Potential of chickpea and psyllium in gluten-free breadmaking: Assessing bread's quality, sensory acceptability, and glycemic and satiety indexes

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Abstract

Currently, it is still a challenge to obtain gluten free breads (GFB) that meet sensory and health requirements of consumers. Thus, this study investigated the effects of chickpea flour (CF) and psyllium (PSY) on GFB quality, sensory acceptability, glycemic and satiety indexes. The control bread was prepared with rice flour (RF) and cassava starch (75:25). Replacing RF with CF improved bread quality by yielding a better loaf volume and crumb texture, enhancing the appearance, texture, and overall acceptability scores, with no change on flavor and aroma scores. Likewise, a double increase in protein, dietary fiber and resistant starch contents was obtained, reducing the glycemic index, and increasing satiety. PSY addition (5.5% flour weight basis) slightly changed the physical properties of bread and did not impair acceptability compared to control. However, the combination of CF and PSY positively influenced all parameters assessed and thus is a promising alternative for GFB with improved nutrient content and reduced glycemic response together with sensory appeal.

Keywords: Gluten-free, Glycemic response, Satiety index, Sensory acceptability, Texture, Mixolab

1. Introduction

The growing demand and consumption of gluten-free (GF) products has transformed the gluten-free food market from a niche of specialized products into a trend in the global food sector (Capriles et al., 2020). This growth occurred mainly due to the increase in the prevalence of gluten-related diseases, such as celiac disease, non-celiac sensitivity to gluten (also called non-celiac sensitivity to wheat), and allergy to wheat, whose treatment is to keep the gluten-free or wheat-free diet for life in order to avoid symptoms and complications (Brouns et al., 2019). However, wheat gluten imparts viscoelasticity to the doughs, which is important for the baking process and for the quality of the resulting bread. Due to its exclusive functionality, the substitution of gluten in bread remains a challenge for scientists and food producers (Bender & Schönlechner, 2020) .

Refined rice flour and corn, potato and cassava starches are the major ingredients frequently used in the manufacture of commercially available gluten-free breads (GFB), which are characterized as starchy foods, with low protein, dietary fiber and micronutrients (Roman et al., 2019; Santos et al., 2019) and, consequently, a high glycemic profile (Capriles & Arêas, 2016). Frequent consumption of products with this nutritional profile can increase the risk of health problems, such as nutritional deficiencies and chronic non-communicable diseases (Capriles & Arêas, 2016).

GFB formulations generally require greater amounts of water (which can vary from 70 to 218% in flour weight basis - fw) to guarantee crumb structure and texture. Thus, GFB doughs are often liquid, with low viscoelasticity and gas holding capacity, baked in pans to obtain a product with the appropriate structure and shape (Capriles et al., 2020). All of these factors contribute to the characterization of GFBs as products with undesirable appearance, flavor and texture, little nutritional content and short shelf life, with limited availability and high price compared to wheat breads (Capriles et al., 2020). To overcome these challenges,

several alternative raw materials, sources of nutrients and bioactive compounds, protein ingredients, hydrocolloids, emulsifiers, enzymes, and new processing and storage techniques have been tested, with the aim of improving quality parameters at levels comparable to gluten-containing breads, as reported in recent literature reviews (Bender & Schönlechner, 2020; Capriles et al., 2020).

The inclusion of nutrient-dense alternative raw materials in GFB formulations has the potential to decrease the glycemic response, which is very important for individuals with celiac disease concomitant with type 1 diabetes mellitus, who require the maintenance of stringent glycemic control and adherence to a gluten-free diet (Capriles & Arêas, 2016). In addition, foods with a low glycemic index (GI) are associated with a high satiety index (SI), which can assist in maintaining the health of diabetic and/or overweight individuals (Shishehbor et al., 2020). Despite the growing interest in the GI of GFB (Capriles & Arêas, 2016), research to determine the GI and/or SI in human trials is still scarce.

Thus, approaches to the formulation of GFB with better technological properties, nutritionally improved and sensorially accepted, have impelled scientists to search for alternative ingredients, and, in this sense, legumes, such as chickpea flour (CF), and soluble fibers such as psyllium (PSY), offer an innovative and more natural approach, gaining importance in recent years due to their functional and nutritional characteristics, important for GF breadmaking (Bender & Schönlechner, 2020; Capriles et al., 2020).

Some researchers have reported that partial and total replacement of conventional raw materials by CF (20-100% in the flour/ starch basis) increases the volume, the content of proteins and dietary fiber, and the acceptability of GFB (Burešová et al., 2017; Ouazib et al., 2016; Rostamian et al., 2014; Santos et al., 2018) and direct consumption of chickpeas is associated with reduced postprandial glucose levels and increased satiety (Zafar & Kabir, 2017). Other researchers have shown the potential of adding PSY mainly composed of

arabinoxylans to the GFB formulation, which can contribute to increasing the viscosity and gas retention of the dough, and improving the volume, structure, texture, appearance and acceptability of GFB (Fratelli et al., 2018; Mancebo et al., 2015; Ziemichód et al., 2019), in addition to increasing the dietary fiber content and reducing the product's glycemic response (Fratelli et al., 2018).

Recently, Santos et al. (2020) applied factorial design to prove the potential of CF and PSY for GF breadmaking, reporting that combining 75 CF and 5.5 PSY (% fwb) results in favourable dough consistency for increasing loaf volume and crumb softness, and leads to GFB with a good appearance and physical properties, reaching values comparable to fresh and stored commercially available counterparts. Nevertheless, this promising formula was established based only on dough and bread instrumental parameters. Then, deeper physical properties characterization along with further sensory and nutrition evaluation could help to validate the potential of CF and PSY for gluten-free breadmaking.

Therefore, the aim of this study was to investigate the effects of CF and PSY on dough parameters, and GFB physical properties, sensory acceptability, and the postprandial glycemic and satiety response in healthy subjects.

2. Material and methods

2.1 Ingredients

Chickpea flour (CF) as dry basis %, contains: 58.8 available carbohydrates, 14.3 dietary fiber, 14.0 protein, 7.3 lipids, 2.9 resistant starch and 2.7 ash. CF was purchased from Radha Mangala Farinhas Ltda., Brazil. Cassava starch (CS), as dry basis %, contains: 97.5 available carbohydrates, 1.5 dietary fiber, 0.7 resistant starch, 0.2 lipid and 0.1 ash. CS was produced by General Mills Brasil Alimentos Ltda., Brazil. Rice flour (RF), as dry basis %, contains: 82.2 available carbohydrates, 9.7 protein, 5.2 dietary fiber, 1.2 resistant starch, 1.1

lipids and 0.6 ash. These contents were determined using standard methods (AOAC 2005), as described in 2.5 section.

RF was produced by Urbano Agroindustrial Ltda., Brazil, were acquired at the local trade. PSY, a concentrate obtained from ground husk, with 95% purity (VITACEL® Psyllium P95) and about 80% dietary fiber. PSY was donated by JRS Latinoamericana Ltda., Brazil. CF, CS and PSY composition were reported previously by Santos et al. (2020).

Xanthan gum (Ziboxan, F80, Deosen Biochemical Ltd., China) was donated by the company Sweetmix Ind. Com. Imp. Exp. Ltda, Brazil, and carboxymethylcellulose (Denvercel FG-2504A, Denver Especialidades Químicas Ltda, Brazil) was donated by the manufacturer itself. The preservatives, powdered calcium propionate (INS 282, Pantec Additives and Ingredients, Brazil) and bread spray mold-inhibitors (Conserv, TFF Produtos Alimentícios, Brazil), as well as water, eggs, white cane sugar, soybean oil, salt and dry yeast were purchased in local commerce.

2.2 Formulation and production of gluten-free breads

Four GFB formulations named 1-RF, 2-CF, 3-RF:PSY and 4-CF:PSY were produced under the same processing conditions. Breads were prepared with a mixture of 75% RF or CF with 25% CS in the fw. 1-RF and 2-CF breads contained combinations of 0.3% xanthan gum and 0.3% carboxymethylcellulose in fw, while 3-RF:PSY and 4-CF:PSY breads contained 5.5% PSY. The other ingredients were used in the same proportions for the four formulations, and contained % fw: 6 white cane sugar, 2 salt, 0.8 dry yeast, 0.1 calcium propionate, 25 whole eggs, 6 soybean oil and 125 water.

The breadmaking process was carried out by the straight dough method, as described by Santos et al. (2020). Briefly, the ingredients were mixed at 110 rpm for 7 min, followed by fermentation for 90 min at 40 °C and 85% relative humidity, baking at 140 °C for 30 min,

cooling for 2 h followed by spraying with mold-inhibitors and packed in polyethylene bags and maintained under controlled conditions (22-25 °C and 50-70% relative humidity) until further analysis.

2.3 Dough thermomechanical properties

The analysis was performed using the Chopin+90 protocol in Mixolab (Chopin Technologies, France). The method 54-60.01 of AACC (2010) was adapted, allowing to know the ingredients' effect on dough properties subject to mixing and temperature variation, similar to what occurs during breadmaking. Thus, all ingredients and their levels used in the bread preparation, except yeast, were used, totaling 90 g of dough. The parameters (expressed in Newton meters, Nm) were obtained for: initial consistency (C1), weakening of protein network (C2), maximum (C3) and minimum (C4) peak during the heating phase, related to gelatinization and starch stability, and the value obtained after cooling (C5), related to starch retrogradation (Matos & Rosell, 2013). Two repetitions were performed for each trial.

2.4 Physical properties of the breads

Breads were evaluated for specific volume, crumb cell structure, moisture content and instrumental texture. Three random breads from each experimental test were used for the specific volume analysis, according to the method 10-05.01 of AACC (2010), and another three breads were used for the other analyses.

Crumb cell structure was evaluated as described by Santos et al. (2020). The monitored parameters were number of cells, average cell size (mm²), and cell area fraction (%). Three slices were analyzed per treatment.

To assess the moisture content, the bread crust and crumb were first separated using a blade based on the light color of the crumb and the dark color of the crust. Subsequently, the

crust and crumb moisture of each bread was determined by drying in an oven at 105 °C, according to method 44-15A of AACC (2010).

The instrumental texture analysis was performed on crust and crumb using a texturometer (TA.XT *plus* texture analyser, Stable Micro Systems, United Kingdom) and Exponent 7.1 software.

Crust hardness was measured by means of a 5 mm puncture test using a cylindrical aluminum probe 3 mm in diameter at a speed of 2 mm/s. The peak of maximum strength (N) was measured at five pre-selected points on the crust of each bread, totaling 15 points per formulation, as recommended by Rinaldi et al. (2017).

For the crumb, firmness was determined according to method 74-09 of AACCI (2010) performed by compressing up to 40% of the original height in two central slices (25 mm thick), whose firmness corresponded to the force necessary to compress the sample at 25% of the original height obtained from three loaves of each experimental formulation (six values) using a 36 mm diameter cylindrical aluminum probe at a speed of 1.7 mm/s. With the same probe, texture profile analysis was performed by double compression in twelve 20x20x20 mm cubes extracted from six slices of each sample, as described by Rinaldi et al. (2017), with a speed of 1 mm/s up to 50% of the cubes height, and then the parameters hardness, chewiness, cohesiveness and springiness were analyzed.

2.5 Proximate composition

Moisture, ash, protein, and lipid contents were determined according to the respective methods 950.46, 923.03, 960.52, 920.39 of AOAC (2005). Soluble and insoluble fiber levels were determined using the enzymatic-gravimetric method 991.43 of AOAC and analytical kit K-ACHDF (Megazyme International Ireland Ltd., Ireland). Resistant, digestible and total starch content were determined using the enzymatic-colorimetric method 2002.02

of AOAC (2005) and the analytical kit K-RSTAR (Megazyme International Ireland Ltd., Ireland). Available carbohydrates were quantified by difference. The data correspond to the average of three determinations.

The energy value was calculated based on the conversion factors per g: 4 kcal (17 kJ) for proteins and carbohydrates, 9 kcal (37 kJ) for lipids, and 2 kcal (8 kJ) for dietary fiber (FAO, 2003).

2.6 Sensorial evaluation

Sensory analysis of the bread was performed by 65 consumers (53 women and 12 men aged 18-50 years) recruited on university campus through internal advertisements. All of them agreed to taste the samples before the tests were carried out, and stated that they consumed breads and had no allergies or intolerances to any of the ingredients present in the products and were informed that they were trying GFB.

Consumers evaluated the acceptability of the appearance, aroma, texture, flavor and overall liking of the breads on a 9-point structured hedonic scale, ranging from 1 = disliked extremely to 9 = liked extremely (Giménez et al., 2012). Bread slices (12.5 mm thick) of the four samples were offered in complete blocks in a monadic manner, adopting the balanced order of presentation. The samples were packed in polyethylene bags encoded with three-digit numbers.

The evaluation was carried out in a sensory analysis laboratory with climate control (20–25 °C) and equipped with individual cabins with white light. Participants were instructed to drink water between samples to minimize residual effects.

2.7 Glycemic and satiety response

GI was determined based on the method proposed by Brouns et al. (2005). SI was assessed using the 7-point scale anchored at the ends with the terms 1 = extremely hungry and 7 = extremely satiated (Holt et al., 1995).

Exclusion criteria were smoking, pregnancy, lactation, allergy to any ingredient used in breads, diabetes or diagnosed gastrointestinal disease, and use of medications that affect the glycemic response, gastric emptying, and appetite. Thus, this study included only healthy adults individuals (12 in total: 2 men and 10 women, aged 20 to 60 years), eutrophics (Body Mass Index from 19.14 to 24.22 kg/m²), non-smokers and who followed an unrestricted diet.

Four sessions were carried out to jointly evaluate the glycemic response and satiety, two to evaluate 1-RF bread as control food (which has the same GI value as white wheat bread, commonly used as a reference food, as reported by Fratelli et al. (2018) and two others to evaluate the 2-CF and 4-CF:PSY formulations. All samples were produced in the laboratory the day before and were storage to the next test day (around 13 to 16 h between baking and consumption). The sessions were held in the morning, according to volunteers' availability in an ambient room, with interval from seven to twelve days between evaluations. All participants were instructed to maintain their eating habits and physical activities, and not to consume alcohol in the days before the test.

Volunteers' blood glucose was collected with the aid of a lancing device (Accu-Check® Multiclix, Roche Diagnostics, Mannheim, Germany) and evaluated on a digital glucometer (Accu-Check® Performa, Roche Diagnostics, Mannheim, Germany) after overnight fast (10-12 h). Then, the volunteers were instructed to consume in 10 min portions of 25 g available carbohydrate from each GFB to avoid an unrealistically large portion size, as suggestions by Brouns et al. (2005) to food with moderate carbohydrate content, as bread. The available carbohydrate contents was previously calculated from the results obtained in the proximate composition analyses, and up to 500 ml of water as a liquid accompaniment was

also offer, according to Wolever et al. (2008) and Capriles & Arêas (2013). The subsequent digital punctures were performed at 15, 30, 45, 60, 90 and 120 min after the first bite of the evaluated food. The volunteers filled out the scale to report the feeling of hunger and satiety immediately after each puncture.

The calculation of the Incremental Area Under the Curve (iAUC) for blood glucose and satiety was performed geometrically by applying the trapezoidal rule, which considers only the area above the baseline, fasting value (Brouns et al., 2005). The mean value of the area of glycemia and satiety of the control bread was considered equal to 100, and the calculation of GI was done through the relation of the areas of the glycemic curves of the test breads on the control bread for each individual and then the average value was obtained. The same calculation was performed for the areas of satiety curves to obtain the SI.

The mean individual GI of the tested GFB was multiplied by 0.7 for conversion of the values on the glucose scale as standard food (glucose GI = 100) (Wolever et al. 2008). This value was used to calculate the Glycemic Load (GL) determined as a product of the GI of the food and the amount of available carbohydrate in the portion of the food (Atkinson et al., 2008). Considering glucose as the standard food, the GI of foods is classified as low (≤ 55), moderate (56-69) and high (≥ 70), and GL as low (≤ 10), moderate (11-19), and high (≥ 20) (Atkinson et al., 2008).

2.8. Ethical considerations

The Research Ethics Committee of the UNIFESP (Brazil) approved this study (number 3.317.683/2019) and all the participants signed an informed consent form before enrollment in the research.

2.9 Statistical analysis

For comparison of means, the data were submitted to one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Multiple factor analysis (MFA) relating the dough and bread properties were performed using the XLSTAT 2020.2 software (Addinsoft, USA, 2020).

3. Results and discussion

3.1 Effects of ingredients on the dough and bread properties

3.1.1 Dough properties

Figure 1 shows the curves corresponding to the four GF dough formulations evaluated by Mixolab. The doughs studied have low consistency in the initial stage ($C1 < 1.1$ Nm, target for wheat flour), and low values for C2 referring to protein weakening, which is expected for GF ingredients (Matos & Rosell, 2013). However, only the 4-CF:PSY trial showed a slight increase in consistency in the initial stage.

During heating, the dough consistency increases due to the starch gelatinization, increasing the torque in C3. The difference in the peaks observed in this step results from the amylose content in each sample, since RF has higher amylose content than CF (Horstmann et al., 2017). Thus, the amylose chains leach out into the aqueous intergranular phase promoting granules swelling (Matos & Rosell, 2013), consequently increases C3 values of samples based on RF. When PSY was added promoted the highest peak, especially in the 3-RF:PSY sample probably due their strong interaction with amylose chains of this system.

On other hand, the replacement of RF with CF caused a delay in the time to obtain C3, due to the difference in the temperature of starch gelatinization, and also decreased the torque in C3 and C4, referring to the stability of the starch gel (comparison between trials 1-2, and 3-

4, Figure 1) probably due to the starch dilution in the system due to the higher levels of proteins, lipids and fibers present in CF. According to Codina et al. (2019), gelatinization and stability of the starch gel formed are influenced by the starches characteristics and their interactions with the other compounds present in the system, leading to a decrease in the water available to starch granules and consequently the decrease in gelatinization and stability of the gel formed.

During cooling, the consistency represented by the C5 torque is associated with starch retrogradation, and the 2-CF trial showed the lowest peak in the final part of the curve (Figure 1). According to Matos & Rosell (2013), this occurs due to amylose chains crystallization, which depends on the nature of the starch and the presence of gelling ingredients or the water-binding ability. This parameter indicates the potential of CF to reduce hardening during the GFB storage period, as already reported in the instrumental parameters evaluated by Santos et al. (2020).

PSY addition caused an increase in the dough consistency during the heating and cooling stages, as can be seen in Figure 1, due to the competitive activity for water between starch and hydrocolloid, their interactions, and the hydration conditions. Besides, the arabinoxylan polysaccharides present in PSY affect control of the water availability of the starch, it could be associated with increase retrogradation (Belorio et al., 2020). Thus, dough parameters shown on the Mixolab curve are dependent on the characteristics of the starch sources used and on the relationship of the starch with the hydrocolloid present in the system, which can influence the quality parameters of the products, among them, appearance.

[Figure 1]

3.1.2 Breads properties

Regarding the appearance of the products (Figure 2), it is observed that bread 1-RF has a lighter crumb and crust color than the other formulations, due to the absence of pigments in RF and CS. In addition, it presents a more compact structure, and crust with cracks because of low gas holding capacity during the baking process.

The replacement of RF with CF results in a crumb with a more yellowish color, as reported by Santos et al. (2018), who suggested that could be due to the carotenoid pigments, mainly β -carotene present in CF, which give the characteristic color of this food. The crust darkening of these breads, on the other hand, can be ascribed to the increase in the Maillard reaction during baking due to the reaction between the reducing carbohydrates and the proteins present in the matrix containing CF, particularly lysine, which is deficient in RF (Rostamian et al., 2014).

PSY addition improved the crust appearance, which has a homogeneous aspect and no cracks with more rounded edges and top, close to the shape expected for a traditional sandwich bread made with wheat. According to Fratelli et al. (2018), PSY addition can result in an increase in the dough consistency, particularly as heating progresses during baking, which contributes to an increase in the gas holding capacity, preventing cracking in GFB crusts and promoting bread expansion. PSY addition made the 3-RF:PSY bread darker, by influence of the light brownish color of the powdered PSY husk, as previously explained by Fratelli et al. (2018). Nevertheless, bread darkening was attenuated when PSY was combined with CF in the 4-CF: PSY bread by influence of CF pigments.

However, the joint use of CF and PSY (bread 4) resulted in GFB with greater expansion, better shape with a rounded slice top, characteristics generally expected by consumers, as a consequence of the functionality of these ingredients in the dough, resulting in improved internal and external appearance of GFB. Thus, the combination of these ingredients is a promising alternative to solve some of the quality defects commonly observed

in GFB, such as compact structure, lack of cellular structure, crumb with hard and crumbly texture, and cracked crust (Capriles et al., 2020).

[Figure 2]

The characterization of the developed GFBs are shown in Table 1. Replacing RF with CF caused an increase in the loaf-specific volume (Table 1, Figure 1). Despite the differences in composition of the formulations, the values observed for the loaf-specific volume 2-CF and 4-CF:PSY are higher than those reported for other GFBs containing 75% or 100% CF, which varied between 0.6 and 2.2 cm³/g (Ouazib et al., 2016; Santos et al., 2018). According to the literature, the content and nature of the starch-protein matrix of chickpeas increases the gas retention capacity of the dough, favoring bread expansion (Santos et al., 2018), whereas the 4-CF: PSY bread stands out for having the largest volume, possibly due to the dough consistency that favored gas retention capacity, enabling greater bread expansion.

PSY addition improved the crumb cell structure, increasing the number of cells, an evident effect in the combination with CF (bread 4), with a reduction in average cell size compared to bread 2-CF, possibly due to the influence of dough consistency, since the characteristics of the system's starch-protein-hydrocolloid network during heating alter the properties of gas cells walls, and consequently the cells characteristics (Buresova et al., 2017). Under the conditions studied, the CF:PSY combination enabled the formation of a more homogeneous crumb. Meanwhile, the 1-RF bread has smaller cells area, which, according to Hager et al. (2012), indicates a denser structure that can compromise its expansion, as seen in Figure 2.

No significant difference in crust moisture was observed between the four breads. Crumb moisture was not altered by replacement of RF by CF (breads 1-2, and 3-4), nor by the PSY addition (breads 1-3, and 2-4 – Table 1). It is worth mentioning that the moisture content of the product is influenced by the water level incorporated and retained, which depends on the starch/flour and hydrocolloids base, as well as the type and level of dietary fiber in the formulation (Capriles et al., 2020). However, this was not observed in the studied conditions since the ingredients retained the water amount incorporated in the same way.

Lower crust hardness was observed for the 2-CF bread, but these values are in agreement with the results obtained by Rinaldi et al. (2017). According to these authors, crust hardness is inversely related to the moisture content of the crust, however, in this study, this relationship was not observed.

We observed that replacement of RF with CF (breads 1-2, and 3-4) or PSY addition (breads 1-3, and 2-4) does not change crumb firmness (Table 1). However, these values are lower than the bread optimized by Santos et al. (2018), prepared with 75% CF and 25% CS, which was 15.8 N; and close to those reported by Fratelli et al. (2018) for GFB optimized with 2.86 and 17.1% PSY, which showed a crumb firmness of 6.8 and 7.9 N, respectively.

Bread 1-RF showed high values of hardness and chewiness, meaning lower softness, and greater resistance to chewing of these breads in the mouth, and were not changed when PSY was added (breads 1-RF and 3-RF:PSY). However, replacement of RF with CF significantly reduced these parameters. The hardness values of breads 2-CF and 4-CF:PSY (1.7 and 2.7 N, respectively) are lower than those reported by Minarro et al. (2012), who added 8% CF (4.7 N), and Buresova et al. (2017), who used 70% CF in combination with 30% RF (30 N), indicating greater softness, which can impact on sensory perception and shelf life. The chewiness values are lower than the GFB prepared based on CF by Ouazib et

al. (2016) and Buresova et al. (2017). The low chewiness values observed in the 2-CF bread indicates easy disintegration of bread in the mouth and was attenuated when combined with PSY (bread 4).

PSY addition caused an increase in cohesiveness and springiness (breads 3-RF:PSY and 4-CF:PSY). Bread with greater cohesiveness is desirable because it forms a bolus instead of disintegrating during chewing, while low cohesiveness and springiness indicate an increased susceptibility of bread to crumble (Ouazib et al., 2016). The increase in cohesiveness and springiness was also observed in the GFB made with 5% of whole or ground PSY seeds in the study by Ziemichód et al. (2019).

Regarding acceptability (Table 1), replacement of RF with CF (comparison between breads 1-2 and 3-4) resulted in an increase in appearance, texture and overall acceptability scores (from 5.1-5.9 to 6.8-7.9), without changing the acceptability of aroma and flavor (values between 5.9 and 6.6). PSY addition did not alter the acceptability of GFB made with RF or CF (comparison between breads 1-3 and 2-4).

Acceptance scores of the evaluated attributes of the 2-CF and 4-CF:PSY breads are satisfactory (6.2-7.9), superior to the appearance, texture, flavor, aroma and overall scores observed in the study by Ouazib et al. (2016), who evaluated different types of CF pre-processing (raw, sprouted, toasted and cooked) in GFB, ranging from 3.4 to 6.2 on a 9-point scale and are similar to crumb appearance, aroma, flavor and overall scores of GFB by Buresova et al. (2017), prepared with 30%, 50%, 70% and 100% CF in combination with RF, ranging from 6.5 to 7.9, on the 9-point scale. Therefore, breads 2-CF and 4-CF:PSY could meet the expectations of consumers who need or choose these products as part of a GF diet, since these consumers are dissatisfied with the quality of the products available, especially about flavor and texture (Capriles et al. 2020).

As for proximate composition of breads (Table 1), replacing RF with CF almost doubled the levels of ash, lipids and proteins and increased the levels of resistant starch and total dietary fiber by 2.5 times, and consequently reduced available carbohydrate content by 36%. PSY addition caused an average increase of 1.7 g of total fiber, maintained the ash and protein levels, and reduced lipid (only in 3-RF:PSY) and resistant starch. The 4-CF:PSY bread brings together the beneficial effects of CF on the GFB composition, plus the increase in fiber content influenced by PSY, which made it possible to triple the fiber content compared to 1-RF bread.

GFBs are generally considered to be foods that are low in dietary fiber (Capriles et al. 2020). However, PSY addition in the formulation based on RF resulted in a product that can receive the claim of “fiber source” as it contains more than 3% of dietary fiber, according to the Codex Alimentarius (2013), while the replacement of RF with CF, and when associated with the addition of PSY, results in products with 7.2% and 8.7% of fibers, respectively, which can be considered “high in fiber,” since they exceed the minimum criterion of 6% of dietary fiber established for food (Codex Alimentarius, 2013). In general, 2-CF and 4-CF:PSY breads show a nutritional quality increase as compared to GFB commercially available in the different European and North and South American countries reported in the review study by Capriles et al. (2020), since they have a 2- to 4-fold reduction in the carbohydrate and lipid content, and an almost double increase in the protein content and can reach up to ten times the dietary fiber content.

[Table 1]

3.1.4 Relationships between the properties of the doughs and of the breads

The MFA presented in Figure 3 shows that the sum of the two factors explained 96.11% of the data variation. The coefficients of the studied variables $RV_{\text{Thermomechanical-physical}} = 0.80$, $RV_{\text{Physical-acceptability}} = 0.70$, $RV_{\text{Physical-composition}} = 0.77$, $RV_{\text{Acceptability-composition}} = 0.98$, indicate that the results are strongly related, and the ingredients RF, CF and PSY (supplementary variable) are related to the thermomechanical, physical, acceptability and composition properties ($RV = 0.72, 0.82, 0.87$ and 0.93 , respectively).

[Figure 3]

F1 explains 67.25% of the data, and are negatively discriminated by RF use that contributed to increase of values in C3 and C4, as well as available carbohydrate content, digestible and total starch, culminating in general in higher values of all instrumental texture parameters, except cohesiveness (Figure 3a) characterizing breads 1-RF and 3-RF:PSY (Figure 3b). Also in F1, CF is positively discriminated, which contributes to obtaining GFB with greater specific volume, large cells size, higher moisture, ash, lipid, protein, resistant starch, and total and insoluble fiber content, factors that contributed to better appearance, texture and overall acceptability (Figure 3a) characterizing 2-CF bread (Figure 3b).

F2 explains 28.86% of the data and shows that the addition of PSY contributed to the elevation of the torques in C1, C2 and C5, an increase in the number of cells, cohesiveness, and soluble fiber content (Figure 3a), characterized by 4-CF:PSY bread (Figure 3b).

Thus, the joint evaluation of these data shows that the quality of these products is dependent on the dough thermomechanical parameters, influenced by the nature and composition of the ingredients. However, the CF use, especially when combined with PSY, results in an increase in dough consistency, with a consequent improvement in the GFB quality.

3.2 Glycemic and satiety response

For this stage of the study, the breads based on CF, well accepted by consumers, were selected to evaluate the glycemic response and satiety, in comparison to white GFB (1-RF), which was used as a control food. Thus, all participants consumed portions of food that contained 25 g of available carbohydrates, corresponding to 71 g of 1-RF bread, 110 g of 2-CF bread, and 111 g of 4-CF:PSY bread, and these portions had 136, 201 and 202 kcal (or 1.92, 1.83 and 1.82 kcal/g of bread), respectively. The postprandial glycemic response and satiety curves are shown in Figure 4.

[Figure 4]

The fasting blood glucose concentration did not differ between tests (average value 4.80 mmol/L) and is consistent with those reported in the literature (Brand-Miller et al. 2009).

Figure 4a shows that replacement of RF with CF caused a reduction in the glycemic response curve, after 30 minutes of testing. PSY addition did not significantly alter the glycemic response curve.

We observed that the peak glycemic response of the control bread occurred at 45 minutes (7.3 mmol/L) and represents an incremental peak of the glycemic response (difference between peak and baseline concentration) of 2.4, consistent with that observed for standard bread made with wheat flour from the studies by Capriles and Arêas (2013) and Zafar et al. (2015), which also occurred after 45 minutes of testing.

For CF bread, the peak glucose concentration occurred at 30 minutes (6.2 mmol/L) and remained the same at 45 minutes, with an increase in the glycemic response of 1.5. Both the time and the incremental peak are in accordance with the wheat bread replaced by 25%

CF in the study by Zafar et al. (2015). For CF:PSY bread, the peak glycemic concentration occurred at 45 minutes and was 6.3 mmol/L, and an increase in the glycemic response of 1.4.

CF-based breads with or without PSY addition showed a reduction in the glycemic response, between 30 and 90 minutes of testing compared to the control bread, although there was no difference in blood glucose after 90 minutes.

Figure 4b shows that replacement of RF with CF increased and prolonged the satiety time when consuming GFBs, with a significant difference after 60 minutes of testing. The consumption of the control bread causes a slight satiety for up to 30 minutes (score of 4.7), and after that time, the volunteers were hungrier. The consumption of CF bread promotes a peak of satiety after 30 minutes, remaining up to 45 minutes (scores of 5.4), with a subsequent gradual decline. The consumption of CF:PSY bread made it possible to extend satiety, with a peak of 5.9 after 60 minutes postprandial, and a gradual reduction in values until the end of the test.

Table 2 shows the parameters obtained for glycemic response and satiety of GFBs. The mean values of iAUC for the glycemic response differed mainly between the control and CF:PSY breads.

Replacing RF with CF makes it possible to obtain GFB with low GI (≤ 55) and GL (≤ 6) with a reduction of 21% in GI and 49% in GL, while the combined effect of CF and PSY reduces GI by 25%, and GL by 52%, similar to the GFBs developed with sourdough Novotni et al. (2012) and GFB enriched with 17.1% PSY (Fratelli et al. 2018).

The results also showed that the average values of iAUC for satiety of the control bread is in agreement with the interval observed by Johnson et al. (2005) and Shishehbor et al. (2020) for white wheat bread. Replacing RF with CF increased the iAUC by 48% and the

SI by 58%, while the combined effect of CF and PSY increased the iAUC by 48% and the SI by 60%.

[Table 2]

To date, no studies have been found evaluating the effect of CF on glycemic response or satiety by GFB. However, it was observed in wheat flour breads that the replacement with 24% of raw or extruded CF did not reduce the glycemic response or promote satiety (Johnson et al., 2005). In another study with breads where wheat flour was replaced with 25% and 35% CF, a glycemic reduction of 29-36% was observed compared to white bread, but the individuals were hungrier after 90 minutes (Zafar et al., 2015).

However, the joint evaluation of these data suggests that the replacement of RF by CF results in GFB with low GI and GL and promotes satiety. The CF use regardless of the PSY addition almost doubled the lipid, protein and resistant starch content and increased the fiber content almost three times as compared to rice-bread. Replacement of RF with CF in GFB, therefore, contributed to the increase of digestive viscosity, delaying the digestion and absorption of carbohydrates, mechanisms of which impact to the reducing the GI and diluting the amount of available carbohydrates, thus contributing to GL reduction and increase of subjective postprandial satiety. Therefore, future experimental studies are required to investigate this products effects on physiology of appetite and satiety.

4. Conclusion

Replacing RF with CF improves GFB technological, sensorial and nutritional quality, besides reducing GI and GL and increasing SI. PSY addition (5.5% fwb) slightly changes bread physical properties and does not impair acceptability compared to RF bread. However,

when combined with CF, PSY positively influences GFB's dough consistency and all parameters assessed, including glycemic response and satiety ratings.

The findings suggest that CF, and its combination with PSY, can be an alternative for GFB with improved nutrient content and reduced glycemic response together with sensory appeal. Thus, an option of healthier and tastier GFB for consumers with gluten-related disorders, and those who choose to adhere to a GF diet. Thereby, the consolidation of GFB research allied to industrial application, as well as commercial availability is essential and integrates our ongoing studies.

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The authors declare no conflict of interest

CRedit authorship contribution statement

Fernanda G. Santos: Investigation, Formal analysis, Validation, Writing – Original Draft.

Etiene V. Aguiar: Investigation. **Cristina M. Rosell:** Writing - Review and Editing. **Vanessa**

D. Capriles: Supervision, Project administration, Funding acquisition, Writing - Review and Editing.

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Figure Captions

Figure 1. Effect of replacing rice flour (RF) with chickpea flour (CF) and the addition of psyllium (PSY) on gluten-free bread dough curves and parameters obtained by Mixolab®.

Figure 2. Effect of replacing rice flour (RF) with chickpea flour (CF) and the addition of psyllium (PSY) on the appearance of crumb (a) and crust (b) of gluten-free breads.

Figure 3. Multiple factor analysis correlating dough thermomechanical parameters and physical properties, acceptability and composition of gluten-free breads made with rice flour (RF) or chickpea flour (CF) and their interactions with psyllium (PSY).

(a) Map of dough parameters (in orange), and characteristics of gluten-free bread: physical properties (in pink), acceptability scores (in red), composition data (in green), and ingredients as supplementary variables (in blue).

(b) Map of gluten-free breads, made on a flour weight basis, with blend of 25% cassava starch and 75% 1-RF; 2-CF; and added 5.5% PSY: 3-RF: PSY; and 4-CF: PSY.

Figure 4. Effect of replacing rice flour (RF) with chickpea flour (CF) and the addition of psyllium (PSY) on the glycemic (a) and satiety (b) response curves of gluten-free breads.

Means and standard error of 12 healthy individuals. All tests were done with bread portions containing 25 g of available carbohydrates. Superscript letters in the same time range indicate significant difference ($P < 0.05$).

Table 1. Effect of replacing rice flour (RF) with chickpea flour (CF) and the addition of psyllium (PSY) on the physical properties, acceptability, and composition of gluten-free breads.

Characteristics evaluated	Gluten-free bread			
	1 - RF	2 - CF	3 - RF:PSY	4 - CF:PSY
<i>Physical properties</i>				
Loaf-specific volume (cm ³ /g) ^a	1.86 ^c ±0.03	2.43 ^b ±0.03	1.70 ^d ±0.03	2.53 ^a ±0.03
Number of cells ^a	50.67 ^{bc} ±1.53	45.00 ^c ±3.00	53.00 ^{ab} ±1.00	58.00 ^a ±4.00
Average cell size (mm ²) ^a	0.10 ^c ±0.01	0.24 ^a ±0.02	0.16 ^b ±0.01	0.19 ^b ±0.02
Cell area fraction (%) ^a	15.11 ^b ±1.26	31.94 ^a ±2.68	29.18 ^a ±1.98	30.17 ^a ±3.48
Crust moisture (%) ^a	24.24 ^a ±2.53	26.09 ^a ±1.57	26.93 ^a ±0.65	24.14 ^a ±0.39
Crumb moisture (%) ^a	58.02 ^{ab} ±0.20	58.55 ^a ±0.65	57.63 ^b ±0.15	58.43 ^{ab} ±0.04
Crust hardness (N) ^b	1.24 ^a ±0.37	0.94 ^b ±0.19	1.24 ^a ±0.25	1.18 ^a ±0.24
Crumb firmness (N) ^c	8.10 ^{ab} ±0.85	6.96 ^b ±0.59	9.01 ^a ±1.06	8.15 ^{ab} ±0.74
Hardness (N) ^d	4.66 ^a ±0.65	1.73 ^c ±0.36	4.26 ^a ±0.77	2.73 ^b ±0.39
Chewiness (N) ^d	2.54 ^a ±0.24	0.70 ^c ±0.12	2.80 ^a ±0.53	1.69 ^b ±0.25
Cohesiveness ^d	0.60 ^b ±0.04	0.51 ^c ±0.03	0.68 ^a ±0.02	0.67 ^a ±0.03
Springiness ^d	0.92 ^b ±0.02	0.81 ^c ±0.04	0.96 ^a ±0.01	0.93 ^b ±0.01
<i>Acceptability scores (9-point structured hedonic scale) ^e</i>				
Appearance	5.51 ^b ±2.32	7.92 ^a ±1.31	5.86 ^b ±2.09	7.54 ^a ±1.60
Aroma	5.86 ^a ±2.28	6.17 ^a ±2.07	6.25 ^a ±1.97	6.46 ^a ±2.10
Flavor	6.25 ^a ±2.09	6.60 ^a ±2.13	6.26 ^a ±1.99	6.34 ^a ±1.73
Texture	5.14 ^b ±2.08	7.45 ^a ±1.70	5.74 ^b ±2.19	7.40 ^a ±1.39
Overall	5.62 ^b ±1.99	6.80 ^a ±1.82	5.94 ^b ±1.87	6.82 ^a ±1.42
<i>Composition (g/100 g) of bread as eaten</i>				
Moisture ^a	52.86 ^b ±0.12	53.93 ^a ±0.20	52.84 ^b ±0.38	53.24 ^b ±0.22
Ash ^a	1.11 ^b ±0.03	1.82 ^a ±0.08	1.12 ^b ±0.03	1.81 ^a ±0.03
Lipid ^a	2.78 ^b ±0.24	4.89 ^a ±0.28	1.89 ^c ±0.07	4.59 ^a ±0.04
Protein ^a	4.18 ^b ±0.07	7.49 ^a ±0.48	3.99 ^b ±0.01	7.41 ^a ±0.43
Insoluble fiber ^f	2.20 ^d ±0.09	6.15 ^b ±0.27	3.84 ^c ±0.33	7.13 ^a ±0.36
Soluble fiber ^f	0.69 ^c ±0.01	1.06 ^b ±0.11	1.03 ^b ±0.08	1.54 ^a ±0.19
Total dietary fiber ^f	2.89 ^d ±0.10	7.21 ^b ±0.30	4.87 ^c ±0.26	8.67 ^a ±0.36
Resistant starch ^a	0.82 ^c ±0.04	2.00 ^a ±0.04	0.65 ^c ±0.05	1.69 ^b ±0.12
Digestible starch ^a	29.65 ^a ±0.94	17.96 ^b ±0.78	29.10 ^a ±1.36	19.44 ^b ±0.90
Total starch ^a	30.47 ^a ±0.95	19.96 ^b ±0.77	29.75 ^a ±1.32	21.13 ^b ±1.02
Available carbohydrates ^g	35.36	22.66	34.68	22.58
Energetic value (kcal)	191	183	183	182
Energetic value (kJ)	805	767	771	763

Values correspond to mean ^a *n* = 3, ^b *n* = 15, ^c *n* = 6, ^d *n* = 12, ^e *n* = 65, ^f *n* = 4 ± standard deviation. Values followed by a different letter on each line are significantly different (*p* < 0.05).

^g Available carbohydrates were calculated by difference [100 – (moisture + ash + protein + lipid + insoluble fiber + soluble fiber + resistant starch)]

Table 2. Effect of replacing rice flour (RF) with chickpea flour (CF) and its combination with psyllium (PSY) on the glycemic and satiety response of gluten-free bread.

Parameters	Gluten-free bread		
	RF	CF	CF:PSY
iAUC _(glycemic response mmol/L x min)	133.5 ^a ± 10.9	103.8 ^{ab} ± 8.6	96.3 ^b ± 7.9
Glycemic index _(bread)	100 ^a ± 0.0	79.2 ^b ± 4.9	74.6 ^b ± 5.7
Glycemic index _(glucose = 100)	70 ^a ± 0.0	55.4 ^b ± 3.5	52.2 ^b ± 4.0
Glycemic load _(50 g portion)	12.38 ^a ± 0.00	6.3 ^b ± 0.4	5.9 ^b ± 0.5
iAUC _(satiety unit x min)	160.7 ^b ± 29.1	310.0 ^a ± 43.9	314.17 ^a ± 30.4
Satiety index (%)	100 ^b ± 0.0	240.7 ^a ± 44.4	250.0 ^a ± 33.7

iAUC = incremental area under the curve

Values are mean (n = 12 healthy individuals) ± standard error. Values followed by a different superscript letter on each line are significantly different ($p < 0.05$).

Figure 1. Effect of replacing rice flour (RF) with chickpea flour (CF) and the addition of psyllium (PSY) on gluten-free bread dough curves and parameters obtained by Mixolab®.

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