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Physicochemical, rheological and sensory characterization of a gluten-free English bread added with *Oxalis tuberosa* flour

Caracterización fisicoquímica, reológica y sensorial de un pan ingles sin gluten adicionado con harina de *Oxalis tuberosa*

R.G. Campos-Montiel[1], M. Vicente-Flores[2], J. J. Chanona-Pérez[3], S. O. Espino-Manzano[2]*,
M. J. González de los Montero-Sierra[2]

[1]*Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Av. Rancho Universitario s/n Km. 1., Tulancingo Hidalgo C.P. 43600, México.*
[2]*Área Agroindustrial-Alimentaria, Universidad Tecnológica de Xicotepec de Juárez, Av. Universidad Tecnológica No. 1000, Tierra Negra, Xicotepec de Juárez, Puebla C.P. 73080, México.*
[3]*Departamento de Ingeniería Bioquímica. Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional. Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu Esq. Cda. Miguel Stampa s/n. C.P. 07738. Delegación Gustavo A. Madero, CDMX, México.*

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Abstract

Physicochemical, rheological, structural and sensory parameters of the gluten-free English bread added with pregelatinized red potato flour (RPF) (*Oxalis tuberosa*) were studied. Five treatments were performed with 0, 6.6%, 13.2%, 19.8% and 26.4% of RPF. In a first stage the texture, physical parameters, and crumb structure were evaluated. According to the results, the best treatments were selected (6.6%, 13.2% of RPF) where they improved the texture of the product without compromising the physical characteristics. In the second stage, the selected treatments were subjected to microstructure analysis (environmental scanning electron microscopy and confocal laser scanning microscopy), nutritional analysis, measurement of the glycemic index (*in vivo* assay), determination of fatty acids and sensory evaluation of the breads. The results show that the addition of RPF in gluten-free English bread increases the extensibility of the dough, decreases the hardness, increases the volume, gives a more uniform crumb without fractures. In the nutritional aspect, the content of protein and ash increased, the content of saturated fatty acids decreased and the glycemic index of the product decreased with significant differences compared to the control ($p<0.05$). In addition, the treatment with 13.2% of RPF obtained a higher preference in the sensory evaluation. The incorporation of RPF is a good option to elaborate a gluten-free English bread with a higher nutritional and structural quality and with a better acceptance in the sensory evaluation.

Keywords: Gluten-free, red potato, microstructure, nutrition, sensory evaluation.

Resumen

Se estudiaron los parámetros fisicoquímicos, reológicos, estructurales y sensoriales del pan inglés sin gluten adicionado con harina de papa roja pregelatinizada (FRP) (*Oxalis tuberosa*). Se realizaron cinco tratamientos con 0, 6,6%, 13,2%, 19,8% y 26,4% de RPF. En una primera etapa se evaluó la textura, los parámetros físicos y la estructura de la miga. Según los resultados, se seleccionaron los mejores tratamientos (6,6%, 13,2% de RPF) donde mejoraron la textura del producto sin comprometer las características físicas. En la segunda etapa, los tratamientos seleccionados fueron sometidos a análisis de microestructura (microscopía electrónica de barrido ambiental y microscopía de barrido láser confocal), análisis nutricional, medición del índice glucémico (ensayo *in vivo*), determinación de ácidos grasos y evaluación sensorial de los panes. Los resultados muestran que la adición de RPF en pan inglés sin gluten aumenta la extensibilidad de la masa, disminuye la dureza, aumenta el volumen, da una miga más uniforme sin fracturas. En el aspecto nutricional, el contenido de proteína y cenizas aumentó, el contenido de ácidos grasos saturados disminuyó y el índice glucémico del producto disminuyó con diferencias significativas con respecto al control ($p <0.05$). Además, el tratamiento con 13,2% de RPF obtuvo una mayor preferencia en la evaluación sensorial. La incorporación de RPF es una buena opción para elaborar un pan inglés sin gluten con una mayor calidad nutricional y estructural y con una mejor aceptación en la evaluación sensorial.

Palabras clave: Libre en gluten, papa roja, microestructura, nutrición, evaluación sensorial.

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* *Corresponding author. E-mail*: `salvador.espino@utxicotepec.edu.mx`

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1 Introduction

Celiac disease is the permanent intolerance to the consumption of gluten contained in cereals such as: wheat, barley, rye and oats, which currently affects 2% of the world population (Wagner *et al*., 2016). This limitation in the diet of celiac people has opened an important area of opportunity in food science and technology. The development of gluten-free products represents a challenge for the food industry, since the absence of gluten in the production of sourdoughs has the consequence that the quality of gluten-free breads (GFB) is deficient. Nowadays, various sources have been studied for the elaboration of GFB, among which the starches of cereals and tubers or flours of legumes and seeds stand out.

In addition to the gluten-free flours, some food additives such as hydrocolloids, enzymes or protein isolates have been added, in order to mimic the physicochemical properties that gluten gives to the dough (Clapassón *et al*., 2020; Demirkesen *et al*., 2014; Mohammadi *et al*., 2015; Sulieman *et al*., 2018).

Red potato (*Oxalis tuberosa*) is an Andean tuber, with a nutritional content of 4.60% protein, 1.66% fat, 2.16% fiber and 88.19% carbohydrates, most of which is starch, so it can be used as an ingredient in the elaboration of GFB (Barrera *et al*., 2004). An added value to this crop is that being a pigmented tuber, it contains antioxidant compounds, where anthocyanins stand out, potentially considering it as a functional ingredient (Chirinos *et al*., 2009).

The evaluation of the quality of bakery products includes rheological tests in dough and bread, physical analysis, image analysis and sensory evaluation (Alba *et al*., 2020; Espino-Manzano *et al*., 2018). These tests can be complemented with the microstructural analysis of the crumb that will allow to observe the structure and distribution of the components of the bread (Alba *et al*., 2020; Díaz-Ramírez *et al*., 2013; Jekle & Becker, 2011; Sulieman *et al*., 2018); without diminishing the importance of essential components that derive in people's nutrition such as proximal chemical analysis, the glycemic index and the determination of fatty acids (Giuberti *et al*., 2017; Sulieman *et al*., 2019; Vici *et al*., 2016).

The objective of this study was the elaboration of gluten-free English bread added with pregelatinized RPF and to carry out physicochemical and nutritional analysis, microstructural evaluation by environmental scanning electron microscopy (ESEM) and confocal laser scanning microscopy (CLSM) and sensory evaluation of the product to observe the influence of RPF on gluten-free dough and English bread.

2 Materials and methods

2.1 *Preparation of pregelatinized red potato flour (Oxalis tuberosa)*

Red potato tubers (*Oxalis tuberosa*, variety Pauccar oca) were acquired from the local market of Acaxochitlan Hgo., Mexico. This crop is characterized by its red color and its sweet taste, in its physiological ripeness state. Red potato flour was obtained following the methodology previously reported by (Espino-Manzano *et al*., 2018). After the drying process, the moisture content of the RPF was 7.51±0.01 g/100g. In a conventional oven (Kitchen Aid, KOSE500EBS, USA) at 40 °C for 24 h. Subsequently, the flour was conditioned to a humidity of 30% to be pregelatinized in a single screw extruder (Shergun, TSE65-S, China), the heating conditions were 55 °C at the inlet, residence at 65 °C and the output at 75 °C, while the screw speed was 55 rpm with a 3 mm circular geometry matrix. The pellets obtained were crushed in a knife mill (IKA, MF 10.1, USA) and sieved on a No. 35 mesh. The flour was kept in polypropylene bags for future analysis.

2.2 *Preparation of gluten-free English bread*

Gluten-free flour was formulated based on: rice flour (32%), corn starch (27%), potato starch (17%), tapioca starch (20.5%) and hydroxypropylmethylcellulose (HPMC) as hydrocolloid (3.5 %) (Wellence Gluten Free, DOW Chemical Pharma & Food Solutions, Germany). For the preparation of English bread, yeast (*Saccharomyces cerevisiae*), ovalbumin (food grade), sugar, powdered milk, vegetable fat, salt and water were also added. The treatments consisted of a gluten-free control (GFC) and four treatments (RP1, RP2, RP3, RP4) where corn starch was substituted for RPF in 6.6%, 13.2%, 19.8% and 26.4% respectively based on the reported Sulieman *et al*. (2018) with modifications.

For the English bread, the dough was made by mixing all the ingredients in a mixer (Kitchen Aid, Professional 600, USA) at medium speed for 5 min. It was fermented for 60 min at 30 °C. Later the bread was formed, putting the dough in aluminum rectangular molds and they were fermented for another 30 min. Finally, they were baked at 180°C for 20 min, modified from Onyango *et al*. (2011).

2.3 Physicochemical tests

2.3.1 Rheological measurements of dough and bread

The texture profile analysis (TPA) in the dough was determined in a texturometer (TA.XT plus, Stable Micro System, UK), following the AACC methodology. Firmness (N), adhesiveness (mJ), cohesiveness and springiness (dimensionless) were determined. The extensibility (mm) of the dough was prepared according to the AACC54-10 method, coupling the *Kieffer Dough and Gluten Extensibility Rig* to the texturometer. The *D/R Dough Inflation System* coupled to a texturometer was used, which measures dough extensional rheology under conditions of strain similar to those of baking expansion (AACC 54-30). The parameters of tenacity (P), extensibility (L) and baking strength (W) were evaluated for the composite flours. The TPA in the bread was determined following the same methodology described above (AACC, 2000).

2.3.2 Physical tests and crumb structure

Crumb and crust color was measured by CIELAB system with L* a* b* values (Rodriguez-Lora *et al*., 2020). The volume was determined by seed displacement. For the density, the weight of the product was recorded and the density was calculated in g/cm^3 (AACC10-05) (AACC, 2000). The evaluation of the crumb structure was carried out by image analysis, where 1 cm^2 samples were analyzed, it was calculated: area of the cells (mm^2), dispersed phase (%), average area of the cells (mm^2) and number of cells per cm^2 (Totosaus *et al*., 2013).

2.3.3 Proximal chemical analysis of bread

Protein (46-12.01), fat (30-10.01), moisture (44-19.01), total ash (08-01.01), fiber (32-05.01) of the samples were determined according to the official methods of the AACC (2000). The percent carbohydrates content was calculated by difference using the following equations (Eq.1).

$$\%Carbohydrates = [100 - (\text{moisture + total ash + protein + fiber + fat})] \quad (1)$$

2.3.4 Glycemic index (GI) of bread

Glycemic index (GI) was determined according to the Astawan & Widowati (2011) protocol. The Research and Ethics Committee of the Institute of Health Sciences at the Universidad Autónoma del Estado de Hidalgo, Mexico, approved the study protocol (official letter Cinv/045/2017) and all volunteers gave their informed consent in writing. Twenty volunteers (9 women and 11 men) with an age range of 25.8±2.56 and a body mass index (BMI) of 25.6±2.2 kg/m were included in the study.

Blood samples (capillaries) were taken with a sterile lancet (OneTouch, Johnson & Johnson, Miami FL, USA) at 0 (basal glucose) 15, 30, 45, 60, 90 and 120 min after eating the food. Blood glucose concentration was measured with a glucometer (OneTouch Ultra-Mini meter, Johnson & Johnson, Miami FL, USA). To calculate the GI of each treatment, the methodology proposed by Wolever (2004) was used, which consists of the difference between the area under the curve of the reference food (glucose solution) and the samples (Eq. 2)

$$GI = \frac{\text{Area under the sample curve}}{\text{Area under the curve of the reference food (glucose solution)}} \times 100 \quad (2)$$

2.3.5 Determination of fatty acids in bread

The fatty acid profile of the samples was determined with the methodology of Añorve-Morga *et al*. (2015). For the extraction of the fatty acids, 2 g of dry sample were placed in 5 mL of a chloroform-methanol mixture (2:1 v/v); and they were kept in extraction for 72 h. The extraction and transesterification of the fatty acids was carried out by taking 500 μL of the lipid extract obtained in each sample, 1 mL of BF3 in methanol was added. Each extract was purified by washing with hexane and two more times with water saturated with hexane.

The purified lipid extract was concentrated with N_2 to dryness and trichosanoic acid (C23:0) (Sigma T-6543, Merck, Germany) was added as an internal standard in each of the samples. For quantification, the extract was resuspended in 0.5 mL of dichloromethane and injected into a gas chromatograph equipped with a flame ionization detector (Perkin Elmer®, Autosystem XL, USA) and a capillary column of silica (Supelco SPTM-2560) of 75 mx 0.18 mm, 0.14 μm. 1 μL of extract was injected in spitless mode. Chromatographic grade N_2 was used as carrier gas at a flow rate of 1 mL min^{-1}. Injector and detector temperatures were maintained at 230 °C and 250 °C respectively.

The temperature gradient used was: 150 °C initial temperature, increasing 4 °C min^{-1} to 214 °C, rest time of 2 min. Subsequently, it increased 2.5 °C min^{-1} to 244 °C, finally maintaining it for 5 min. The identification of the methylated esters was compared with the retention times with a mixture of standards (FAME Mix C4-C24, Supelco®). The samples were analyzed in triplicate.

2.4 *Evaluation of the dough and crumb microstructure*

For microstructure analysis, previously lyophilized doughs were scanned with a focused electron beam (5.00kV) in an environmental scanning electron microscope (ESEM) (Quanta 3D FEG, FEI inc. Hillsboro, USA) at 120x and 500x (Díaz-Ramírez *et al.*, 2013; Morales-Hernández, *et al.*, 2019). The microstructure of dough and crumb was also observed by confocal scanning laser microscopy (CLSM). The preparation of the samples consisted in the use of two fluorochromes: fluorescein 5-isothiocinate at 0.5% that has protein binding and rhodamine B at 0.15% (Sigma Chemicals Co, St. Louis MO, USA) that has fixation with carbohydrates. The methodology proposed by Morales-Hernández *et al.* (2019), using a confocal scanning laser microscope (LSM 710, Carl Zeiss, Germany) at 20x with excitation wavelengths of 488 and 518 nm for fluorescein and rhodamine respectively. The maximum emission was 568 to 625 nm for both stains.

2.5 *Sensory evaluation*

The sensory evaluation of the bread consisted of a preference test to 80 untrained judges. A 5-point hedonic scale was used (1 point to "I dislike it a lot" and 5 to "I like it very much." The parameters of firmness, flavor, aroma, crumb and crust color, general acceptance were evaluated (Espino-Manzano *et al.*, 2018; Hager *et al.*, 2012).

3 Results and discussions

3.1 *Rheological tests of dough and bread*

Table 1 shows the results of the rheological tests for the dough. In dough, firmness was one of the most affected parameters with significant differences in all treatments ($p<0.05$). The incorporation of RPF in the treatments shows a gradual increase in firmness with respect to the concentration of RPF, being RP1 and RP2 whom had the lowest firmness; contrary to the RP3 and RP4 treatments, which were the ones that had more firmness. In cohesiveness, the use of RPF presented similar values with the control, without significant differences ($p>0.05$) except for RP1. It is observed that with an addition of between RP1 to RP2 of RPF the doughs are softer and retain their cohesiveness, characteristics that can predict the quality of the bread once baked.

In the adhesiveness of doughs, values of 0.94 and 0.96 mJ were observed in the RP1 and RP2 treatments respectively without significant differences ($p>0.05$) with the control. However, for RP3 and RP4 the value of this parameter decreases, which can be caused by the lack of water absorption in the dough. In springiness, no significant differences ($p>0.05$) were found between the treatments. It was observed that the gluten-free doughs (GFD) showed a behavior of cookie dough, being harder and not maintaining their internal binding forces. It has been studied that the lack of sulfur amino acids (methionine and cysteine) present in the gluten network does not allow the formation of a mass with viscoelastic properties (Mariotti *et al.*, 2013).

In general, it was observed that RPF in different amounts infers on the texture parameters. It has been studied that the incorporation of protein concentrates obtained from *Lupinus albus* and *Jatropha curcas*, modify the texture parameters in relation to wheat dough, increasing the firmness. It is possible that the nature of these proteins does not allow the full development of the gluten network, remaining dissolved in the dough (Totosaus *et al.*, 2013). The use of different sources and additives to improve the characteristics of GFDs have been studied in various works. Brites *et al.* (2010), elaborated GFB based on corn flour, finding average values of 24.5 N in the dough firmness, without the incorporation of additives, showing that without their use hard doughs are obtained. On the other hand, most of the studies carried out for GFB are based on a batter and not a dough, which makes it difficult to evaluate the texture parameters as it behaves more like a liquid and not a viscoelastic fluid (Brites *et al.*, 2010; Demirkesen *et al.*, 2014; Mariotti *et al.*, 2013; Mohammadi *et al.*, 2015; Onyango *et al.*, 2011; Sulieman *et al.*, 2018).

Table 1. Rheological tests of English gluten-free dough and bread added with pregelatinized red potato flour (*Oxalis tuberosa*).

Test	GFC	RP1	RP2	RP3	RP4
Dough TPA					
Firmness (N)	9.32±0.10c	4.66±0.13e	5.06±0.06d	13.52±0.17a	10.49±0.17b
Cohesiveness	0.34±0.06ab	0.34±0.07ab	0.27±0.01c	0.31±0.01bc	0.35±0.03a
Adhesiveness (mJ)	0.96±0.07a	0.96±0.06a	0.94±0.03a	0.78±0.03c	0.87±0.03b
Springiness	0.98±0.01a	0.96±0.01a	0.97±0.02a	0.97±0.01a	0.98±0.00a
Extensibility (mm)	13.34±0.37b	16.12±0.64a	15.39±0.37a	11.23±0.42bc	12.48±0.46b
Tenacity*					
Baking strength W ($J*10^4$)	523.5±8.72c	887.3±4.44b	850.1±19.97b	1287.93±28.94a	1277.94±4.39a
Bread TPA					
Firmness (N)	21.10±0.07c	15.64±0.05e	18.85±0.09d	28.53±0.16b	33.21±0.03a
Cohesiveness	0.83±0.01d	0.86±0.01bc	0.85±0.01c	0.87±0.01b	0.89±0.01a
Adhesiveness (mJ)	0.01±0.00a	0.02±0.00a	0.01±0.00a	0.03±0.00a	0.01±0.00a
Springiness	0.90±0.01e	0.95±0.00a	0.87±0.00b	0.81±0.01c	0.95±0.00d

TPA: texture profile analysis. RPF: Pregelatinized red potato flour (*Oxalis tuberosa*). GFC: gluten-free control. RP1: adding RPF 6.6 %. RP2: adding RPF 13.2 %. RP3: adding RPF 19.8 %. RP4: adding RPF 26.4 %. *Tenacity test was only done on a mixture of flours. Different letters in the same row indicate significant differences ($p < 0.05$) according to the Tukey test.

The extensibility test measures the tension of the dough until it breaks, it allows to evaluate the quality of the gluten present in the dough. The extensibility results show that in GFD this behavior is diminished due to the weak forces of attraction in the composition of the dough, which shows that the presence of gluten is essential to obtain an elastic dough (Alba *et al*., 2020; Poutanen *et al*., 2009). Also, the addition of extra ingredients, such as fats and other carbohydrates, break into the network and make it less extensible. It can also be observed that the use of RPF in the different treatments increases the dough extensibility, where RP1 and RP2 obtained the highest values with respect to the control (16.12 ± 0.64 mm and 15.39 ± 0.37 mm respectively). Studies by Al-Saleh & Brennan (2012) show that the extensibility dough made with weak wheat flours is in a range of 12 to 20 mm, which correlates it with the GFD of the control and the treatments. In baking, studies show that it is required to use a flour whose extensibility is 23 to 35 mm (Jekle & Becker, 2011) that will subsequently allow good expansion during baking. In a study by Schober *et al*. (2008) in GFDs made with zein, corn starch and hydroxypropylmethylcellulose (HPMC), extensibility values of 37 mm were obtained, justifying that the use of food additives intervenes in the formation of elastic doughs.

For the dough tenacity by Dough Inflation system, the mechanical properties of the dough subjected to a biaxial extension where the expansion force is in two perpendicular directions in the same plane were evaluated. Baking strength (W) is a physical property correlated with the protein content of wheat, which indicates that the parameters of said test define the quality of the flour, where values of $W<200$ are considered weak flours destined for the biscuit industry, values of $300>W>200$ are considered bakery quality flours and values of $W<300$ are flours intended for the production of pasta (Dobraszczyk & Salmanowicz, 2008). The P and L values also show the balance that the flour has for the production of an elastic dough and the CO_2 that it can retain during fermentation. The values for the flours used in this study show that the incorporation of the RPF increases the tenacity of the dough with significant differences ($p<0.05$) compared to the control. There is a proportional increase in baking strength (W) with respect to the higher RPF content, where RP2 and RP4 have distant values showing that the GFDs will form doughs with higher CO_2 retention that can interfere with the final expansion of the bread.

Regarding bread, the TPA shows (Table 1) that there is an increase in firmness in the treatments. This increase is caused while baking, where the loss of water increases the hardness of the crust. In turn, this is related to the tenacity dough test, where the firmness of the treatments with a higher content of RPF have higher values (RP3 = 28.53 ± 0.16 N and RP4 = 33.21 ± 0.03 N) with significant differences ($p<0.05$). In the cohesiveness parameter, the treatments showed an increase compared to the control. This constant shows that the use of hydrocolloids causes a gummy effect on the GFB. Baking the product transforms the GFD into a thermoset gel that acquires high hardness and cohesiveness, which makes it a gummy food, without forming a suitable crumb. In general, for bakery products, hardness is considered one of the most important quality parameters, since it is inversely correlated with the shelf life of the product. According to studies carried out by Mohammadi *et al*. (2015) and Ziobro *et al*. (2013), the loss of moisture in the GFB, follows a linear behavior when the hardness increases by more than 200% from its initial measurement until 72h, so its useful life is relatively short. The research carried out on GFB has as one of its objectives to obtain a low initial hardness to prolong the shelf life of the product. With this objective, there are studies where different types of GFD mixtures are carried out, Onyango *et al*. (2011) found that a mixture of tapioca starch and sorghum flour produces a softer product compared to other mixtures. On the other hand, the use of additives, such as emulsifiers of protein bases, enzymes and hydrocolloids have proven to have good results in the elaboration of GFB.

3.2 Physical tests and bread structure

Table 2 shows the results of the evaluation of the physical tests (color in crumb and crust, volume and density) and evaluation of the crumb structure. Color in food is considered one of the most important parameters, since consumer acceptance is derived mainly from it (Cappa *et al*., 2013; Sulieman *et al*., 2018). The results show that in the crumb there is a darkening with the incorporation of RPF in the treatments, where the luminosity decreases (-L) and the yellowness increases (+b*). In crust there is a similar behavior. There is a higher browning due to the Maillard reaction produced during the last stage of baking (Ziobro *et al*., 2013).

Table 2. Physical and structural tests of gluten-free English bread added with pregelatinized red potato flour (*Oxalis tuberosa*).

Test	GFC	RP1	RP2	RP3	RP4
Color					
Crumb					
L*	79.60±0.13a	75.86±0.11b	75.28±0.03b	73.19±0.06c	72.32±0.12c
a*	-0.35±0.02f	0.65±0.01e	2.61±0.00a	1.95±0.01c	1.46±0.02d
b*	23.28±0.09c	30.77±0.03b	29.32±0.03b	32.40±0.01a	33.43±0.03a
Crust					
L*	81.33±0.08a	74.13±0.10b	72.95±0.04c	72.80±0.04c	69.43±0.30d
a*	1.77±0.00e	2.09±0.02d	3.64±0.30a	2.90±0.05b	2.27±0.01c
b*	34.74±0.09c	36.77±0.11b	31.74±0.01d	36.63±0.01b	37.87±0.08a
Volume (cm^3)	93.33±1.54a	80.00±0.00b	63.33±3.77c	53.33±5.77d	50.00±0.00d
Density (g/cm3)	0.49±0.06e	0.58±0.00d	0.66±0.06c	0.89±0.08b	0.92±0.00a
Crumb structure (Image analysis)					
Cell area (mm^2)	21.62±4.67a	23.81±3.44a	19.90±2.81a	20.95±4.00a	18.03±1.49a
Dispersed phase (%)	21.47±4.68a	23.68±3.41a	19.74±2.80a	20.82±3.97a	17.92±1.47a
Average area of the cells (mm^2)	0.89±0.18a	1.00±0.19a	1.04±0.10a	1.13±0.29a	0.76±1.47a
Number of cells per cm^2	24.25±1.89a	23.75±1.70a	19.00±0.81b	18.75±2.21b	23.75±1.89a

RPF: Pregelatinized red potato flour (*Oxalis tuberosa*). GFC: gluten-free control. RP1: adding RPF 6.6 %. RP2: adding RPF 13.2 %. RP3: adding RPF 19.8 %. RP4: adding RPF 26.4 %. Different letters in the same row indicate significant differences ($p <0.05$) according to the Tukey test.

Various investigations show that with the incorporation of protein isolates and flours from sources with high fiber content, as well as the addition of hydrocolloids greater than 2%, they influence the color of the crumb and crust, making it darker (Mohammadi *et al*., 2015; Sulieman *et al*., 2018; Ziobro *et al*., 2013). In sweet bread, Güémes-Vera *et al*. (2008) demonstrated, with a panel of trained judges, that the most widely accepted crumb color is a light yellow to creamy yellow hue, determined in the parameter b*.

In the volume, in the control it was higher with respect to the other treatments, due to the higher CO_2 retention, produced in fermentation and expansion during baking. A decrease in volume inversely proportional to density was observed with a higher addition of RPF in the treatments, presenting significant differences ($p<0.05$). This shows that, in GFBs, the main problem is the lack of a stable and elastic structure that retains CO_2, so the volume is limited and the structure of the crumb is unstable. Volume is an evaluation parameter for bakery products, as consumers have a preference for light and high-volume breads. Another physical aspect is the density, the relationship that exists between the weight of the dough (g) with respect to the volume (cm^3). This parameter defines whether the bread has a porous structure, which will be directly related to the texture. The density of the control (0.49 ± 0.06 g/cm^3) was lower compared to the other treatments where the RP4 was the one with the highest density (0.92 ± 0.00 g/cm^3) that shows a very compact crumb. Regarding these properties, Demirkesen *et al*. 2010) mention that a bread with a density less than 0.50 g/cm^3 will retain the appropriate quality for a commercial bread.

For the evaluation of the crumb structure by image analysis, it is shown that there are no significant differences ($p>0.05$) in the parameters evaluated, except for the number of cells per cm^2. Therefore, it can be concluded that the treatments are within the standard, however, the control presented fractures in the crumb structure. Various studies show the variation that exists between the distribution of cells in GFBs, being 4 mm^2 cells, in breads made with batter (Alba *et al*., 2020; Mariotti *et al*., 2013; Pongjaruvat *et al*., 2014; Sánchez-Pardo *et al*., 2012; Totosaus *et al*., 2013). Demirkesen *et al*. (2014) evaluated the distribution of the cells and the structure of gluten-free breads using various hydrocolloids, finding that the lowest porosity and the highest number of cells were obtained with the addition of hydrocolloids such as CMC and HPMC, relating them to a fine crumb structure (cells smaller than 0.7 mm^2), in addition, firmness and cohesiveness were correlated with the internal structure in terms of porosity, number and size of cells.

3.3 Nutritional content of bread

Treatments RP1 and RP2 were selected according to the results of the previous tests, where they showed a higher quality of GFC compared to the rest of the treatments. Regarding the nutritional content of the breads (Table 3), it was found that the treatments added with RPF had an increase in protein content of 20 and 33% in RP1 and RP2 with respect to the control, finding significant differences ($p<0.05$). Other parameters where an increase in the nutritional content of the breads was found was in the increase of ash (related to micronutrients) and dietary fiber, where the control had the lowest content. Finally, there was a decrease in the content of total fat and carbohydrates with significant differences in RP2 ($p<0.05$) compared to the control. Based on the nutritional evaluation of the treatments, it is suggested that a bakery product has a lower content of fats and simple carbohydrates, in turn of a higher content of protein and dietary fiber, as shown in the addition of RPF to GFB.

Table 3. Nutritional facts of gluten-free English bread added with pregelatinized red potato flour (*Oxalis tuberosa*).

Treatment	Humidity	Protein	Fat	Ash	Fiber	Carbohydrates
GFC	25.49±0.13a	5.35±0.20c	8.86±0.13a	1.81±0.08c	0.89±0.18b	57.60±0.19a
RP1	26.7±0.85b	6.38±0.14b	5.60±0.23c	2.06±0.13b	1.20±0.19a	58.06±0.67a
RP2	28.9±0.39c	7.32±0.26a	6.20±0.25b	2.30±0.06a	1.34±0.08a	53.94±0.67b

RPF: Pregelatinized red potato flour (*Oxalis tuberosa*). GFC: gluten-free control. RP1: adding RPF 6.6 %. RP2: adding RPF 13.2 %. Different letters in the same column indicate significant differences ($p <0.05$) according to the Tukey test. * The proximal chemical analysis was determined as a percentage of 100 g of product.

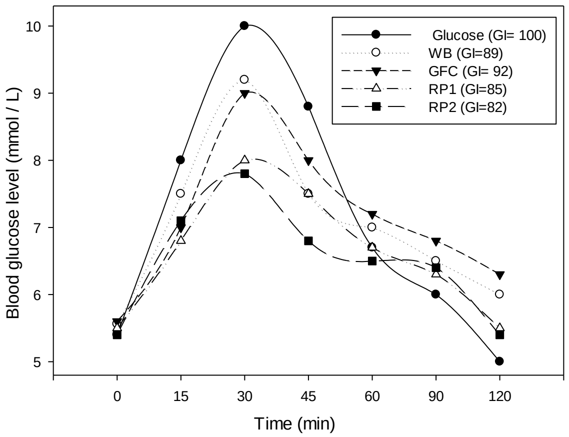

Fig. 1 Glycemic response and calculated glycemic index (GI) of gluten-free English bread with different amounts of pregelatinized red potato flour (*Oxalis tuberosa*). WB (White bread) as a control, GFC, RP1 and RP2 with 0, 6.6 and 13.2% RPF respectively. The times of each measurement were: 0 (basal glucose, 8 h fasting), 15, 30, 45, 60, 90 and 120 min. The cutoff time was determined based on postprandial glucose.

Previous studies showed the nutritional value of GF bakery products added with RPF modify the composition of the food (Espino-Manzano *et al.*, 2018) where it was shown that the content of resistant starch and Slowly digestible starch increased, which can modify the glycemic index of the food. RPF was previously analyzed, finding values of 6.03% protein, 0.19% fat, 1.66% fiber, 3.21% ash and 85.15% carbohydrates on a dry basis (g/100g), which are similar to that reported by Barrera *et al.* (2004).

3.4 Glycemic response and glycemic index (GI) of bread

The glycemic response and glycemic index (GI) of bread are shown in Fig. 1, where the glucose curve is taken as the reference food. In breads, the control (WB) showed a maximum peak of 9.30 ± 0.40 mmol/L at 30 min of consumption, which relates it to a higher content of rapidly digested carbohydrates. In RP2 treatment (7.49 ± 0.20 mmol/L) the glycemic response showed a lower value and a constant decrease from 30 min to 120 min, which relates it to an adequate balance of carbohydrates (fast and slow digestion) (Espino-Manzano *et al.*, 2018). Treatments RP2 and RP1 show a similar behavior, however, at 45 min RP1 showed a slight increase in blood glucose, followed by a gradual release until 120 min. According to Eq. 2, the GI of the treatments was calculated where it is shown (Fig. 1) that in relation to the control food (glucose GI = 100), GFC and the treatments had a GI of 92, 85 and 82 respectively. These results classify all high GI treatments that have a GI ≥ 70. Treatments RP1 and RP2 that were added with RPF showed a lower glycemic response than the control counterpart. According to several studies, the GI of a food can be directly conditioned to the composition of the formulation and the process. To date, several investigations have been carried out in which the glycemic response of gluten-free products is studied, showing similar data to this work (Fratelli *et al.*, 2018; Packer *et al.*, 2000; Scazzina *et al.*, 2016). Lau *et al.* (2015) showed that the type of cooking and the type of formulation directly affect the glycemic response, where the breads made with steam cooking had a lower GI than the baked breads, this due to the fact that the content of slow-digesting starch is higher in steamed breads and directly affects the postprandial glycemia of the food.

3.5 Fatty acid profile

According to the fatty acid profile (Table 4), those with the highest presence are saturated fatty acids. Saturated fatty acids such as caproic (C6: 0), caprylic (C8: 0) and margaric (C17: 0) were found in small amounts in all treatments. These types of fatty acids found in fats of animal origin, taking as a reference the milk used in making bread. The saturated fatty acids with the highest content were palmitic acid (C16: 0) and arachidic acid (C20: 0), in higher concentrations at 100mg/100g of sample. Finding in GFC, 308.77 ± 8.24 and 356.31 ± 3.41 mg/100g the highest content of these fatty acids, in RP1 with 122.44 ± 2.49 and 176.89 ± 3.43 mg/100g the lowest content of these fatty acids respectively. The unsaturated fatty acids identified in the samples were myristoleic (C14: 1), oleic (C18: 1n-9), linoleic (C18: 2n-6) and linolenic (C18: 3n-3). Linoleic acid (C18: 2n-6) was found in the highest quantity in the samples, where the GFC treatment had the highest content (275.30 ± 2.65 mg/100 g) and finally RP2 (111.28 ± 1.22 mg/100 g). According to the total content of saturated fatty acids, there are significant differences ($p<0.05$) between the treatments, where the GFC formulation, which is made without RPF, had the highest content of saturated fatty acids (711.28 ± 3.32 mg/100 g), secondly and the treatments RP1 (331.13 ± 2.62 mg/100 g) and RP2 (455.59 ± 5.01 mg/100 g), showed a decrease in the content of saturated fatty acids.

Table 4. Fatty acid profile of gluten-free English bread added with pregelatinized red potato flour (*Oxalis tuberosa*).

Fatty acid	GFC	RP1	RP2
Saturated fatty acids (mm/100 g)			
Caproic (C6:0)	9.87±0.11a	6.46±0.16c	7.18±0.04b
Caprylic (C8:0)	11.28±0.29a	6.84±0.12c	8.23±0.09b
Palmitic (C16:0)	308.77±8.24a	122.44±2.49c	161.24±0.03b
Margaric (C17:0)	16.92±1.03a	11.13±1.22b	13.62±0.44ab
Stearic (C18:0)	8.14±0.28a	7.38±1.43b	8.09±0.56a
Arachidic (C20:0)	356.31±3.41a	176.89±3.43c	257.22±1.47b
Unsaturated fatty acids			
Monounsaturated fatty acids (mm/100 g)			
Myristoleic (C14:1)	16.48±1.12a	10.06±0.25c	12.20±0.03b
Oleic (C18:1n-9)	116.25±1.56a	41.10±2.24c	53.96±0.10b
Polyunsaturated fatty acids			
Linoleic (C18:2n-6)	275.30±2.65a	111.28±1.22c	152.14±0.52b
Linolenic (C18:3n-3)	54.10±2.84a	29.72±3.57c	46.73±0.68b
Σ Saturated fatty acids	711.28±3.32a	331.13±2.62c	455.59±5.01b
Σ Unsaturated fatty acids	462.13±2.51a	192.16±2.50c	265.04±2.06b
Unsaturated fatty acids /saturated fatty acids ratio	0.65	0.58	0.58
Ratio n-6 / n-3	5.1	3.74	3.26

RPF: Pregelatinized red potato flour (*Oxalis tuberosa*). GFC: gluten-free control. RP1: adding RPF 6.6 %. RP2: adding RPF 13.2 %.Different letters in the same row indicate significant differences ($p < 0.05$) according to the Tukey test.

This indicates that the content of unsaturated fatty acids is decreased by almost 50% in relation to saturated ones. The decrease and increase in the previous values on saturated and unsaturated fatty acids could be related to the concentration of the ingredients used in each formulation, since it has been mentioned that the use of flours obtained from different sources such as: vegetables, seeds, fruits, herbs, tubers, etc., its composition will be variable, finding different concentrations of the fatty acids present in the lipid matrix (Giaretta *et al.*, 2018; Hernández-Uribe *et al.*, 2020). Finally, the ratio of omega-6 fatty acids and omega-3 fatty acids (Ω6 / Ω3) is shown, where there is a decrease with respect to the treatments compared with GFC (5.10), up to RP2 (3.26). This important indicator in the content of unsaturated fatty acids, because it shows the balance it contains, and in turn will have a better beneficial effect on the body. In addition to the impact on the nutritional quality of foods and their caloric content, lipids have been shown to be related to cardiovascular diseases. Therefore, the type of fatty acids present in food does not have to be limited only to the amount that they have, but to the type and relationship in proportion to being consumed (Hoenselaar, 2012; Sulieman *et al.*, 2019). According to Costantini *et al.* (2014), the addition of ingredients, such as seeds, which in addition to nourishing, have bioactive compounds that impact human health, such as omega 3 fatty acid, which has been shown to have benefits in their consumption, especially those related to cardiovascular diseases, inflammation, hyperlipidemia and cancer, so they must be consumed in the diet as they are not synthesized by the body. Various studies have shown the use of various sources to increase the content of unsaturated fatty acids in the preparation of bakery products. Giaretta *et al.* (2018) and Costantini *et al.* (2014) studied breads added with chia seeds (Salvia hispánica) and found an increase in the content of polyunsaturated fatty acids as the use of these increased, which improved the ratio with respect to saturated fatty acids. Also, the Ω6 / Ω3 ratio was improved by finding proportions of 6: 1 and 4: 1 (data similar to the present study).

3.6 Microstructure analysis of dough and crumb

In dough microstructure analysis by ESEM with a magnification of 120x, it is observed that in GFC dough (Fig. 2a) a compact structure, poorly defined and with micro-fractures, caused by the absence of

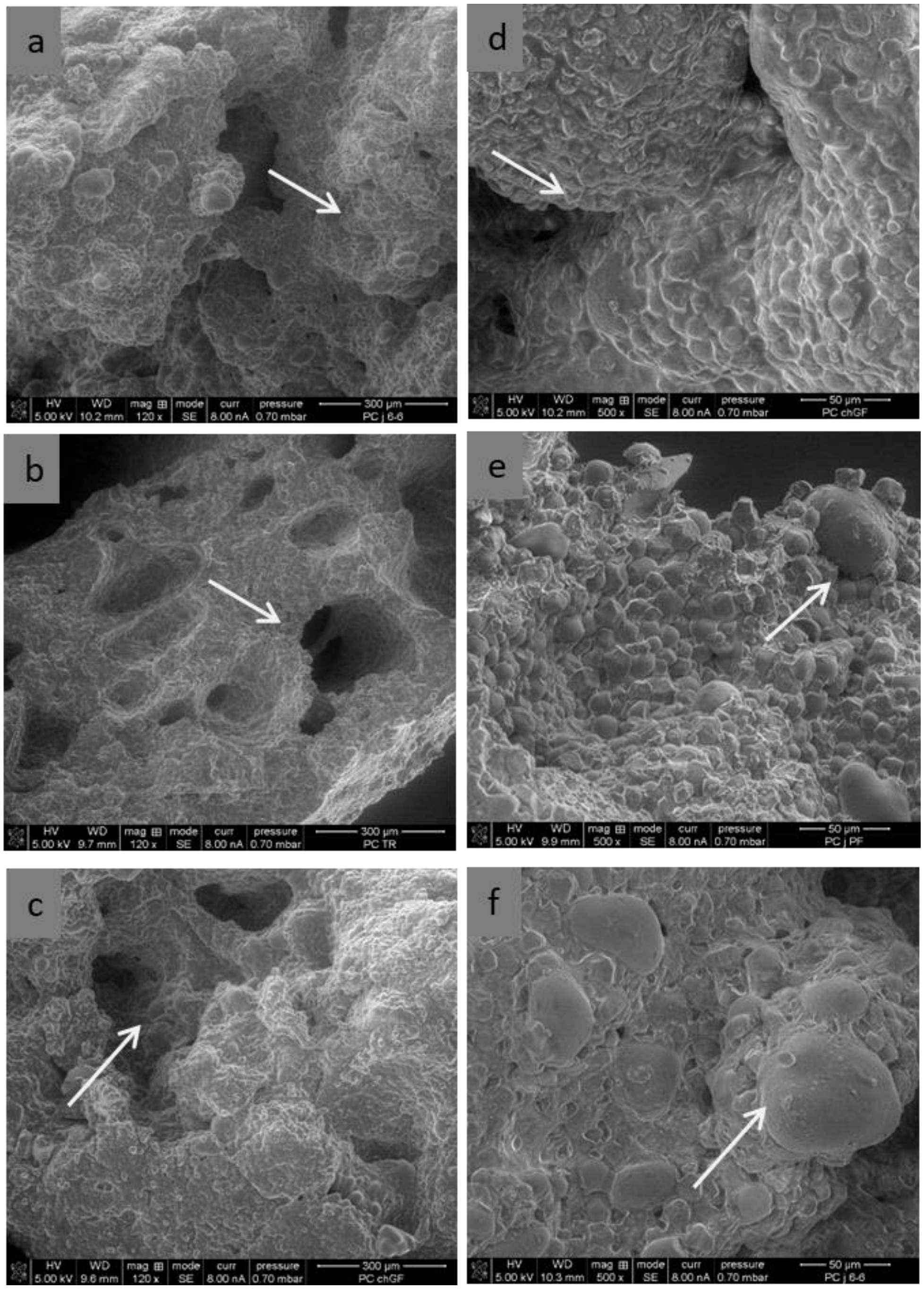

Fig. 2 Environmental Scanning Electronic Microscopy (ESEM) images of the dough. (a, b, c) Dough with 0, 6.6 and 13.2% RDP at 120x; (d, e, f) Dough with 0, 6.6 and 13.2% RPF at 500x. The arrows show some cells formed in the microstructure of the doughs, as well as the distribution of the components, among which the starches of different sizes of the gluten-free bread components stand out, with the larger starches of pregelatinized red potato flour (*Oxalis tuberosa*) standing out.

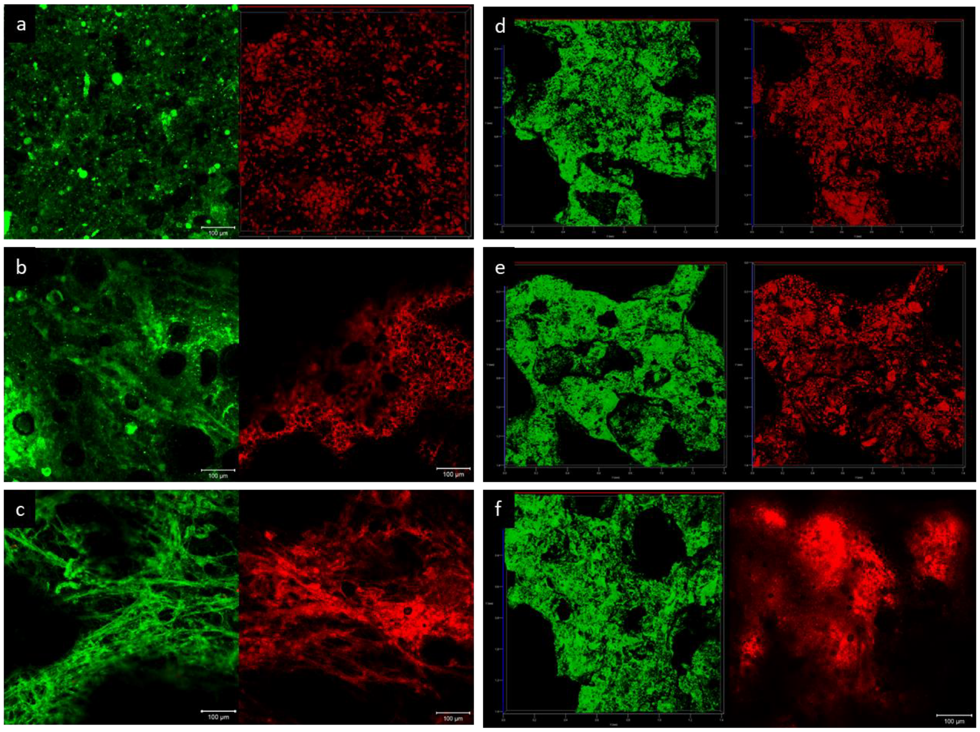

Fig. 3 Confocal Scanning Laser Microscopy (CSLM) images of dough and crumb. (a, b, c) Doughs with 0, 6.6 and 13.2% of pregelatinized red potato flour (*Oxalis tuberosa*); (d, e, f) Gluten-free bread crumbs with different amounts of pregelatinized red potato flour (*Oxalis tuberosa*) with 0, 6.6 and 13.2%. The images show the binding of fluorochrome (fluorescein) to proteins in green and the binding of rhodamine B to carbohydrates in red.

gluten, is observed. In RP1 (Fig. 2b) and RP2 (Fig. 2c) doughs the formation of some cells can be observed in the structure that can be caused by the addition of RPF that provides certain viscoelastic properties to the dough. With a magnification of 500x it is observed that the control dough (Fig. 2d) presents a compact structure, where there is an agglomeration of the components, mainly starches. The images corresponding to RP1 (Fig. 2e) and RP2 (Fig. 2f) it can be seen that the sizes of the starch granules are variable in shape and size coming from the RPF, which implies the structure of the crumb. Fig. 3 shows the CLSM microscopies for the dough and crumb. The fluorochromes used have an affinity for proteins (green) and carbohydrates (red). The distribution and dispersion of carbohydrates is observed in GFC dough (Fig. 3a), where it does not show the formation of the gluten matrix. While in RP1 (Fig. 3b) and RP2 (Fig. 3c) doughs the formation of a more stable structure (similar to a structured matrix) is observed, which may be due to the presence of proteins in the dough. On the other hand, in the images that highlight the carbohydrates (red), the starch granules that are attached to these structures are observed. The images (Fig. 3d-3f) show the stiffness that the crumb acquires. In proteins (green) there is the formation of cells resulting from fermentation and baking. While carbohydrates (red), mainly starches, remain attached to the formed crumb. In GFC (Fig. 3d) cracks in the crumb and a compact distribution of carbohydrates are observed, while in treatments RP1 (Fig. 3e) and RP2 (Fig. 3f), although the behavior is similar, the structure shows more cells. structured, however, carbohydrates have a very similar distribution, which deduces that the crumb is compact. Previous studies in the analysis of bread microstructure, through the use of CLSM show how the interaction between the complex structure of the gluten network and starches is presented, the CLSM allows to visualize the internal behavior of the different parts that make up the bread (proteins and carbohydrates) and relate them to the parameters previously studied. The relationship of the texture with the formation of the crumb can be observed even in the distribution of the different sizes of starches in GFD and compared with the treatments (Baier-Schenk *et al*., 2005; Díaz-Ramírez *et al*., 2013; Jekle & Becker,

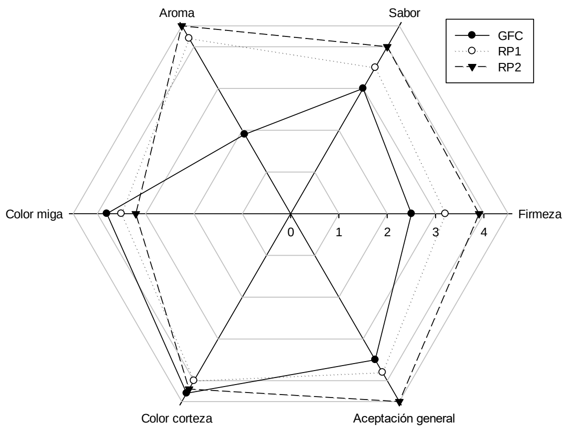

Fig. 4 Sensory evaluation of gluten-free English bread with different amounts of pregelatinized red potato flour (*Oxalis tuberosa*) with 0, 6.6 and 13.2%. 5-point hedonic scale (1 point "I dislike it a lot" and 5 "I like it a lot", using untrained judges.

2011; Morales-Hernández *et al*., 2019; Schober *et al*., 2008).

3.7 *Sensory evaluation*

The sensory evaluation results are shown in Fig. 4. Regarding the evaluated parameters (firmness, flavor, aroma, crumb color, crust color and general acceptance), a relationship was found with the physical and rheological parameters mentioned above. The firmness (hardness) of the treatments (RP1 and RP2) had a better acceptance with the addition of RPF, where the judges argued that these were softer compared to the control. For flavor and aroma, RP2 had a higher preference where the judges mentioned that those with higher content had a sweeter and more pleasant aroma, as well as a more balanced flavor. In the aroma GFC had a low evaluation because it showed to have influence of the starches and the hydrocolloid used, having a "synthetic" aroma. Regarding the color of the crumb and crust, there was a higher preference for the GFC where the judges argued that the typical color of an English bread (golden crust and slightly yellow crumb) is preserved. And the treatments added with RPF had a more intense color in the crumb and crust, although they did not reject the product. Finally, in the evaluation of general acceptance, GFC obtained the lowest evaluation (3.5), followed by RP1 (3.8) and with the highest acceptance the treatment RP2 (4.8). Some sensory evaluation studies, with trained judges, show that the quality deficiencies of GFB are the lack of aroma, excessive hardness and sometimes flavors foreign to bread, where the final acceptance is lower than wheat (Alba *et al*., 2020; Hager *et al*., 2012; López-Fernández *et al*., 2021; Sulieman *et al*., 2018). In studies where trained and untrained judges suffered from celiac disease, the prevalence of global acceptance of gluten-free products was high (Clapassón *et al*., 2020; Espino-Manzano *et al*., 2018; Laureati *et al*., 2012; Milde *et al*., 2012; Pagliarini *et al*., 2010; Sulieman *et al*., 2018).

Conclusions

This study shows that the use of red potato flour (*Oxalis tuberosa*) is an alternative from an unconventional source in the production of gluten-free bread, since it improves the texture properties, reducing its initial firmness. The RPF partially replaced the corn starch used, obtaining a more uniform structure. In addition, it increases the content of protein, ash, dietary fiber, decreases the glycemic index, the content of saturated fatty acids and improves the balance of polyunsaturated fatty acids (Ω6 / Ω3) in bread. The sensory evaluation showed a better acceptance in the 13.2% RPF treatment.

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