Development of gluten free breads from *Colocasia esculenta* flour blended with hydrocolloids and enzymes

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Abstract

Colocasia esculenta, belonging to the Araceae family, represents an attractive alternative as gluten-free (GF) main ingredient owing its healthy pattern. The aim was to explore the GF breadmaking potential of *Colocasia* spp. cormels flour, thermally treated or blended with hydrocolloids (HPMC, xanthan gum, guar gum), enzymes (glucose oxidase or proteases) or potato starch. A total of eight formulations were used to obtain GF bread-like products. Resulting breads were characterized based on their technological quality, but also on their functional quality by *in vitro* starch digestion. *Colocasia* spp. cormels flour-based breads displayed similar quality parameters observed in previous reported GF formulations. The addition of an endoprotease allowed developing breads with higher specific volume, but the alcalase type protease increased crumb softness. In general, resulting GF breads contained higher SDS and RS fraction than RDS fractions. A better starch digestibility pattern than those previously reported in GF breads was also observed, which confirm the potential of *Colocasia* spp. cormels flour as novel nutritive source of GF flours.

Keyword: bread; *Colocasia esculenta*; digestibility; gluten-free; glycemic index

1. Introduction

Over the last decades, the popular belief that gluten free (GF) diet is a healthier option along with gluten-related disorders are driving an increasing number of consumers to opt for a GF diet as a lifestyle choice. Consequently, numerous researches have been conducted on GF products to improve their technological and nutritional properties (Capriles & Arêas, 2014; Capriles, dos Santos, & Arêas, 2016). Nevertheless, diverse studies stated that consumers remain unsatisfied with the quality of GF products (do Nascimento, Fiates, dos Anjos, & Teixeira, 2014), while others highlighted their lack of nutritional values compared to their gluten-containing counterparts (Pellegrini & Agostoni, 2015). Therefore, a balance between technological properties and nutritional value is necessary to match the consumer's requests.

Currently, refined flours or starches are the main ingredients used to produce commercial GF products, specifically GF breads. As a result, breads have poor technological quality including dry crumbling crumb, poor mouthfeel and poor flavor (Gallagher, Gormley, & Arendt, 2003). These GF breads also show deficient nutritional values including low protein content and higher carbohydrate than the recommended intake (Segura & Rosell, 2011). The inclusion of alternative GF flours into GF breads recipes is one of the main approaches to improve their claimed poor technological and nutritional quality (Alvarez-Jubete, Arendt, & Gallagher, 2010). Alternative flours from pseudocereals, roots, tubers and legumes sources have been applied successfully to improve the nutrient profiles of the GF products (Capriles et al., 2014). Nevertheless, their minimal structure-building potential requires the use of additives or ingredients. Thus far, hydrocolloids, proteins, enzymes have been mixed with blends of GF flours and starches to improve the technological quality of end-products (Masure, Fierens, & Delcour, 2016). Apart from mentioned inferior technological properties, the inclusion of

all these alternative flours into GF breads is limited owing its detrimental effect on the sensory properties of end-products (Capriles et al., 2016). Therefore, further searches on other nutritive GF flours are needed.

The *Colocasia esculenta* (L.) Schott (*Colocasia* spp.) rhizome is grown largely in Cuba for their edible corms and cormels (Calle, Benavent-Gil, Garzón, & Rosell, 2019). Its nutritional components make this material very attractive to improve the nutritional value of foods (Kaushal, Kumar, & Sharma, 2015). The *Colocasia* spp. has been stated as a good source of protein (11 - 16%), crude fiber (5 - 9%) and potassium (2271-4276.06 mg/100g), but also other minerals including iron, calcium, sodium, magnesium, phosphorus, zinc and copper (Arıcı, Yıldırım, Özülkü, Yaşar, & Toker, 2016). Furthermore, the *Colocasia* spp. flour also contains vitamins and has antioxidant activity (Chandrasekara & Josheph Kumar, 2016).

Despite the evidence of positive nutritional value of *Colocasia* spp. flour and its promising economic value, as far as authors knowledge, there are no studies about the utilization of this flour in GF breadmaking. Ammar, Hegazy, and Bedeir (2009) incorporated 5,10,15 and 20% *Colocasia* spp. flour into a wheat flour-based dough and concluded that the adding of 10% *Colocasia* spp. flour results in bread with similar rheological and organoleptic properties than those observed in wheat flour bread (Emmanuel, Osuchukwu, & Oshiele, 2010). Sanful (2011) also replaced wheat flour with different percentages of *Colocasia* spp. flour, increasing the amounts of ash, total carbohydrates and fiber as increasing the taro flour level in the breads.

The main objective of the present study was to explore the GF breadmaking potential of *Colocasia* spp. flour. Furthermore, based on the aforementioned complex GF systems found in the literature, the effect of different additives including hydrocolloids, enzymes and starch was also evaluated to overcome the technological challenge involved in the

gluten removal. The suitability of *Colocasia* spp. flour individually, thermally pretreated or blended with different additives for application in a GF systems was investigated and their effects on the technological and nutritional properties of the GF bread products were evaluated.

2. Materials and methods

2.1. Materials

Cormels from freshly *Colocasia* spp. MC-2012, harvested at 9 months of maturity, were collected from National Institute of Tropical Food Research Farms in Cuba. The rhizomes were cleaned to remove all foreign matter, peeled and cut into slices of 1 cm. After mixing with water and a solution of sodium metabisulfite (20 mg/kg) during 30 min to avoid oxidation reaction, the slices were dehydrated with a forced convection tray dryer (Keller, Ihne & Tesch KG No. 3709, Lampertheim, Germany) at 45°C during 24 h. Then, the dry slices were milled and the obtained flour kept at 4°C for subsequent analyses. Hydroxypropylmethylcellulose (HPMC, Methocel™ K4M) was generously donated by Dow Pharma & Food Solutions (La Plaine Saint Denis, France). Guar gum – 3500 and xanthan gum food grade were obtained from EPSA (Valencia, Spain) and Jungbunzlauer (Wulzeshofen, Austria), respectively. Gluzyme Mono 10000 BG (EC 1.1.3.4) containing 10,000 glucose oxidase U/g, iZyme BA (EC 3.4.21.1) containing 0.15 AU/g endo-protease activity, and Alcalase 1.5 MG Type FG (EC 3.4.21.62) containing 1.5 AU/g alcalase activity (Subtilisin type) were provided by Novozymes (Bagsværd, Denmark). Potato starch was purchased from Tereos Syral (Marckolsheim, France). All other ingredients were acquired in the local market. All reagents were of analytical grade and used without further purification.

2.2. Flour characteristics

Standard methods were used to determine the flour characteristics (AACC, 1999; AOAC, 1990). Moisture was measured by oven drying at 130°C for 90 min (AACCI Method 44-15.02). Total nitrogen content was analyzed according to the Kjeldahl method (AACCI methods 46-12.01) using a nitrogen-to-protein conversion factor of 6.25. Fat content was quantified following the Soxhlet method (AACC Method 30-25.01). Ash content was determined by incinerating samples in a muffle at 900°C for 2 h (AACC Method 08-01.01). The crude fiber content of the samples was analyzed in accordance with the AOAC Method 973.18. Carbohydrate content was estimated by difference.

Water binding capacity (WBC) was analyzed according to the method described by Cornejo and Rosell (2015). Results were expressed as grams of water retained per gram of solid.

2.3. *Baking process*

A total of eight reported GF bread formulations were selected (Calle, Villavicencio, Rosell, & Bernabé-Marques, 2014; Gujral & Rosell, 2004; Marco & Rosell, 2008; Morreale, Garzón, & Rosell, 2018; Renzetti & Arendt, 2009a) and adapted to *Colocasia* spp. cormels flour characteristics. All of them were prepared relying on a simple GF formulation based on *Colocasia* spp. cormels flour, present individually, pretreated or blended with different ingredients (starches, enzymes and other hydrocolloids) widely used in the design of GF bread. To obtain pretreated *Colocasia* spp. cormels flour, 50 g of resulting *Colocasia* spp. cormels flour were mixed during 5 min with 113.5 mL of boiled water, which corresponded to the water binding capacity required for hydrating 50 g of flour. This partially pregelatinized flour was directly used in the recipe and it was referred as pretreated flour. The formulations used are summarized in Table 1, which were based on flour as follows: 100% of rhizome flour (F1); 50 % of flour

blended with 50% of pregelatinized flour (F2); 100% of flour blended with hydrocolloids (F3 and F4); 100% of flour blended with enzymes (F5, F6 and F7); and 80% of flour blended with 20% of potato starch (F8). GF doughs were prepared by mixing the dry ingredients and then by adding the oil and water with the compressed yeast previously dissolved at 20°C. Mixing was carried out in a Robot Coupe RM8 (Barcelona, Spain) at speed 3 for 8 min. 50 g-Dough pieces were put into pans and placed into a proofer (Lezo, Spain) for 50 min at 30°C and a relative humidity of 85%. The breads were baked in an electric oven (F106, FM Industrial, Córdoba, Spain) at 185°C, 80% of humidity for 20 min. After baking, bread loaves were removed from the pans and cooled at room temperature for 45 min. Loaves packed in polyethylene bags to prevent drying were stored at 24°C for 24 h and then used for further analysis. Baking was performed on 2 independent trials and 5 loaves were prepared for each bread type at each baking trial.

2.4. Quality assessment of the GF breads

Resulting GF breads were evaluated in terms of quality parameters as previously described by Matos and Rosell (2013). Bread moisture content was determined in two steps following the AACC (1999). Bread volume was measured by the rapeseed displacement method. Specific volume (cm^3/g) was calculated as the ratio between the volume of the bread and its weight. Weight loss during baking was assessed by weighing the pans before and after baking. These measurements were carried out in three breads of each batch.

The texture parameters including hardness (g), cohesiveness, chewiness (g) resilience and springiness were evaluated using a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) equipped with a 5 Kg load cell, which compresses the bread crumb with a 25 mm aluminum cylindrical probe. Bread samples were sliced into 10

mm slices and analyzed with a texture profile analysis (TPA) double compression test. During the test, samples were compressed twice up to 50% strain (penetration of its original height) at a cross head speed of 1 mm/s and 30 s gap between compressions with a trigger force of 5 g. Data was acquired using Texture expert software and showed as the average value taking three breads from each batch.

Crumb color was recorded using a Minolta colorimeter (Chromameter CR-400/410 Konica Minolta, Japan) after standardization with a white calibration plate ($L^* = 96.9$; $a^* = -0.04$; $b^* = 1.84$). The data collected from three slices of each bread measured at three different locations of the slices were averaged and expressed using CIE- $L^*a^*b^*$ scale where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b^* indicates hue on a blue (-) to yellow (+) axis.

2.5. *In vitro* digestion

Digestibility of GF breads was assayed following the method described by (Benavent-Gil & Rosell, 2017b). The amount of starch fractions based on the hydrolysis rate of starch was calculated and expressed in amount of glucose released (mg/100 mg) by using the method of Englyst, Veenstra, and Hudson (1996). Rapidly digestible starch (RDS) was defined as the starch fraction that was hydrolyzed within 20 min of incubation, slowly digestible starch (SDS) was the starch fraction hydrolyzed within 20 and 120 min, and resistant starch (RS) was defined as the starch fraction that remaining unhydrolyzed after 16 h of incubation.

Glucose measure was determined in supernatant samples using a glucose oxidase–peroxidase (GOPOD) kit (Megazyme, Dublin, Ireland). Starch was calculated as glucose (mg) $\times$ 0.9.

Experimental data were fitted to first order equation to study the kinetics of *in vitro* digestion (Goñi, García-Alonso, & Saura-Calixto, 1997): $C = C_{\infty} (1 - e^{-kt})$. C was the

concentration at t time, C_{∞} was the equilibrium concentration or maximum hydrolysis extent, k was the kinetic constant and t was the time chosen. The hydrolysis index (HI) was calculated as the ratio area under the hydrolysis curve (0–180 min) of the sample and area of a standard material (white bread) over the same period of time. The expected glycemic index (eGI) was obtained using the equation $eGI = 8.198 + 0.862HI$ (Granfeldt, Björck, Drews, & Tovar, 1992).

2.6. Statistical analysis

The data reported in the tables and figures are average values of duplicates and expressed as a mean $\pm$ standard deviation. Data was subjected to analysis of variance (ANOVA) to separate the effect of different additives. Fisher's least significant test was used for assessment of significant differences among experimental mean values with a significance level of 0.05. Pearson correlation coefficient (r) and p-value were used to indicate correlations and their significance using Statgraphics Centurion XVII software (Bitstream, Cambridge, N).

3. Results and discussion

3.1. Proximate composition of raw *Colocasia esculenta* cormels flour

The composition of the *Colocasia* spp. flour used in the present study expressed in percentage (based on dry basis) was as follows: moisture: $6.33 \pm 0.02\%$, protein: $8.28 \pm 0.07\%$, ash: $5.04 \pm 0.00\%$, fat: $0.53 \pm 0.00\%$, crude fiber: $4.38 \pm 0.23\%$ and carbohydrates: 75.44 ± 0.35 . Data obtained are in agreement with literature (Temesgen & Retta, 2015a). As expected, *Colocasia* spp. cormels flour displayed high carbohydrate content, but it also proved to be a good source of minerals and fiber. The protein content was lower than that found in teff (12.84 ± 0.51) and buckwheat (12.39 ± 0.38) flours (Hager & Arendt, 2013). However, it was higher than that found in the most commonly used GF flours such as maize (5.50%), rice (7.33%), cassava (1.4%) or

sweet potato (6.3%) flours (Hager et al., 2013; Pasqualone et al., 2010a, 2010b; Yadav, Guha, Tharanathan & Ramteke, 2006).

3.2.Characterization of *Colocasia* spp. cormels flour-based breads

The suitability of *Colocasia* spp. cormels flour (F1) for GF breadmaking was evaluated testing the different alternatives that the literature offers for building up inner structures. Nevertheless, the amount of water used for making GF breads was based on the water binding capacity of the flour (2.27 g/g flour), since that parameter has been previously confirmed as a good indicator of the optimum amount of water to be used in gluten free systems (Espinosa-Ramírez, Garzon, Serna-Saldivar, & Rosell, 2018). Resulting GF breads were characterized regarding their moisture content, weight loss, specific volume and crumb color (Table 2). The observed moisture content (44.29 - 50.64%), specific volume and weight lost in F1 breads were comparable to GF rice-based breads such as oat, quinoa and so on (Hager et al., 2012). Likely, the higher water binding capacity of *Colocasia* spp. flour (Calle et al., 2019) compared to other common GF flours and starches (Martínez & Gómez, 2017), might be responsible for those high values of moisture content.

The color of bread is one of the first characteristics observed by consumers, determining choice and preference. Results from the crumb color parameters are summarized in Table 2. As expected, the color of F1 breads was visually dark, which was further confirmed by their lower L^* value. This value could be attributed to the natural color of raw *Colocasia* spp. flour, which displayed 81.05 ± 0.36 , 1.17 ± 0.07 and 14.63 ± 0.45 for L^* , a^* and b^* , respectively. Kumar, Sharma, Kaushal and Singh (2014) highlighted that *Colocasia* spp. flour can contain naturally colored pigments and those affected the color characteristics of end products.

To describe the texture of the *Colocasia* spp. -based breads (F1), crumb hardness, cohesiveness, chewiness, resilience and springiness are depicted in Table 3. Resulting breads revealed much lower crumb texture values than those reported for commercial GF bread (Matos & Rosell, 2012), probably due to the higher moisture contents (de la Hera, Rosell & Gomez, 2014).

3.3. Effect of additives in *Colocasia* spp. cormels flour-based breads

Different strategies previously reported to build up inner structures in gluten free breads or improve flour functionality were applied to increase the technological quality of *Colocasia* spp. breads. The effect of thermally treated *Colocasia* spp flour blended with the raw flour (F2), hydrocolloids (F3, F4), enzymes (F5-F7) or potato starch (F8) in the quality of *Colocasia* spp. based breads were evaluated (Table 2). Those formulations have been selected from literature to show the different alternatives applied to improve GF breads quality. Among them, hydrocolloids of different nature -HPMC (Marco et al., 2008), mixture of HPMC, xanthan gum and guar gum (Calle et al., 2014), enzymes with strengthening (glucose oxidase) or weakening action (proteases), and potato starch. The statistical analysis revealed significant differences ($p < 0.05$) regarding moisture content, weight loss and specific volume. In general, the effect of treated *Colocasia* spp. flour (F2) as well as hydrocolloids (F3, F4) and enzymes addition (F5-F7) resulted in higher moisture content than those observed in F1 breads. Overall, F2 and F7 samples displayed the highest moisture content. F2 bread was obtained replacing *Colocasia* spp. cormels flour by pretreated *Colocasia* spp. cormels flour. The pretreatment involved the heating of the flour in excess of water to cause a partial or complete gelatinization of the starch granules, which increase its ability to bind water (Njintang & Mbofung, 2006). On the other hand, F7 breads that incorporates a protease (Alcalase 1.5 MG Type FG) in its recipe, might bind more water due to the hydrophobicity reduction of the flour. Some

224 authors explained the effect of the protease on gluten free flours due to its hydrolytic
225 action on the proteins, that led to a decrease in the hydrophobicity of the system in
226 specific flours (Renzetti & Arendt, 2009b). Nevertheless, no general trend could be
227 defined regarding proteases, since that effect was not observed in F6 that also contained
228 a protease type enzyme. Conversely, F8 breads displayed lower moisture content, likely
229 due to the low capacity of potato starch to bind water (Benavent-Gil & Rosell, 2017a).
230 In line with previous findings (Cornejo et al., 2015; Matos et al., 2013; Renzetti et al.,
231 2009a; Shin, Gang & Song, 2010), the specific volume values ranged from 1.11 ± 0.05
232 to 2.71 ± 0.13 mL/g, values were significant dependent on the recipe applied. Compared
233 to F1 breads, differences were only observed in the case of F6, F7 and F8 breads.
234 Among them, F6 breads displayed the highest specific volume, likely due to the enzyme
235 ability to modify protein functionality. Nevertheless, proteases effect on GF breads are
236 really dependent on the type of flour (Renzetti & Rosell, 2016). In fact, it seems that
237 iZyme improves breadmaking performance of cormels' flour. In opposition, the other
238 protease tested (alcalase) led to the smallest specific volume (F7). Small statistical
239 differences were also observed in the case of weight loss, particularly the lower weight
240 loss of F2 and F6 breads compared to F1 breads. However, in the case of F2 the water
241 ability retention of starch would be responsible of that effect, conversely in F6, it might
242 be ascribed to the more hydrophilic structure resulting from proteins hydrolysis.
243 The L^* , a^* and b^* values for crumb color showed significant ($p < 0.05$) differences
244 among the different formulations used to obtain the GF breads. The lowest value of L^*
245 (lightness) was obtained for F8, which could be expected due to the opacity that confers
246 the potato starch. Regarding a^* and b^* values, all samples exhibited positive values,
247 indicating hue on red and yellow axis for all bread samples. However, significant
248 variation was observed among the different formulations. HPMC present in F3 was the

unique additive that did not modify the a^* value, compared to the control. The rest of recipes showed a decrease in the a^* values. Regarding the b^* parameter, again could be distinguished the effect of HPMC, leading to brownish crumb in F3; in contract, more pale crumbs were obtained when adding potato starch. Therefore, crumbs color was not only dependent on the flour color, but on the interaction of ingredients and additives. As expected, crumb texture was significantly ($p < 0.05$) influenced by the recipe applied (Table 3). Hardness values ranged from 191 ± 9 to 361 ± 17 g. Overall, F2 and F7 breads displayed the highest and lowest values, respectively. The hardness of F3 and F4 increased, despite the hydrocolloid addition usually tends to decrease hardness (Liu et al., 2018). Nonetheless, their effect seems to be also dependent on the flour used Sasaki (2018). Again, enzymes effect on crumb hardness was really erratic, particularly in the case of proteases that increase (F6) or decrease (F7) it, depending on the type of protease (Kawamura-Konishi, Shoda, Koga & Honda, 2013). It should be emphasized that although general consensus exists about the inverse relationship between specific volume and crumb hardness, recipes tested in this study confirmed that additives/enzymes modify constituents network and in consequence the crumb structure, breaking down that general rule. Noticeable differences were also showed in chewiness. In general, the ingredients addition increased this parameter, except in the case of F7 breads, which did not modify it. In the case of cohesiveness, all breads exhibited similar behavior, except in the case of F2, F5 and especially F8 bread that increased this parameter, suggesting a more integrated matrix. Among them, F8 breads showed the highest value. Consumer's acceptance is greatly influenced by the cohesiveness, which quantifies the internal resistance of material. Therefore, starch addition led to more compact structure, since it decreased the crumbling. Starch addition (F8) also affected significantly the resilience

and springiness of the breads. Springiness has been commonly related with resilience values, which reduction indicates the loss of crumb elasticity (Onyango, Mutungi, Unbehend & Lindhauer, 2011). Considering the overall texture results, it seems that starch addition it is advisable to improve the texture properties of *Colocasia* spp. based breads.

3.4. Digestibility of GF breads

Relevant starch fractions including RDS, SDS and RS were evaluated and categorized depending on its rate of digestion (Table 4) (Englyst et al., 1996). *Colocasia* spp. cormels flour-based breads (F1) exhibited high SDS and RS fractions and low RDS fraction. Considering the great impact of RDS on the glycemic response (Englyst et al., 1996), the production of GF breads with higher amounts of SDS and RS fractions are interesting from a nutritional point of view. It has been already known the healthy profile of *Colocasia* spp. starch due to their small size (Temesgen & Retta, 2015b), but what this study shows, is that the healthy profile is even present in the *Colocasia* spp. based breads.

In general, the starch fraction pattern observed in F1 breads was also found in the rest of the breads, except in the case of potato starch addition (F8). Nevertheless, the different recipes caused changes in α -amylase susceptibility, resulting in significantly ($p < 0.05$) differences within RDS contents without significantly affecting the SDS and RS content (Table 4). The largest increase of RDS fraction was found in F8 samples, which is consistent with the pattern previously reported for starchy foods (Poutanen, Flander, & Katina, 2009). Furthermore, studies conducted by Segura et al. (2011) highlighted that RDS is the most predominant fraction in available commercial GF breads mainly based on corn starch. This effect could be attributed to the starch gelatinization (Shumoy, Van

Bockstaele, Devecioglu & Raes, 2018), which results in a rapid degradation of starch (Poutanen et al., 2009).

3.5. *In vitro* and expected glycemic index of GF breads

The different gluten free breads were subjected to *in vitro* enzymatic hydrolysis in order to simulate starch digestibility. At specific intervals of *in vitro* reaction, starch hydrolysis was measured as glucose released and Figure 1 shows the resulting plots. Furthermore, primary and secondary parameters derived from the *in vitro* digestion were also analyzed. Results obtained including the kinetic constant (k), equilibrium concentration of hydrolyzed starch (C_{∞}), area under the hydrolysis curve after 180 min (Liu et al.), hydrolysis index (HI) and estimated glycemic index (eGI) are summarized in Table 4. Starch hydrolysis draws plots characterized by a linear increase of glucose released during the early stage of digestion, which can be maintained over time or reach the plateau (Blazek & Gilbert, 2010). In this regard, a typical digestion pattern was observed for F1 breads, which at the early stage of hydrolysis exhibited a linear increase in the amount of glucose released. Nevertheless, the kinetic constant (k) for the amylolysis evidenced slower hydrolysis kinetics than those observed by Segura et al. (2011) when evaluated different commercial GF breads. After 90 min of *in vitro* digestion, F1 breads reached the plateau, showing the maximum hydrolysis (C_{∞}) to a lower extent than those previously reported (de la Hera et al., 2014). Following this trend, lower eGI was found compared with reported GF breads (de la Hera et al., 2014; Liu et al., 2018; Segura et al., 2011; Wolter, Hager, Zannini & Arendt, 2013). Some authors reported that GF breads display significantly higher eGI compared to traditional breads (Segura et al., 2011). These results suggest that *Colocasia* spp. flour might provide end products with higher nutritional properties.

Concerning the influence of the different recipes, varying susceptibilities to enzyme hydrolysis were observed (Figure 1). Starch hydrolysis exhibited different rate and extent for *Colocasia* spp. based breads obtained from the diverse recipes (Table 4). The kinetic constant (k) of amylolysis ranged from 0.0249 ± 0.0028 to 0.0116 ± 0.0053 , displaying similar hydrolysis kinetics than F1 breads. Nevertheless, F8 samples evidenced faster hydrolysis kinetics than those observed for the other samples, which agrees with the increase of RDS above described. The maximum hydrolysis (C_{∞}) was not significantly affected, thus all studied GF breads displayed similar extent of starch hydrolysis than F1 breads. Nevertheless, ingredients addition had a significant effect ($p < 0.05$) on the HI, AUC and eGI parameters, which showed similar trend. Results obtained revealed that only the addition of potato starch increased the HI, AUC and eGI parameters, while the addition of pretreated flour, gums or enzymes did not change these parameters. Considering that *in vitro* glycemic index has been previously correlated with RDS content (Liu et al., 2018), the greatest eGI presented by F8 breads could be explained by their large RDS content. In fact, a strong positive correlation was observed between RDS content and eGI ($r = 0.9498$, $p < 0.0100$) in the present study. Nevertheless, it is worth noting that the observed eGI was lower than those previously reported, even using potato starch in the formulation (de la Hera et al., 2014; Liu et al., 2018; Segura et al., 2011; Wolter et al., 2013). These results suggest that *Colocasia* spp flour could maintained its nutritional properties in complex food matrix.

4. Conclusions

The positive nutritional value of *Colocasia* spp. flour including high protein, minerals and fiber content, as well as low fat content makes this ingredient attractive to enhance the nutritional value of GF breads. However, to build up a light bread crumb structure from *Colocasia* spp. flour requires the development of specific strategies. For doing so,

different recipes have been tested in this study. By compiling results, it can be concluded that proteases had a significant effect on the specific volume of breads, but their effect was dependent on the type of proteases. Izyme increased the specific volume, whereas alcalase type protease decreased it, but lead to softer crumbs. All GF breads showed very appropriate pattern regarding starch digestibility, with high amount of SDS and RS, which were not significantly affected with the recipes tested, and neither the *in vitro* glycemic index. Within the tested recipes, the addition of potato starch was the least advisable. Overall, *Colocasia* spp. flour can be used to produce GF breads with similar technological quality parameters than those previously reported with common GF flours, but with significantly better estimated glycemic index.

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519 **FIGURE CAPTIONS**

520 **Figure 1.** Starch hydrolyzed during *in vitro* starch digestibility of gluten-free breads
521 based on *Colocasia* spp. cormels flour, pretreated or blended with different additives.
522
523

524 **Table 1.** Gluten free bread recipes

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Flour (g)	100	100	100	100	100	100	100	80
Water (g)	227	227	227	227	227	227	227	227
Salt (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Compressed Yeast (g)	3	3	3	3	3	3	3	3
Sugar (g)	2	2	2	2	2	2	2	2
Oil (g)	2	2	2	2	2	2	2	2
HPMC (g)			2	0.29				
Xanthan gum (g)				0.21				
Guar gum (g)				0.50				
Gluzyme Mono 10000 BG (g)					0.01			
iZyme BA (g)						0.1		
Alcalase 1.5 MG Type FG (g)							0.01	
Potato starch (g)								20

525

526 Ingredients: 100% of rhizome flour (F1); 50 % of *Colocasia* spp. cormels flour blended with 50% of pre-treated *Colocasia* spp. cormels flour
527 (F2); 100% of flour blended with hydrocolloids (F3 and F4); 100% of flour blended with enzymes (F5, F6 and F7); and 80% of flour blended
528 with 20% of potato starch (F8).

529 **Table 2.** Different quality characteristics of gluten-free breads based on raw *Colocasia* spp. cormels flour, pretreated or blended with different
530 ingredients, additives or processing aids.

	Moisture (g/100 g)	Weight loss (%)	Specific volume (mL/g)	L^*	a^*	b^*
F1	57.63 ± 0.26 b	15.49 ± 0.69 bc	1.74 ± 0.03 b	57.01 ± 0.37 c	6.88 ± 0.38 e	22.29 ± 0.63 c
F2	60.23 ± 0.18 ef	12.78 ± 0.60 a	1.63 ± 0.02 b	57.48 ± 0.49 c	5.18 ± 0.10 b	20.34 ± 0.25 b
F3	58.74 ± 0.61 c	14.93 ± 0.97 bc	1.70 ± 0.05 b	56.87 ± 0.66 bc	7.09 ± 0.82 e	23.97 ± 0.66 d
F4	58.80 ± 0.41 cd	13.80 ± 0.81 ab	1.67 ± 0.02 b	56.09 ± 0.49 bc	6.13 ± 0.13 d	21.04 ± 0.53 b
F5	59.69 ± 0.29 de	13.98 ± 1.57 ab	1.65 ± 0.09 b	55.02 ± 1.98 ab	5.85 ± 0.30 cd	21.75 ± 0.96 b
F6	59.30 ± 0.68 cd	13.07 ± 1.78 a	2.71 ± 0.13 c	57.21 ± 0.80 c	5.92 ± 0.49 cd	20.70 ± 0.52 b
F7	60.96 ± 0.17 f	13.89 ± 1.03 ab	1.11 ± 0.05 a	57.41 ± 2.27 c	5.63 ± 0.16 bc	20.74 ± 0.40 b
F8	55.56 ± 0.20 a	16.30 ± 1.53 c	1.20 ± 0.06 a	57.21 ± 0.80 a	5.92 ± 0.49 a	20.70 ± 0.52 a
p-value	0.0000	0.0031	0.0000	0.0029	0.0000	0.0000

531

532 Values followed by different letters within a column denote significant differences. Ingredients: 100% of rhizome flour (F1); 50 % of *Colocasia*
533 spp. cormels flour blended with 50% of pre-treated *Colocasia* spp. cormels flour (F2); 100% of flour blended with hydrocolloids (F3 and F4);
534 100% of flour blended with enzymes (F5, F6 and F7); and 80% of flour blended with 20% of potato starch (F8).

535

536 **Table 3.** Analysis of crumb texture of gluten-free breads based on *Colocasia* spp. cormels flour alone, pretreated or blended with different
537 ingredients, additives or processing aids.

	Hardness (g)	Cohesiveness	Chewiness (g)	Resilience	Springiness
F1	263 ± 38 c	0.337 ± 0.044 a	24 ± 3 a	0.104 ± 0.019 c	0.419 ± 0.075 a
F2	361 ± 17 e	0.384 ± 0.041 b	69 ± 17 e	0.126 ± 0.013 d	0.484 ± 0.107 ab
F3	316 ± 12 d	0.348 ± 0.028 ab	50 ± 4 cd	0.095 ± 0.010 a-c	0.441 ± 0.087 ab
F4	323 ± 23 d	0.313 ± 0.044 a	45 ± 6 cd	0.091 ± 0.009 ab	0.434 ± 0.135 a
F5	209 ± 16 ab	0.373 ± 0.020 b	39 ± 14 bc	0.103 ± 0.009 c	0.546 ± 0.193 b
F6	330 ± 18 d	0.331 ± 0.037 a	54 ± 3 de	0.101 ± 0.012 bc	0.473 ± 0.116 ab
F7	191 ± 9 a	0.334 ± 0.030 a	27 ± 4 ab	0.086 ± 0.008 a	0.473 ± 0.111 ab
F8	233 ± 17 b	0.500 ± 0.031 c	65 ± 6 e	0.143 ± 0.019 e	0.697 ± 0.090 c
p-value	0.0000	0.0000	0.0000	0.0000	0.0001

538

539 Values followed by different letters within a column denote significant differences.

540 Ingredients: 100% of rhizome flour (F1); 50 % of *Colocasia* spp. cormels flour blended with 50% of pre-treated *Colocasia* spp. cormels flour
541 (F2); 100% of flour blended with hydrocolloids (F3 and F4); 100% of flour blended with enzymes (F5, F6 and F7); and 80% of flour blended
542 with 20% of potato starch (F8).

543

544 **Table 4.** *In vitro* starch digestibility and its kinetic parameters of gluten-free breads based on *Colocasia* spp. cormels flour alone, pretreated or
545 blended with different ingredients, additives or processing aids.

	RDS (mg/100 mg)	SDS (mg/100 mg)	RS (mg/100 mg)	<i>K</i>	C_{∞}	AUC	HI	<i>eGI</i>
F1	4.98 ± 0.51 ab	7.316 ± 0.035	6.032 ± 0.344	0.0127 ± 0.0020 ab	15.82 ± 0.48	1715 ± 81 ab	19.01 ± 0.89 ab	24.58 ± 0.77 ab
F2	5.32 ± 0.48 a-c	6.232 ± 1.391	5.959 ± 0.370	0.0173 ± 0.0028 ab	13.32 ± 2.77	1643 ± 245 ab	18.21 ± 2.71 ab	23.90 ± 2.34 ab
F3	4.38 ± 0.40 a	6.895 ± 1.309	6.730 ± 0.393	0.0116 ± 0.0053 a	16.10 ± 4.90	1565 ± 92 a	17.35 ± 1.02 a	23.15 ± 0.88 a
F4	7.01 ± 0.40 bc	5.809 ± 0.094	6.492 ± 0.362	0.0243 ± 0.0016 ab	13.56 ± 0.17	1885 ± 57 ab	20.89 ± 0.64 ab	26.20 ± 0.55 ab
F5	6.05 ± 2.17 a-c	7.298 ± 0.692	7.228 ± 1.085	0.0161 ± 0.0091 ab	16.66 ± 1.75	1897 ± 286 b	21.02 ± 3.17 b	26.32 ± 2.74 b
F6	5.25 ± 0.49 a-c	5.632 ± 0.652	7.178 ± 1.184	0.0189 ± 0.0043 ab	12.25 ± 0.92	1560 ± 7 a	17.29 ± 0.08 a	23.10 ± 0.07 a
F7	7.29 ± 0.56 c	5.864 ± 0.313	5.153 ± 0.387	0.0249 ± 0.0028 b	13.87 ± 0.01	1939 ± 59 b	21.49 ± 0.66 b	26.72 ± 0.57 b
F8	12.36 ± 0.84 d	3.815 ± 1.174	7.207 ± 0.329	0.0486 ± 0.0100 c	16.24 ± 0.40	2576 ± 7 c	28.55 ± 0.08 c	32.81 ± 0.07 c
p-value	0.0005	0.0514	0.1006	0.0031	0.3868	0.0017	0.0017	0.0017

546

547 Ingredients: 100% of rhizome flour (F1); 50 % of *Colocasia* spp. cormels flour blended with 50% of pre-treated *Colocasia* spp. cormels flour
548 (F2); 100% of flour blended with hydrocolloids (F3 and F4); 100% of flour blended with enzymes (F5, F6 and F7); and 80% of flour blended
549 with 20% of potato starch (F8).

550 Values followed by different letters within a column denote significant differences

551 C_{∞} and k were quantified following the equation, $C = C_{\infty}(1 - e^{-kt})$

552 *eGI* was estimated as reported Goñi et al. (1997)

Figure 1.

556

557