Gluten-free sorghum pasta: starch digestibility and antioxidant capacity compared with commercial products

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Abstract

Background: The development of new products with an additional focus on nutrition, beyond technological quality, is fundamental to improve the celiac diet quality. Nutritional attributes of white and brown sorghum gluten-free pasta developed in a previous work were analyzed. The extent and kinetics of starch *in vitro* digestion, estimated glycemic index (eGI), potentially bioaccessible and dialyzable polyphenols and antioxidant activity were evaluated and compared with commercial products.

Results: Sorghum flour samples allowed to obtain pasta with high protein (≈170 g kg^{-1}), dietary fiber (≈80 g kg^{-1}), polyphenols (2.6 g GA kg^{-1} pasta) and antioxidant activity. This sorghum pasta showed a slower starch *in vitro* digestion than the other gluten-free pasta, with a high level of protein hydrolysis (76%). The highest eGI was observed in rice sample (69.8) followed by corn-based pasta (66.4). White and brown sorghum gluten-free pasta showed 2.9 and 2.4 times, respectively, higher potentially bioaccessible polyphenol content compared to that in cooked pasta. In addition, no significant variation in antioxidant activity was found in sorghum pasta after digestion and around 48 and 36% of activity was detected in dialysate.

Conclusion: Both types of sorghum gluten-free pasta have demonstrated their nutritional value and represent a high potential alternative to current commercial pasta.

Keywords: gluten-free pasta, starch digestibility, potentially bioaccessible, dialyzability, antioxidant activity.

1.- Introduction

Pasta is one of the most common cereal food products due to its long shelf-life, easy transportation, simple cooking and good palatability. In the last years, pasta has been recognized by its nutritional quality and as an excellent option for enrichment with functional ingredients, mainly to provide sources of fiber, antioxidants and polyphenols [1].

Due to the fundamental role of gluten in pasta products, its replacement in gluten-free counterparts is problematic and represent a major challenge when trying to obtain a product with acceptable technological quality [2]. In addition, the evaluation of nutritional attributes is very important since most of the additives used to replace gluten are proteins, modified starches, gums and lipids that greatly influence these properties in the final product. Besides this, studies are scarce and only a few evaluate the nutritional quality of gluten-free pasta [3,4].

The glycemic index is a way of ranking carbohydrate food according to the postprandial glucose increase generated in blood after consumption. In this regard, as the gluten network entraps starch material limiting its hydrolysis by the digestive enzymes, lack of gluten in gluten-free carbohydrate rich foods may increase the glycemic response, especially in pasta [5]. Another aspect worth considering is the fact that the strategies most commonly used for gluten role replacement include modified starches like pre-gelatinized ones or application of high temperature that gelatinizes the starch within non-gluten flour. This allows the creation of a matrix that gives good texture properties and retains components during pasta cooking [6] yet, it increases the proportion of starch available for enzymes, and consequently, the glycemic index of final product. In that sense, the evaluation of kinetic starch digestibility becomes crucial to provide celiac people with products having not only acceptable technological quality but also acceptable nutritional attributes.

Most of the commercial gluten-free pasta available is produced with rice, corn or soy flour. With that in mind we recently published a study using sorghum flour as a raw material for gluten-free pasta, with a combination of additives, in which we obtained a final product with good technological quality [7]. Sorghum (*Sorghum bicolor* (L.) Moench) is a crop drought-resistant and heat tolerant in semi-arid conditions and has been traditionally used primarily as animal feed in Western countries; nevertheless, nearly 40% of the world sorghum production is used for human food in Africa and India [8]. In addition to these advantages, sorghum is gluten-free and has a high content of polyphenols [9] which are related with a positive impact on human health [10].

Polyphenols in sorghums are mainly phenolic acids and flavonoids, which have gained interest due to their antioxidant activity, cholesterol-lowering properties and other potential health benefits [11]. Tannins are the most important phytochemical components of sorghum since they possess properties that have also been associated with various positive impacts on human health [12]. On the other hand, tannin decreased digestibility and bioavailability of proteins due to the formation of complexes [13]. All these characteristics reinforce the potential novel use of sorghum flour in the development of gluten free products with a considerable proportion of bioactive compounds. In these sense, we developed a gluten-free pasta using decorticated sorghum flours resulting in a product with good cooking properties [7] avoiding treatments as fermentation or sprouting of grains [14].

The objective of the present research was to study the nutritional quality of sorghum gluten-free pasta throughout the analysis of *in vitro* starch digestibility, antioxidant activity of final product and dializability of components of interest, compared with commercial gluten-free pasta from rice, corn and soy flour.

2.- MATERIALS AND METHODS

2.1.- MATERIALS

Gluten-free pasta from white (*Sorghum bicolor* L. Moench, Pannar-8706 W) and brown (*Sorghum bicolor* L. Moench, Pioneer-81G67) sorghum flour was made according the optimized formulation developed in our laboratory including xanthan gum, egg albumen, egg powder and pregelatinized starch as ingredients [7]. Rice pasta (RP) (Blue Patna pastas, Coopar S.A., Uruguay), soy pasta (SP) (Elca Alimentos Saludables, FRI-DIET, Argentina), corn pasta (CP) made with white corn (var. Capia) (CAUQUEVA., Argentina) and corn pasta with vegetables made with white corn (var. Capia) (CAUQUEVA, Argentina), were purchased from the local market. Table I shows the nutritional value for sorghum (determined in our lab) and commercial gluten-free pasta (as stated on the product packaging). All samples were dry pasta and were cooked at the optimal cooking time according to the AACC method 66-50 [15].

Amylase from porcine pancreas (A3176), pepsin from porcine gastric mucosa (P7000), pancreatin from porcine pancreas (P7545), bile salts (B8756), Trolox (238813) and serine (68353) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). The chemicals used in this study were of analytical grade.

2.2 - METHODS

2.2.1.- Determination of main compounds of nutritional interest

2.2.1.1.- Total dietary fiber

Freeze-dried cooked pasta (1 g) was milled for total dietary fiber content determination according to method 32-05 [15]. Two replicates were analyzed and the results were expressed as grams of total dietary fiber per kg of cooked pasta in dry basis.

2.2.1.2.- Protein content

The protein content of cooked pasta was determined by Kjeldahl method and the nitrogen conversion factor used was 6.25 [15]. Pasta samples were cooked and freeze-dried and then milled prior to analyses. Two replicates were analyzed and percentage of protein was expressed as grams of proteins per kg of cooked pasta in dry basis.

2.2.1.3.- Total polyphenol content

Four different solvent mixtures were prepared in order to extract the major content of bioactive compounds from cooked pasta previously freeze-dried. One hundred milligrams of freeze-dried cooked pasta were mixed with 1.5 mL of each solvent mixture: methanol, methanol: water (70:30), acetone and acetone: water (70:30), in all cases with a final concentration of 0.1% HCl. The solvent/sample mixtures were mixed for 10 min and then centrifuged at 12,000 x g for 15 min. The supernatant was recovered and the extraction was repeated once. The solvent with better extraction performance based on the total polyphenol content method was acetone: water (70:30) (data not shown). Hence, the extraction was performed in duplicate to determine total polyphenol content and antioxidant activity analyses.

Total polyphenols were determined using the Folin-Ciocalteu method, with gallic acid (GA) as a calibration standard [16] in duplicate in each replicate of solvent extraction performed. The total polyphenols content (TPC) was expressed as g GA per kg of cooked pasta in dry basis.

2.2.2.- Antioxidant activity determinations

2.2.2.1.- $ABTS^{\bullet+}$ radical cation scavenging activity

$ABTS^{\bullet+}$ radical cation scavenging activity (ABTS-RCSA) was measured according to Re *et al* [17] using trolox as standard. Two determinations were performed in each solvent extraction replicate and results were expressed as mmol of Trolox equivalent per kg of cooked pasta in dry basis.

2.2.2.2.- Ferric reducing ability

Ferric reducing activity (FRA) of gluten-free pasta was determined by FRAP assay according to Pulido *et al.,* [18] using gallic acid as a standard. Two determinations were performed in each solvent extraction replicate and results were expressed as GA g per kg of cooked pasta in dry basis.

***2.2.3.- In vitro* digestion of gluten-free pasta samples**

2.2.3.1.- Estimated glycemic index

In vitro digestion was performed in duplicate using the multi-enzymatic method of Bustos *et al.* [19] using white bread as reference. Briefly, samples of gluten-free cooked pasta (4 g) were mixed with 0.01 M phosphate saline buffer (0.12 M NaCl, 2.7 mM KCl), pH 6.9. Afterwards, a pepsin digestion was performed followed a porcine pancreatic alpha amylase hydrolysis both carried out at 37 ºC. The rate of starch digestion was expressed as the percentage of total starch hydrolyzed at different times (30, 60, 90, 120 and 180 min). Expected GI was then estimated applying a first order non-linear model.

2.2.3.2.- *In vitro* digestion of pasta

In vitro digestion of the gluten-free pasta samples was performed in duplicate according to Bustos *et al.,* [20] to evaluate starch and polyphenol dialyzability and antioxidant activity in dialyzable fraction. Briefly, the ratio used was 50/50 w/v for: pasta/Simulated Salivary Fluid (SSF); oral content/Simulated Gastric Fluid (SGF) and gastric content/Simulated Intestinal Fluid (SIF) corresponding to three stages: oral, gastric and intestinal.

Aliquots of 1 mL were withdrawn at time 0, after oral digestion, at 10, 30, 60 and 120 min of the gastric digestion and at 10, 30, 90 and 180 min of the intestinal step to monitor the hydrolysis degree of starch and its kinetic parameters.

Total starch in cooked gluten-free pasta was measured in duplicate in each sample according to AACC 32-40 [15].

<u>2.2.3.3.- Monitoring starch hydrolysis during *in vitro* digestion and kinetic analysis</u>

Starch hydrolysis was monitored by the analysis of reducing sugar content in each aliquot using 3,5 dinitrosalicylic acid (DNS) method. Two non-linear models were applied to describe separately oral-gastric and intestinal digestion for starch hydrolysis (Eqs. 1 and 2, respectively). Parameter estimation was carried out using the Sigma Plot software (version 12, Systat Software Inc.). The rate of starch digestion was expressed as the percentage of total starch present in sample hydrolyzed at different times.

$$C_g = C_{g\infty}\,(1 - e^{-K_g\,t}) \qquad (1)$$

$$C_i = C_0 + C_{i\infty}\,(1 - e^{-K_i\,t}) \qquad (2)$$

Where parameters from oral-gastric digestion are identified with subscript *g* and from the intestinal phase with *i*; *C* is the percentage of starch hydrolyzed at time *t* during digestion, C_∞ is the percentage of starch hydrolyzed at infinite time, *K* is the kinetic constant and C_0 is the percentage of starch hydrolyzed at the beginning of the intestinal phase.

Starch classifications based on Englyst et al., [21] were also determined.

<u>2.2.3.4.- Protein digestibility</u>

The protein digested during *in vitro* method was measured in duplicate by OPA method according to Nielsen *et al.* [22] using OPA reagent (P1378, Sigma-Aldrich) and, serine as standard and deionized water for blank value.

<u>2.2.3.5.- Potentially bioaccessible polyphenols and their dialyzability and antioxidant activity</u>

Potentially bioaccessible polyphenols and antioxidant activity was assessed by analyzing an aliquot at the end of *in vitro* digestion of gluten-free pasta samples as was indicated in Bustos et al. [20].

2.2.4.- Statistical analysis

Two batch of white and brown sorghum pasta were made and two lots of commercial pasta was analyzed in duplicate each one. Results of each analysis were compared by DGC means-comparison test [23], using multivariate analysis of conglomerates in a matrix obtained from the sample mean. This allowed samples to be grouped according to descending levels of preference (A, B and C) and with a degree of significance of 95 %. For these, the InfoStat Statistical software was used. Pearson correlation coefficients (*r*) were calculated with a $p<0.05$.

2.3.- RESULTS AND DISCUSSION

2.3.1.- Determination of main compounds of nutritional interest and antioxidant activity

One way of increasing complex carbohydrate content in pasta is to incorporate dried-vegetables in formulation, as the case of commercial sample: Corn Pasta with Vegetables (CPV). Considering that, a high percentage of non-gluten products are made with corn and rice flour which have low dietary fiber content (Table II), so that, the use of partially decorticated sorghum and soy flour became a good alternative [24].

The lowest protein content was found in rice pasta probably due to lack of ingredients such as egg and albumin, although, this observation was different from declared in package due to we determined the protein content in cooked pasta (Table II) while nutritional facts are calculated in raw product. Both corn and soy pasta samples showed intermediate levels, while sorghum pasta showed the highest protein content in both samples (Table II), since inclusion of egg in formulation [7].

In recent years many researchers have focus on increasing the content of bioactive compounds with antioxidant activity in different food matrixes. One approach on this, is to determine total polyphenol content by the Folin-Ciocalteu method, which besides it showed some interferences with other food components [16] it still shows high correlation with specific antioxidant activity methods to address different mechanisms [25].

One of the remarking characteristics of sorghum flour is its high polyphenols content and antioxidant activity [9,11], thus, the study of the content of those in gluten-free pasta after cooking is particularly important. In this regard sorghum pasta samples showed the highest polyphenol content in cooked pasta (Table II). According to the supplier the only difference between Corn Pasta (CP) and CPV formulation was vegetable inclusion, leading to an increase in total polyphenol content (TPC). No significant differences were found between CP and SP.

Antioxidant activity was addressed by two methods to evaluate radical scavenging activity and reducing power mechanisms, the first predominating in all selected gluten-free pasta samples (Table II). Total polyphenol content showed a high correlation with radical scavenging activity (r=0.98, $p<0.05$) and reducing ability (r=0.99, $p<0.05$), Brown Sorghum Pasta (BSP) being the one with the highest values. On the other hand, sorghum flour and vegetable inclusion in pasta formulation lead to products with a better profile than rice, improving the options of gluten-free pasta flavors in agreement with observed by Marti et al.[26].

2.3.2.- Estimated glycemic index

The estimated glycemic index of gluten-free pasta was very similar between samples, as seen in Figure 1. Pasta made from rice and white corn showed the highest values. All samples can be classified as moderate glycemic index (IG), RP being in the limit of high glycemic index (69.8). These results are in agreement with others reported in which rice and corn pasta presented higher glycemic index than others [26,27]. Many celiac people also suffer diabetes or sugar metabolism disorders, requiring the development of gluten-free pasta products with a reduced glycemic index.

2.3.3.- *In vitro* digestion of gluten-free pasta samples

In vitro digestion of gluten-free pasta was performed to assess the extent and kinetics of starch hydrolysis that explained the estimated glycemic index observed. Table III and IV show the results found. The experimental values were fitted to equations 1 and 2 and the R^2 values of the fitted curves were above 0.97 in all cases, which demonstrates that the model described the data adequately.

The highest degree of starch hydrolysis during the oral phase of *in vitro* digestion was observed for both sorghum pasta samples, and the lowest value was found for corn pasta with vegetables (Table III). The low degree of starch hydrolysis of CPV sample could be due to the vegetable fiber that retards amylase action [28]. On the other hand, the highest degree of starch digestion from sorghum pasta is in agreement with the fact that these samples were made with a domestic extruder that generated a less compact structure [7] easily accessible for enzymes, compared to the other gluten-free pasta tested, also both containing pregelatinized corn starch.

After the oral-phase, pH was lowered and pepsin added, although some α-amylase could remain active [20,29], in agreement with the very low kinetic constant observed in comparison to intestinal phase, except for sorghum pasta (Table IV). During gastric phase, corn and soy pasta showed higher starch hydrolysis than the other samples, and the lowest values were observed for sorghum pasta samples ($p<0.05$). In addition to that observed for oral phase, the lowest level of starch hydrolysis during gastric phase was found for sorghum pasta samples, probably due to the increase in viscosity caused by hydrocolloids used in formulation, which are known to retards enzyme action [30].

The intestinal phase showed similar (although significantly different) values for starch hydrolysis for sorghum and rice pasta samples, while in soy and corn pasta it presented considerable lower values, particularly for corn pasta with vegetables addition (Table IV). These observations could be explained by the action of polyphenols released at low pH during gastric digestion, affecting the action of digestive enzymes as reported [10].

The low degree of hydrolysis observed for corn pasta with vegetables in their formulation is related to the high proportion of dietary fiber that could reduce the *in vitro* susceptibility of starch to amylase, in addition to improved health benefits as exposed. This phenomenon is associated with the change in pasta microstructure and/or the limitation of water availability for starch gelatinization generated by fiber hydration [28,30].

Additionally, total starch hydrolysis values are reported in Table IV, where RP and CPV showed the highest and lowest values, respectively. On the other hand, sorghum pasta samples presented intermediate values of starch hydrolysis which is an important nutritional characteristic beyond rice and corn pasta, the most common in market.

Another important nutritional aspect to evaluate is the extent of protein hydrolysis during *in vitro* digestion. As can be seen in Table IV, the extent of protein hydrolysis at the end of the *in vitro* method was maximum for rice pasta, probably attributed to the denaturalization of proteins during processing [26,31]. White sorghum pasta showed higher protein digestibility than the pasta made with brown sorghum flour, similarly to exposed for starch hydrolysis it is possible to consider that the same factors could also influence amino acid and peptide release such as polyphenols and fiber and tannin-protein complexes [12]. The lowest protein digestion extent was observed in soy gluten-free pasta, followed by corn pasta and corn pasta with vegetables. The low degree of starch hydrolysis in conjunction with the high level of protein digestion in soy pasta could be explained by the presence of enzyme inhibitors [32], and the inverse relation in CPV may be due to the open structure that usually generates the incorporation of vegetable fibers to the formulation, making proteins more accessible for enzymes [4]. White sorghum gluten-free pasta showed high level of protein *in vitro* digestion compared to the other selected samples, which is another important nutritional attribute that could help to improve the quality of diet for celiac people.

From starch hydrolysis curves the three fractions of starch defined according to its digestibility by Englyst et al. [21] were calculated and Table V shows the results. The applied *in vitro* digestion method is different from that proposed by those authors which is approved to substantiation of a health claim related to “slowly digestible starch in starch containing foods” and “reduction of post-prandial glycaemic responses” [33]. Although, the method used in this research is more realistic, including the same enzymes and allowed the study of the kinetic of hydrolysis of nutrients during in vitro digestion, since that, we calculated the fractions proposed by Englyst method in our curves without considering the application of health claims.

The highest fraction of digested starch corresponds to rapidly digested starch, with a depreciable amount of slowly digested starch in all non-sorghum samples (Table V). That means that a high and quick increase in blood sugar is expected after ingestion of commercial gluten-free pasta, which is a serious problem in celiac people who frequently have associated problems in sugar metabolism [24]. In this sense, it is remarkable that sorghum pasta samples have shown a good proportion of slowly digested starch, in relation to the interactions of polyphenols and proteins already mentioned or due to the addition of egg and albumin that could delay enzyme action, as in the gluten matrix.

As far as we know, there is no study concerning the effect of digestion on polyphenol content or the antioxidant activity in dialysates from gluten-free pasta. As shown in Figure 2, all products tested showed higher contents of polyphenols potentially bioaccessible than the ones observed after cooking , which means that all samples are characterized by a considerable amount of bound phenolics released during *in vitro* digestion, as reported in polyphenol-enriched bread [34]. From Figure 2 it is clear that the high content of polyphenol in CPV samples generated by the addition of vegetables showed the highest potentially bioaccessible content, while RP and CP presented the lowest values according to those observed in cooked pasta.

Scavenging activity measured with ABTS radical cation showed no differences between cooked pasta content and bioaccessible fraction in both sorghum pasta samples; yet, all other gluten-free pasta samples showed around twice the content of the cooked pasta. Similar results were found in ferric reducing ability that was three times reduced after sorghum pasta digestion and increased in all other gluten-free pasta samples (Figure 2). These observations could be explained due to the fact that both types of sorghum pasta showed the lowest increase in polyphenols after digestion (2.9 and 2.4-times), leading to no modification in scavenging activity at the end of digestion and a decrease in ferric reducing capacity. In sorghum most polyphenols are esterified to cell wall components and could be extracted in alkaline conditions [11], thus the conditions used in our *in vitro* digestion model were mild, producing polyphenol release. Another possible explanation is that polyphenols interact with pasta matrix in phenolic-enriched bread, as described by Świeca *et al.* [34]. On the other hand, the commercial gluten-free pasta tested showed high release of polyphenol that probably contributed to the increase in antioxidant activity (Figure 2). These observations indicate that phenolics from the pasta samples analyzed were highly bioaccessible *in vitro*.

Dialyzability values indicate that the potentially bioaccessible polyphenols from the evaluated gluten-free pasta were poorly dialyzable *in vitro* (Figure 2). In this sense, the highest values were found for both types of sorghum pasta with 8% of bioaccessible compounds being dialyzable, whereas the lowest value was determined for SP (<1% of bioaccessible content).

Considering the antioxidant activity in dialyzable fraction, the values were much lower than those potentially bioaccessible, as observed in total polyphenol content. Scavenging activity was minimum for RC and SP (32% and 20% of bioaccessible activity, respectively) and maximum in CPV with 68% of activity in dialysate compared to potentially bioaccessible activity. Sorghum pasta showed intermediate values with 48% and 36% of activity compared to those detected after digestion for white and brown sorghum pasta samples, respectively. Finally, CPV pasta showed the maximum value of reducing power in dialyzable fraction (16% with respect to bioaccessible activity) and WSP and BSP intermediate values with 19% and 22% with respect to potentially bioaccessible activity.

Dialyzability results showed that bioactive compounds with antioxidant activity are poorly potentially bioavailable *in vitro*, indicating that the polyphenols released during *in vitro* digestion are not able to permeate the dialysis tube probably due to polarity, size or interaction with the food matrix [13,34].

It is clear that the food matrix affected potentially bioaccessible and dialyzable compounds; however, its mechanism remains unclear and have to be thoroughly studied. Particularly, gluten-free products usually include hydrocolloids in their formulation which probably increase viscosity during *in vitro* digestion, delaying the passage of compounds throughout the dialysis tube. These results indicated that sorghum pasta samples showed greater potentially bioaccessible polyphenols, scavenging activity and reducing power, in addition to higher dialyzable values than those compared to rice and corn pasta which are the most common available gluten-free pasta.

3.4 - CONCLUSIONS

Despite the growing population of celiac people that must follow a gluten-free diet, there are few publications available on gluten-free food digestibility attributes. Our research showed that pasta based on sorghum flour presented not only good technological and sensorial quality, as we reported previously [7], but also remarkable nutritional attributes. Results indicated that the sorghum flour used to produce gluten-free pasta conferred interesting nutritional attributes to the commercial pasta products tested, particularly considering starch hydrolysis rate and extent, which increased the level of slowly digested starch. White and brown sorghum cooked pasta had higher radical cation scavenging activity and ferric reducing ability than other tested samples in relation to the highest Total Polyphenol Content determined. Moreover, polyphenols were potentially high bioaccessible with good dialyzability, i.e., white and brown sorghum pasta showed intermediate levels of bioaccessible polyphenols and the lowest ferric reducing capacity; however, it showed the maximum radical scavenging activity. Considering dialyzability results, the selection of white or brown sorghum flour to develop gluten-free pasta samples generated the highest level of potentially available polyphenols with high scavenging activity, without significant differences between sorghum varieties. Finally, the rice pasta presented the highest value of estimated glycemic index followed by CP, while the other samples showed slightly lower values. Further clinical experiments are required to establish whether similar trends can also be observed *in vivo*.

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Table I.- Nutritional value for sorghum and commercial gluten-free pasta per portion (80 g).

Sample	Ingredients	Energy (kcal) – (kJ)	Fat (g)		Carbohydrate	Fiber	Protein
			Total	Saturated	(g)	(g)	(g)
WSP	White sorghum decorticated flour, egg albumen, egg powder, pregelatinized corn starch	295 (1235)	5	-*	51	5.1*[1]	14.8
BSP	Brown sorghum decorticated flour, xanthan gum, egg albumen, egg powder, pregelatinized corn starch	315 (1318)	5.3	-*	57.2	7.3*[1]	13.0
RP	Rice flour, water, egg and beta-carotene	273 (1147)	0	0	62	0.7	5.3
SP	Soy flour, maize and cassava, egg, salt and beta-carotene	292 (1239)	2.8	1.2	58	0.8	8.8
CP	White corn (var. Capia) and corn starch	204 (863)	3.3	0.5	41	3.4	2.6
CPV	White corn (var. Capia), corn starch and dehydrated celery, onion and leek	205 (865)	4.3	0.5	41	3.6	2.7

* Not determined. *[1]: dietary fiber values. WSP: white sorghum pasta, BSP: brown sorghum pasta, RP: rice pasta, SP: soy pasta, CP: corn pasta, CPV: corn pasta with vegetables.

Table II.- Dietary fiber, protein, total polyphenols and antioxidant activity of cooked gluten-free pasta.

Gluten-free pasta sample	Total Dietary Fiber (g kg^{-1} cooked pasta db.)	Protein content (g kg^{-1} cooked pasta db.)	Total Polyphenol Content (g GA kg^{-1} cooked pasta db.)	$ABTS^{•+}$ radical cation scavenging activity (mmol Trolox kg^{-1} cooked pasta db.)	Ferric reducing ability (g GA kg^{-1} cooked pasta db.)
WSP	64 ± 3^{b}	174 ± 0 ^{f}	2.41 ± 0.07^{d}	8.19 ± 0.41^{d}	0.571 ± 0.034^{e}
BSP	91 ± 4^{d}	162 ± 1^{e}	2.88 ± 0.05^{e}	11.29 ± 0.35^{e}	0.721 ± 0.011^{f}
RP	31 ± 3^{a}	22 ± 1^{a}	0.37 ± 0.01^{a}	1.33 ± 0.01^{a}	0.063 ± 0.004^{b}
SP	78 ± 4^{c}	60 ± 2^{c}	1.37 ± 0.03^{c}	5.46 ± 0.16^{c}	0.243 ± 0.007^{d}
CP	34 ± 3^{a}	91 ± 2^{d}	0.52 ± 0.02^{b}	3.08 ± 0.02^{b}	0.034 ± 0.002^{a}
CPV	104 ± 5^{e}	52 ± 1^{b}	1.31 ± 0.03^{c}	5.10 ± 0.27^{c}	0.209 ± 0.004^{c}

db.: dry basis. WSP: white sorghum pasta, BSP: brown sorghum pasta, RP: rice pasta, SP: soy pasta, CP: corn pasta, CPV: corn pasta with vegetables. *Values with different letters within the same column indicate significant differences (P<0.05).

Table III.- Starch hydrolyzed during oral *in vitro* digestion of gluten-free pasta*

Gluten-free pasta sample	Starch hydrolyzed (%)
WSP	5.9 ± 0.2[c]
BSP	5.2 ± 0.2[c]
RP	3.2 ± 0.8[b]
SP	3.1 ± 0.9[b]
CP	4.0 ± 0.4[b]
CPV	2.7 ± 0.4[a]

WSP: white sorghum pasta, BSP: brown sorghum pasta, RP: rice pasta, SP: soy pasta, CP: corn pasta, CPV: corn pasta with vegetables. *Different letters in the columns indicate significant difference $P<0.05$.

Table IV.- Adjusted parameters obtained with kinetic equations for starch and protein hydrolysis during *in vitro* oral-gastric and intestinal phases of gluten-free pasta*

Gluten-free sample	Starch hydrolyzed at oral-gastric phase (%) (C_g)[1]	Kinetic constant at oral-gastric phase (min $^{-1}$) (K_g)[1]	Initial starch concentration at intestinal phase (%) (C_0)[2]	Starch hydrolyzed at intestinal phase (%) (C_i)[2]	Kinetic constant at intestinal phase (min $^{-1}$) (K_i)[2]	Total starch hydrolysis (g kg^{-1} of starch)	Protein digested (%)
WSP	24.9 ± 1.1^{b}	0.025 ± 0.002^{a}	25.3 ± 0.9	30.0 ± 0.8^{c}	0.029 ± 0.002^{a}	550 ± 9^{b}	86 ± 4^{d}
[illegible]SP	20.8 ± 0.3^{a}	0.044 ± 0.007^{c}	23.9 ± 0.3	33.2 ± 0.5^{d}	0.017 ± 0.002^{a}	543 ± 6^{b}	66 ± 2^{c}
RP	37.6 ± 0.6^{d}	0.030 ± 0.001^{b}	36.9 ± 1.6	35.9 ± 1.1^{e}	0.115 ± 0.012^{b}	735 ± 14^{d}	100 ± 3^{e}
SP	43.7 ± 1.0^{f}	0.026 ± 0.002^{a}	41.9 ± 1.5	23.0 ± 1.5^{b}	0.229 ± 0.009^{c}	667 ± 14^{c}	19 ± 2^{a}

CP	42.1 ± 1.0^{e}	0.023 ± 0.004^{a}	40.1 ± 0.8	23.5 ± 0.6^{b}	0.230 ± 0.007^{c}	656 ± 13^{c}	34 ± 3^{b}
CPV	34.2 ± 0.9^{c}	0.020 ± 0.001^{a}	30.3 ± 1.1	14.6 ± 0.9^{a}	0.119 ± 0.015^{b}	487 ± 5^{a}	64 ± 4^{c}

Note: [1]Parameters of the kinetic equation $C = C_g\ (1 - e^{-K_g t})$. [2]Parameters of the kinetic equation $C = C_0 + C_i\ (1 - e^{-K_i t})$. Parameter C_0 has no statistical analysis since it is equivalent to $C_{g\infty}$ and values are presented to demonstrate the accurate adjustment of both equations. WSP: white sorghum pasta, BSP: brown sorghum pasta, RP: rice pasta, SP: soy pasta, CP: corn pasta, CPV: corn pasta with vegetables. *Different letters in the columns indicate significant difference $P<0.05$.

Table V.- Rapidly (RDS), slowly (SDS) and resistant digestible starch (RS) fractions in gluten-free pasta tested*.

Gluten-free pasta sample	Rapidly Digested Starch (g kg^{-1} pasta)	Slowly Digested Starch (g kg^{-1} pasta)	Resistant Starch (g kg^{-1} pasta)
WSP	384 ± 4^{b}	160 ± 5^{d}	456 ± 5^{c}
BSP	333 ± 9^{a}	194 ± 3^{e}	473 ± 9^{c}

RP	692 ± 24^{e}	37 ± 10^{c}	271 ± 20^{a}
SP	647 ± 16^{d}	2 ± 0^{a}	351 ± 16^{b}
CP	633 ± 5^{d}	2 ± 0^{a}	365 ± 5^{b}
CPV	435 ± 6^{c}	14 ± 4^{b}	552 ± 4^{d}

WSP: white sorghum pasta, BSP: brown sorghum pasta, RP: rice pasta, SP: soy pasta, CP: corn pasta, CPV: corn pasta with vegetables. *Different letters in the columns indicate significant difference *P*<0.05.

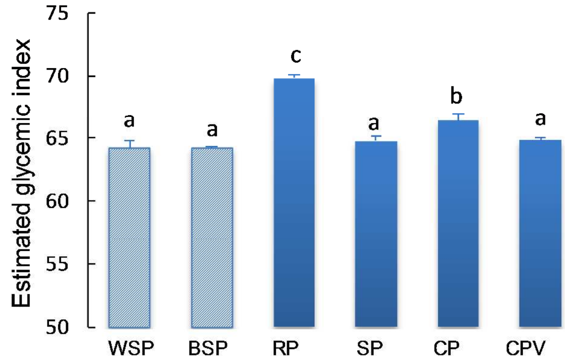

Figure 1.- Estimated glycemic index of gluten-free pasta samples.

*Different letters in the columns indicate significant difference *P*<0.05. Non-stripped bars indicate commercial gluten-free pasta.

WSP: white sorghum pasta, BSP: brown sorghum pasta, RP: rice pasta, SP: soy pasta, CP: corn pasta, CPV: corn pasta with vegetables.

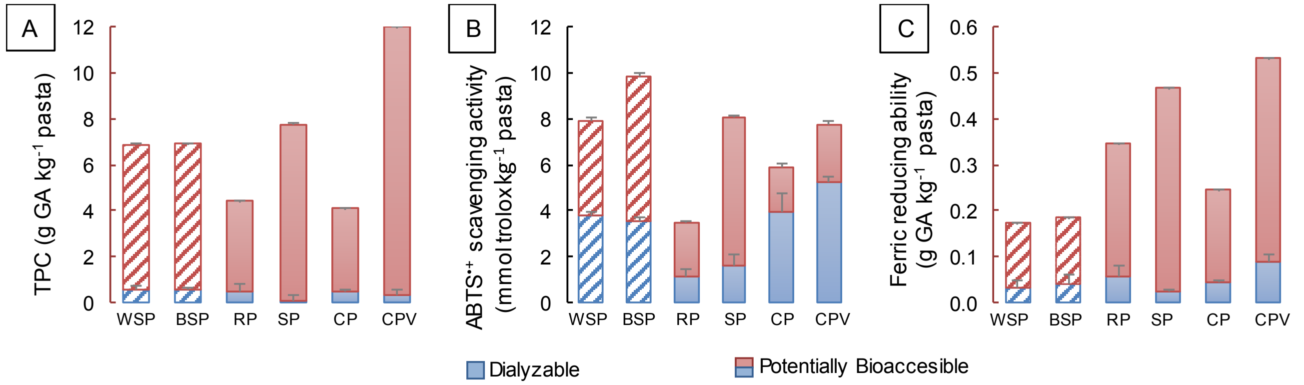

Figure 2.- Potentially bioaccessible and dialyzable total polyphenol content (TPC, A), ABTS$^{\bullet +}$ radical cation scavenging activity (B) and ferric reducing ability (C).

Non-stripped bars indicate commercial gluten-free pasta. Low error in some samples made the bars not appreciable.