**Starch and antioxidant compounds release during *in vitro* gastrointestinal digestion
of gluten-free pasta**

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Abstract

The microstructure produced in cooked gluten-free pasta depends of the ingredients used and it affect the starch hydrolysis, the release of phenolic compounds and their antioxidant capacity. The aim of this study was to evaluate the starch hydrolysis and bioaccessibility of antioxidant compounds during *in vitro* gastrointestinal digestion of

gluten-free pasta and its relationship with the microstructure. The highest starch hydrolysis was in the intestinal phase, but pasta with the highest content of unripe plantain and chickpea presented the lowest released of phenolics. The insoluble dietary fiber in the pasta can be responsible. The cooked pasta showed high antioxidant capacity in the intestinal phase. Regions with gelatinized starch granules in a less dense protein network, and other ones with intact or swollen starch granules surrounded by and extensive structural protein network were observed. The starch digestion and bioaccessibility of antioxidant compounds were related to the structure of the gluten-free matrix.

Key words: antioxidants; gluten-free pasta; microstructure; starch; simulated digestion.

1. Introduction

Pasta is a highly consumed product that is made from wheat semolina, which has been appreciated for its relatively low glycemic index (GI); *in vitro* and *in vivo* studies demonstrated that starch digestion of pasta is lower compared to other foods made with starchy flours (Zou, Sissons, Gidley, Gilbert, & Warren, 2015). The mechanism by which pasta presents this behavior is attributed to two aspects of its structure. The first one is that semolina pasta presents a compact and relatively dense microstructure, which limits the surface of the digestive enzymes that can access to the available starch (Jenkins, Wolever, Jenkins, Lee, Wong, & Josse, 1983) and prevents the starch granules from swelling during cooking (Heneen & Brismar, 2003; Sissons, Aravind, & Fellows, 2010)

and the second one is the presence of a continuous gluten matrix that traps starch granules and reduces the accessibility of the α -amylase for its hydrolysis (Sissons et al., 2010). On the other hand, for gluten-free pasta, the starch structure (e.g. B- or C-type X-ray diffraction pattern), the type of proteins that can produce a network and the phenolics compounds, can affect the starch digestibility. This effect can be ascribed to some interactions, arrangements of these components in the food matrix and inhibition of amylases by phenolic compounds (Bordenave, Hamaker, & Ferruzzi, 2014; Camelo-Mendez, Agama-Acevedo, Sanchez-Rivera, & Bello-Pérez, 2016).

Microstructurally, in a food matrix, such as cooked pasta, starch granules can be found in different morphological states. Granules may be either fully gelatinized or partially swollen and intact in the outer, central and internal regions (Heneen & Brismar, 2003). The rate of starch digestion can be characterized by measuring the hydrolyzed percentage as a function of time. One of the most used methods is the one proposed by Englyst, Englyst, Hudson, Cole, & Cummings (1999), in which fast, slow and resistant starch fractions are determined. Some authors suggest that these methods are limited in the quantitative measure of the speed of starch digestion, and cannot be used to detect constant changes in the rate of digestion. In this sense, the first-order model suggested by Goñi, Garcia-Alonso, & Saura-Calixto (1997) have been previously applied to characterize the rate of starch hydrolysis (Butterworth, Warren, Grassby, Patel, & Ellis, 2012). However, there are few studies on the relationship between the microstructure of a gluten-free pasta and its relation to the digestibility of starch and bioaccessibility of bioactive compounds.

The use of *in vitro* models has gained attention to study the behavior of the food matrix in the release, bioaccessibility and digestibility of compounds with nutritional interest. Specifically, phenolic compounds interact with the other components of the matrix and lead to the formation of complexes that directly affect their bioaccessibility (Bordenave et al., 2014). Those interactions are affected by the physicochemical properties of the food matrix, processing conditions (temperature, moisture, oxygen presence, etc.) and gastrointestinal conditions (*e.g.*, such as pH, temperature, bile salts and digestive enzymes) (Domínguez-Avila et al., 2017). Despite this, studies on the application of models to describe the release of phenolic compounds from the food matrix are still limited.

The aims of this study were: 1) to characterize microstructurally gluten-free pasta made with chickpea, blue maize and unripe plantain flours, and 2) to evaluate starch digestibility and bioaccessibility of antioxidant compounds during *in vitro* gastrointestinal digestion.

2. Material and methods

2.1. Plant material

Unripe plantain (*Musa paradisiaca* L.), chickpea (*Cicer arietinum* L.) and blue maize (*Zea mays* L.) flours were used to make gluten-free pasta. Unripe plantain, stage 2 (Aurore, Parfait, & Fährasmane, 2009), was purchased from a local market in Cuautla, Morelos, Mexico, and the flour was obtained from the pulp of the fruit following the procedure described by Ovando-Martínez, Sáyago-Ayerdi, Agama-Acevedo, Goñi, & Bello-Pérez (2009). Chickpeas were provided by Universidad de Sinaloa (Mexico) and

milled by the procedure proposed by Flores-Silva, Berrios, Pan, Agama-Acevedo, Monsalve-González, & Bello-Pérez (2015). Commercial pigmented (blue) maize was provided by the experimental field of INIFAP Texcoco, (México) during the 2012-2013 season. Maize flours were prepared according to Camelo-Méndez et al. (2016).

2.2. Spaghetti formulation and processing

Gluten-free pasta were prepared with unripe plantain, chickpea, blue maize flours (Table 1), where the proportions of each flour were selected from a previous study (Flores-Silva et al., 2014). Thirty-two mL of water and 1.5 g carboxymethylcellulose were added for each 100 g of the blend, the mixture was homogenized for 5 min to allow hydration. Then it was extruded (1.5-mm die) using a laboratory extruder (Beutelspacher, México, D.F) at 50 °C in the three heating zones (entry, medium, and outlet) to 72 rpm. The pasta was dried at 45 °C for 16 h in a forced air oven. The cooking quality was performed for each pasta sample in order to determine the optimum cooking time (OCT) (AACC 66-50 method, AACC, 2000) and the cooking loss (CL) that was determined gravimetrically by weighing the residue after evaporating the cooking water.

2.3. Confocal scanning laser microscopy (CSLM)

Samples were analyzed using Confocal Scanning Laser Microscopy (CSLM) (LSM 710, Carl Zeiss, Oberkochen, Baden- Württemberg, Germany) using the methodology proposed by Salgado-Cruz, Ramírez-Miranda, Díaz-Ramírez, Alamilla-Beltran, & Calderón-Domínguez (2017) with slight modifications. Briefly, cooked pasta samples (0.5 g) were placed on heparin (1 mL, 5000 units, Inhepar® PiSA) for 5 min, rinsing the

sample by adding 5 mL of water, the excess of water was removed with an absorbent material (100% polyester), and two dyes were subsequently added: 25 min with fluorescein 5-isothiocyanate (FITC, 0.05% in water) for starch and 5 min with rhodamine B (0.015% in water) for protein. The samples were rinsed in distilled water and then mounted on a histological assembling medium (Fluoroshield™, Sigma) on glass plates to avoid dehydration of the sample. Normally, double-staining of such systems with a mixture of rhodamine B and FITC leads to staining of proteins by rhodamine B and starch granules by FITC. However, rhodamine B and FITC can also bind non-covalently with starch and protein respectively, depending upon the concentration of the components in the system. In a low protein system, such as pasta, when using high concentration of FITC (FITC 1% vs. rhodamine B 0.1%), it complexes with both starch and protein. In contrast, rhodamine B binds more specifically with protein allowing the visualization of both starch and protein simultaneously (Zheng, Gidley, & Dhital, 2016). The excitation laser wavelength was at 488 and 568 nm for FITC and Rhodamine B respectively, and the maximum emissions were at 518 and 625 nm.

2.4. *In vitro* gastrointestinal digestion

The *in vitro* gastrointestinal digestion was carried out simulating oral, gastric and intestinal phases. The oral phase was simulated following the methodology proposed by Grandfeldt, Björck, Drews, & Tovar (1992) with some modifications. One gram of freshly prepared food (dry basis) was chewed 15 times for about 15 seconds, then the material was put in a beaker and each person rinsed the mouth with 5 mL of phosphate buffer (0.05 M, pH 6.9) Six healthy subjects participated in the chewing phase of the

experiments. Then, 10 mL of HCl-KCl buffer (0.2 M, pH 1.5) was added, the pH adjusted to 1.5, and 0.2 mL of a pepsin (P700; 800-2500 units, Sigma-Aldrich) solution (300mg / mL HCl-KCl reagent) was added and incubated at 40 °C in a water bath for 60 min. Afterwards, the pH was adjusted to 7.5 prior to the addition of a mixture of enzymes: 1 mL of pancreatin (P7545, 8 x USP, Sigma-Aldrich) solution: 5 mg / mL in 0.1 M phosphate buffer, pH 7.5, 2 mL lipase solution: 7 mg / mL in 0.1 M phosphate buffer, pH 7.5, 2 mL of bile extract: 17.5 mg / mL in 0.1 M phosphate buffer, pH 7.5 and the samples were incubated for 1 h at 37 °C in a water bath with constant agitation. Later, nine mL of 0.1 M tris-maleate buffer pH 6.9 was added and the pH adjusted to 6.9. Then, the samples were transferred to dialysis tubing with a pore size of 12000 Da and 1 mL of pancreatic α -amylase solution (120 mg / mL tris-maleate buffer) and the dialysis bag was closed and the samples were incubated at 37 °C (Saura-Calixto, García-Alonso, Goni, & Bravo, 2000; Lucas-González, Navarro-Coves, Pérez-Álvarez, Fernández-López, Muñoz, & Viuda-Martos, 2016.) After each phase (oral, gastric and intestinal), samples were centrifuged at 8000 x g for 12 min, 4 °C. The supernatants and the aliquots (2 mL) taken during the intestinal phase (0, 10, 20, 40, 60, 120, 180, 240, 300, 360, 420 and 480 min) were used to quantify reducing sugars content with DNS, total polyphenol content, and antioxidant capacity. The reducing sugar concentrations were multiplied by a conversion factor of 0.95 to obtain starch concentrations.

2.5. Determination of total phenolic content

Total phenolic content (TP) was determined by the Folin-Ciocalteu procedure (Bello-Pérez, Flores-Silva, Camelo-Méndez, Paredes-López, & Figueroa-Cárdenas, 2015)

expressed as milligrams of gallic acid equivalents per gram of dry matter by using a calibration curve.

2.6. Antioxidant Capacity

The antioxidant capacity was evaluated by the inhibition of free radical using the ABTS method (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999) as well as ferric reducing antioxidant power (FRAP method) (Benzie & Strain, 1996). Three independent experiments were performed for each assayed extract and results were expressed in milligram equivalent of Trolox per gram of dry weight, using a Trolox standard curve.

2.7. Fitting to first-order kinetics

The experimental data of this study were adjusted to three first order models kinetics, described below:

Digestibility curves were fitted to a familiar first-order equation proposed by Goñi et al. (1997):

$$C_t = C_{\infty}(1 - e^{-kt}) \quad (1)$$

C_t is the concentration of product or reactant at time t , C_{∞} is the corresponding concentration at the end point and k is a pseudo-first order rate constant. For ease of plotting, the equation is usually cast in the form:

$$\ln \left[\frac{(C_{\infty} - C_t)}{C_{\infty}} \right] = -kt \quad (2)$$

Thus, a plot of $\ln[(C_\infty - C_t)/C_\infty]$ against t is linear with a slope of $-k$. The rate constant is a function of the fixed amylase concentration used in the digestion and is therefore pseudo-first order.

The second model, Guggenheim has been used to determinate the rate constant of 1st order reactions where C_∞ is unknown and the method has been widely used by chemists for decades (Frost & Pearson, 1961; Niebergall & Sugita, 1968). C is measured at times that are separated by a constant time increment Δt (i.e., the intervals between, t_1, t_2, t_3 etc. are all Δt).

Then it can be shown that:

$$C_{i+1} - C_i = C_\infty e^{-kt} (1 - e^{-k\Delta}) \quad (3)$$

Hence:

$$\ln(C_{i+1} - C_i) = \ln[C_\infty (1 - e^{-k\Delta})] - kt \quad (4)$$

The first term on the right hand side of the equation is constant because both C_∞ and the time interval are constant, and so a plot of $\ln(C_{i+1} - C_i)$ against t is linear with a slope of $-k$. In practice a plot is made using data points of $\ln(C_2 - C_1), \ln(C_3 - C_2), \dots, \ln(C_{n+1} - C_n)$, etc. against $t_1, t_2, \dots, t_{n-1}$ etc., respectively.

Poulsen et al., (2003) extended the Guggenheim approach to situations where the interval between measurements does not have to be constant. These authors also point out that their method will demonstrate whether the data are truly of logarithmic form and should also reveal whether the rate constant remains unchanged throughout the whole of the reaction.

$$\frac{dC}{dt} = C_\infty k e^{-kt} \quad (5)$$

This first derivative represents the slope of a digestibility curve at time t . Expressing Eq. (5) in logarithmic form gives:

$$\ln\left(\frac{dC}{dt}\right) = \ln(C_{\infty}k) - kt \quad (6)$$

Thus, a plot of $\ln(dC/dt)$ against t is linear with a slope of $-k$. The intercept on the y axis equals $\ln(C_{\infty}k)$ and so C_{∞} can be calculated from the value of k obtained from the slope of the plot. Poulsen et al. (2003) refer to this plot as a ‘log of slope’ or logarithm of the slope (LOS) plot, and as has already been mentioned, the slope is sensitive to changes in k occurring during a reaction. Such changes will be revealed by discontinuities in the linear plot. To use the LOS plot requires determination of the slope of a digestibility curve at several time points. Provided that the time points on the curve are not too far apart, the slope may be estimated from the fraction $(C_2 - C_1)/(t_2 - t_1)$, $(C_3 - C_2)/(t_3 - t_2)$ etc. and the natural logarithms plotted against the relevant, meantime, i.e., $(t_2 + t_1)/2$, $(t_3 + t_2)/2$ etc. A spread sheet can be set up to perform these relatively simple calculations.

2.8. Statistical analysis

Statistical analysis was done using the Statgraphics Plus version 5.1® (Manugistics, Inc., Rockville, MA, USA) software. Mean (at least three replicates) and standard deviations were determined for all measurements. One-way analysis of variance (ANOVA) was employed to establish significant differences. In all cases, the statistically significant level was considered at $p < 0.05$.

3. Results and discussion

3.1. Microstructural analysis

After hydration in hot water, the surface of pasta became smooth, probably due to the leaching of unbounded compounds to hot water from pasta surface. Samples were cooked at their optimum cooking time (Table 1), which ranged between 7.3 and 7.7 minutes and is in agreement with the cooking loss because it increased with increase in blue maize content.

Fig. 1 exhibits the cross-sectional microstructure (internal and external regions) of hydrated gluten-free pasta, the pasta strand diameter became larger due to the absorption of water and the inside revealed a spongy but regular structure for all formulations. The process of cooking is an essential step in the occurrence of the greatest number of structural transformations. In those images, granules of starch fused with the protein matrix were observed, similar to those reported by Tudorică, Kuri, & Brennan (2002). The amount of blue maize flour did not affect the cross-sectional microstructure, which for all pasta products was rather similar. Microstructure was very compact inside, with starch-protein matrix and few aggregates visible at high magnification. This structure was attributed to the effect of the extrusion-cooking process that produced starch gelatinization and consistent structure (Wójtowicz & Mościcki, 2009), supporting the low values of cooking losses found in our study (Table 1).

A notable difference was observed in the distribution of the starch granules in the internal and external region of the pasta. On the outer region, gelatinized or dispersed starch granules were observed in a less dense protein network. In contrast, the inner region has a more compact structure with starch granules surrounded by a widespread protein network. Microstructural studies of gluten-free pasta are limited; Zheng, Gidley, & Dhital (2016) analyzed gluten-free pasta made with immature banana flour added with different

ingredients as xanthan gum, guar, and egg. Authors reported two regions (external and internal) in the gluten-free pasta observed by CSLM, the outer and inner surfaces clearly showed that the outer one was less dense compared to the inner one and that both had varying proportions of intact (ungelatinized) starch granules. Zou et al. (2015) observed wheat starch and semolina pasta by CSLM after cooking, describing completely swollen granules in starch samples, whereas those in the cooked pasta remained largely intact and did not fully swell. The authors reported that the appearance of the starch granules varied from the surface to the central regions of the pasta, starch granules completely swollen in the external region, partially swollen in the intermediate region and almost intact in the center, being attributed this behavior to the gluten present in the pasta.

Regarding the impact of composition, starch granules with an average diameter of 10 μm were observed in pasta where blue maize flour was in the highest level in the formulation (Table 1, F3), with round shapes and sizes between 5 and 20 μm as was reported in white maize samples (Román, Martínez, Rosell, & Gómez, 2017); while pasta with the highest ratio of unripe plantain flour (F1), presented elongated granules with average size of 40 μm . The physical entrapment of starch in the network of protein and gum of the pasta hinders enzymatic diffusion and further hydrolysis of starch as well as swelling of starch granules, which in consequence decreases the starch availability to digestive enzymes (Sissons et al., 2010).

Starch gelatinization and protein denaturation are the main structural changes during cooking of wheat pasta. In the spaces between the granules, the protein that traps the starch granules was observed. When gelatinization occurs, the space between the granules and the protein network decreased (Lucisano, Cappa, Fongaro, Mariotti, 2012).

Petitot, Abecassis, Micard (2009) indicated that the protein network limits the swelling of the starch granules favoring a highly compact structure that restrict the starch hydrolysis. In this work, due to the lack of gluten for formation of the pasta matrix, carboxymethylcellulose (CMC) was used. It has been reported that the inclusion of gums in the pasta can have diverse effects. Some studies have showed that xanthan gum can interact with galactomannans, as guar gum, resulting in an increase in the viscosity of the pasta dough (Linlaud, Puppo & Ferrero, 2009). The gums may also interact with starch granules by creating a surrounding layer that provides a physical barrier for the enzymes to access the starch granules and produce their hydrolysis. Furthermore, the amylase may form a complex with gums and the resulting gum-amylase complex may lack of catalytic activity, generating non-competitive inhibition (Slaughter, Ellis, Jackson, & Butterworth, 2002). In the same sense, the high dietary fiber content, between 11.2 and 13.5 % (Camelo-Mendez, Flores-Silva, Agama-Acevedo, Tovar & Bello-Perez, 2018) present in the spaguettis is mainly insoluble fiber (arabinoxylans, pectins, hemicellulose) of the three flours and this kind of fiber is associated with high water retention, restricting starch gelatinization that is in agreement with the higher resistant starch content (5.03 %, Camelo-Mendez et al., 2018). Also, the insoluble fiber modify the pasta structure that affect the rate of starch hydrolysis (Bustos, Perez & Leon, 2011).

3.2. Evaluation of gluten free pasta on the starch content and antioxidant capacity during in vitro digestion

Figure 2 shows the percentage of starch, polyphenols and antioxidant capacity of gluten-free pasta during the different phases (oral, gastric and intestinal) of *in vitro* gastrointestinal digestion.

The starch granules in the outer region of pasta were rapidly digested, as they were fully swollen and dispersed (and therefore more available) to the enzymes, while the starch granules in the central region remained undigested since were ungelatinized so they were not accessible to the enzyme. This phenomenon has been observed in pasta made with semolina wheat, where the authors suggested that the granules found in the external, intermediate and central region are complete, partially hydrolyzed and not hydrolyzed, respectively (Zou et al., 2015). This is related with the slowly digestible starch (12-27 g/100g) and resistant starch (3-5 g/100g) contents previously reported (Camelo-Méndez, Flores-Silva, Agama-Acevedo, Tovar & Bello-Pérez, 2017) and with the low pGI (<64) (Table 1).

Independently of the pasta composition, all the samples followed the same trend on starch hydrolysis, total phenolic compounds released and antioxidant capacity. Specifically, the highest digestion was observed during intestinal phase, followed by gastric and in minor extent the oral phase. Some differences were observed associated to the various pasta composition; particularly an increase in the available starch, total phenolic compounds and antioxidant capacity (ABTS, FRAP) as the content of blue maize increased (Table 1).

After the oral digestion, the release of reducing sugars (quantified as maltose), total phenol content and antioxidant capacity was less than 6%. In this phase, minimum modifications of the food matrix were expected due to the decrease in particle size by chewing, short exposure time and low hydrolytic activity of the salivary amylase

(Mosele, Macià, Romero, Motilva, & Rubió, 2015). In this phase, only free polyphenols were released from the matrix, ie those no linked to other molecules such as proteins, lipids, and carbohydrates (Bordenave et al., 2014). The low content of hydrolyzed starch was expected, because it has been reported that during this phase only 5% hydrolysis occurs (Lee, Bello-Pérez, Lin, Kim & Hamaker, 2013). Gastric digestion had higher effect ($p<0.05$) on the release of antioxidant compounds and reducing sugars than the oral phase. Phenolic compounds released during this phase could be associated to the breakdown of complexes with proteins, fiber residues or sugars, as previously reported (Gullón, Pintado, Barber, Fernández-López, Pérez-Álvarez, Viuda-Martos 2015), which in the present study, seems to be higher when increasing the amount of blue maize flour (F2 and F3). The low pH and enzymatic activity favors the release of phenolic compounds, mainly flavonoids, in the food matrix (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013).

In the last phase of gastrointestinal digestion, at the intestinal phase, the percentage of phenolic compounds released (CF) and starch hydrolysis was 40% and 30% of the initial values of the samples, respectively (Table 1). Overall, a proportion of phenolic compounds are transformed into several structures with different chemical and biological (bioaccessibility) characteristics when the food matrix is exposed to gastrointestinal conditions (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007). Considering that phenolic bioaccessibility is defined as the amount of ingested polyphenols that are available for absorption in the small intestine (Ahmad-Qasem, Cánovas, Barrajon-Catalán, Carreres, Micol, & García-Pérez 2014), in the present study, the bioaccessibility

of the phenolic compounds in pasta was up to 80% (Figure 2). The lowest amount was obtained with the pasta that contained the lowest amount of blue maize (F1).

Gullón et al. (2015) reported that during digestion phenolic compounds undergo (i) modification of chemical structure, (ii) increase or reduction of solubility or (iii) interaction with other compounds influencing directly their bioaccessibility; but also to the partial hydrolysis of total phenolic compounds (Hsu, Hurang, Chen, Wenig, & Tseng 2004). However, other studies attributed the antioxidant capacity of digested intestinal samples to the deprotonation of the hydroxyl residues present in the aromatic rings or to the formation of derived products with higher antioxidant capacity than that of its precursors (Pineda-Vadillo et al., 2016). This higher antioxidant capacity of the digested samples could explain the results obtained with the different samples, because despite the higher amount of blue maize flour in F2 and F3, only significantly higher antioxidant capacity was observed with F3.

In vitro digestion was fitted to different models to understand the kinetic of the pasta digestion (Figure 3, Table 2). Table 2 shows the values of the kinetic parameters after fitting the experimental values of maltose release, total phenolic compounds and antioxidant capacity. Taking into account the values of R^2 (>0.9), best fitting was obtained by applying Goñi model (Goñi et al., 1997). The values of R^2 and k were similar among samples since the values of total starch and phenolic compounds are similar (Table 1). The values of k for these samples were higher than those reported by other authors, who only simulated intestinal phase using α -amylase for enzymatic hydrolysis (Goñi et al., 1995; Butterworth et al., 2012). The differences might be attributed to the digestion sequence of the pasta due to that a reduction of the food size (food matrix)

increase the surface area and consequently the hydrolysis rate of the enzyme. In fact, in hydrolysis of semolina pasta k value increased when it was digested with pepsin prior to the hydrolysis with α -amylase (Zou et al., 2015).

In addition, it has been reported that α -amylase can complex with gums and the resulting gum-amylase complex may lack catalytic activity, an example of non-competitive inhibition as postulated by Slaughter et al. (2002); the presence of both gums and egg protein have a synergistic effect since it generated a strong dough giving better pasta. In addition, the presence of anthocyanins affect the amylolysis (Camelo-Méndez et al., 2016), and different interactions have been proposed: polyphenol- α -amylase, polyphenol-starch (Zhu, 2015) and polyphenol-protein (Bordenave et al., 2014) that directly affect bioaccessibility of phenolic compounds and the digestibility of the starch in the food complex matrix.

4. Conclusions

Pasta cooking was not sufficient to gelatinize the starch granules in the inner region, and differences were observed depending on the composition. Gastrointestinal digestion of the pasta was significantly stronger during the intestinal phase. Pasta containing increasing amounts of blue maize flour showed significant differences in the amount of phenolic compounds and antioxidant capacity during digestion phase. Goñi's model could be a useful model to follow the digestion kinetics of phenolic compounds and antioxidant capacity. The use of *in vitro* models allows to propose new technological platforms for easy use with a practical unlimited experimental capacity and without limitations based on ethical concerns.

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Conflicts of interest

The authors have declared no conflicts of interest.

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Figure 1. Confocal scanning laser microscopy of cooked gluten-free pasta with whole flours. The samples were stained with FITC and Rhodamine B for starch (shown in green) and protein (shown in red), respectively. S: starch granules, P: protein. For spaghetti formulation (F1, F2 and F3) see Table 1.

Figure 2. Starch, total phenolic, and antioxidant capacity content of gluten-free pasta during gastrointestinal digestion *in vitro*. For spaghetti formulation (F1, F2 and F3) see Table 1. Letters indicates significant differences within each digestion phase.

Figure 3. Release of starch, total phenolics, and antioxidant compounds (experimental data) of gluten free-pasta in an *in vitro* gastrointestinal digestion system. For spaghetti formulation (F1, F2 and F3) see Table 1.

571 Table 1. Formulations and properties of gluten-free pasta prepared with blue maize, chickpea, and unripe plantain flours.

Code	Ingredients (%) ^a			Cooked pasta properties									
	BM	CH	UP	OCT (min)	CL (%)	Protein (%) ^b	DF (%) ^b	TS (%) ^b	AS (%) ^b	TP ^{b*}	ABTS ^{b**}	FRAP ^{b**}	pGI ^b
F1	25	37.5	37.5	7.3	9.6	10.5	11.20	63.3	58.8	82.5	117.9	168.7	64
F2	50	25	25	6.7	9.8	10.9	13.51	65.8	61.0	176.0	133.6	174.8	63
F3	75	12.5	12.5	7.7	10.7	11.1	11.52	68.3	63.2	210.4	145.0	194.7	60

572 ^a1.5 g of carboxymethylcellulose and 32 mL of water were added per 100g of mixture, ^bData previously reported (Camelo-Méndez *et*
573 *al.*, 2017). *mg of gallic acid/g, **mmol trolox equivalents/g. ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), AS:
574 available starch, BM: blue maize flour, CH: chickpea flour, CL: cooking loss, DF: dietary fiber, FRAP: ferric reducing antioxidant
575 power, OCT: optimum cooking time, pGI: predicted glycemic index, TS: total starch, TP: total phenolic content, UP: unripe plantain
576 flour.

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582 Table 2. First order rate coefficients of starch, phenolic compounds, and antioxidant capacity release of gluten-free pasta in an *in vitro*
583 gastrointestinal digestion system

Model	Sample		Starch		TPC		ABTS		FRAP	
			k (min ⁻¹)	R^2	k (min ⁻¹)	R^2	k (min ⁻¹)	R^2	k (min ⁻¹)	R^2
Goñi	F1		0.0077	0.919	0.0076	0.951	0.0076	0.940	0.0078	0.966
	F2		0.0077	0.951	0.0078	0.950	0.0078	0.966	0.0077	0.978
	F3		0.0076	0.976	0.0092	0.902	0.0088	0.954	0.0088	0.978
Guggenheim	F1		0.0036	0.714	0.0050	0.938	0.0031	0.467	0.0035	0.322
	F2		0.0037	0.864	0.0037	0.863	0.0036	0.688	0.0034	0.608
	F3		0.0033	0.304	0.0033	0.304	0.005	0.651	0.0043	0.424
LOS	F1	Fast phase	0.0035	0.940	0.0038	0.804	0.0038	0.496	0.0131	0.873
		Slow phase	3×10^{-5}	0.709	5×10^{-5}	0.796	5×10^{-5}	0.573	2×10^{-4}	0.427
	F2	Fast phase	0.0033	0.726	0.0048	0.791	0.0037	0.408	0.0038	0.599
		Slow phase	5×10^{-5}	0.704	5×10^{-5}	0.796	6×10^{-5}	0.822	7×10^{-5}	0.717
	F3	Fast phase	0.003	0.757	0.0045	6×10^{-5}	0.0041	0.956	0.0041	0.585
		Slow phase	9×10^{-5}	0.615	6×10^{-5}	0.572	9×10^{-5}	0.367	1×10^{-4}	0.699

584 Confidence intervals of the fitting modelling $\alpha=95\%$. For spaghetti formulation (F1, F2 and F3) see Table 1. TPC: total phenolic
585 content. k : digestion rate constant.

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