PAPER

CHARACTERIZATION OF PASTA WITH THE ADDITION OF *CICER ARIETINUM* AND *SALVIA HISPANICA* FLOURS ON QUALITY AND ANTIOXIDANT PARAMETERS

A.G. COTA-GASTÉLUM, M.G. SALAZAR-GARCÍA, A. ESPINOZA-LÓPEZ, L.M. PEREZ-PEREZ, F.J. CINCO-MOROYOQUI, O. MARTÍNEZ-CRUZ, F.J. WONG-CORRAL and C.L. DEL-TORO-SÁNCHEZ*

Department of Research and Postgraduate in Food (DIPA), University of Sonora. Blvd, Luis Encinas y Rosales S/N, Colonia Centro 83000, Hermosillo, Sonora, Mexico

*Corresponding author: Tel: +526622592208; Fax: +526622592208

E-mail address: carmen.deltoro@unison.mx

ABSTRACT

Quality parameters, antioxidant properties, *in vitro* digestion and consumer acceptance were determined in pasta prepared with chickpea and chia flours. Pastas fortified with chia and chickpea increased protein, fiber content, total phenols, and antioxidant capacity with respect to the control. More than 85% of the antioxidant capacity and over 90% of the phenolic compounds in cooked pasta were retained after *in vitro* digestion, which is considered high. Pasta prepared with 25% wheat semolina, 10% chia flour, and 65% chickpea flour has high quality parameters, phenol content, antioxidant capacity and consumer acceptance.

Keywords: *Cicer arietinum*, pasta, phenols, *Salvia hispanica*

1. INTRODUCTION

Pasta products are basic foodstuffs that are important in human food consumption (GELENCSÉR *et al*., 2008). The simplicity of pasta production (traditionally manufactured from durum wheat semolina), in addition to its ease-of-handling and storage stability has facilitated its popularity and widespread consumption around the world (CHILLO *et al*., 2008). Nevertheless, pasta is considered insufficient with regard to its nutritional value, due to a poor source of protein (unless supplemented) and its protein has low amounts of essential amino acids such as lysine. Hence, pasta is an excellent vehicle for supplementation with fiber, proteins, and many other valuable healthy components. It could be appropriately designed and would operate as a functional food if healthy components were to be incorporated into its formulation (BORNEO and AGUIRRE, 2008). Some of these ingredients could be chickpea (*Cicer arietinum*) and chia (*Salvia hispanica*) seeds.

Chickpea is an important grain legume because of its nutritional quality. It is a rich source of complex carbohydrates, protein, vitamins, and minerals (JUKANTI *et al*., 2012; HIRDYANI, 2014). Polyphenols are also present in chickpeas. Some researches indicate that one of the disadvantages of the presence of phenolic compounds (denominated anti-nutritionals) is that they bind to proteins through non-covalent interactions (electrostatic and hydrophobic interactions, and hydrogen bonding), which reduces their nutritional availability (MONDOR *et al*., 2009). However, these types of compounds are currently of great interest because they are bioactive compounds that can confer significant long-term health benefits (AGUILERA *et al*., 2011). Polyphenols have been recognized as the most abundant source of antioxidants in our diet due to their activities and beneficial properties such as antihypertensive and antibacterial actions as well as anticarcinogenic properties (YANG *et al*., 2001; THOMASSET *et al*., 2007; XU and CHANG, 2010). In majority of the cases, free radicals are responsible for degenerative diseases such as cancer and diabetes, among others (HAN *et al*., 2007), and polyphenols have the ability to scavenge free radicals, thereby preventing associated ailments.

The phenolic composition of the chickpea is represented by phenolic acids (*p*-hydroxybenzoic acid, vanillic acid, ferulic acid, and *p*-coumaric acid) and flavonols (quercetin, kaempferol, and myricetin), as well as isoflavones (daidzein and genistein) (MONDOR *et al*., 2009; SREERAMA *et al*., 2010; FARES and MENGA, 2012). One research was found that studied the effects of the toasting of chickpeas on the antioxidant properties of chickpea flour added to durum wheat pasta. In that research, it was concluded that toasting the chickpea increases its phenolic content and antioxidant activity (FARES and MENGA, 2012). However, to the best of our knowledge, there is no information on these compounds in fortified pastas after *in vitro* digestion. The majority of investigations focused on the health benefits accruing from the digestibility of starch, such as a lower glycemic index for persons with diabetes (GOÑI and VALENTÍN-GAMAZO, 2003; PETITOT *et al*., 2010; FLORES-SILVA *et al*., 2014) and for the prevention of cancer, as well as protection against cardiovascular diseases due to the dietary-fiber content of starch (CHILLO *et al*., 2008). The remainder of studies on chickpea-fortified pasta focused on improving its nutritional, cooking, and sensory qualities, in which the authors of these studies concluded that chickpea confers good quality and nutritional properties on pastas (ZHAO *et al*., 2005; SABANIS *et al*., 2006; WOOD, 2009; ABOU-ARAB *et al*., 2010; BASHIR *et al*., 2012; PADALINO *et al*., 2015).

Furthermore, chia (*Salvia hispanica*) which is native to southern Mexico and northern Guatemala has become an important raw material for obtaining functional foods due to its high content of fatty acids, protein, fiber, and secondary metabolites such as phenolic compounds (COATES and AYERZA, 1996; SANDOVAL-OLIVEROS and PAREDES-

LÓPEZ, 2013; MARTÍNEZ-CRUZ and PAREDES-LÓPEZ, 2014). Chia seeds are very rich in phenolic compounds and possess a high antioxidant capacity, suggesting that phenolic acids (gallic, caffeic, ferulic, chlorogenic, and rosmarinic), flavonols (quercetin and kaempferol), and isoflovonols (Genistein, Daidzin, and Glycitein) may decrease the invasiveness of cancer cells, remove ROS (Reactive Oxygen Species), and improve clinical outcome (SANDOVAL-OLIVEROS and PAREDES-LÓPEZ, 2013; MARTÍNEZ-CRUZ and PAREDES-LÓPEZ, 2014; REYES-CAUDILLO *et al*., 2008). According to MARINELI *et al*. (2014) and COELHO and SALAS-MELLADO (2014), the consumption of chia seed can therefore serve as an important alternative for improving consumer health, suggesting its use as a functional food in the human daily diet. Previous studies have shown that pasta made with chia flour (7.5% and 10%) had a higher nutritional value and superior technological characteristics (OLIVEIRA *et al*., 2015; MENGA *et al*., 2017).
Due to the many properties that chickpea and chia possess, they are good candidates for use in the fortification of pasta to improve the nutritional quality in pasta formulations. Thus, the purpose of this study was to characterize pasta fortified with chickpea flour and chia flour in terms of their quality parameters, antioxidant properties and *in vitro* digestion.

2. MATERIAL AND METHODS

2.1. Materials

Commercial wheat (*Triticum durum*) semolina, Kabuli-type chickpea (*Cicer arietinum*) and chia (*Salvia hispanica*) were purchased at a local store. Chickpea grains were manually cleaned and dried at 60°C for 6 h. To obtain chickpea flour, a Perten model LM3100 (PerkinElmer, USA) mill was used. The chia grains were ground in an analytical mill (Braun Aromatic Coffee grind KSM2, USA) to obtain chia flour. Both flours were then standardized to a particle size of 60 mesh (0.25 mm).
All chemical reagents were purchased from local laboratory suppliers and were of analytical grade.

2.2. Pasta elaboration

Each blend was made with different proportions of commercial wheat (*T. durum*) semolina, chickpea flour, and chia flour, as shown in Table 1. Chia flour was the same in all formulations (10%) because, in preliminary studies, this amount was optimal for the sensory quality of pasta (data not shown). For its preparation, 100 g of flour was utilized. Distilled water was added at 35% absorption (considering the moisture of each mixture, between 35 and 42 mL/100 g of the mixture) and were mixed at room temperature in a mixer machine (professional model 600 HD, USA) at low speed (set 1) for 10 min. Afterwards, the dough was allowed to rest for 10 min in a plastic bag at room temperature. First, the proofed dough was laminated in the pasta machine (IMPERIAL R 220 model RMN, Italy) at setting 3, and finally at setting 1. The pasta was hand-cut into strips approximately 20 cm in length (fresh pasta) using a scissors. The pastas were kept to air-dry for 2 days at ambient temperature according to CLEARY and BRENNAN (2006). The six pasta samples were placed individually in sealed containers to avoid moisture exchange and were then stored at 5°C.

Table 1. Formulations for pasta made with the wheat semolina, chia flour and chickpea flour.

	Formulations (g/100 g)					
Flour	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Wheat semolina	100	85	65	45	25	0
Chia	0	10	10	10	10	10
Chickpea	0	5	25	45	65	90

2.3. Chemical analysis

The testing was performed according to Association of Official Analytical Chemists (AOAC) Official Methods. Semolina, chia, and chickpea flours and each pasta sample were analyzed for crude protein (method 955.04) and ash (method 920.153) using the AOAC (2000) methods. Total dietary fiber and fat were determined by methods 985.29 and 920.85, respectively according to AOAC (1997). Carbohydrates were determined by difference.

2.4. Cooking quality determinations

The cooking quality of the pasta, such as optimal cooking time, water absorption, weight gain by pasta, and solid loss during cooking, were evaluated using method 66-50 of the American Association of Cereal Chemists (AACC) Official Methods (AACC, 2000).

2.5. Determination of color

Color was evaluated utilizing the CIELAB system (Hunter Lab MiniScan EZ 45/0-L Model, USA). The analyzed parameters included the following: L^* (which represents the percentage of brightness, where black is 0% and white is 100%); a^* (where $+a^*$ is red and $-a^*$ is green), and b^* (where $+b^*$ is yellow and $-b^*$ is blue). The readings were taken at room temperature on the surface of the pastas, with 10 repetitions for each evaluated sample (OLIVEIRA *et al*., 2015).

2.6. Antioxidant activity

In determining the antioxidant activity of the different pasta formulations, the extracts were obtained first. All samples (raw and cooked) were adjusted to 0.17 g/mL with 1% hydrochloric acid in methanol (REHMAN and SHAH, 2005; LI *et al*., 2007). Using an orbital shaker (Thermo Scientific, USA) for 3 h at room temperature, the super natants of the samples were separated by centrifugation (10,000 rpm, 15 min, and 4°C). DPPH [2,2-DiPhenyl-1-PicrylHydrazyl] and ABTS [2,2′-Azino-Bis-(3 ethylbenzoThiazoline-6-Sulfonic acid)] assays were employed to quantify the antioxidant activity. Additionally, quantification of total phenolic content was determined.

2.6.1 DPPH assay

The antioxidant properties of the pasta samples using the DPPH assay were measured by the method described by MOLYNEUX (2004). Briefly, a 0.1 mL aliquot of the sample solutions was mixed with 3.9 mL of a free radical DPPH solution (6×10^{-5} mol/L). The reaction mixtures were incubated for 30 min in darkness and their absorbance was measured at 515 nm. All analyses were carried out in triplicate and a Trolox standard

curve was utilized for quantification. The results were reported as micromole of Trolox Equivalent per gram of sample (μmol TE/g).

2.6.2 ABTS assay

The ABTS assay was performed using the procedure of RE *et al*. (1999). A 2.97 mL of the cation radical solution was combined with 0.03 mL of the extract. The samples were incubated for 30 min at room temperature, and later, absorbance was measured at 734 nm. A control was prepared containing the cation radical solution with no pasta extracts, and another, with the solvent used. All analyses were performed in triplicate and a Trolox standard curve was used for quantification. The results were reported as micromole of Trolox Equivalent per gram of sample (μmol TE/g).

2.6.3 Total phenolic content

Quantification of total phenols was assayed by a spectrophotometric method utilizing the Folin-Ciocalteau reagent (SINGLETON *et al*., 1999). Thereafter, 50 μL of each extract solution was combined with 3 mL of deionized water and 250 μL of 1 N Folin-Ciocalteau reagent. After 5 min of incubation at room temperature, 750 μL of a 20% sodium carbonate solution and 950 μL of deionized water were added, measuring absorbance at 760 nm. A gallic acid standard calibration curve (0–100 mg/L) was prepared and the results were expressed as mg of Gallic Acid Equivalents per gram of sample (mg GAE/g).

2.7. *In vitro* digestion

This assay was conducted according to GIL-IZQUIERDO *et al*. (2002), but with some modifications. The cooked pastas were the samples used in this analysis. A total of 20 g of pasta was cooked in 100 mL of distilled water for 7.5 min. After that, 1 g of cooked pasta was removed, cut into small pieces of approximately 2 mm, and dissolved in distilled water at a material-to-solvent ratio of 1:15. This preparation was mixed in a vortex mixer (Daigger, Vortex-Genie 2) and was adjusted to pH 2.0 with 1 M HCl. A total of 0.5 mL was removed and combined with 0.75 mL of pepsin (315 U/mL prepared in 0.2 M KCl buffer) and 1.75 mL of deionized water. The samples were neutralized with 1.25 M $NaHCO_3$ after incubation at 37°C in a shaking water bath (Wise Bath, DAIHAN Scientific, WSB-18) at 80 rpm for 2 h. A total of 0.375 mL of pancreatin solution (4 mg/mL, prepared in 0.1 M Phosphate Buffer Saline [PBS]) was added to the samples. Afterward, the samples were transferred onto dialysis membranes (12,000 Da), placed in an Erlenmeyer flask containing 35 mL of 0.1 M PBS solution, and incubated again in the shaking water bath (4 h, 37°C, 80 rpm). Antioxidant activity and total phenols were determined before (initial) and after digestion (inside and outside of the membrane).

2.8. Consumer acceptance test

To evaluate the acceptability of the pasta, a 5-point hedonic scale was used for acceptance testing, in which the upper and lower extremes, respectively, correspond to 5 (liked very much) and 1 (disliked very much). The pasta was placed in boiling water with 5 g of salt and 10 mL of oil, and was subsequently served to tasters. The sensory panel was composed of 100 untrained tasters who were recruited randomly (OLIVEIRA *et al*., 2015).

2.9. Statistical analysis

Statistical analysis of the data obtained in all experiments were performed by Analysis of variance (ANOVA) using the StatGraphics Centurion ver. 17.0 XV ver. 15.2.06 statistical software. Comparison of means was performed by the Tukey least significance test (p<0.05). The experiments were run in triplicate.

3. RESULTS AND DISCUSSION

The chemical composition of the samples is presented in Table 2. Chia and chickpea seeds are rich in protein (27.94 and 22.93%, respectively) and fiber (30.02 and 20.22%, respectively) when compared to other grains such as barley, rice, amaranth, corn, and wheat. Several authors found similar results in protein and fiber content in chia and chickpea (MONDOR et al., 2009; OLIVEIRA *et al*., 2015; SÁNCHEZ-VIOQUE *et al*., 1999; MUÑOZ *et al*., 2012; SARGI *et al*., 2013). Despite the fact that chia had the highest quantity of protein, it has not been marketed, to our knowledge, as a source of protein, due to the fact that the profile of amino acids is limiting for schoolchildren (OLIVEIRA *et al*., 2015). However, it can be mixed with other grains to improve protein balance in formulations for adults (AYERZA and COATES, 2011). This is one of the reasons for the addition of chickpea to the formulation in this study. An increase in protein and fiber content was observed in the pasta formulations by increasing the amount of chickpea, bearing in mind that the amount of chia was the same in all of the formulations. This result is significant because pasta is considered a low nutritional-value product. Hence, chickpea considerably fortified the pasta (from F3 to F5) with protein and fiber in comparison with semolina pasta (F1). However, the sample in which semolina was replaced with chia flour and chickpea flour (F6) had the highest amount of protein and fiber, indicating that both seeds are good options for elaborating pasta rich in this type of compound.

Table 2. Chemical properties of chia flour, chickpea flour, and wheat semolina and the different pasta formulations (% dry base).

	Protein (%)	Fat (%)	Ash (%)	Total Fiber (%)	Carbohydrates (%)
Chia	27.94±0.55^{a}	35.31±0.11^{a}	4.46±0.06^{a}	30.02±0.11^{b}	2.26±0.96^{e}
Chickpea	22.93±0.25^{b}	8.02±0.31^{c}	2.88±0.02^{b}	20.22±1.12^{a}	45.95±0.58^{c}
Semolina	14.9±0.37^{e}	1.06±0.03^{d}	0.93±0.03^{d}	4.72±1.41^{g}	78.39±0.34^{a}
F1	15.14±0.48^{e}	1.14±0.03^{d}	0.94±0.02^{d}	4.25±1.41^{g}	78.53±0.53^{a}
F2	15.01±0.02^{e}	11.31±1.27^{b}	1.06±0.01^{d}	11.42±0.56^{f}	72.62±1.31^{b}
F3	17.26±0.44^{d}	12.38±0.42^{b}	1.81±0.02^{c}	14.02±0.63^{e}	68.55±0.68^{c}
F4	17.96±0.35^{d}	11.07±0.32^{b}	2.08±0.01^{b}	17.66±1.32^{d}	68.89±0.74^{c}
F5	18.92±0.51^{d}	12.35±0.64^{b}	2.65±0.02^{b}	19.29±1.08^{d}	66.08±1.20^{c}
F6	24.12±0.38^{b}	10.90±1.42^{b}	3.03±0.10^{b}	21.04±0.99c	61.95±1.81^{d}

Different letters within the same column indicate statistical differences (p < 0.05). F1: pasta with 100% semolina; F2: pasta with 85% semolina, 10 % chia flour and 5% chickpea flour; F3: pasta with 65% semolina, 10% chia flour and 25% chickpea flour; F4: pasta with 45% semolina, 10% chia flour and 45% chickpea flour; F5: pasta with 25% semolina, 10% chia flour and 65% chickpea flour; F6: pasta with 10% chia flour and 90% chickpea flour. Carbohydrates were determined by difference.

Furthermore, it was observed that fat content for chia-seed is high (35.31%), while fat content (8.02%) for chickpea is low. In comparison with F1, an increase in fat content was observed, from F2 to F6; however, there were no significant differences (p>0.05) between the fat content in these latter samples, indicating that chia could be the principal seed responsible for this increase. Alpha-linolenic acid represents 46.72%-62.44% of the total fatty acids in chia seed (PEIRETTI, 2011). It is an omega-3 fatty acid and is popular for preventing and treating diseases of the heart and blood vessels, among other functions (MANTZIORIS *et al*., 1994; ZIA-UI-HAQ *et al*., 2007). Additionally, alpha-linolenic acid is denominated "essential" because it cannot be synthesized by humans, but can only be consumed through foods. Therefore, pasta formulations with chia comprise a good option for consuming this compound.

On the other hand, ash content in chia is high (4.46%) compared with chickpea (2.88%) and other cereals such as rice, wheat, and sorghum. Magnesium, calcium, iron, and phosphorus are the minerals found in abundance in chia seed and are essential for a healthy diet (OLIVEIRA *et al*., 2015; CAPITANI *et al*., 2012). The quality of many foods depend on the concentration and type of minerals they contain, including their taste, appearance, texture, and stability. In the pasta formulations, the major content of ash was in F5 and F6 (2.65 and 3.03%, respectively), without a significant difference between them (p>0.05). Generally, all pastas have a high content of carbohydrates (61.95–78.53%), especially F1, which had the highest content. From F2 to F6, a decrease in these amounts were observed due to the increment of fiber influencing the calculation of carbohydrates, which is determined by difference. Similar behavior was obtained by PADALINO *et al*. (2014) in pastas enriched with pea flour and by OLIVEIRA *et al*. (2015) with pastas enriched with chia flour.

3.1. Cooking quality and color parameters

Table 3 depicts the results of the cooking quality and color parameters of the pastas. Optimal cooking time was the same (7.5 min) for all pasta types. OLIVEIRA *et al*. (2015) reported from 15-16 min cooking time in pastas with chia flour (7.5, 15, and 30%) instead of wheat flour, which is longer than the time frame used in our study.

Table 3. Cooking quality and color parameters for the different pasta formulations.

	F1	F2	F3	F4	F5	F6
Optimum cooking time (min)	7.5^{a}	7.5^{a}	7.5^{a}	7.5^{a}	7.5^{a}	7.5^{a}
Water absorption (%)	64.1±1.6^{a}	64.5±2.1^{a}	64.7±1.8^{a}	61.1±1.1^{b}	59.0±1.0^{b}	59.6±0.7^{b}
Percentage weight increase (%)	118.2±4.1^{a}	116.7±7.3^{a}	113.5±5.3^{b}	110.3±9.2^{b}	96.2±1.9^{c}	69.6±3.0^{d}
Solid loss (%)	4.5±0.5^{b}	4.6±0.3^{b}	4.6±0.2^{b}	4.9±0.0^{b}	4.7±0.4^{b}	6.3±0.9^{a}
*L**	75.5±0.1^{a}	57.4±1.5^{b}	55.2±1.1^{b}	53.6±0.1^{bc}	51.3±0.7^{c}	48.9±0.6^{c}
a	2.9±0.2^{c}	5.0±0.3^{b}	5.4±0.2^{b}	5.9±0.1^{b}	6.5±0.1^{b}	7.1±0.2^{a}
b	29.5±0.1^{a}	28.6±0.3^{a}	28.1±0.1^{a}	28.1±0.4^{a}	27.0±2.3^{a}	21.5±0.1^{b}

Different letters in the same line differ statistically at the 5% level by the Tukey's test.
F1: pasta with 100% semolina; F2: pasta with 85% semolina, 10 % chia flour and 5% chickpea flour; F3: pasta with 65% semolina, 10% chia flour and 25% chickpea flour; F4: pasta with 45% semolina, 10% chia flour and 45% chickpea flour; F5: pasta with 25% semolina, 10% chia flour and 65% chickpea flour; F6: pasta with 10% chia flour and 90% chickpea flour.

Another study with pastas contained 30% of chickpea obtained the cooking time of 10.5 min (ZHAO *et al*., 2005). Spaghetti with 30% of chickpea flour exhibited an optimal cooking time of 5.75 min (WOOD, 2009), which is lower than that in our study. Thus, our results (7.5 min) are within the average optimal cooking time of pastas fortified with these types of seeds.

Moreover, water absorption, weight increase, and the loss of soluble solids demonstrated significant differences (p<0.05) across treatments. Water absorption was higher, from F1 to F3, without significant differences among them (p >0.05). In this case, the level of substitution did not affect water absorption. Several studies indicated that the presence of chia increased water absorption due to the high fiber content of chia flour. The fibers, when mixed with water, form three-Dimensional (3-D) networks by means of the mucilaginous compounds that this seed contains (OLIVEIRA *et al*., 2015; MUÑOZ *et al*., 2012; CAPITANI *et al*., 2012). However, in our study, water absorption was not affected by the presence of chia. This behavior could be due to the fact that the interactions, mostly with chickpea, possessed fewer free bonds for binding the water molecules, thereby limiting the formation of 3D networks. Within this context, the weight increase in the sample is related to the water absorption, demonstrating similar behavior. Highest weight increase was obtained for samples with the highest content of semolina (F1 and F2). This fact can be explained by the high interaction with the water of the protein gluten (gliadin and glutenin) and the starch derived from the semolina (FEILLET and DEXTER, 1996). Similar results were obtained by FLORES-SILVA *et al*. (2014) in spaghetti made with chickpea, unripe plantain, and maize flours.

In addition, when semolina was replaced with chia flour and chickpea flour (F6), it exhibited a greater loss of soluble solids, which differed statistically from the rest of the samples (p<0.05). However, there were no significant differences from F1 to F5 (p>0.05), showing that semolina and its interaction with chia and chickpea prevents the loss of soluble solids. HUMMEL (1966) classified pastas according to loss of solids: up to 6% is characteristic of very good quality, up to 8% average quality, and values equal to or greater than 10% are low-quality pastas. Hence, according to this classification, pasta enriched with chia and chickpea in the presence of semolina are very good quality pastas. This result can be attributed to the protein contained in the samples, which causes the retention of amylose during cooking (CHILLO *et al*., 2008). Although, F6 possesses the highest protein content, it is evident that the absence of the semolina proteins increases the loss of soluble solids. OLIVEIRA *et al*. (2015) observed that the addition of chia flour improved the quality of the pastas by reducing the loss of solids. On the contrary, ZHAO *et al*. (2005) studied pastas with flours of legumes such as beans and chickpeas, in which the authors observed an increase in loss of solids during cooking. This was attributed to losses in the structural changes of the protein network due to partial substitution of wheat protein by legume protein (TORRES *et al*., 2007). However, in our case, the interaction among semolina, chia, and chickpea conferred better quality pastas.

The addition of chia and chickpea flour to the pasta showed a reduction in brightness (lower L*), more red (higher a*), and less yellow (lower b*), when compared with semolina pasta (F1), principally when the proportion of chickpea flour was increased (Table 3). Results of redness (a*) values reached the maximal values (7.1). The greater intensity of the yellow color is a highly desirable feature in pasta products, because it is one of the most influential visual appeals in the acceptance of pastas (CHANG and FLORES, 2004). F1 pasta differed statistically from others, featuring greater intensity of the yellow color (b* value), thus favoring acceptability. These results were similar to those of several studies of pasta fortified with chia or chickpea (WOOD, 2009; ABOU-ARAB *et al*., 2010; OLIVEIRA *et al*., 2015).

3.2. Antioxidant activity and total phenols

DPPH, ABTS, and total phenol determinations of raw and cooked pasta samples are illustrated in Fig. 1. Antioxidant activity and total phenols content increased when the chickpea concentration increases. Similar results were observed by FARES and MENGA (2012) in durum wheat pasta enriched with chickpea flour and by KHAN *et al*. (2013) in durum wheat pasta enriched with sorghum. The samples that presented highest antioxidant capacity in both radicals were F5 (DPPH 698.54 μmol TE/g, ABTS 1029.16 μmol TE/g), and F6 (DPPH 756.12 μmol TE/g, ABTS 1063.8 μmol TE/g). However, ABTS exhibits more affinity (Fig. 1b) for the pasta compounds than DPPH (Fig. 1a). This could be explained by the ABTS reacting more specifically with an H-atom donor than DPPH, thereby accomplishing the ABTS reaction faster (PRIOR *et al*., 2005; ROGINSKY and LISSI, 2005; ROSA-ALCARAZ *et al*., 2017). Additionally, ABTS can measure hydro- and lipophilic compounds (KRISHNAIAH *et al*., 2011). On the other hand, SHARMA and BHAT (2009) indicated that the DPPH radical reacts to a greater degree with lipophilic compounds, but other researches mentioned that this radical does not react mainly with flavonoids, which contain no hydroxyl groups in the B-ring, as well as with aromatic acids containing only one OH-group (VON GADOW *et al*., 1997; YOKOZAWA *et al*., 1998). The phenolic compounds in chia (rosmarinic acid, protocatechuic acid, caffeic acid, and gallic acid, among others) and chickpea (*p*-hydroxybenzoic acid, vanillic acid, ferulic acid, and *p*-coumaric acid, among others) comprise hydrophilic compounds to a greater extent (FARES and MENGA, 2012; MARTÍNEZ-CRUZ and PAREDES-LÓPEZ, 2014; MENGA *et al*., 2017). Additionally, these compounds have more H-atom donor or aromatic acids containing only one OH-group and, according to what has been previously mentioned, this explains the behavior observed in high affinity for ABTS rather than for the DPPH radical.

In our study, antioxidant capacity is higher than in other uncooked pasta (maximum of 1,063.8 μmol TE/g); for example, in durum wheat pasta enriched with sorghum that has a maximum of 33.7 μmol TE/g (KHAN *et al*., 2013). In cooked pasta, the maximal amount obtained in our study was 800.67 μmol TE/g, a higher amount compared with pasta supplemented with germinated pigeon pea flour (5.8 μmol TE/g) (TORRES *et al*., 2007) and in pasta enriched with bean flour (1.26 μmol TE/g) (GALLEGOS-INFANTE *et al*., 2012). Temperature affects antioxidant activity, with a loss of approximately 50% in DPPH (Fig. 1a) and of 30% in ABTS (Fig. 1b). The same behavior was observed in the concentration of phenolic compounds, these decrease is approximately 30% in cooked pasta (Fig. 1c). Similar results were obtained by KHAN *et al*. (2013) in durum wheat pasta enriched with sorghum, where the authors demonstrated a decrease of 20-55% in antioxidant capacity and a decrease of 21-55% of phenolic compounds in cooked pasta.

There were no significant differences ($p > 0.05$) between 65% and 90% of chickpea in the formulations in each radical and in the amount of total phenols (14.76 and 16.34 mg GAE/g, respectively). These results were higher than those reported by GALLEGOS-INFANTE *et al*. (2012) in pasta enriched with bean flour (0.49 mg GAE/g), by TURCO *et al*. (2016) in pasta with faba bean flour (1.85 mg GAE/g), and by KHAN *et al*. (2013) in wheat pasta with sorghum (1.27-3.22 mg GAE/g). The correlation among antioxidant activities with phenols was carried out, and it was observed that this correlation was high ($R^2 = 0.921$). This indicates that phenols were the principal compounds responsible for antioxidant activity in the pasta, while pasta made only with semolina (F1) presented very low antioxidant activity and phenolic content, confirming the effect of the fortification accomplished with chia and chickpea.

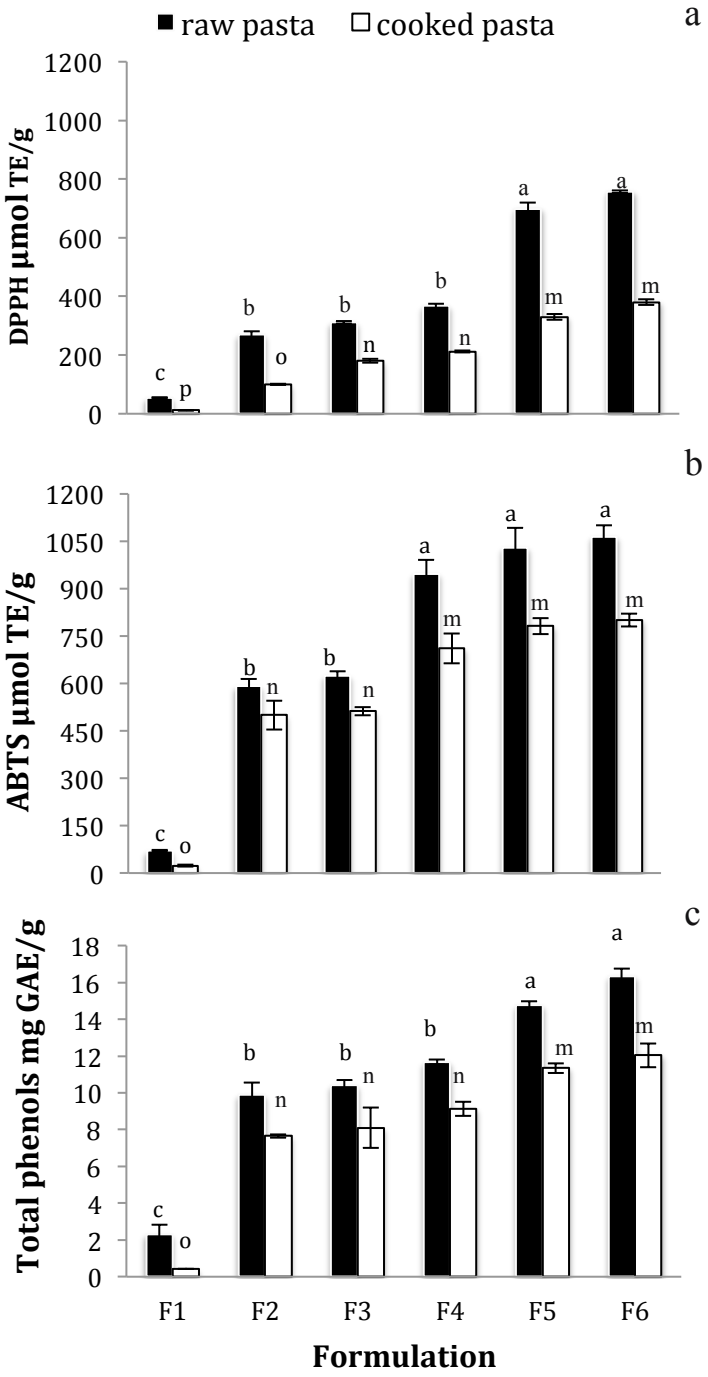

Figure 1. Antioxidant activity by DPPH (a), ABTS (b) and Total phenols (c) of raw and cooked pastas. Columns not sharing a common letter within the same treatment (raw or cooked) of pasta are significantly different ($P< 0.05$). F1: pasta with 100% semolina; F2: pasta with 85% semolina, 10 % chia flour and 5% chickpea flour; F3: pasta with 65% semolina, 10% chia flour and 25% chickpea flour; F4: pasta with 45% semolina, 10% chia flour and 45% chickpea flour; F5: pasta with 25% semolina, 10% chia flour and 65% chickpea flour; F6: pasta with 10% chia flour and 90% chickpea flour.

Cooked F1 pasta demonstrated a decrease of approximately 80% when compared with uncooked pasta, indicating that chia-chickpea-fortified pastas have lower cooking losses.

Some authors came to the same conclusion when chickpea-fortified spaghetti and apple by-product-fortified pasta were compared (WOOD, 2009; LONČARIĆ *et al*., 2014).

3.3. *In vitro* digestion

In vitro digestion was carried out only in cooked pastas using pepsin, pancreatin, and dialysis membranes to simulate the intestine. The most important finding in our study comprised the antioxidant capacity and the amount of phenolic compounds that pass through the membrane, where the outside of the membrane simulates the blood serum. The results revealed major antioxidant capacity in both radicals and the major amount of phenolic compounds outside of the membrane when the amount of semolina is decreased or absent in the formulations (Fig. 2). There were no significant differences (p>0.05) in antioxidant capacity between F5 and F6 (Figs. 2a and 2b). However, the highest antioxidant activity was detected in ABTS radical by F5 (655.1 ± 7.56 μmol ET/g) and F6 (703.4 ± 14.98 μmol ET/g). More than 85% of this antioxidant capacity was maintained outside of the membrane, which is considered high. Compared with the F5 and F6 undigested samples in ABTS radical (781.43 μmol ET/g and 800.67 μmol ET/g, respectively), there was an approximate decrease of 16% in F5 and 12% in F6 of antioxidant capacity.

Also, comparison with the F5 and F6 undigested samples (367 μmol ET/g and 388.21 μmol ET/g, respectively) in the DPPH radical revealed a major decrease of approximately 50% in F5 and 45.8% in F6 on the antioxidant capacity. As discussed previously, phenols were the principal compounds responsible for the antioxidant activity in the pasta; thus, if these compounds were affected by the digestive process, consequently, the antioxidant activity was affected. In our case, the phenolic compounds outside of the membrane in F5 (9.82 ± 0.1 mg GAE/g) and F6 (10.97 ± 0.3 mg GAE/g) exhibited the highest amounts, maintaining approximately more than 90% of these compounds compared with the initial amount (Fig. 2c). Hence, the differences between antioxidant activities in both radicals could be due to the affinity of the compounds in each sample and/or to their bioaccessibility (fraction of bioactive substance that is released from the food matrix) that consequently affects their bioavailability (amount of phenolic compounds reaching the blood circulation system), (RODRÍGUEZ-ROQUE *et al*., 2014). CATTANEO *et al*. (2015) found that after a simulated gastric condition, the antioxidant activity of puffed kernels was high compared to the undigested sample measured by ABTS and FRAP methods. RUFIÁN-HENARES and DELGADO-ANDRADE (2009) obtained similar behavior in flaked wheat-based breakfast cereals measured by ABTS, DPPH and FRAP methods.

In this context, some factors can affect the bioaccessibility and bioavailability of phenolic compounds. Studies indicated that in the cell wall of legume/seeds, these compounds could be present in insoluble-bond forms (covalent bonds, 20–60%) (SHAHIDI and YEO, 2016). These insoluble bonds affect the bioaccessibility and consequently, the bioavailability of the phenolic compounds because these bonds need to be cut by specific enzymes that generally are provided by intestinal microbiota (PEREZ-PEREZ *et al*., 2018). Most native polyphenols in foods are in glycoside form, which cannot be absorbed in the intestinal mucosa; therefore, the release of these performed by human and microbial enzymes is a necessary mechanism needed by them to break through the intestinal barrier (VALDÉS *et al*., 2015). In our study, we focused on the soluble forms (non-covalent bonds) of the phenolic compounds through *in vitro* digestion without the influence of microbiota. Thus, additional research should be carried out on the absorption of phenolics in the human colon. Also, hydrophobicity, the membrane-mediated transport, the stability of the compounds, environmental pH, degree of polymerization, interactions with other

compounds, molecular mass, and the complexity of the food matrix are some other factors that influence the absorption of phenolic compounds (TARKO *et al*., 2009; RODRÍGUEZ-ROQUE *et al*., 2014; SHAHIDI and YEO, 2016).
In our opinion, the majority of *in vitro* digestion studies of different fortified pastas have for long focused on starch digestibility. The principal interest of these studies is that sugars are progressively released from pasta during digestion, leading to a standard increase in postprandial blood glucose and insulin response (GOÑI and VALENTÍN-GAMAZO, 2003; PETITOT *et al*., 2010; FLORES-SILVA *et al*., 2014;PADALINO *et al*., 2015;GELENCSÉR *et al*., 2008; REYES-CAUDILLO *et al*., 2008; ZHENG *et al*., 2016). Our study focused on the bioaccessibility of phenolic compounds and antioxidant capacity after *in-vitro* digestion in order to provide additional and valuable information on the high effect of chia-chickpea-fortified pasta in the scavenging of free radicals. Thus, the antioxidant property of this fortified pasta could be involved in the defense mechanism of the organism against pathologies associated with the attack of free radicals, such as cancer, coronary heart disease, obesity, type 2 diabetes and hypertension, among others (PISOSCHI and NEGULESCU, 2011). With the results obtained in our research, pasta fortified with chia and chickpea can maintain high levels of phenolic compounds and antioxidant capacity after *in- vitro* digestion, therefore, making it capable of providing health benefits.

3.4. Consumer acceptance test

The results of consumer acceptability are presented in Fig. 3. Highest overall acceptability by tasters was the pasta with 100% semolina (F1) and samples containing wheat semolina (45% and 25%), chia (10%), and chickpea (45% and 65%) flours (F4 and F5, respectively), which revealed no significant differences (p >0.05). Similar results were obtained by ZHAO *et al*. (2005) in spaghetti fortified with chickpea. The pasta in which chia and chickpea are utilized instead of wheat semolina (F6) triggered the least acceptance. Therefore, the presence of wheat semolina is important for the sensory properties of fortified pastas. Additionally, PETITOT *et al*. (2010) noted that the addition of a high level of legume flour induced some minor structural changes in pasta. This explains the high acceptability of pastas with a high content of chickpea, containing semolina in their formulation. The addition of chia and chickpea causes inclusion of fibers, dilution of gluten proteins by albumins and globulins, and the largest amount of thin protein films, which may have favored the higher susceptibility of starch to digestive enzymes. Consequently, the acceptability of these types of fortified pastas is presently on the increase. If we were required to choose a pasta sample, it would of necessity be the sample that complies with all of the parameters (high quality parameters, phenol content, antioxidant capacity, and consumer acceptance). In accordance with all of the results in this work, this selected sample would be F5 (25% semolina, 10% chia flour, and 65% chickpea flour).

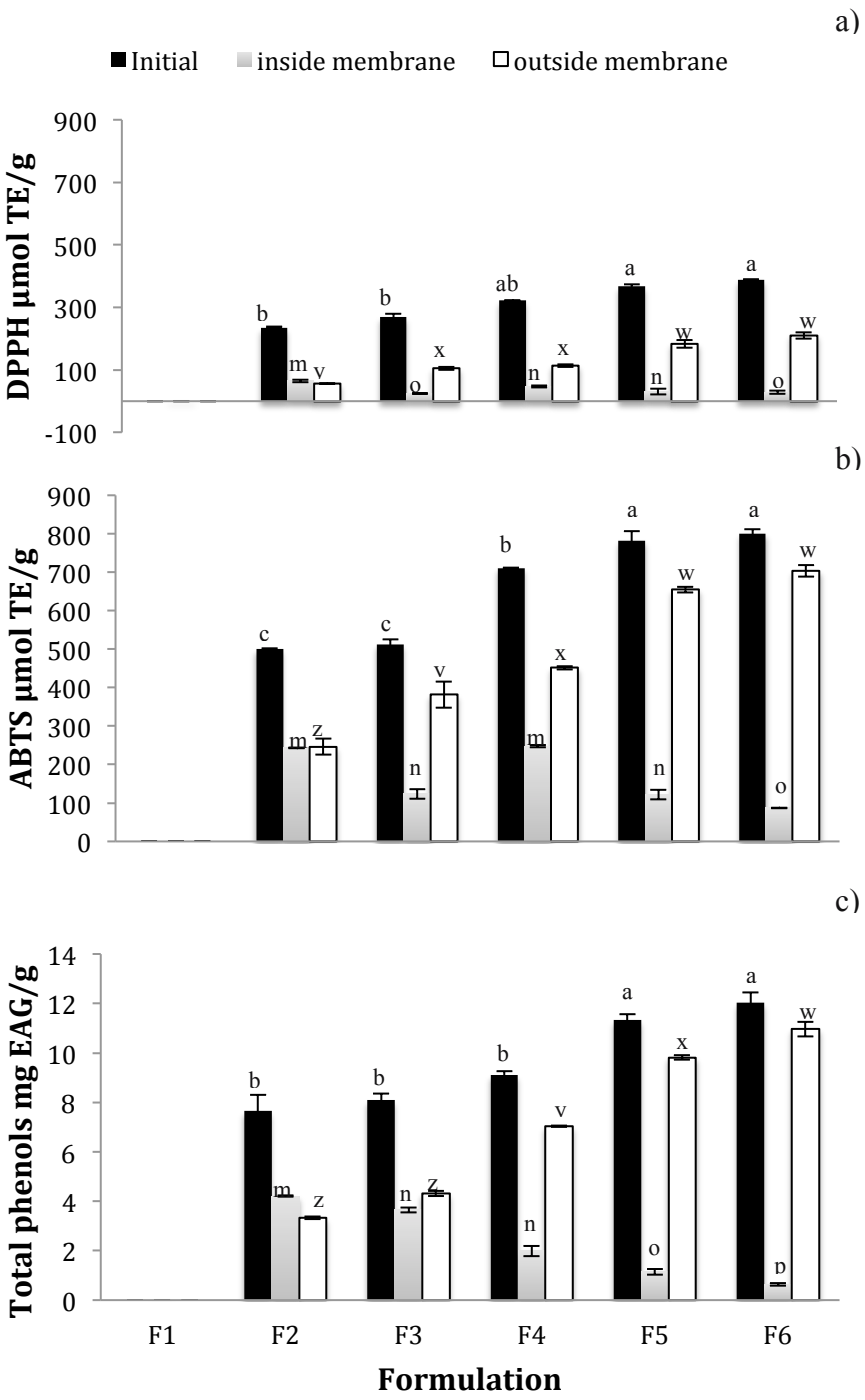

Figure 2. Antioxidant activity measured by DPPH (a) and ABTS (b) and total phenols (c) of digested and undigested (initial) cooked pasta.
Columns not sharing a common letter within the same status (initial, inside membrane and outside membrane) of pasta are significantly different ($P < 0.05$). F1: pasta with 100% semolina; F2: pasta with 85% semolina, 10 % chia flour and 5% chickpea flour; F3: pasta with 65% semolina, 10% chia flour and 25% chickpea flour; F4: pasta with 45% semolina, 10% chia flour and 45% chickpea flour; F5: pasta with 25% semolina, 10% chia flour and 65% chickpea flour; F6: pasta with 10% chia flour and 90% chickpea flour.

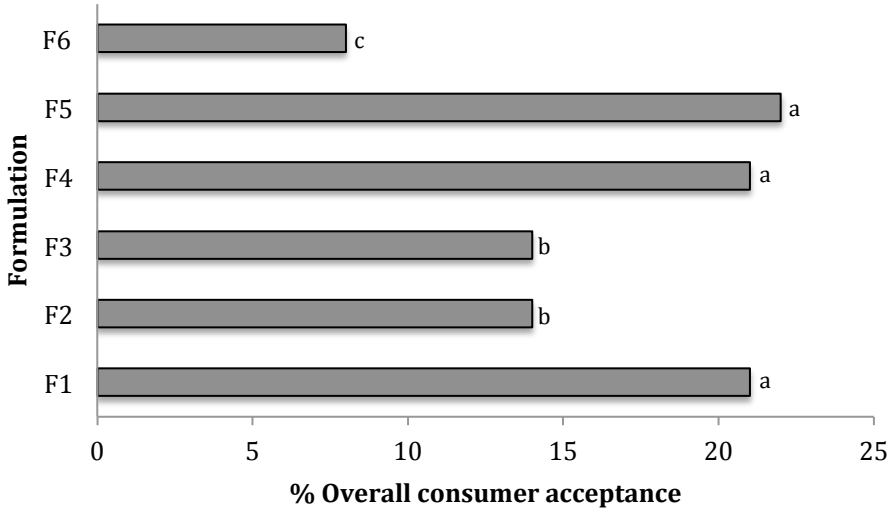

Figure 3. Percentage of consumer acceptance of the different pasta formulations.
The sensory panel was composed of 100 untrained tasters. Identical letters do not differ statistically at the 5% level by the Tukey's test. F1: pasta with 100% semolina; F2: pasta with 85% semolina, 10 % chia flour and 5% chickpea flour; F3: pasta with 65% semolina, 10% chia flour and 25% chickpea flour; F4: pasta with 45% semolina, 10% chia flour and 45% chickpea flour; F5: pasta with 25% semolina, 10% chia flour and 65% chickpea flour; F6: pasta with 10% chia flour and 90% chickpea flour.

4. CONCLUSIONS

In this work, we studied the effect of pasta with the addition of chickpea flour and chia flour in terms of quality parameters, antioxidant properties, and their *in vitro* digestion. The results revealed that the addition of these flours increases protein and fiber content, indicating that both seeds comprise good options for elaborating pasta rich in these types of compounds. Cooking parameters are important for consumers; in our results, the level of substitution of chia and chickpea did not affect water absorption; additionally, the presence of these seeds in the pasta avoided the loss of soluble solids. Thus, the interaction among semolina, chia, and chickpea yielded better quality pastas. Antioxidant activity and total phenols content increased in the fortified pastas. Although, cooking affected the amount of phenols and antioxidant capacity, *in vitro* digestion studies demonstrated that pasta fortified with chia and chickpea can maintain high levels of phenolic compounds and antioxidant capacity after *in-vitro* digestion, thus being able to provide health benefits. However, *in vivo* measurements are needed to confirm any biological effects. Chia-chickpea-fortified pasta would make an inexpensive, attractive, convenient and healthy food that is acceptable to consumers. Therefore, with the results obtained in our research, chia and chickpea can be utilized to develop more value-added products, thus rendering them more economical and affordable for developing countries.

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