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Nutritional characterization of gluten free non-traditional pasta

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Abstract
When a food is formulated, its characterization is important from the chemical and biochemical point of view; even more when non-traditional raw materials are used. Noodles were made with cassava starch and corn flour (4:1), milk, egg, salt and xanthan gum. The chemical composition of the pasta was determined and the total and resistant starch content was quantified. The hydrolysis rate of the starch was measured at different times, from which the hydrolysis index and, subsequently, the predictive glycemic index was calculated. The chemical composition of the noodles showed its high content of total fibers. From the digestibility tests, high values were obtained for proteins (93%), and average values for the starch (52%). The results of the starch hydrolysis kinetics showed a higher proportion of slowly digestible starch with a low glycemic index (46%). Analyzed noodles are within the dietary guidelines that suggest a diet with high total dietary fiber content and low glycemic index.

Keywords: starch digestibility; predictive glycemic index; noodles non-traditional; cassava starch; chemical composition

1. Introduction
Digestibility is a parameter used to measure the nutritional value of different foods, because it is not enough that a nutrient is in high percentages, since it must be digestible so that it can be assimilated and, consequently, used by the organism. From the nutritional point of view, the starches present in food are hydrolyzed and absorbed as glucose in the intestine, while the proteins are digested depending on their origin and food processing before its ingestion, giving as final products of the digestion mainly free amino acids and some dipeptides and tripeptides.

The degree of digestion and absorption of available carbohydrates is affected by several factors: food processing (Jenkins *et al*., 1986, Berti *et al*., 2004) [24, 3]; origin of starch (Gularte & Rosell, 2011) [17]; different dietary matrices with varied physical structures that imply different rate of digestion (Matos Segura & Rosell, 2011, Berti *et al*., 2004) [27, 3]; presence of additives in the formulation (Susanna & Prabhasankar, 2013) [40], which influence the availability of physical access of enzymes to the substrate (Singh, Dartois & Kaur, 2010; Horstmann, Lynch & Arendt, 2017) [39, 22].

The protein digestibility in a food is defined as the proportion of nitrogen in the food that is absorbed after digestion. This can be affected by various anti-nutritional factors: fiber, tannins and phytates that could intervene with proteins or some minerals (Rayas Duarte, Mock & Satterlee, 1996) [35] and due to the effect of thermal processing (Hamaker *et al*, 1987) [18].

Englyst *et al*. (1992) [7] established a classification of the starch fractions, according to their digestion rate: the first fraction that is digested is the rapidly digested starch (RDS), between 20 and 120 minutes corresponds to the slowly digested starch (SDS) and finally, the fraction called resistant starch (RS) that corresponds to what has not been enzymatically digested. The product of digestion is absorbed in the duodenum and proximal regions of the small intestine; RDS rapidly increases glucose levels, while SDS produces a slow and steady increase in postprandial blood glucose levels (Zhang & Hamaker, 2009) [45].

In vitro starch digestibility, is a predictive parameter of the physiological effects of a particular food, measures the susceptibility of starch to digestive enzymes and is commonly used as a rapid method to predict the in vivo glycemic index. The glycemic index (GI) was developed in an attempt to systematically classify the carbohydrates of different foods, according to the effects integrated over time on postprandial glycemia (Jenkins *et al*., 2002) [25]. Foods with high GI values are those that are digested and absorbed quickly, which corresponds to higher proportions of RDS and causes a greater fluctuation of blood glucose per unit of carbohydrates than those foods with lower GI values, which are related to higher proportions of SDS (Brand-Miller *et al*., 2009) [5] and even to RS (Shamai, Bianco-Peled & Simon, 2003, Englyst & Hudson, 1996) [38, 8]. GI is methodologically defined as the incremental area under blood glucose response curve after the consumption of a food in relation to that produced by a reference food, in an equi-carbohydrate portion (50 or 25 g); to this, Brand-Miller & Foster Power (1999) [4] classified the GI of food as low (≤55 g/100 g), medium (56-69 g/100 g) and high (≥70 g/100 g), based on a known concentration of glucose.

Investigations made in pasta with wheat flour or mixtures with other flours, without added additives (Tudorica, Kuri & Brennan, 2002, Osorio-Díaz *et al*., 2008, Goñi & Valentín Gamazo, 2003, Brandt Miller *et al*., 2009) [41, 31, 14, 5], which have a matrix of proteins and carbohydrates that, according to Zhang & Hamaker (2009) [45], limit the access of amylolitic enzymes to the starch components, concluded that they

produce a low postprandial response to blood glucose.

It is important to know the chemical composition and digestibility when a food is formulated, as well as its glycemic index, even more when non-traditional ingredients are used. Hydrocolloids are ingredients added to the food base mix, widely used in food technology as emulsifying agents and, among other functions, to replace gluten in the formulation of bread and pasta elaborated with flours other than wheat (Rojas, Rosell & Benedito, 1999; Gómez *et al*., 2007) [36, 11]. Preichardt *et al* (2011) [34] reported that xanthan gum is among the most used hydrocolloids in the farinaceous baked industry; on the other hand, according to Chung *et al*., (2007) [6], most hydrocolloids are soluble in water, but rarely digested in the intestine, which could retard the starch digestibility in the upper region of the human intestine, providing health benefits. This study was focused on the chemical and biochemical characterization of noodles made from cassava starch, focusing on proteins and starch digestibility, which are critical parameters to determine the nutritional quality of product.

2. Materials and methods

2.1 Ingredients and chemical reagents

All ingredients used for noodles preparation were purchased in local markets and are national brands: cassava starch (Montecarlo), corn flour (Indelma), whole milk powder (Ilolay), salt (Dos Anclas), vegetable fat (Margadan), xantan gum (XG, Parafarm). The chemical reagents were amyloglucosidase (A7420), α-amylase (A3176) and pancreatin (P3292) from Sigma Aldrich; Pepsin, Sodium phosphate dibasic and monobasic and trichloroacetic acid (Biopack), HCl, KOH and Sodium Acetate (Cicarelli), Acetic acid (Raudo), commercial kit K-TDFR-200A (Megazyme) and glucose oxidase-peroxidase assay kit (GOD-POD, Wiener Lab).

2.2 Formulation of pasta

Noodles were made with an own formulation (Milde *et al*, 2009; Milde, Ramallo & Puppo, 2012) [28, 29] optimized for their production, composed of cassava starch and corn flour in a 4:1 proportion, whole milk powder (7 g/100 g); salt (0.5 g/100 g) and XG (0.6 g/100 g). To this solid mixture, vegetable fat (3.5 g/100 g), whole egg (31 g/100 g) and water were added to form homogeneous dough that allowed its lamination and cutting (spaghetti) with Pastalinda brand laminator. As a control, the same formulation without the addition of XG was used. For determinations, noodles were previously cooked in boiling water during their optimal cooking time (5 min).

2.3 Chemical composition

The determinations were made with the Official Method of the Association of Official Analytical Chemists (AOAC, 1995) [2]: ash (923.03), fat (922.06), protein (984.13, nitrogen factors of 6.25); total dietary fiber (TDF, 991.43, kit K-TDFR-200A), phosphorus (P) with the ascorbic acid method and moisture contents (925.09). Calcium (Ca) was evaluated by the titration method with EDTA, NOM-187-SSA1/SCFI-2002.The determinations were made in triplicate.

2.4 *In vitro* protein digestibility

The samples were incubated with pepsin and pancreatin according to the method described by Akeson & Stahmann (1964) [1], with modifications. 2 g of cooked noodles was weighed, then crushed with a mortar to simulate chewing and a solution of pepsin in 0.1 mol equiv/L HCl (37 ºC, 3 h) was added. The samples were incubated with a solution of pancreatin in 0.2 mol/L phosphate buffer and sodium azide was added to avoid contamination during the incubation period (37 ºC, 24 h, pH 8). Finally, 20 g/100 g trichloroacetic acid was added and centrifuged (30 min, 3000 g). The total protein concentration was studied from the supernatant by the Kjeldahl method, on a dry basis. The determinations were made in triplicate.

To calculate the digested noodle proteins digestibility with respect to those that have not been digested, the following formula was used:

$$\text{Proteins digestibility} = \frac{\text{total proteins of digested noodle}}{\text{total proteins of undigested noodle}} \times 100 \quad (1)$$

2.5 *In vitro* starch digestibility

To determinate the *in vitro* starch digestibility, two methods were necessary. To quantify the fraction of total starch (TS) the method of Holm *et al*. (1986) [20] was used and for the resistant starch (RS) the method of Goñi *et al*. (1996) [12] was applied, both with some modifications. 1 g of cooked noodles was weighed, then crushed with a mortar for 1 min to simulate chewing. A solution of pepsin in 0.1 mol equiv/L HCl was added for the hydrolysis of the present proteins (40 °C, 60 min, pH 1.5 in a shaking water-bath). A solution of α-amylase in 0.2 mol/L phosphate buffer (37 °C, 16 h, pH 6.9) was incorporated. To determinate TS, 2 mol/L KOH was added (room temperature, 30 min with stirring), then a solution of amyloglucosidase was added in 0.4 mol/L sodium acetate buffer (60 °C, 45 min, pH 4.7). To quantify the fraction of RS, once the incubation with α-amylase was over, the samples were centrifuged, discarding the supernatant. It was suspended with 2 mol/L KOH (room temperature, 30 min, with stirring). Finally, a solution of amyloglucosidase was added in 0.4 mol/L sodium acetate buffer (60 °C, 45 min, pH 4.7). The samples were centrifuged and the released glucose of the supernatant was measured using the GOD-POD kit. The result obtained was multiplied by the factor 0.9 for the conversion of glucose to starch. Determinations were made in triplicate.

2.6 Starch hydrolysis kinetics and estimation of the predictive glycemic index (pGI)

The hydrolysis index (HI) of starch at different times was measured following the method of Goñi, Alonso & Calixto (1997) [13] with modifications. 1 g of cooked noodles was weighed; a solution of pepsin in 0.1 mol equiv/L HCl (40 °C, 60 min, pH 1.5 in a shaking water-bath) was added. After, a solution of α-amylase in 0.2 mol/L phosphate buffer (37 °C, pH 6.9 in a shaking water-bath) was added, aliquots of 1 mL of the sample were taken at different times (30, 60, 90 and 120 min), these were placed in a bath at 100 °C and agitated vigorously for 5 min to inactivate the enzyme. Tubes were kept in refrigeration until the end of the incubation time. A

solution of amyloglucosidase in 0.4 mol/L sodium acetate buffer (60 °C, 45 min, pH 4.7 in a shaking water-bath) was added to each tube. They were centrifuged for 15 min at 3000 g; from the supernatants, the glucose concentration was determined by the GOD-POD kit in each tube. As control, Goñi & Valentín-Gamazo (2003) [14] used white bread with wheat flour and their hydrolysis values were taken as reference for the present work. The hydrolysis curves were graphed as the percentage of hydrolyzed total starch at different times (30, 60, 90 and 120 min). The in vitro digestion kinetics was described, adjusting the curves to a first order model, established by Goñi, Alonso & Calixto (1997) [13], according to the equation:

$$C = C_{\infty}\left(1 - e^{(-k\,t)}\right) \quad (2)$$

where C is the concentration at a time t, C∞ is the equilibrium concentration; k is the kinetic constant and t is a specific time.
The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the noodles (between 30 and 120 min) by the area under the reference bread curve:

$$HI = \frac{\text{Area under the noodle curve}}{\text{Area under the reference bread curve}} \times 100 \quad (3)$$

The predictive glycemic index (pGI) was calculated using the empirical equation proposed by Granfeldt (1994) [15]:

$$pGI = 0,862\,HI + 8,198 \quad (4)$$

2.7 Statistical analysis

Results obtained from the chemical composition and digestibility were expressed as mean values and standard deviation of three repetitions. Data were analyzed using one-way analysis of variance (ANOVA) to determine the significance of mean differences between groups, by Statgraphics plus 5.1 software, with a significant level of $P < 0.05$ by Fisher's test. The hydrolysis curves of the analyzed samples were plotted using Microsoft EXCEL software and the adjustment of them was carried out through a non-linear regression in Statgraphics.

3. Results and Discussion

3.1 Chemical composition

The addition of XG to the formulation studied positively influenced the values obtained for the chemical composition of the noodles based on cassava starch and corn flour (Table 1). Its presence could fortify the matrix of the food, avoiding the loss of nutrients to the cooking water (Granito, Torres & Guerra, 2003; Yalcin & Basman, 2008; Susanna & Prabhasankar, 2013) [16, 43, 40].

Table 1: Noodles centesimal composition (g/100g)

Noodle	Moisture	Protein*	Ash*	TDF*	Fat*	Ca*	P*
Control	74.0±0.5 [a]	4.7±0.1[a]	1.1±0.1[a]	7.5±0.7[a]	1.6±0.1[a]	164.0±12.0[a]	189.5±4.4[a]
With XG	73.4±0.5[a]	5.5±0.6[b]	1.6±0.1[b]	9.7±0.2[b]	1.6±0.1[a]	182.0±8.5[a]	197.0±3.8[a]

Control: Noodles without XG. Different letters in the same column indicate significant differences (P< 0.05).
* % db: percentage on dry basis.

Other researchers analyzed the chemical composition of gluten free pastas made with different flours (rice, corn), which were replaced with different proportions of bean flours, broad beans, quinoa (Giuberti *et al*., 2015; Giménez *et al*, 2013) [10, 9]; they differ mainly in the high protein content with respect to the present work. While in total dietary fiber content, the results obtained were high compared with other authors (Giménez *et al*, 2013; Zandonadi *et al*, 2012) [9, 44].
The statistical analysis of the micronutrients showed that there are no significant differences (p>0.05) in the concentration of Ca and P; both minerals are very united forming complexes with the milk casein, who contributes them in the analyzed noodles.

3.2 *In vitro* protein digestibility

When analyzing the total protein values of the undigested noodles and after being digested, statistically significant differences ($P < 0.05$) were found between the analyzed noodles (Table 2).

Table 2: Average values standard deviations of total proteins from undigested samples and digested samples, without and with added XG.

Noodles	Total protein from not-digested noodles*	Total proteins of digested noodles*
Control	4.7 ± 0.1[a]	2.9 ± 0.1[a]
With XG	5.5 ± 0.6[b]	5.1 ± 0.5[b]

Control: Noodles without XG. Different letters in the same column represent a statistically significant difference ($P < 0.05$).
* % d.b: percentage on dry basis.

In vitro protein digestibility of analyzed noodles was calculated using equation (1) which resulted in: 62.7 g/100 g of digested proteins for control noodles and 92.7 g/100 g of digested proteins for samples of noodles with XG. The value obtained from the in vitro protein digestibility for noodles elaborated with the addition of XG was similar to that found by Susanna & Prabhasankar (2013) [40] when they analyzed wheat flour pastas (91.34 g/100 g) and gluten free pastas with XG (95.18 g/100 g).
Giménez *et al*. (2013) [9] developed pastas with different flours (corn, quinoa, broad beans and their mixtures); other researchers (Herken *et al*, 2006; Rayas-Duarte, Mock & Satterlee, 1996) [19, 35] studied pasta with wheat flour substituted with seed flours; obtained protein digestibility

values in vitro lower than those reached in the present work.

3.3 *In vitro* starch digestibility

The noodles with XG added had higher starch content which was retained in the food matrix (Table 3). However, the values obtained for RS, were not statistically different between noodles made without and with the XG addition, proving that the starch-protein interactions that make inaccessible to enzymatic attack during digestion, also prevent the cooking loss and remain independently of the XG presence.

Table 3: Mean values ± standard deviation of total starch (TS), resistant starch (RS), available starch (AS) in cooked noodles with and without added XG.

Noodles	TS*	RS*	AS*
Control	32.7 ± 3.9[a]	5.8 ± 1.3[a]	26.9 ± 2.7[a]
With XG	51.9 ± 3.6[b]	5.1 ± 0.4[a]	46.9 ± 3.2[b]

Control: noodles without GX. Different letters in each column denote statistically significant differences ($P < 0.05$).
* % d.b: percentage on dry basis.

Different authors (Rayas-Duarte, Mock & Satterlee, 1996; Larrosa *et al*, 2016) [35, 26] reported that during the cooking of pastas is produced the leaching of components to cooking water, mainly amylose, other polysaccharides (not starch), proteins and even some minerals.

Osorio-Díaz *et al*. (2014) [32] studied noodles of wheat flour substituted with different concentrations of banana flour; found an increase in the content of TS and RS and a decrease in AS with the addition of higher proportions of banana flour, also observing higher cooking loss. Susanna & Prabhasankar (2013) [40] characterized gluten free pastas made with different flours (soy, chana, sorghum) with the addition of additives (xanthan gum, guar, HPMC) observed that in some cases there were no significant differences when incorporating additives. These results differ from those obtained in the present work.

3.4 Starch hydrolysis kinetics and estimation of the predictive glycemic index (pGI)

The noodles analyzed were digested significantly more slowly than in the case of the reference white bread (Figure 1), coinciding with the results obtained for other researchers (Osorio-Díaz *et al*., 2003; Goñi & Valentin-Gamazo, 2003; Grandfelt, 1994) [30, 14, 15]. This could be a consequence of several factors: matrix with different ingredients resulting in a complex formulation (Schakel *et al*., 2008) [37]; physical condition or microstructure of starch (Parada & Rozowski, 2008) [33]; amylose/amylopectin ratio (Wolever, 1990) [42].

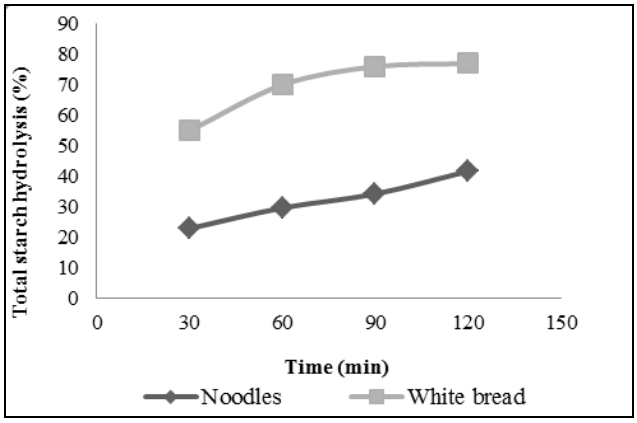

Fig 1: *In vitro* total starch hydrolysis curve in function of time in cooked noodles and reference white bread.

According to the starch hydrolysis curve presented and considering the classification of Englyst *et al*. (1996) [8], the values obtained are 22.9 g/100 g for the RDS and 41.6 g/100 g for SDS. This indicates that noodles elaborated from cassava starch and corn flour have a higher proportion of slowly digestible starch.

Studies conducted by Hong *et al*. (2015) [21] about the influence of an additive (guar gum) with starch concluded that high proportions of guar gum in relation to starch (1:20) prevents the action of enzymes and therefore, decreases the rate of hydrolysis, obtaining more SDS and RS; while lower relations between them (1:80-1:90) favors the enzymatic interaction contributing more RDS and SDS. In the study presented, the XG/starch relation used, stimulate the formation of a loose microstructure, with greater molecular mobility, with weaker hydrogen bonding interactions, which allowed an enzymatic action that generated a greater proportion of SDS.

According to Gularte & Rosell (2011) [17], those who study the effect of the addition of hydrocolloids on different starches (corn and potatoes), observed that when they added XG, the RDS values increased significantly regarding to the control (without hydrocolloids); however, the values of SDS remained constant in the case of potato starch and decreased in corn starch; results that differ from those found in this research.

The primary and secondary parameters derived from the in vitro digestion kinetics of noodles and white bread are listed in Table 4. These parameters include the hydrolysed starch equilibrium concentration (C∞), the kinetic constant (k), the hydrolysis index (HI) and the predictive glycemic index (pGI).

Table 4: Equilibrium concentration (C_∞), kinetic constant (k) hydrolysis index (HI) and predictive glycemic index (pGI) for noodles in relation to white bread.

Samples	C_∞*	K*	%HI	pGI (%)**
Noodles	42.2	0.02	42.25	45.95
Reference white bread	78.19	0.04	100	94

*C_∞ and k were determined by the equation $C = C_\infty(1-e^{-kt})$.
**pGI was calculated from equation proposed by Granfeldt (1994).

To describe the hydrolysis curve for the noodles, was chosen an exponential model of the first order, with a correlation coefficient R^2= 0.98. The value of C_∞ represents the maximum concentration of hydrolysis when it has reached a plateau. The values of C_∞ for the studied noodles were lower than those of the reference white bread. Gularte & Rosell (2011) [17], observed significant differences in the C_∞ values between potato starch and corn starch and their mixtures with different hydrocolloids, but did not show a general tendency based on the levels of hydrocolloids added. They concluded that, depending on the hydrocolloid-starch interaction, the enzymatic action could be facilitated or not. The kinetic constant (k), which reflects the hydrolysis rate at the beginning of the reaction, is lower for the studied noodles than for the reference bread, which would indicate a slower digestion.

Brand-Miller & Foster Powell (1999) [4] classification, showed values lower than 55 g/100 g of GI corresponds to a low glycemic index; in later studies, Brand Miller *et al*. (2009) [5] found that pasta has low GI (43 g/100 g) when they compared the postprandial glycemic responses with that of different foods. The results obtained in the present work coincide with these authors.

Jang, Bae & Lee (2015) [23] found that the incorporation of different hydrocolloids in formulations of noodles with common and whole wheat flour produced a decrease in pGI, but not when they evaluated with buckwheat flour; they expressed that the starch digestibility and the quality of the noodles are affected by the nature of the flours used, the levels and type of hydrocolloids included, and the relation between the flour and the hydrocolloid.

4. Conclusions

Noodles made with a mixture of cassava starch and corn flour showed a significant percentage of TDF, high protein digestibility and a higher proportion of slowly digestible starch (SDS). The hydrolysis rate of the noodles was low, which is an indicator of the capacity of the developed pastas to generate low glycemic responses. It was demonstrated that chemical composition and digestibility, as well as the pGI, depend on the formulation, the processing, the cooking of the food and the presence or not of hydrocolloids, which can significantly affect the accessibility of the digestive enzymes. The pastas studied are within the dietary guidelines that suggest a diet rich in fibers and slow-digesting carbohydrates that produce a low glycemic index; they are also an option for people with celiac disease. All this makes the present formulation into a food product of interest to the industry, because it provides a direct application of cassava starch, which is considered a product of low commercial value. It also provides advantages to both farmers and pasta producers, as it represents a possibility to diversify production and expand in the market.

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