Effect of added psyllium and food enzymes on quality attributes and shelf life of chickpea-based gluten-free bread

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Highlights

- Obtaining high-quality gluten-free bread remains a challenge with increasing demand
- Combination of promising ingredients evaluated with full factorial design
- Experimental breads were compared with fresh and stored commercial counterparts
- Chickpea flour and psyllium modified dough parameters and bread physical properties
- Combining 75 chickpea and 5.5 psyllium (g/100g) improve bread quality and shelf life

Abstract

A 2^4 full factorial design with four center points was used to investigate the effects of chickpea flour (CF), psyllium (PSY), cyclodextrin glycosyltransferase (CGTase), and transglutaminase (TGase) on dough Mixolab® parameters and fresh and stored gluten-free bread (GFB) physical properties. Results show that CF and PSY have the greatest effects on the investigated variables. CF increases the loaf-specific volume and crumb firmness values of fresh GFB, effects of increased starch stability (C4) and tendency to starch retrogradation (C5). Both PSY and PSY-CF interactions reduce the loaf-specific volume and increase the crumb firmness of GFB during storage, effects of an increased initial consistency (C1). CF - CGTase interaction reduced crumb firmness during storage, and TGase had no effect. High CF-levels (75 and 100 g/ 100 g flour weight basis, fwb) combined with low PSY-levels (4.5 and 5.5 g/ 100 g fwb) resulted in favorable dough consistency for increasing loaf volume and crumb softness. Results also show that the combination of 75 CF and 5.5 PSY (g/ 100 g fwb) produces a GFB with good physical properties and appearance, reaching values comparable to commercially available fresh and stored GFB.

Keywords: Gluten-free bread; Shelf life; Mixolab; Response Surface Methodology; Multiple Factor Analysis.

1. Introduction

Wheat bread has an important meaning in a human nutrition to many cultures and it is a global staple food. Wheat gluten is a key structure-building protein, essential in leavened baked goods, and its central role in breadmaking and bread quality is irreplaceable. The absence of gluten has great impact on dough, breadmaking process and final bread quality (Matos & Rosell, 2015). Therefore, obtaining high-quality gluten-free bread (GFB) remains a major challenge for scientists and producers, with increasing demand due to the growing number of gluten-intolerant and gluten-tolerant individuals following a gluten-free (GF) diet (Capriles et al., 2020).

Notwithstanding the huge research efforts in bread research and the impactful growth of the GF market in the last decade, important issues stay unaddressed, like unattractive appearance, notably, cracked crust and low loaf volume lacking cellular structure; dry and crumbly crumb texture; undesirables mouthfeel and flavor; shorter shelf life; and low nutritional content (Capriles et al., 2020). To overcome these problems, various ingredients, process conditions and technologies have been investigated, as summarized by recent reviews (Capriles et al., 2020). These confirm the complexity of the GFB to reach a nutritious and aerated structure resembling gluten containing breads. Promising results have been acquired with several ingredients like chickpea flour (CF), a nutrient-dense raw material, psyllium (PSY), a natural bioactive soluble fiber extracted from the husks of *Plantago ovata* seeds, and some processing aids like cyclodextrin glycosyltransferase (CGTase) and transglutaminase (TGase) enzymes to improve GFB quality and shelf life.

Among the alternative nutrient-dense raw materials, pulses may represent a new forward-looking frontier in GFB development, because of its functional and nutritional characteristics (Melini et al., 2017). From a nutritional point of view, chickpeas (*Cicer arietinum* L.), contributes to nutrition and health, being an important source of nutrients like proteins, fibers, minerals and bioactive compounds whose consumption benefits human health and can reduce the risk of chronic diseases (Jukanti et al., 2012; Rachwa-Rosiak et al., 2015). From a technological point of view, CF has emulsifier, foaming and gelation properties, as well as high water and oil absorption capacities and viscosity (Du et al., 2014), important in food preparation, like GFB.

Recent studies show that CF can be used in high proportions (20-100 g/ 100 g) on the flour weight basis (fwb), replacing conventional raw materials when preparing acceptable GFB. However, its high crumb firmness (Burešová et al., 2017; Ouazib et al., 2016; Rostamian et al., 2014; Santos et al., 2018) could compromise the consumer acceptance and shelf life of these products.

PSY addition to GFB preparation can improve volume, structure, texture, appearance, acceptance and shelf life (Mancebo et al., 2015; Fratelli et al., 2018; Ziemichód et al., 2019), along with fiber enrichment, which decreases glycemic index (Fratelli et al., 2018). PSY consumption can improve health, aiding in intestinal transit, cholesterol control, glycemia and satiety (Franco et al., 2020).

The CGTase (EC 2.4.1.19, *Bacillus spp.*) enzyme hydrolyzes starch in cyclodextrins and its molecular structure, with a polar surface and a hydrophobic internal cavity, can act as an emulsifier, forming complexes with lipids and proteins (Gujral, et al., 2003a; 2003b; Basso et al., 2015). Previous studies show that adding CGTase to GFB increases loaf-specific volume and crumb softness (Gujral et al., 2003a, 2003b; Basso et al., 2015) and reduces the firming rate (Gujral et al., 2003b) by inhibiting starch-protein matrix interaction, and diminishing amylopectin retrogradation, which Fadda et al. (2014) state as responsible for increasing crumb firmness during storage.

The TGase (EC 2.3.2.13, *Streptomyces spp.*) enzyme catalyzes transfer reactions between lysine and glutamine residues, transforming soluble proteins into insoluble polymers of high molecular weight. This protein crosslink can improve the viscoelastic behavior of the dough by increasing its ability to retain gases during the baking process, resulting in expansion, structure, texture improvement and GFB acceptance (Marco & Rosell, 2008; Gusmão et al., 2019).

Previous studies show the potential of CF, PSY, CGTase, and TGase in improving GFB quality parameters, but the combined effect of these promising ingredients is yet unknown. The present study aimed at verifying the potential of PSY, CGTase, and TGase to improve quality attributes and shelf life of chickpea-based GFB, understanding possible interactions in this complex matrix. A full factorial design was applied to investigate main and interaction effects between these ingredients, both on the dough thermomechanical properties and GFB physical properties, allowing the relationship between these variables to be evaluated and the establishment of promising formulas, which were compared with two commercially available GFBs.

2. Material and methods

2.1 Ingredients

The CF (containing, g/ 100 g as dry basis: 2.7 ash, 7.3 lipids, 14.0 protein, 14.3 dietary fiber, 2.9 resistant starch and 58.8 available carbohydrates) was purchased from Radha Mangala Farinhas Ltda. (Brazil). The cassava starch (CS), (containing, g/ 100 g as dry basis: 0.2 lipid, 0.1 ash, 1.5 fiber, 0.7 resistant starch and 97.5 available carbohydrates) produced by General Mills Brasil Alimentos Ltda. (Brazil), was acquired at the local trade.

PSY, a concentrate obtained from ground husk, with 95% purity (VITACEL® Psyllium P95) and containing about 80% dietary fiber was donated by JRS Latinoamericana Ltda. (Brazil).

CGTase (NS 27068, EC 2.4.19 *Bacilus lichenoformis*), with 3 KNU-CP/g specific activity (Kilo Novozymes Unit/CGTase Product, Novozymes A/S, Denmark), was donated by Novozymes Latin America Ltda. (Brazil). TGase (Activa BF, EC 2.3.2.13, *Streptoverticillium mobaraense* var. S-8112) with 109 U/g specific activity was donated by Ajinomoto do Brasil Ind. and Com. de Alimentos Ltda. (Brazil).

Calcium propionate (INS 282, Pantec Aditivos e Ingredientes, Brazil), bread spray mold-inhibitors solution composed by sorbic acid, calcium propionate and ascorbic acid diluted in alcohol and water (Conserv, TFF Alimentos, Brazil) and other ingredients (water, eggs, sugar, soybean oil, salt and dry yeast) were purchased in the local market.

2.2 Gluten-free breadmaking process and storage

For breadmaking, some previous test were performed and all formulas were defined on the fwb (g/ 100 g) in: 125 water, 25 whole eggs, 6 white cane sugar, 6 soybean oil, 2 salt, 0.8 dry yeast and 0.1 calcium propionate. In the experimental design (Table 1), flour and starch basis consisted of 100 CF or blends of 75CF: 25CS, or 50CF: 50CS g/ 100g, PSY levels ranged from 4.5 to 12.5 g/ 100 g fwb, CGTase from 0 to 40 µL/ 100 g fwb, and TGase from 0 to 1 g/ 100 g fwb.

The GFB was produced following a straight dough process. First, all ingredients were weighed, except the CGTase that was previously diluted in 30 ml of water, and added to the mixer bowl (BPS-05-NSkymsen, Metalúrgica Siemsen Ltd., Brazil), and then combined by mixing with a dough hook for 7 min at 110 rpm. After mixing, 300 g of dough were set into greased and floured bread pans (19 x 7.5 x 5 cm) and proofed for 90 min at 40 °C and 85% relative humidity (CFK-10, Klimaquip S/A – Tecnologia do Frio, Brazil), then baked at 140 °C for 30 min (HPE-80, Prática Produtos S.A., Brazil). After cooling for 2 h, the loaves were sprayed with mold-inhibitors, packaged in polyethylene bags and stored under controlled conditions (21-25 °C and 50-70% relative humidity) for 96 h (4 days). A total of twelve loaves were prepared from three batches for each bread experimental trial.

2.3 Commercial gluten-free bread samples

Two commercially available white GFB products (P1 and P2) were collected directly from each manufacturer (São Paulo, Brazil) on the production day and stored for up to 96 h in our laboratory, under the same storage conditions and analysis applied to the experimental GFBs. Ingredient list and nutrition facts of P1 and P2 are described in Table S1, Supplementary Material.

2.4 Dough thermomechanical properties

Performed using the Chopin+ protocol in Mixolab®2 (Chopin Technologies, France), this analysis followed the 173 ICC (2008) and 54-60.01 AACC (2010) methods, establishing water level at 125 g/ 100 g fwb. The CF, PSY, CGTase and TGase, combined in the levels described by the experimental design (Table 1), together with CS and water were subjected to agitation for 45 minutes, at 80 rpm, at three temperatures, 30, 90 and 50 °C. The total weight of the sample analyzed was 75 g. The evaluated parameters correspond to the torques (expressed in Newton meters, Nm) obtained for: initial consistency (C1), weakening of the protein network (C2), maximum (C3) and minimum (C4) torque during the heating stage, concerning starch gelatinization and stability, and the torque obtained after cooling (C5) related to starch retrogradation. The secondary parameters were obtained by the difference between the peak torques of the primary parameters C1(at 8 min)-C2; C3-C2, C3-C4 and C5-C4, referring to protein weakening, starch gelatinization, breakdown and retrogradation of starch (Matos & Rosell, 2013; Švec & Hrušková, 2015) rates, respectively. Two repetitions were performed for each test.

2.5 Bread evaluation

Fresh bread characterization consisted of loaf-specific volume, crumb moisture and firmness, following the AACC methods 10-05.01, 44-15.02 and 74-09.01 (AACC, 2010) described by Santos et al. (2018). The crumb cell structures were evaluated by digital image analysis, as described by López et al. (2013). Slices (12.5 mm thick) images were captured at 1200 dpi using a flatbed scanner (Epson L355, Epson do Brasil Indústria e Com. Ltda, Brazil), then processed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). A view field of 945 x 710 pixels (2.0 cm wide x 1.5 cm high) was evaluated for each image and an alveolar threshold of 0.0005 mm^2 applied. The crumb structure analysis included number of cells, average cell size (mm^2) and cell area fraction (%). Analyzes of loaf-specific volume, moisture and crumb porosity were performed in triplicate, while crumb firmness represents the average of six values. Effects of storage time on crumb moisture and firmness were monitored. For this, three random loaves of each preparation were evaluated after 0, 24 and 96 h of production. The firming rate was calculated using Equation (1):

$$Firming\ rate\ = (crumb\ firmness\ time\ 96h - crumb\ firmness\ time\ 0)/\ crumb\ firmness\ time\ 0 \qquad (1)$$

2.6 Experimental design

A 2^4 full factorial design with four center points was used to verify the main and interaction effects between x_1= CF, x_2= PSY, x_3= CGTase and x_4= TGase on the dough and GFB properties. CF levels were selected based on the results of Santos et al. (2018), PSY in Fratelli et al. (2018), CGTase in Gujral et al. (2003a) and TGase in Marco and Rosell (2008). The lower and upper limits of the factors (coded at -1 and +1, respectively) were confirmed from previous bakery trials. The entire experimental design comprised 20 trials; four of them were repetitions of the center point, randomly performed to reduce the impact of systematic errors on the results. Table 1 shows the real and coded levels of the studied ingredients.

[Table 1]

Equation (2) was used to evaluate main and interaction effects.

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 \beta_1 x_1 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 + \beta_{123} x_1 x_2 x_3 + \beta_{124} x_1 x_2 x_4 + \beta_{134} x_1 x_3 x_4 + \beta_{234} x_2 x_3 x_4 + \beta_{1234} x_1 x_2 x_3 x_4 \quad (2)$$

Where Y_i represents the responses of variables, β_i regression coefficients, and x_i coded factors.

2.7 Statistical analysis

Means of trials differences were identified by one-way analysis of variance (ANOVA) and Tukey's test. Response surface methodology was applied, and the model adequacies were checked by adjusted coefficient of determination ($R^2_{adj} > 70\%$), lack of fit ($P > 0.05$), and residual analysis by residual plots. These analyses were performed using the Statistica 13.5 software (Tibco Inc., USA, 2018).

Multiple Factor Analysis (MFA), the Regression Vector (RV) and coefficient and Pearson's linear correlation (*r*) were also calculated using XLSTAT 2020.2 software (Addinsoft, USA, 2020).

3. Results and discussion

3.1 Ingredients effect on dough and bread properties

Detailed information regarding GFB physical characteristics and dough thermomechanical properties (descriptive statistics, Pareto and response surface charts), as well as loaves appearance and crumb cell structure of factorial design trials are described in the Supplementary Material.

Although hydration adjustment is recommended for protein and fiber-rich materials (Conte et al., 2019; Capriles et al., 2020) like CF and PSY, this could turn recognizing if the improvement effect was because of the water or the ingredients difficult. Thus, to avoid confusion in the ingredients effect, 20 bread experiments received fixed hydrations based on previous tests.

Loaf volume and crumb softness are the main desirable bread characteristics, so instrumental parameters such as loaf-specific volume, crumb firmness and moisture are investigated, which can predict product acceptance (Conte et al., 2019). Some ingredients effects on dough properties are explained by the thermomechanical parameters obtained with Mixolab, which may be involved in bread quality (Matos & Rosell, 2013).

Table 2 shows the coefficients obtained for factorial design regression models and the model adequacy to the experimental data.

[Table 2]

Regarding GFB physical properties, significant models fit and high adjusted coefficients of determination for crumb firmness (0 and 96h) and moisture (0 and 24h) were obtained, with the 83% to 99% variation being explained by the models. In short, CF and PSY were the factors with greater effects on these responses.

Significant coefficients showed that CF increases loaf-specific volume, crumb firmness values of fresh product, and decreases firming rate, probably because of the higher content and nature of the CF proteins on the formula (Kaur & Singh 2005; Du et al., 2014; Santos et al., 2018). Thus, CF may have contributed to protein crosslinking, which increases dough gas retention capacity, consequently increasing GFB volume. Having good gelation capacity, chickpea starch-proteins ratio result in a firm gel structure (Kaur & Singh, 2005), which can influence the increase of crumb firmness values in formulas with higher CF levels.

PSY reduced the loaf-specific volume and increased crumb firmness values during storage with a parallel reduction of the crumb moisture. Occurring in the bread staling, the water migration from crumb to the crust provides a drier and more hardened crumb (Fadda et al., 2014). High PSY levels exacerbated this process probably because of its high water absorption capacity (Ziemichód et al., 2019). Fratelli et al. (2018) reported a crumb softening effect resulting from the PSY functioning as GFB texture improver, higher water content and especially the PSY-water interaction. The discrepancy observed in the present study relates to the fixed hydration of the experiments, limiting the plasticizer effect of the water. Nevertheless, PSY is the factor that exerted the greatest influence on crumb cell structure and interacts with CF increasing the number of cells and decreasing its mean area, which enables more homogeneous crumb characteristics (see also Figure S7, Supplementary Material).

The antagonistic interaction observed between CGTase and CF reduces crumb firmness and firming rate throughout the storage period. Despite differences in the breadmaking, these findings are consistent with previously reported reductions in crumb firmness in GFB produced with 20 and 30 μL CGTase in fwb, that have been related to its amylase activity and the formation of cyclodextrins-lipids complexes (Gujral et al., 2003a; Basso et al., 2015).

While CF-TGase interaction increased fresh GFB crumb moisture, CGTase-TGase interaction decreased it. After a 24 h storage period, only the interaction between PSY and TGase influenced crumb moisture, decreasing its values. CF-TGase interaction had no effect on crumb firmness and increased the firming rate. Renzetti et al. (2008) state that protein crosslinking by TGase addition is important for forming internal networks in GF systems; however, excessive crosslinks can result in a structure that compromises expansion during proofing and baking, and softness in the final product, which may explain the effects observed by TGase inclusion on CF-based GFB (see also Figure S8, Supplementary Material).

Significant models for dough thermomechanical properties were obtained with the 75% to 99% explained by the models. PSY increases dough consistency (C1) and starch gelatinization (C3). CF increases starch stability (C4) and retrogradation (C5), parameters that may explain the CF effect of increasing crumb firmness in fresh bread. Other significant coefficients that might be stressed were the decrease in starch retrogradation (C5) induced by CGTase, again related to amylase activity and cyclodextrins release.

Significant correlations ($P < 0.001$) were observed between increase of C1 with reduction in average cells size ($r = -0.783$); increase in C2 torque with increase in the number of cells ($r = 0.765$) and with the reduction of average cells size ($r = -0.860$). Matos and Rosell (2013) state that high consistency dough limit the expanding cells during proofing, damaging volume and crumb softness. C2 torque reduction because of protein denaturation at heating start was not observed in these trials (supplementary Figure S4), suggesting that high PSY levels have strong ability to form complexes with systems proteins through both ionic and nonionic interactions, affecting dough strength, preventing this effect (Pejcz et al., 2018). These data show that high PSY levels increase dough consistency, limiting expansion and consequently resulting in a denser structure, with low loaf-specific volume and high crumb firmness. Again, the diminished effects of high PSY levels in these physical properties was associated with the fixed hydration level. Fratelli et al. (2018) reported the importance of PSY and water interaction to obtain proper dough consistency, enabling GFB expansion, structure and softness.

MFA was performed using GFB physical properties and dough thermomechanical parameters. The first two MFA dimensions explain a 77.1% total variance (Figure 1), and the coefficient $RV_{\text{thermomechanical-physical}} = 0.71$ indicate these variables significant relationship.

[Figure 1]

Figure 1a presents a variable relationship map where second factor comprises loaf-specific volume, firming rate, and CF, thus starch performance was significantly affecting this response. While first factor relates to PSY, thermomechanical parameters and crumb porosity, moisture and firmness of crumb during all storage period. CGTase and TGase are not discriminated any axes and, therefore, no influence the investigated responses.

Each chart point in Figure 1b represents the 20 experimental trials with each quadrant corresponding to grouped trials. Trials 3, 7,11 and 15 prepared with 50 CF and 12.5 PSY show higher C1 and C2 torques and higher firming rates. Trials 4, 8, 12 and 16 prepared with 100 CF and 12.5 PSY, present higher C3, C4 and C5 torques; possibly, the combination of the highest CF and PSY levels increased dough consistency during heating and cooling, resulting in its greater number of cells and crumb firmness of fresh bread (0h). While trials 1, 5, 9 and 13 prepared with 50 CF and 4.5 PSY have higher crumb moisture content during storage period. On the other hand, trials 2, 6, 10 and 14 containing 100 CF and 4.5 PSY presented concomitantly higher loaf-specific volume and lower firming rate, possibly from the lower consistency observed in C1. Presenting intermediate response values, because made with intermediate factor levels, trials 17-20 do not stand out among the studied variables. All experiments had different enzyme levels in their formulas.

To the best of our knowledge, this was the first study to evaluate the addition of PSY, CGTase, and TGase to CF-based GFB in conjunction. Therefore, future studies evaluating formula microstructure and water level variation can help to understand the functional and physicochemical properties of the chickpeas starch-protein matrix, especially in a formula modified by improvers (like enzymes, emulsifiers and hydrocolloids).

3.2 Selection of experimental formula and comparison with commercial GFBs

Considering the data analysis, and to incorporate the highest CF levels without damaging the physical properties and structure of GFB, promising combinations were found between factors CF, PSY and CGTase.

Based on the fitted model (Table 2), the experiment containing (g/ 100 g fwb) 75 CF and 5.5 PSY was selected because it results in lower crumb firmness during 4-days of storage, which Capriles et al. (2020) state may result in sensory-accepted products. Trials 2 (100CF: 4.5PSY) and 6 (100CF: 4.5PSY: 40µLCGTase) of factorial design were selected for their higher loaf-specific volume and lower crumb firmness during storage, according to MFA (Figure 1). The results of the confirmatory experiments performed were similar to the predicted values (Table S3, Supplementary Material).

To prove the potential of these approaches, the promising trials A-C (A – 75CF: 5.5PSY, B - 100CF: 4.5PSY, and C – 100CF: 4.5PSY: 40µLCGTase) were compared with two fresh and stored commercially available GFB products (P1 and P2). Table 3 and Figure 2 presents these results.

[Table 3]

Comparing trials B and C, the added CGTase affects only the firming rate, practically doubling its value, which is an undesirable effect. Among the experimental breads, trial A showed the lower crumb firmness throughout storage period, with values comparable to P1.

P1 presented higher loaf-specific volume and number of cells and lower fresh crumb firmness. However, its crumb firmness values were similar to trial A after 24 h, and to trials A and B after 96 h of storage. On the other hand, P2 showed the lowest loaf volume and higher crumb firmness during storage period, and average cells size similar to experimental breads A and B.

Excepting bread P2, all other experimental breads presented reduction in crumb moisture after 96 h of production. The crumb moisture of commercial breads is significantly lower than the experimental ones, probably because of the difference in hydration levels.

[Figure 2]

P1 presented a finer crumb, with higher number of cells and lower average cells size, while P2 presented crumb cell characteristics similar to the experimental GFBs (Table 3, Figure 2). The carotenoid pigments present in CF gave the experimental CF-based GFB a yellowish crumb color (Jukanti et al., 2012), while commercial P1 and P2 made with refined flours and starches, presented a white crumb color. Trial A showed a rounded top and more homogeneous crumb than trials B and C, desirable characteristics for breads. No sample showed cracks in the crusts, which is a technological defect often found in GFB. CGTase addition impaired the loaves structure in trial C. From the tested values, the 75 CF and 5.5 PSY (g/ 100 g fwb) combination achieved better results in both fresh and stored conditions, reaching values comparable to commercially available GFB.

4. Conclusion

The factorial design helped prove the potential of CF and PSY for GF breadmaking. High CF levels (75 and 100 g/ 100 g fwb) combined with low PSY levels (4.5 and 5.5 g/ 100 g fwb) result in favorable dough consistency for increasing loaf volume and crumb softness. Under the experimental domain, the CF-CGTase interaction reduced crumb firmness during storage, while TGase had no effect.

GFB with a good appearance and physical properties, reaching values comparable to fresh and stored commercially available GFB, can be achieved by combining 75 CF and 5.5 PSY (g/ 100 g fwb).

This is a promising approach which simultaneously improves GFB physical properties, nutrient composition, and shelf life. Its industrial application is paramount and integrate our ongoing research, aiming meet consumers expectations who choose to, or must adhere to a GF diet.

Appendix A. Supplementary data

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Author's Contribution

Fernanda Santos: Investigation, Formal analysis, Validation, Writing - Original Draft. **Etiene Aguiar and Ana Centeno**: Investigation. **Cristina Rosell**: Writing - Review and Editing. **Vanessa Capriles:** Supervision, Project administration, Funding acquisition, Writing - Review and Editing.

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Table 1. Independent variables and respective levels according to the 2^4 full factorial design.

Trials	**Coded levels**				**Real values (flour and starch basis [a])**			
	CF (x_1)	**PSY (x_2)**	**CGTase (x_3)**	**TGase (x_4)**	**CF (g/100g)**	**PSY (g/100g)**	**CGTase (µL/100g)**	**TGase [b] (g/100g)**
1	-1	-1	-1	-1	50	4.5	0	0
2	1	-1	-1	-1	100	4.5	0	0
3	-1	1	-1	-1	50	12.5	0	0
4	1	1	-1	-1	100	12.5	0	0
5	-1	-1	1	-1	50	4.5	40	0
6	1	-1	1	-1	100	4.5	40	0
7	-1	1	1	-1	50	12.5	40	0
8	1	1	1	-1	100	12.5	40	0
9	-1	-1	-1	1	50	4.5	0	1
10	1	-1	-1	1	100	4.5	0	1
11	-1	1	-1	1	50	12.5	0	1
12	1	1	-1	1	100	12.5	0	1
13	-1	-1	1	1	50	4.5	40	1
14	1	-1	1	1	100	4.5	40	1
15	-1	1	1	1	50	12.5	40	1
16	1	1	1	1	100	12.5	40	1
17	0	0	0	0	75	8.5	20	0.5
18	0	0	0	0	75	8.5	20	0.5
19	0	0	0	0	75	8.5	20	0.5
20	0	0	0	0	75	8.5	20	0.5

[a] Basis comprise chickpea flour (CF) and cassava starch blends with a 100 (g/100g) sum; PSY: psyllium; CGTase: cyclodextrin glycosyltransferase; TGase: transglutaminase.

[b] Amount of g TGase / g protein: 0.02 for trials 17-20; 0.03 for trials 12,14 and 16; 0.04 for trial 10; 0.05 for trials 13 and 15; 0.06 for trials 9 and 11.

Table 2. Regression coefficients and models quality obtained from the responses variables (Y_i) of the 2^4 full factorial design

Regression coefficients and model quality	**Physical properties of gluten-free bread**											**Dough termomechanical parameters**								
	Loaf specific volume (cm^3/g)	Crumb structure analysis			**Crumb firmness (N)**				**Crumb moisture (%)**			**Primary parameters (Nm)**					**Secondary parameters (Nm)**			
		Number of cells	**Average cells size (mm^2)**	**Area fraction (%)**	**0**	**24 h**	**96 h**	**Firming rate**	**0**	**24 h**	**96 h**	**C1**	**C2**	**C3**	**C4**	**C5**	**C1-C2**	**C3-C2**	**C3-C4**	**C5-C4**
Constant	2,21	65,28	0,16	28,20	11,91	18,02	19,84	0,91	56,63	56,56	55,82	0,56	0,43	0,96	0,75	1,16	0,10	0,53	0,21	0,41
CF (x_1)	0,15	12,15	-	1,57	3,70	1,56	-	-0,63	-0,27	-0,36	-0,29	-0,02	0,02	0,06	0,33	0,44	-0,03	0,04	-0,27	0,11
PSY (x_2)	-0,18	23,90	-0,07	-	1,98	5,47	5,81	0,47	-0,69	-0,62	-0,52	0,35	0,24	0,08	0,13	0,17	0,08	-0,15	-0,04	0,04
CGTase (x_3)	-0,10	-	-	-1,20	1,37	4,49	4,16	0,20	-	-	-	-	-	-	-0,03	-0,07	0,004	-	0,02	-0,04
TGase (x_4)	-0,04	-	-	-	1,82	3,44	-	-0,37	-	-	-	-0,01	-	-	-0,03	-	-0,003	-	0,03	0,01
CF*PSY	-0,13	10,60	-0,01	-	3,36	2,08	2,49	-0,42	-	-	-	-0,03	0,01	0,04	0,07	0,07	-0,033	0,03	-0,03	0,01
CF*CGTase	0,05	-	-	-	-2,50	-4,96	-5,98	-0,21	-	-	-	-	-	-	0,02	0,02	-0,01	-	-0,02	-
CF*TGase	0,04	-	-0,01	-1,21	-	-	-	0,34	0,12	-	-	-	-	0,02	0,05	0,03	-0,01	-	0,02	0,03
PSY*CGTase	0,05	-	-	1,18	-1,09	-	-	0,30	-	-	-	-	-	-	-0,03	0,04	-	-	-0,04	-0,01
PSY*TGase	-0,09	-	-	-1,17	1,24	2,76	-	-0,38	-	-0,13	-	-	0,01	-	0,02	-0,03	-0,003	-	0,02	0,01
CGTase*TGase	-0,02	-	-0,01	-	-	1,89	-	-0,17	-0,12	-	-	-	-0,01	-	0,02	-	0,01	-	-0,03	-0,02
CF*PSY*CGTase	-0,05	-	-	-	0,60	-	-1,92	-0,33	-	-	-	-	-	-	-	-	-0,01	-	-0,02	-0,01
CF*PSY*TGase	-	-	-	-	-	-1,28	-	0,40	-	-	-	-	-	-	-	-	-0,01	-	0,02	0,02
CF*CGTase*TGase	0,05	-	-0,01	-1,06	-0,60	-2,00	-	0,16	-	-	-	-	0,01	-	0,02	-	-0,01	-0,02	-0,03	-0,03
PSY*CGTase*TGase	-0,01	-	0,02	-	-	2,18	-	-	-	-	-	-	-0,01	-	-	-	0,01	-	-	-0,02
R^2_{adj} (%)	83,96	66,13	75,21	56,82	99,48	82,69	83,41	86,82	93,12	89,16	63,96	99,66	98,34	87,61	87,98	93,45	74,89	84,87	86,37	87,63
Model (P)	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Lack-of-fit (P)	0,000	0,200	0,024	0,140	0,678	0,027	0,167	0,021	0,243	0,193	0,187	0,068	0,011	0,110	0,006	0,008	0,000	0,029	0,004	0,002

CF: chickpea flour; PSY: psyllium; CGTase: cyclodextrin glycosyltransferase; TGase: transglutaminase.
R^2_{adj} adjusted coefficient of determination. Model significance and Lack of fit. P = probability level. - No significant effect at the 5% level

Table 3. Comparison of physical properties of fresh and stored promising experimental chickpea-based gluten-free breads with their commercial counterparts.

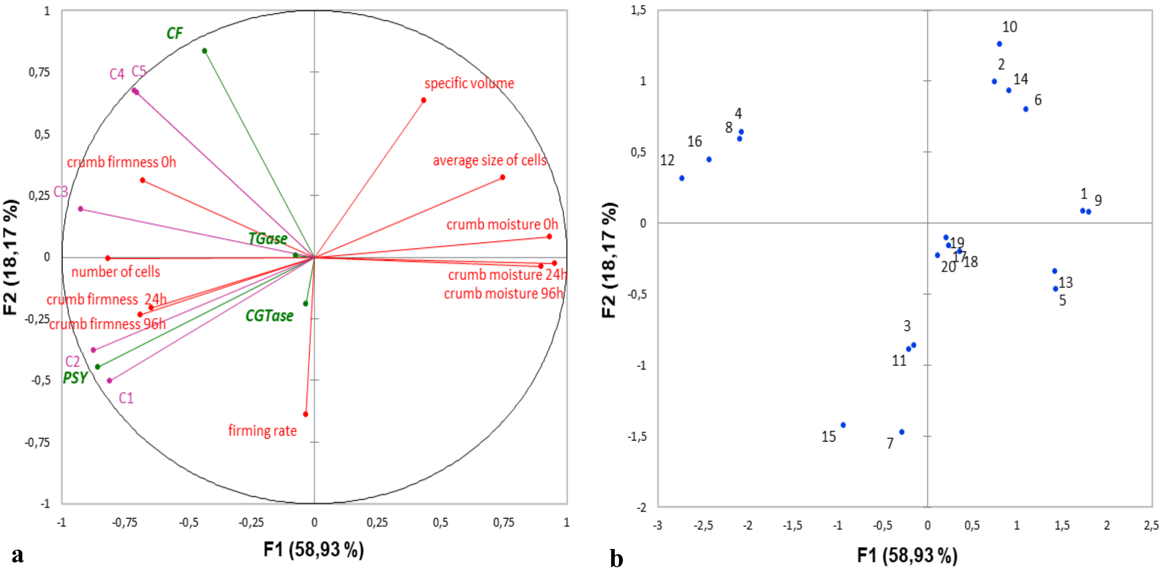
Bread physical properties	**Experimental trials**[a]			**Commercial breads**[b]	
	A **75CF:5.5PSY**	**B** **100CF:4.5PSY**	**C** **100CF:4.5PSY: 40CGTase**	**P1**	**P2**
Loaf specific volume (cm^3/g)	2,58 ^{b} ±0,02	2,59 ^{b} ±0,03	2,50 ^{b} ±0,05	3,12 ^{a} ±0,06	2,20 ^{c} ±0,03
Crumb structure analysis					
Number of cells	46,00 ^{bc} ±2,65	44,33 ^{bc} ±6,43	29,67 ^{c} ±5,77	124,33 ^{a} ±11,55	57,33 ^{b} ±3,05
Average cells size (mm^2)	0,22 ^{ab} ±0,02	0,22 ^{ab} ±0,04	0,33 ^{a} ±0,09	0,06 ^{c} ±0,01	0,16 ^{bc} ±0,01
Area fraction (%)	33,09 ^{a} ±1,63	32,47 ^{a} ±2,41	31,78 ^{a} ±7,37	25,37 ^{a} ±1,81	30,88 ^{a} ±1,12
Crumb firmness (N)					
0	7,25 ^{Cc} ±0,15	9,93 ^{Bb} ±0,34	9,42 ^{Bb} ±0,40	2,08 ^{Cd} ±0,10	12,33 ^{Ca} ±0,63
24h	8,69 ^{Bcd} ±0,18	10,81 ^{Bb} ±0,89	10,13 ^{Bbc} ±0,54	8,04 ^{Bd} ±0,36	26,84 ^{Ba} ±2,34
96h	10,74 ^{Ac} ±0,36	12,55 ^{Abc} ±1,61	14,43 ^{Ab} ±1,07	10,13 ^{Ac} ±0,27	34,94 ^{Aa} ±3,66
Firming rate	0,48 ^{c} ±0.07	0,26 ^{c} ±0.16	0,53 ^{c} ±0.13	3,88 ^{a} ±0.26	1,83 ^{b} ±0.36
Crumb moisture (%)					
0	57,62 ^{Aa} ±0,08	56,86 ^{Aa} ±0,29	56,93 ^{Aa} ±0,04	47,87 ^{Ab} ±0,03	45,25 ^{Ac} ±0,93
24h	57,64 ^{Aa} ±0,20	56,40 ^{ABb} ±0,09	56,86 ^{Aab} ±0,07	47,50 ^{Bc} ±0,07	44,97 ^{Ad} ±0,61
96h	57,13 ^{Ba} ±0,23	55,98 ^{Ba} ±0,14	56,12 ^{Ba} ±0,43	47,19 ^{Cb} ±0,06	44,99 ^{Ac} ±1,18

[a] Basis comprise chickpea flour (CF) and cassava starch blends with a 100% sum; PSY: psyllium; CGTase: cyclodextrin glycosyltransferase.

[b] Breads provided by manufacturers after production.

Values indicate mean ± standard deviation. Lower case letters in the same row indicate differences between formulas ($P < 0.05$ Tukey's test). Capital letters on the same column indicate differences in the formulas at different time intervals ($P < 0.05$ Tukey's test).

Figure 1. Multiple factor analysis correlating dough thermomechanical parameters and physical properties of gluten-free bread prepared with a 2^4 full factorial design to study the effects of chickpea flour (CF); psyllium (PSY); cyclodextrin glycosyltransferase (CGTase) and transglutaminase (TGase)



(a) map of active variables and supplementary data in italic; (b) distribution of the 20 developed trials: **1-** 50CF: 4.5PSY; **2-** 100CF: 4.5PSY; **3-** 50CF: 12.5PSY; **4-** 100CF: 12.5PSY; **5-** 50CF: 4.5PSY: 40CGTase; **6-** 100CF: 4.5PSY: 40CGTase; **7-** 50CF: 12.5PSY: 40CGTase; **8-** 100CF: 12.5PSY: 40CGTase; **9-** 50CF: 4.5PSY: 1TGase; **10-** 100CF: 4.5PSY: 1TGase; **11-** 50CF: 12.5PSY: 1TGase; **12-** 100CF: 12.5PSY: 1TGase; **13-** 50CF: 4.5PSY: 40CGTase: 1TGase; **14-** 100CF: 4.5PSY: 40CGTase: 1TGase; **15-** 50CF: 12.5PSY: 40CGTase: 1TGase; **16-** 100CF: 12.5PSY: 40CGTase: 1TGase; **17-20**: 75CF: 8.5PSY: 20CGTase: 0.5TGase

Figure 2. Crumb cell characteristics and representative images of experimental gluten-free bread prepared with different enzyme levels on flour weight basis (fwb) of chickpea flour (CF, g/ 100g), psyllium (PSY, g/ 100g), cyclodextrin glycosyltransferase (CGTase, µL/ 100g) and commercial gluten-free bread products (P1 and P2).

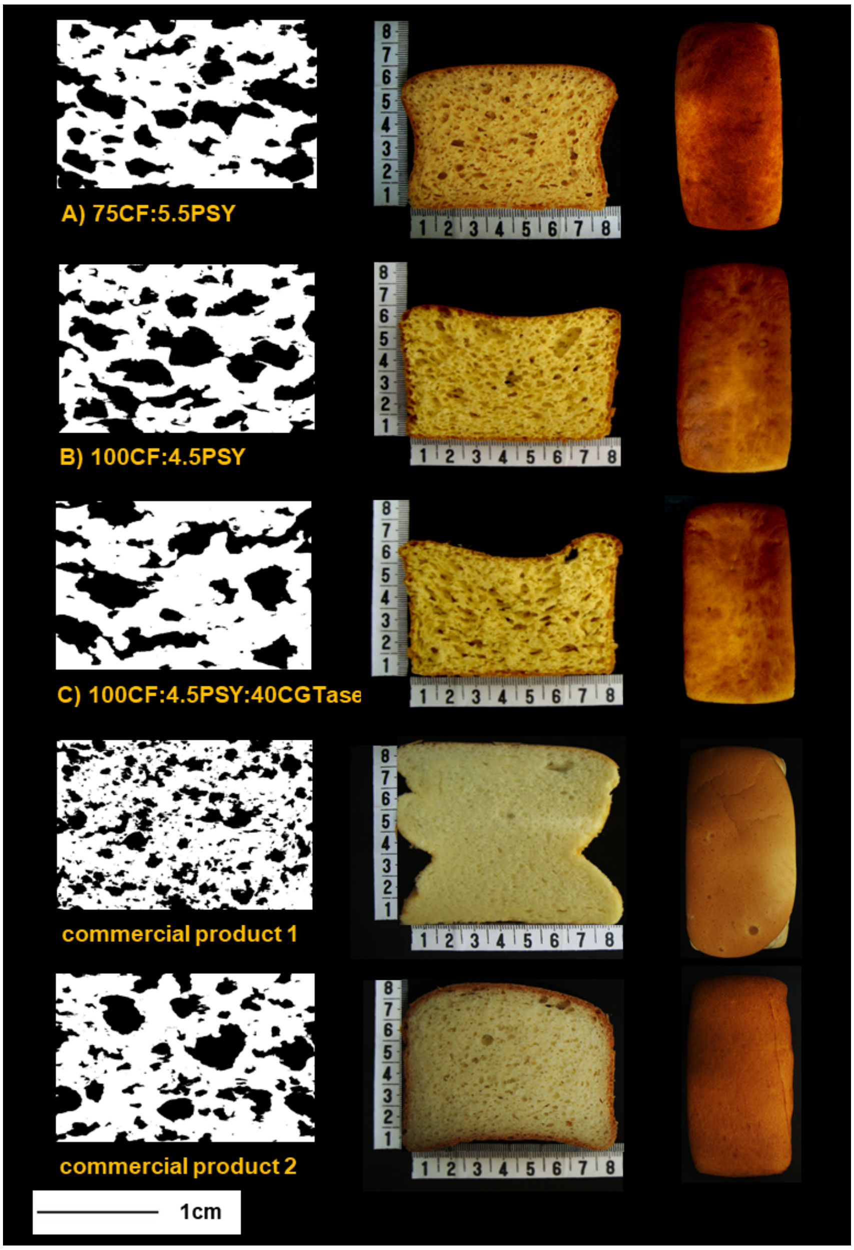


Table S1. Label information of commercial gluten-free breads evaluated

Product label information	**P1**		**P2**	
Ingredient list	rice flour, potato starch, cassava starch, corn starch, modified starch, sunflower oil, egg white, bakers' yeast, HPMC, sea salt, monoglycerides of distilled fatty acids and calcium propionate		rice flour, cassava starch, potato starch, dehydrated egg, palm vegetable fat, sugar, bakers' yeast, salt, xanthan gum and water	
Nutrition facts	50g (2 slices)		50g (1 ½ slice)	
	Amount per serving	**% DV [a]**	Amount per serving	**% DV [a]**
Energy value	121 kcal = 508 kJ	6	104 kcal = 437	5
Carbohydrates	22 g	7	19 g	6
Proteins	1,4 g	2	1,6 g	2
Total fat	2,8 g	5	2,3 g	4
Saturated fat	0 g	0	-	0
Trans fat	0 g	*	-	*
Dietary fiber	0,4 g	2	-	0
Sodium	169 mg	7	240 mg	10

[a] Daily value of reference based on a 2000 kcal or 8400 kJ diet.

* DV not established

- No contain

Table S2. Physical properties of gluten-free breads obtained according to the 2^4 full factorial design.

Trials	Coded levels (Real values - flour and starch basis ^{a})				Variables investigated in fresh bread ^{b}				Variables investigated during bread storage						
					Specific volume (cm³/g)	Crumb cell characteristics			Crumb firmness (N) ^{c}				Crumb moisture (%) ^{b}		
	CF (x_1)	PSY (x_2)	CGTase (x_3)	TGase (x_4)		Number of cells	Average size (mm$^{2)}$	Area fraction (%)	0	24h	96h	Firming rate	0	24h	96h
1	-1 (50)	-1 (4.5)	-1 (0)	-1 (0)	2.30 ^{cd} ± 0.03	45.50 ^{hijk} ± 2.12	0.19 ^{abcdefg} ± 0.01	29.28 ^{ab} ± 0.93	4.11 ^{Bjk} ± 0.44	5.92 ^{Ai} ± 0.34	6.60 ^{Aj} ± 1.35	0.61	57.63 ^{Aa} ± 0.02	57.68 ^{Aa} ± 0.04	56.92 ^{Ba} ± 0.25
2	1 (100)	-1 (4.5)	-1 (0)	-1 (0)	2.59 ^{b} ± 0.03	44.33 ^{hijk} ± 6.43	0.22 ^{abcdef} ± 0.04	32.47 ^{b} ± 2.41	9.93 ^{Bgh} ± 0.34	10.81 ^{Bgh} ± 0.89	12.55 ^{Agh} ± 1.61	0.26	56.86 ^{Abc} ± 0.29	56.40 ^{ABfg} ± 0.09	55.98 ^{Bbc} ± 0.14
3	-1 (50)	1 (12.5)	-1 (0)	-1 (0)	2.09 ^{e} ± 0.03	58.00 ^{fghi} ± 4.24	0.14 ^{cdefg} ± 0.02	29.50 ^{ab} ± 2.93	2.64 ^{Bk} ± 0.24	9.94 ^{Ah} ± 1.52	9.67 ^{Ahij} ± 0.33	2.66	56.06 ^{ABdef} ± 0.14	56.37 ^{Afg} ± 0.03	55.80 ^{Bcd} ± 0.19
4	1 (100)	1 (12.5)	-1 (0)	-1 (0)	2.22 ^{d} ± 0.03	89.50 ^{bcd} ± 7.78	0.10 ^{fg} ± 0.02	30.95 ^{ab} ± 2.78	18.58 ^{Cc} ±0.43	22.98 ^{Bc} ± 0.75	29.32 ^{Ac} ±0.64	0.58	55.37 ^{Bf} ± 0.10	55.78 ^{Ah} ± 0.13	55.25 ^{Bde} ± 0.14
5	-1 (50)	-1 (4.5)	1 (40)	-1 (0)	1.85 ^{gh} ± 0.03	30.50 ^{jk} ± 0.71	0.25 ^{abcde} ± 0.12	18.84 ^{b} ± 2.12	14.07 ^{Be} ± 1.31	22.37 ^{Ac} ± 1.27	25.06 ^{Ad} ± 2.82	0.78	57.62 ^{Aa} ± 0.22	57.29 ^{Aabc} ± 0.22	56.18 ^{Bab} ± 0.28
6	1 (100)	-1 (4.5)	1 (40)	-1 (0)	2.50 ^{b} ± 0.05	29.67 ^{k} ± 5.77	0.33 ^{a} ± 0.09	31.78 ^{a} ± 7.37	9.42 ^{Bh} ± 0.40	10.13 ^{Bh} ±0.54	14.43 ^{Afg} ±1.07	0.53	56.93 ^{Aabc} ± 0.04	56.86 ^{Ade} ± 0.07	56.12 ^{Bbc} ± 0.43
7	-1 (50)	1 (12.5)	1 (40)	-1 (0)	2.20 ^{d} ± 0.03	65.00 ^{efgh} ± 1.41	0.12 ^{cdefg} ± 0.01	26.26 ^{ab} ± 1.93	5.49 ^{Cij} ± 1.30	15.78 ^{Bef} ± 2.83	35.27 ^{Aab} ± 4.04	5.42	56.86 ^{Abc} ± 0.13	56.72 ^{Adef} ± 0.11	55.93 ^{Bbc} ± 0.07
8	1 (100)	1 (12.5)	1 (40)	-1 (0)	2.01 ^{ef} ± 0.02	88.00 ^{bcde} ± 8.49	0.12 ^{defg} ± 0.03	30.90 ^{ab} ± 3.12	16.75 ^{Bcd} ± 0.75	22.23 ^{Ac} ± 1.86	22.24 ^{Ade} ± 1.00	0.33	55.89 ^{Aef} ± 0.10	55.41 ^{Bh} ± 0.05	54.31 ^{Cf} ± 0.13
9	-1 (50)	-1 (4.5)	-1 (0)	1 (1)	2.39 ^{c} ± 0.03	37.67 ^{ijk} ± 5.03	0.25 ^{abce} ± 0.06	30.66 ^{ab} ± 4.21	4.04 ^{Cjk} ±0.24	6.04 ^{Bi} ±0.38	9.55 ^{Ahij} ±0.18	1.36	57.53 ^{Aab} ± 0.07	57.59 ^{Aa} ± 0.15	56.58 ^{Bab} ± 0.19
10	1 (100)	-1 (4.5)	-1 (0)	1 (1)	2.78 ^{a} ± 0.03	33.00 ^{jk} ± 7.07	0.30 ^{ab} ± 0.07	31.12 ^{ab} ± 1.13	11.94 ^{Bf} ± 1.18	14.58 ^{Aefg} ± 2.28	16.46 ^{Af} ± 1.12	0.38	57.27 ^{Aabc} ± 0.06	57.01 ^{Abcd} ± 0.07	56.07 ^{Bbc} ± 0.23
11	-1 (50)	1 (12.5)	-1 (0)	1 (1)	1.96 ^{f} ± 0.03	74.67 ^{cdef} ± 3.06	0.09 ^{fg} ± 0.02	23.24 ^{ab} ± 4.37	6.71 ^{Bi} ±0.88	7.85 ^{ABhi} ±1.32	9.09 ^{Aij} ±0.68	0.35	56.06 ^{Adef} ± 0.78	56.27 ^{Ag} ± 0.12	55.72 ^{Acd} ± 0.32
12	1 (100)	1 (12.5)	-1 (0)	1 (1)	1.93 ^{fgh} ± 0.00	153.00 ^{a} ± 7.81	0.05 ^{g} ± 0.01	26.21 ^{ab} ± 2.27	26.62 ^{Ca} ±0.55	33.59 ^{Bb} ± 1.20	37.56 ^{Aa} ±0.33	0.41	55.61 ^{Af} ± 0.05	55.43 ^{Bh} ± 0.09	54.77 ^{Cef} ± 0.04
13	-1 (50)	-1 (4.5)	1 (40)	1 (1)	1.83 ^{h} ± 0.03	29.00 ^{k} ± 4.58	0.27 ^{abc} ± 0.04	25.56 ^{ab} ± 4.43	16.30 ^{Cd} ± 1.32	19.65 ^{Bcd} ± 2.64	20.99 ^{Ae} ± 0.57	0.29	57.48 ^{Aab} ± 0.22	57.36 ^{Aab} ± 0.09	55.81 ^{Bcd} ± 0.41
14	1 (100)	-1 (4.5)	1 (40)	1 (1)	2.70 ^{a} ± 0.04	48.00 ^{hijk} ± 10.00	0.18 ^{bcdefg} ± 0.05	28.29 ^{ab} ± 4.00	9.92 ^{Cgh} ±1.06	14.37 ^{Bfg} ± 0.72	11.93 ^{Aghi} ± 0.98	0.20	57.08 ^{Aabc} ± 0.31	56.91 ^{Acde} ± 0.04	56.36 ^{Babc} ± 0.06
15	-1 (50)	1 (12.5)	1 (40)	1 (1)	1.64 ^{i} ± 0.03	51.33 ^{hij} ± 6.51	0.13 ^{bcdefg} ± 0.00	27.94 ^{ab} ± 6.29	12.61 ^{Cef} ± 1.85	47.59 ^{Ba} ± 5.10	34.74 ^{Aab} ±2.51	1.75	55.80 ^{Aef} ± 0.17	55.74 ^{Ah} ± 0.45	55.25 ^{Ade} ± 0.08
16	1 (100)	1 (12.5)	1 (40)	1 (1)	1.93 ^{fg} ± 0.00	100.50 ^{b} ± 6.36	0.07 ^{g} ± 0.01	24.69 ^{ab} ± 3.76	21.98 ^{Bb} ±0.20	31.49 ^{Ab} ± 0.92	32.70 ^{Ab} ±2.03	0.49	55.64 ^{Af} ± 0.16	55.45 ^{Ah} ± 0.07	54.71 ^{Bef} ± 0.27
17	0 (75)	0 (8.5)	0 (20)	0 (0.5)	2.32 ^{c} ± 0.06	69.33 ^{defg} ± 5.51	0.12 ^{defg} ± 0.02	28.26 ^{ab} ± 2.58	11.41 ^{Bfgh} ± 0.81	16.15 ^{Adef} ±0.78	16.82 ^{Af} ±0.64	0.47	56.57 ^{Acde} ± 0.05	56.69 ^{Adef} ± 0.05	56.12 ^{Bbc} ± 0.10
18	0 (75)	0 (8.5)	0 (20)	0 (0.5)	2.34 ^{c} ± 0.03	92.00 ^{bc} ± 9.54	0.10 ^{fg} ± 0.01	29.89 ^{ab} ± 2.10	11.77 ^{Bfg} ± 1.32	15.35 ^{Aef} ±1.75	15.17 ^{Afg} ±1.22	0.29	56.87 ^{Abc} ± 0.07	56.90 ^{Acde} ± 0.05	56.50 ^{Bab} ± 0.12
19	0 (75)	0 (8.5)	0 (20)	0 (0.5)	2.33 ^{c} ± 0.03	75.67 ^{cdef} ± 1.53	0.11 ^{fg} ± 0.00	27.89 ^{ab} ± 0.65	11.43 ^{Bfg} ± 0.97	15.34 ^{Aef} ±0.96	16.26 ^{Af} ±0.71	0.42	56.72 ^{Acd} ± 0.18	56.75 ^{Adef} ± 0.06	55.97 ^{Bbc} ± 0.13
20	0 (75)	0 (8.5)	0 (20)	0 (0.5)	2.34 ^{c} ± 0.03	91.00 ^{bc} ± 2.65	0.10 ^{fg} ± 0.01	30.33 ^{ab} ± 3.20	12.46 ^{Bef} ±1.07	18.33 ^{Ade} ±1.06	20.45 ^{Ae} ±2.00	0.64	56.65 ^{Acd} ± 0.17	56.56 ^{Aefg} ± 0.01	56.03 ^{Bbc} ± 0.11

[a] Basis comprise chickpea flour (CF) and cassava starch blends with a 100 (g/100 g) sum. PSY: psyllium (g/100 g). CGTase: cyclodextrin glycosyltransferase (µL/100 g) and TGase: transglutaminase (g/100 g). [b] n=3; [c] n=6.

Values indicate mean ± standard deviation. Lower case letters in the same column indicate differences between formulas ($P < 0.05$ Tukey's test). Capital letters on the same line indicate differences in formulas at different time intervals ($P < 0.05$ Tukey's test).

Figure S1. Pareto charts obtained to evaluate the effects of (1) CF: chickpea flour; (2) PSY: psyllium; (3) CGTase: cyclodextrin glycosyltransferase and (4) TGase: transglutaminase in the physical properties of gluten-free breads

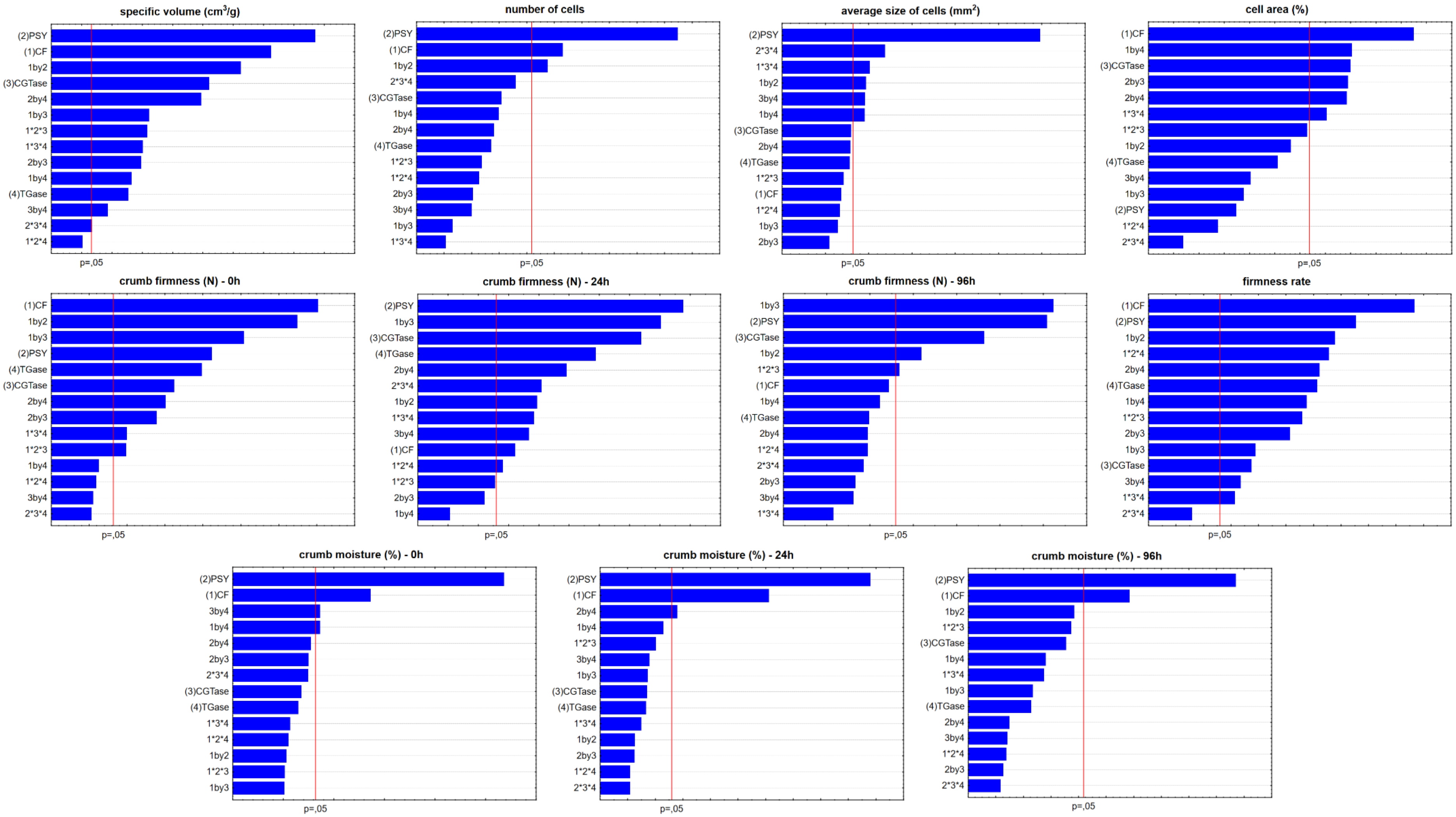


Figure S2. Response surfaces for crumb firmness (N) at 0 (a-f) and 96 (g-i) hours after gluten-free bread production

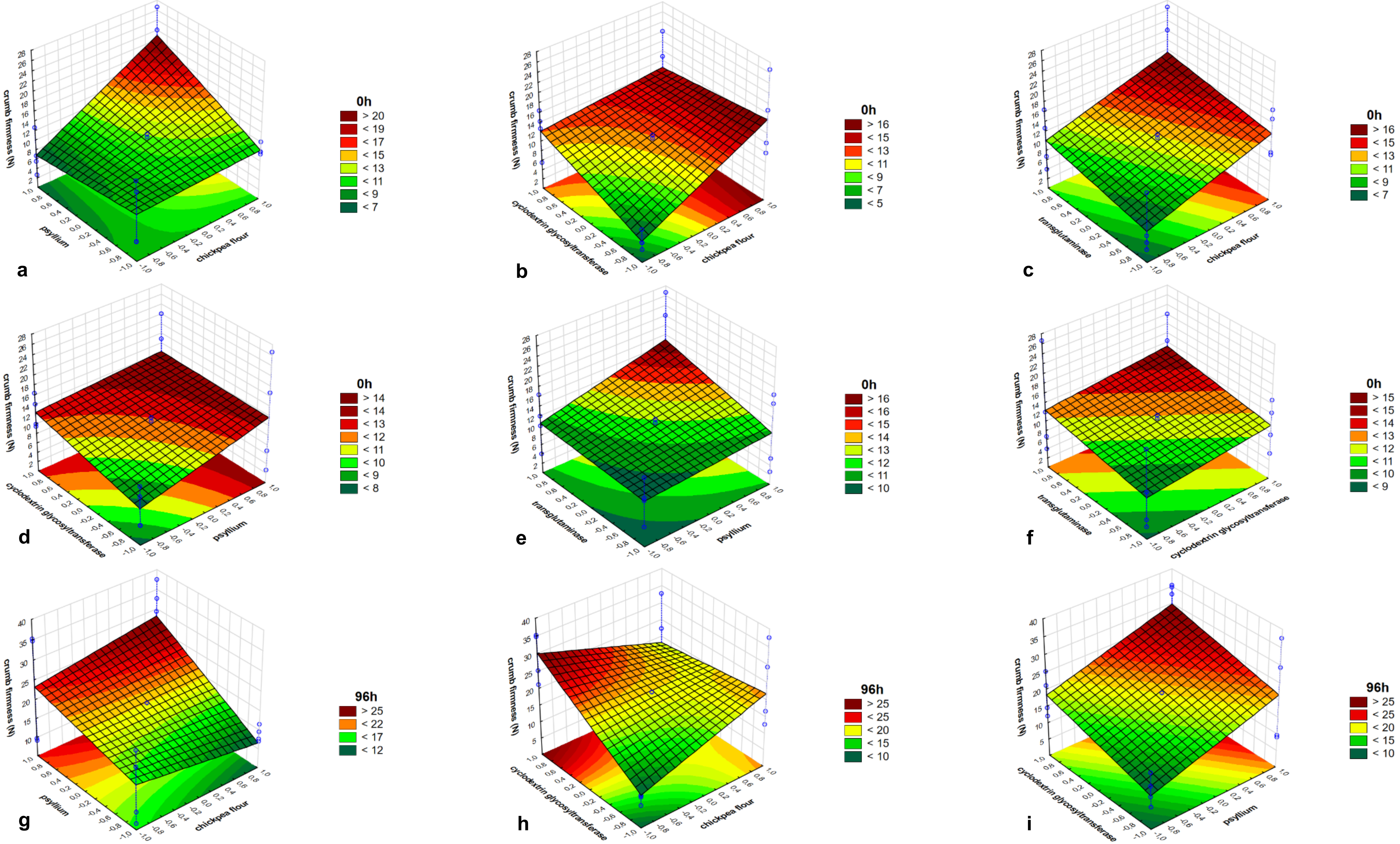


Figure S3. Response surfaces for crumb moisture (%) at 0 (a-c) and 24 (d and e) hours after gluten-free bread production

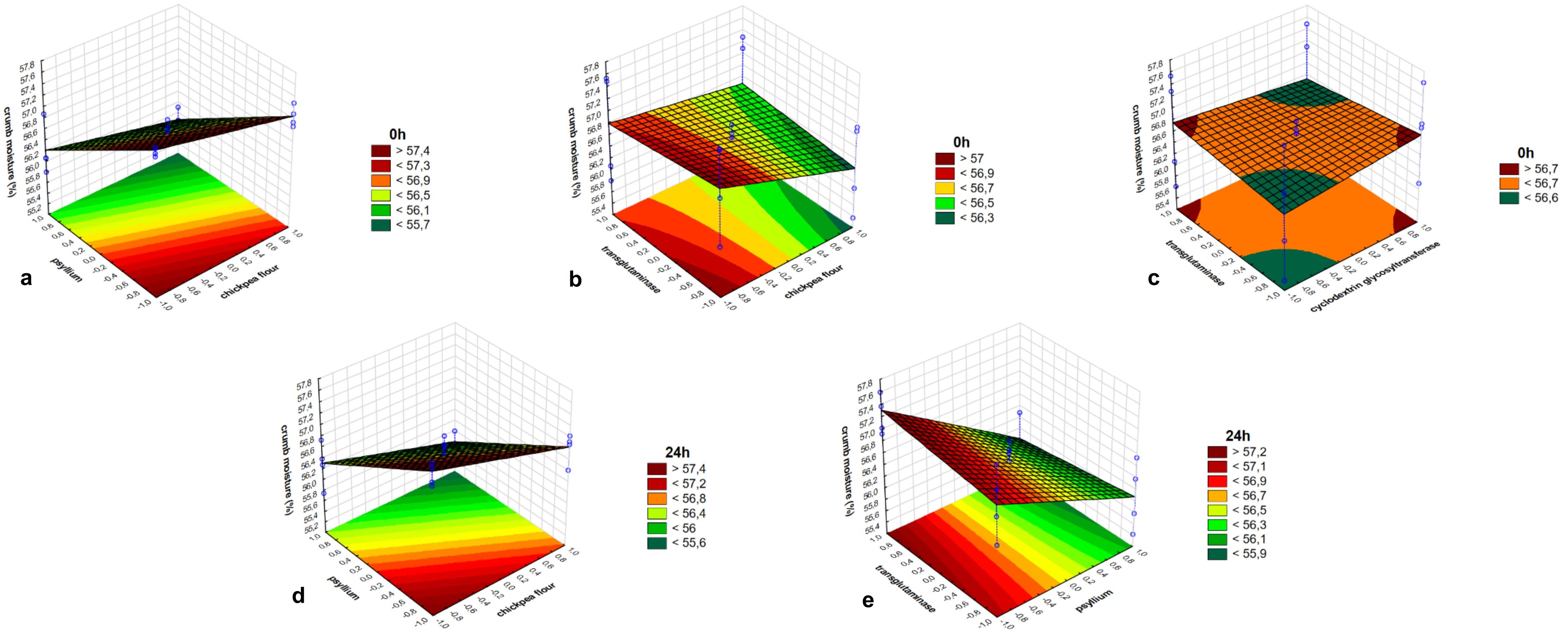
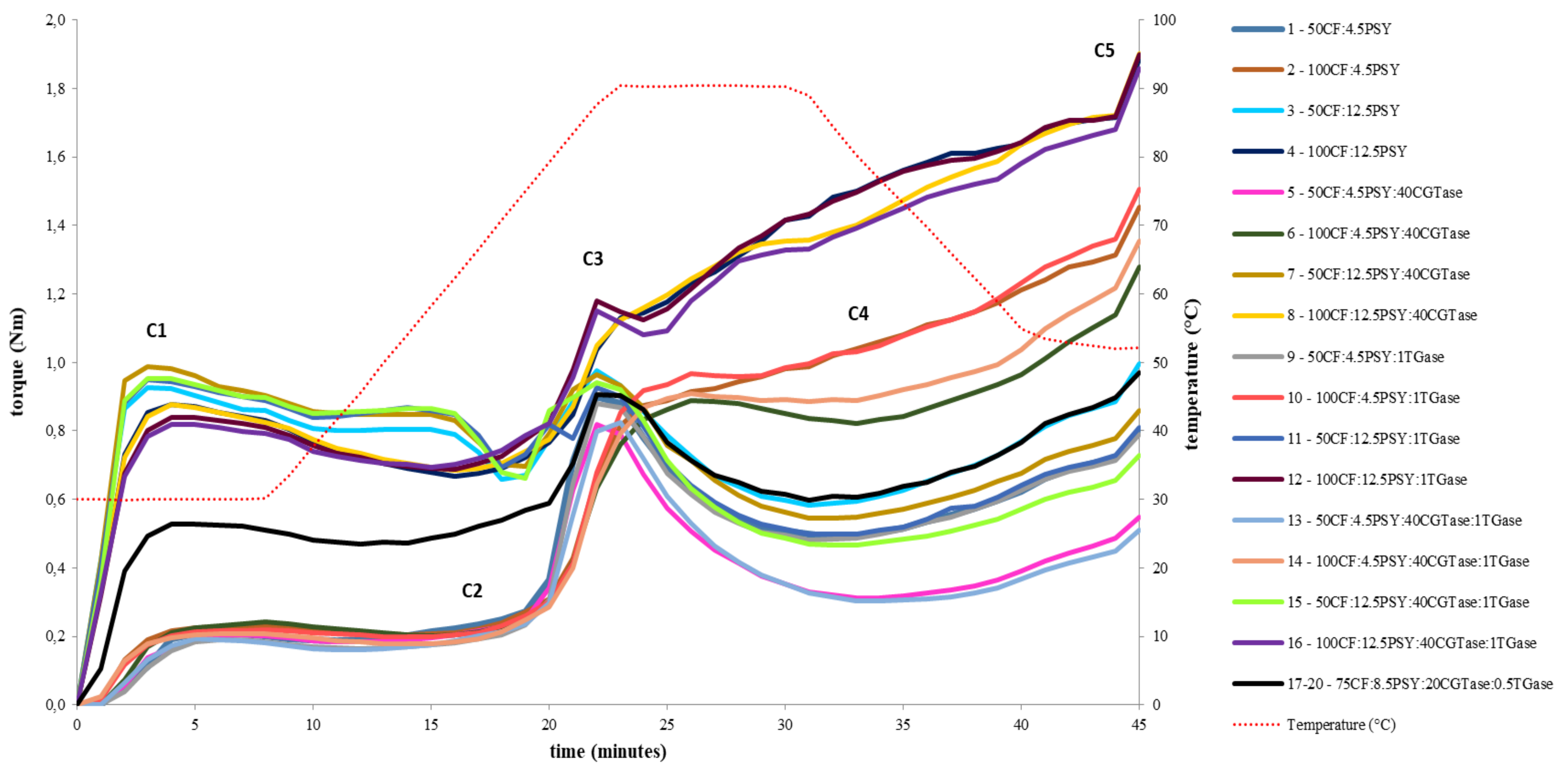


Table S3. Dough thermomechanical parameters obtained by Mixolab for the 2^4 full factorial design

Trial	Coded levels (Real values – flour and starch basis [a])				Primary parameters (Nm)					Secondary parameters (Nm)			
	CF (x_1)	PSY (x_2)	CGTase (x_3)	TGase (x_4)	C1	C2	C3	C4	C5	C1-C2	C3-C2	C3-C4	C5-C4
1	-1 (50)	-1 (4.5)	-1 (0)	-1 (0)	0.21 ^{g} ± 0.01	0.18 ^{e} ± 0.00	0.90 ^{bcde} ± 0.01	0.49 ^{fg} ± 0.03	0.80 ^{g} ± 0.03	0.02 ^{e} ± 0.00	0.72 ^{ab} ± 0.01	0.41 ^{c} ± 0.02	0.31 ^{fg} ± 0.00
2	1 (100)	-1 (4.5)	-1 (0)	-1 (0)	0.23 ^{g} ± 0.00	0.20 ^{e} ± 0.00	0.86 ^{cde} ± 0.01	0.99 ^{c} ± 0.01	1.45 ^{bc} ± 0.01	0.03 ^{e} ± 0.00	0.66 ^{b} ± 0.01	-0.12 ^{g} ± 0.00	0.46 ^{bcd} ± 0.02
3	-1 (50)	1 (12.5)	-1 (0)	-1 (0)	0.94 ^{bc} ± 0.03	0.60 ^{bc} ± 0.00	0.99 ^{b} ± 0.08	0.58 ^{ef} ± 0.07	1.00 ^{ef} ± 0.02	0.26 ^{b} ± 0.01	0.38 ^{de} ± 0.07	0.41 ^{c} ± 0.01	0.42 ^{cde} ± 0.05
4	1 (100)	1 (12.5)	-1 (0)	-1 (0)	0.88 ^{cd} ± 0.00	0.67 ^{a} ± 0.02	1.14 ^{a} ± 0.00	1.42 ^{a} ± 0.01	1.89 ^{a} ± 0.01	0.16 ^{c} ± 0.01	0.47 ^{cd} ± 0.02	-0.28 ^{i} ± 0.01	0.47 ^{bcd} ± 0.02
5	-1 (50)	-1 (4.5)	1 (40)	-1 (0)	0.21 ^{g} ± 0.04	0.18 ^{e} ± 0.02	0.83 ^{e} ± 0.01	0.31 ^{h} ± 0.02	0.55 ^{h} ± 0.04	0.02 ^{e} ± 0.01	0.65 ^{b} ± 0.01	0.52 ^{a} ± 0.01	0.24 ^{gh} ± 0.02
6	1 (100)	-1 (4.5)	1 (40)	-1 (0)	0.24 ^{g} ± 0.01	0.20 ^{e} ± 0.02	0.89 ^{bcde} ± 0.01	0.82 ^{d} ± 0.00	1.28 ^{d} ± 0.05	0.04 ^{e} ± 0.00	0.69 ^{ab} ± 0.02	0.07 ^{e} ± 0.00	0.46 ^{bcd} ± 0.05
7	-1 (50)	1 (12.5)	1 (40)	-1 (0)	0.99 ^{a} ± 0.01	0.66 ^{ab} ± 0.01	0.97 ^{b} ± 0.02	0.54 ^{efg} ± 0.01	0.86 ^{fg} ± 0.02	0.24 ^{b} ± 0.02	0.31 ^{ef} ± 0.03	0.43 ^{bc} ± 0.01	0.32 ^{efg} ± 0.01
8	1 (100)	1 (12.5)	1 (40)	-1 (0)	0.88 ^{de} ± 0.02	0.68 ^{a} ± 0.02	1.12 ^{a} ± 0.02	1.36 ^{a} ± 0.00	1.90 ^{a} ± 0.02	0.14 ^{cd} ± 0.00	0.44 ^{cd} ± 0.00	-0.23 ^{hi} ± 0.02	0.55 ^{b} ± 0.02
9	-1 (50)	-1 (4.5)	-1 (0)	1 (1)	0.19 ^{g} ± 0.01	0.16 ^{e} ± 0.01	0.89 ^{bcde} ± 0.02	0.48 ^{g} ± 0.02	0.79 ^{g} ± 0.00	0.02 ^{e} ± 0.01	0.73 ^{ab} ± 0.01	0.41 ^{c} ± 0.01	0.31 ^{fg} ± 0.02
10	1 (100)	-1 (4.5)	-1 (0)	1 (1)	0.22 ^{g} ± 0.00	0.19 ^{e} ± 0.01	0.97 ^{b} ± 0.01	1.00 ^{c} ± 0.01	1.51 ^{b} ± 0.00	0.03 ^{e} ± 0.01	0.77 ^{a} ± 0.00	-0.03 ^{f} ± 0.02	0.51 ^{bc} ± 0.01
11	-1 (50)	1 (12.5)	-1 (0)	1 (1)	0.96 ^{ab} ± 0.01	0.67 ^{a} ± 0.03	0.93 ^{bcde} ± 0.03	0.49 ^{fg} ± 0.03	0.81 ^{g} ± 0.06	0.22 ^{b} ± 0.01	0.26 ^{f} ± 0.00	0.44 ^{bc} ± 0.00	0.32 ^{efg} ± 0.02
12	1 (100)	1 (12.5)	-1 (0)	1 (1)	0.84 ^{de} ± 0.00	0.68 ^{a} ± 0.01	1.19 ^{a} ± 0.02	1.11 ^{b} ± 0.01	1.90 ^{a} ± 0.02	0.13 ^{cd} ± 0.01	0.50 ^{c} ± 0.01	0.08 ^{e} ±0.01	0.79 ^{a} ± 0.01
13	-1 (50)	-1 (4.5)	1 (40)	1 (1)	0.20 ^{g} ± 0.01	0.16 ^{e} ± 0.02	0.84 ^{de} ± 0.02	0.30 ^{h} ± 0.03	0.51 ^{h} ± 0.03	0.02 ^{e} ± 0.00	0.68 ^{ab} ± 0.00	0.54 ^{a} ± 0.01	0.21 ^{h} ± 0.00
14	1 (100)	-1 (4.5)	1 (40)	1 (1)	0.21 ^{g} ± 0.01	0.18 ^{a} ± 0.00	0.91 ^{bcde} ± 0.01	0.88 ^{d} ± 0.03	1.35 ^{cd} ± 0.06	0.03 ^{e} ± 0.01	0.74 ^{ab} ± 0.01	0.03 ^{e} ± 0.02	0.47 ^{bcd} ± 0.02
15	-1 (50)	1 (12.5)	1 (40)	1 (1)	0.96 ^{ab} ± 0.00	0.58 ^{c} ± 0.03	0.95 ^{bc} ± 0.05	0.46 ^{g} ± 0.02	0.73 ^{g} ± 0.04	0.32 ^{a} ± 0.03	0.37 ^{de} ± 0.08	0.49 ^{ab} ± 0.02	0.27 ^{gh} ± 0.01
16	1 (100)	1 (12.5)	1 (40)	1 (1)	0.82 ^{e} ± 0.01	0.69 ^{a} ± 0.01	1.15 ^{a} ± 0.00	1.35 ^{a} ± 0.04	1.86 ^{a} ± 0.04	0.10 ^{d} ± 0.01	0.46 ^{cd} ± 0.00	-0.20 ^{h} ± 0.03	0.50 ^{bc} ± 0.00
17	0 (75)	0 (8.5)	0 (20)	0 (0.5)	0.54 ^{f} ± 0.01	0.47 ^{d} ± 0.00	0.92 ^{bcde} ± 0.01	0.61 ^{e} ± 0.01	0.99 ^{ef} ± 0.03	0.05 ^{e} ± 0.00	0.45 ^{cd} ± 0.01	0.31 ^{d} ± 0.00	0.38 ^{def} ± 0.02
18	0 (75)	0 (8.5)	0 (20)	0 (0.5)	0.54 ^{f} ± 0.01	0.47 ^{d} ± 0.01	0.91 ^{bcde} ± 0.00	0.56 ^{efg} ± 0.01	0.95 ^{ef} ± 0.02	0.05 ^{e} ± 0.00	0.43 ^{cd} ± 0.01	0.34 ^{d} ± 0.01	0.39 ^{def} ± 0.02
19	0 (75)	0 (8.5)	0 (20)	0 (0.5)	0.52 ^{f} ± 0.00	0.46 ^{d} ± 0.01	0.93 ^{bcd} ± 0.02	0.62 ^{e} ± 0.01	1.01 ^{e} ± 0.05	0.05 ^{e} ± 0.01	0.47 ^{cd} ± 0.01	0.31 ^{d} ± 0.02	0.39 ^{def} ± 0.05
20	0 (75)	0 (8.5)	0 (20)	0 (0.5)	0.53 ^{f} ± 0.00	0.46 ^{d} ± 0.00	0.89 ^{bcde} ± 0.02	0.59 ^{e} ± 0.03	1.00 ^{ef} ± 0.04	0.05 ^{e} ± 0.00	0.43 ^{cd} ± 0.02	0.30 ^{d} ± 0.00	0.40 ^{def} ± 0.02

[a] Basis comprise chickpea flour (CF) and cassava starch blends with a 100 (g/ 100 g) sum. PSY: psyllium (g/ 100 g), CGTase: cyclodextrin glycosyltransferase (µL/ 100 g) and TGase: transglutaminase (g/ 100 g). N=2; Values indicate mean ± standard deviation. Lower case letters in the same column indicate differences between formulas ($P < 0.05$ Tukey's test).

Figure S4. Curves [a] and dough thermomechanical parameters obtained by Mixolab from trials made on the flour and starch basis with levels variation of chickpea flour (CF,%) combined with cassava starch, psyllium (PSY,%), cyclodextrin glycosyltransferase (CGTase, µL), tranglutaminase (TGase, %) and 125% water



[a] Curves represent the mean of trials performed in duplicate

Figure S5. Pareto charts obtained by Mixolab parameters (Nm) to evaluate the effects of (1) CF: chickpea flour; (2) PSY: psyllium; (3) CGTase: cyclodextrin glycosyltransferase and (4) TGase: transglutaminase in gluten-free dough

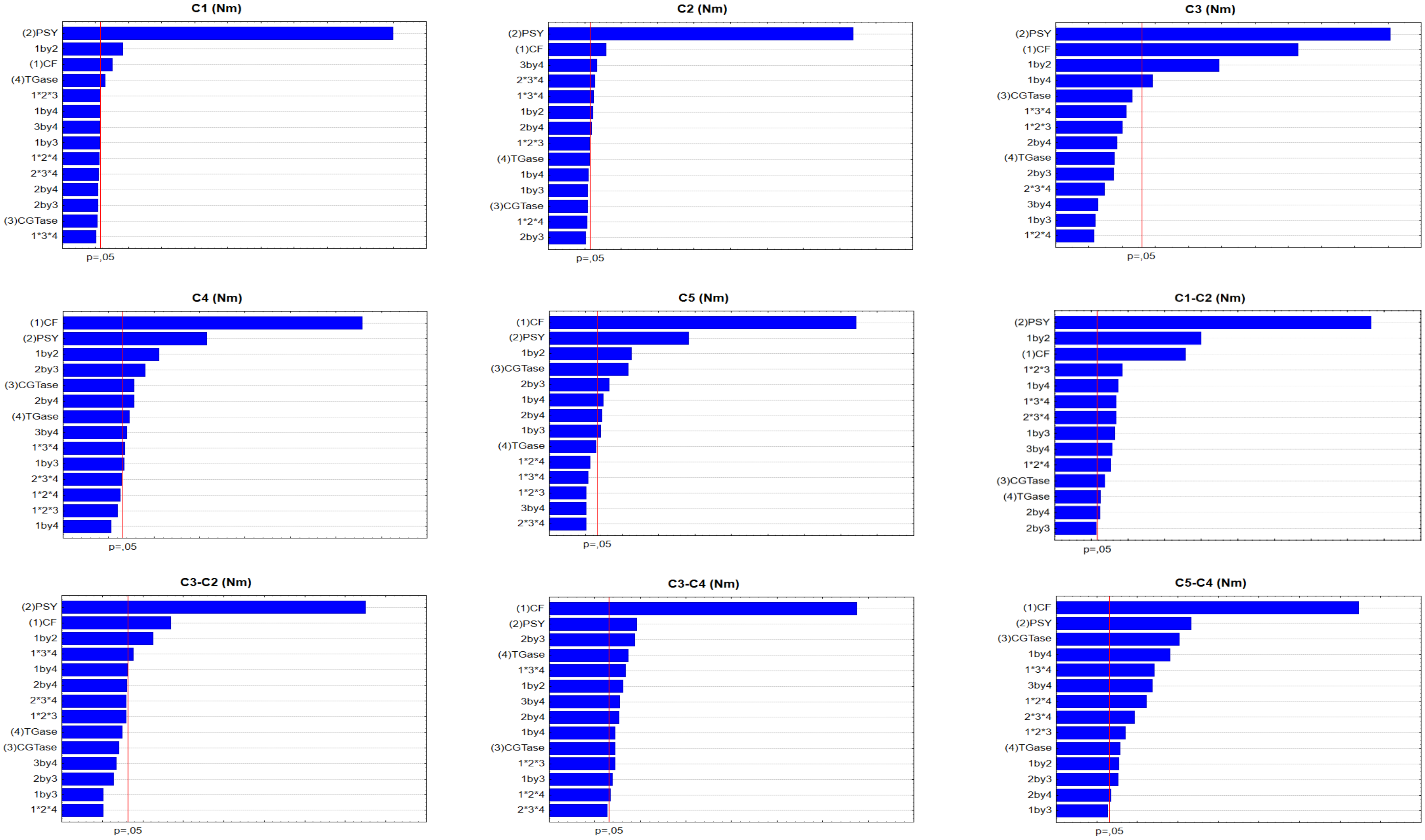


Figure S6. Response surfaces for torques (Nm) C1 (a-c) and C3 (d and e) of trials evaluated by Mixolab

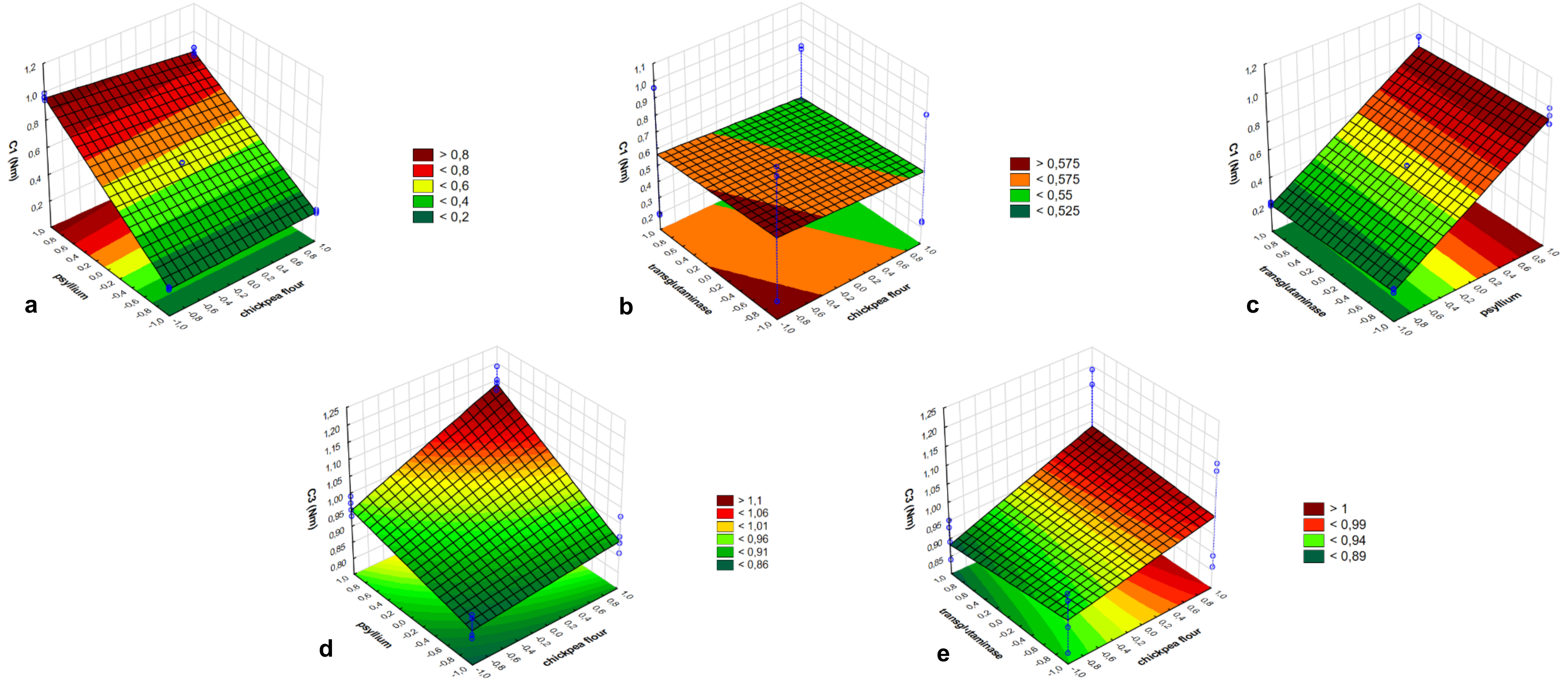
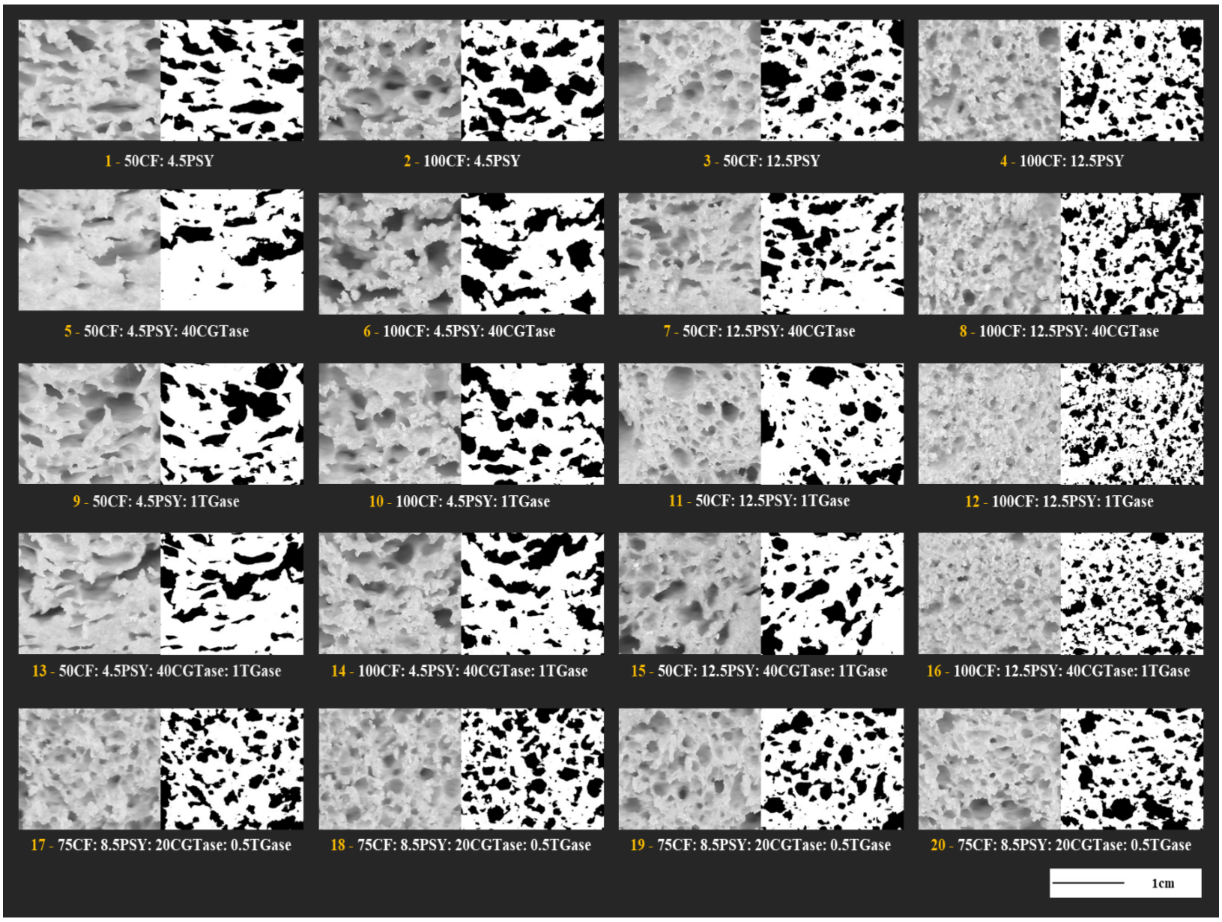


Figure S7. Crumb cell appearance of gluten-free breads prepared on the flour weight basis of CF: chickpea flour (g/ 100g); PSY: psyllium (g/ 100g); CGTase: cyclodextrin glycosyltransferase (µl / 100g) and TGase: transglutaminase (g/ 100g) according to the 2^4 full factorial design trials



Images 945 x 710 pixels in gray scale (left) and binary (right).

Figure S8. Representative images of gluten-free bread formulas made with 2^4 full factorial design to evaluate the effects of CF (chickpea flour, g / 100g); PSY (psyllium, g / 100g); CGTase (cyclodextrin glycosyltransferase, μL/ 100g) and TGase (transglutaminase, g / 100g).

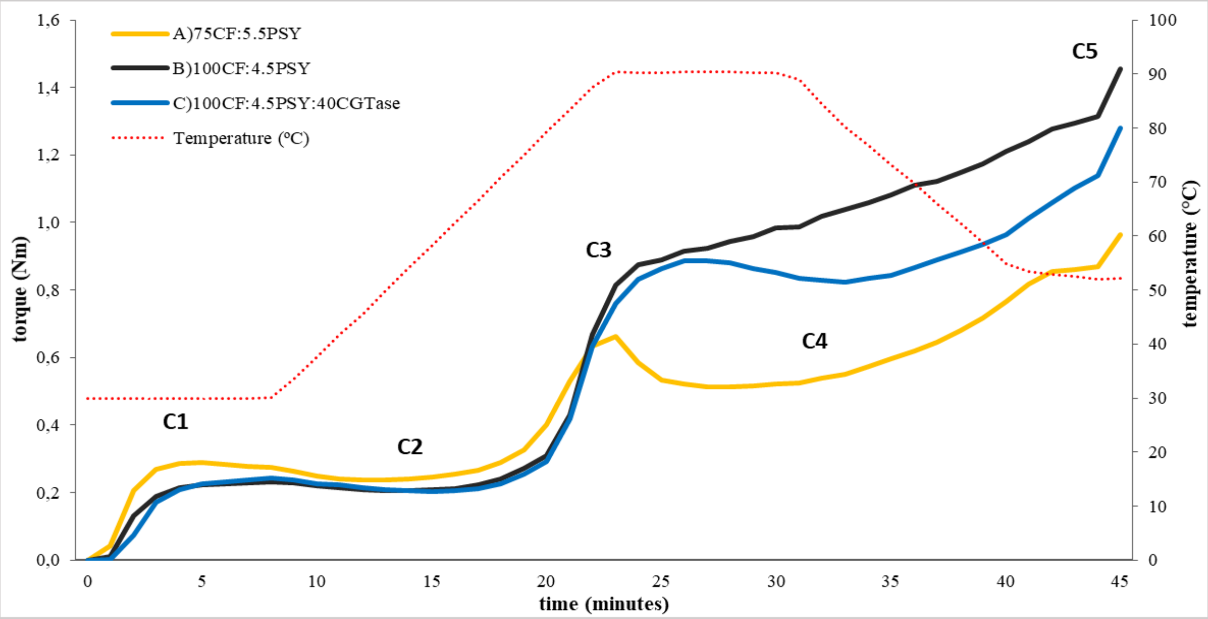

1 - 50CF: 4.5PSY
2 - 100CF: 4.5PSY
3 - 50CF: 12.5PSY
4 - 100CF: 12.5PSY
5 - 50CF: 4.5PSY: 40CGTase
6 - 100CF: 4.5PSY: 40CGTase
7 - 50CF: 12.5PSY: 40CGTase
8 - 100CF: 12.5PSY: 40CGTase
9- 50CF: 4.5PSY: 1TGase
10- 100CF: 4.5PSY: 1TGase
11- 50CF: 12.5PSY: 1TGase
12- 100CF: 12.5PSY: 1TGase
13- 50CF: 4.5PSY: 40CGTase: 1TGase
14- 100CF: 4.5PSY: 40CGTase: 1TGase
15- 50CF: 12.5PSY: 40CGTase: 1TGase
16- 100CF: 12.5PSY: 40CGTase: 1TGase
17- 75CF: 8.5PSY: 20CGTase: 0.5TGase
18- 75CF: 8.5PSY: 20CGTase: 0.5TGase
19- 75CF: 8.5PSY: 20CGTase: 0.5TGase
20- 75CF: 8.5PSY: 20CGTase: 0.5TGase

Table S4. Expected and observed responses based on the adjusted models to validate the physical and thermomechanical properties of the trials

Coded levels (Real values – flour basis ^{a})				Expected responses						Observed responses					
Trial	CF	PSY	CGTase	Crumb firmness (N)		Crumb moisture (%)		Torques (Nm)		Crumb firmness (N)		Crumb moisture (%)		Torques (Nm)	
				0h	96h	0h	24h	C1	C3	0h	96h	0h	24h	C1	C3
A	0	-0.75	-1	7.34	11.32	57.03	56.93	0.31	0.90	7.25 ^{c}	10.74 ^{c}	57.62 ^{a}	57.64 ^{a}	0.29 ^{a}	0.67 ^{b}
	(75)	(5.5)	(0)	(6.52-8.17)	(8.52-14.12)	(56.60-57.46)	(56.45-57.40)	(0.30-0.32)	(0.83-0.96)	(7.10-7.41)	(10.36-11.12)	(57.43-57.81)	(57.14-58.18)	(0.24-0.34)	(0.52-0.82)
B	1	-1	-1	9.71	11.45	56.81	56.69	0.23	0.88	9.93 ^{a}	12.55 ^{b}	56.86 ^{b}	56.40 ^{c}	0.23 ^{b}	0.87 ^{a}
	(100)	(4.5)	(0)	(8.43-10.99)	(7.06-15.83)	(56.59-57.04)	(56.47-56.91)	(0.22-0.24)	(0.85-0.91)	(9.58-10.28)	(10.87-14.24)	(56.14-57.59)	(56.16-56.63)	(0.21-0.25)	(0.80-0.93)
C	1	-1	1	9.66	11.63	57.05	56.69	0.23	0.88	9.42 ^{b}	14.43 ^{a}	56.93 ^{b}	56.86 ^{b}	0.24 ^{b}	0.89 ^{a}
	(100)	(4.5)	(40)	(8.37-10.94)	(7.24-16.01)	(56.82-57.27)	(56.47-56.91)	(0.22-0.24)	(0.85-0.91)	(9.00-9.84)	(13.30-15.56)	(56.84-57.02)	(56.69-57.04)	(0.18-0.30)	(0.83-0.95)

[a] Basis comprise a blend of chickpea flour (CF) with cassava starch at 100 (g/100 g) sum. PSY: psyllium (g/100 g) and CGTase: cyclodextrin glycosyltransferase (µL/100g).
Values indicate the mean and 95% confidence interval. Expected responses obtained from the coefficients of the adjusted models.
Means followed by lowercase letters in the same column differ ($P < 0.05$ Tukey's test).

Figure S9. Curves [a] and thermomechanical parameters obtained by Mixolab from trials made on the flour and starch basis, with the levels variation of chickpea flour (CF,%) in combination with cassava starch, psyllium (PSY,%), cyclodextrin glycosyltransferase (CGTase, µL) and 125% water.



[a] Curves represent the mean of trials performed in duplicate

Table S5. Dough thermomechanical parameters obtained by Mixolab for the promising experimental trials made on the flour and starch basis, with the levels variation of chickpea flour (CF g/ 100 g) in combination with cassava starch, psyllium (PSY, g/ 100 g), cyclodextrin glycosyltransferase (CGTase, µL/ 100 g) and 125 g/ 100 g water.

Trials	C1	C2	C3	C4	C5	C1-C2	C3-C2	C3-C4	C5-C4
A 75CF: 5.5PSY	0.29^{a} ±0.01	0.23^{a} ±0.01	0.67^{b} ±0.02	0.51^{c} ±0.03	0.96^{c} ±0.00	0.04^{a} ±0.01	0.44^{b} ±0.01	0.17^{a} ±0.01	0.46^{a} ±0.03
B 100CF: 4.5PSY	0.23^{b} ±0.00	0.20^{a} ±0.00	0.86^{a} ±0.01	0.99^{a} ±0.01	1.45^{a} ±0.01	0.03^{a} ±0.00	0.66^{a} ±0.01	-0.12^{c} ±0.00	0.46^{a} ±0.02
C 100CF: 4.5PSY: 40CGTase	0.24^{b} ±0.01	0.20^{a} ±0.02	0.89^{a} ±0.01	0.82^{b} ±0.00	1.28^{b} ±0.05	0.04^{a} ±0.00	0.69^{a} ±0.02	0.07^{b} ±0.00	0.46^{a} ±0.05

N=2; Values indicate mean ± standard deviation. Lower case letters in the same column indicate difference between formulas ($P < 0.05$ Tukey's test).