly related to the formation of complexes between proteins and cyclodextrins. Furthermore, CGT presented a greater effect in dough behavior when subjected to heat. A delay in temperature at the beginning of starch gelatinization, as well as a least viscosity increment are preferred to ensure gluten-free laminated baked products of optimum quality. As a conclusion,

CGT played a central role in gluten-free laminated structure obtainment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human/animal rights This article does not contain any studies with human or animal subjects.

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