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NUTRACEUTICALS & FUNCTIONAL FOODS | RESEARCH ARTICLE

Influence of the addition of *Amaranthus mantegazzianus* flour on the nutritional and health properties of pasta

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Abstract: To improve the nutritional and functional properties of pasta, bread wheat flour was substituted with wholemeal flour from *Amaranthus mantegazzianus* (WMAF) at 15, 30, 40 and 50% w/w. Proteins, dietary fibre, glycemic index (GI) and carbohydrate and protein digestibility were determined in the resulting pasta. Squalene content was also measured, in view of the beneficial health properties of this compound. With the addition of 30% of WMAF, where the technological quality of pasta remained acceptable, protein and fibre contents resulted in 23 and 50% higher than control pasta. Pasta presented a significant enrichment of squalene content at all levels of substitution with WMAF. In addition, all samples remained in the range of low GI foods, and protein digestibility values were even higher than control pasta.

Subjects: Breads, Cereals & Dough; Food Analysis; Nutraceuticals & Functional Foods

Keywords: amaranth flour; pasta; carbohydrate digestibility; protein digestibility; glycemic index; squalene

1. Introduction

Pasta is a cereal-based food product consumed across the world. For consumers, the cooking quality parameters are the most important characteristics; however, pasta has favourable nutritional properties as well (Gelenesér, Gál, & Salgó, 2010). There is a growing interest in pasta as a source of carbohydrates—74–77%, dry basis—(Petitot, Boyer, Minier, & Micard, 2010), and as a low glycemic

ABOUT THE AUTHORS

Our team consists of 12 PhD researches and several doctoral thesis students, all of us focused on science and technology of cereal food.

For more than 20 years we have participated in over 50 research and development projects, most of them supported by the National Agency of Scientific and Technological Promotion (ANPCyT), the Ministry of Science, Technology and Productive Innovation of Argentina (MINCyT) and other international agencies, from which numerous publications have resulted.

One of such project was the "Study of Physicochemical properties related to technological, sensory and nutritional quality of pasta. The addition of ingredients as functional foods". In particular, parts of the results obtained from this project are shown in the manuscript that we have sent to you.

PUBLIC INTEREST STATEMENT

WMAF improved the nutritional and health properties of pasta. Protein and fibre content notably increased with the addition of amaranth flour. Amaranth flour provided a significant enrichment of squalene in the pasta produced. All pasta samples remained classified as low glycemic index. Protein digestibilities of all pasta produced were higher than control sample.

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index food (GI), eliciting a low postprandial blood glucose and insulin response (Chillo, Ranawana, & Henry, 2011); characteristic attributed to its compact structure resulting from the formation of starch–protein network during the pasta production process (Menon, Padmaja, & Sajeev, 2015).

Like most food proteins, pasta proteins are not ideal proteins, but in combination with other food sources it can turn into an excellent alternative. Pasta is limited in two of the eight essential amino acids: lysine and threonine (Rayas-Duarte, Mock, & Satterleei, 1996). In addition, since pasta is basically made from durum semolina or wheat flour, water and salt, it is claimed to lack other essential nutritional components, such as dietary fibre, vitamins and minerals, which are lost during semolina or flour refinement (Choo & Abdul Aziz, 2010). Therefore, several studies (Baiano, Lamacchia, Fares, Terracone, & La Notte, 2011; Chillo et al., 2011; Gallegos-Infante et al., 2010; Marconi & Carcea, 2001), have attempted to improve the nutritional properties of pasta through the incorporation of non-traditional raw materials.

Amaranth was a staple food of the pre-Columbian Aztecs that shows rapid growth, with tolerance to arid climate conditions and poor soils where traditional cereals cannot be grown (Roa, Santagapita, Buera, & Tolaba, 2014). Interest in amaranth has been aroused due to its high nutritional value (Plate & Arêas, 2002). It contains between 15 and 22% proteins (Tosi, Ré, Lucero, & Masciarelli, 2001). The amino acid composition and the important proportion of lysine and sulphur amino acids of its proteins make it particularly suitable to complement proteins of cereals and legumes (Castel, Andrich, Netto, Santiago, & Carrara, 2012).

Amaranth has between 9 and 16% of dietary fibre, and its lipid content ranges between 3.1 and 11.5% (Tosi et al., 2001). Amaranth grain oil has significant amounts (8%) of squalene, which has many important direct and indirect beneficial effects on human health (Kaur, Singh, & Rana, 2010). Squalene has been found to act as an anticancer agent (Warleta et al., 2010), and to have hypocholesterolemic action (He, Cai, Sun, & Corke, 2002; Xia et al., 2015), antihypertensive effect (Pogojeva, Gono, Kulakova, Miroshnichenko, & Martirosyan, 2006) and cardioprotective activity (Sabeena Farvin et al., 2004).

Most of the studies have focused on amaranth grain mainly obtained from *Amaranthus hypochondriacus* L., *Amaranthus cruentus* L.and *Amaranthus caudatus* L. (Xia et al., 2015), but there are fewer studies focusing on *Amaranthus mantegazzianus* Passer (GBIF, 2013). Moreover, considering the nutritional and functional properties of amaranth grain, the aims of this work were: (1) to determine the feasibility of using wholemeal flour from *A. mantegazzianus* in pasta as a source of protein, dietary fibre and squalene to improve pasta nutritional quality and provide functional properties; (2) to study how wheat flour substitution for amaranth flour affects carbohydrate and protein digestibility as well as GI, in order to assess whether this substitution could compromise the inherent low GI properties of pasta or its digestibility.

2. Materials and methods

2.1. Materials
Wholemeal amaranth flour (WMAF) was obtained by milling cleaned grains of *A. mantegazzianus* Pass. from a commercial variety (Pass cv Don Juan Inta Anguil, La Pampa, Argentina) with a hammer mill Fritsch Pulverisette 16 (Germany) and sieving it through a US No. 30 (590 μm) with a Zony Test MR (Argentina) sifter. WMAF presented (dry basis) 17.5 ± 0.1 g/100 g of protein, 7.8 ± 0.1 g/100 g of lipid, 12.2 ± 0.5 g/100 of dietary fibre, 3.33 ± 0.05 g/100 g of ash and 60.5 ± 0.8 g/100 g of total starch.

Commercial bread wheat flour (CBWF), without additives, was provided by Molino Campodónico (Argentina). CBWF presented (dry basis) 12.2 ± 0.1 g/100 of protein, 0.9 ± 0.2 g/100 of lipid, 3.7 ± 0.1 g/100 g of dietary fibre, 0.76 ± 0.03 g/100 g of ash and 76.0 ± 0.4 g/100 g of total starch.

All measurements were done according to AACC Method 46–13, 30–25, 32–05 and 08–01 (AACC International, 2000), respectively. Total starch was measured according to AACC Method 32–40 (AACC International, 2000), using K-RStar assay kit (Megazyme International Ireland Ltd., Bray, Ireland).

2.2. Samples
In this work samples have been named "pasta", although they were made from bread wheat flour by a process of sheeting and cutting. Pasta samples were produced using CBWF as a base plus the addition of WMAF at four different percentages, 15, 30, 40 and 50 g/100 g (A15, A30, A40 and A50, respectively). Plain pasta (100% CBWF) served as control sample (Co). Flour blends (50 g) were mixed, with optimum amount of distilled water determined experimentally, and salt (1 g/100 g of flour), with a hand mixer (Philips, 190 W, Argentina) at maximum speed, for 3 min. Dough was sheeted after a 13 min resting time, using a pasta maker (Pastalinda®, Argentina). Pasta strips of 2 mm wide and 2 mm thick were dried by means of a pre-drying step at 30°C, 40% relative humidity (RH), for 30 min, followed by a drying step at 30°C, 65% RH, for 17 h. Each formulation was prepared in three replicate batches and processed on different days. Pasta was cooked in boiling distilled water in minimum pasta:water ratio (1:10).

2.3. Analytical methods

2.3.1. Cooking quality
Optimum cooking time and cooking loss were evaluated according to AACC Method 66-50 (AACC International, 2000). Firmness score of cooked pasta was calculated from a texture profile analysis obtained through two deformation cycles of 70%, with a texture analyser (TA-XT2, Stable Micro System, Surrey, UK), with the blunt side of HDP/BS probe, on one strand of cooked pasta. Each value of firmness represents the mean of 12 replicates, measured in each single sample of each of the three batches prepared for every substitution level.

2.3.2. Protein and dietary fibre of cooked pasta
Protein was measured according to AACC Method 46-13 (AACC International, 2000), by the Kjeldhal method (N × 6.25). Dietary fibre of cooked pasta was determined by an enzymatic–gravimetric method (AACC Method 32-05), (AACC International, 2000). Total dietary fibre was considered as the residue obtained after submitting a gram of cooked, dry and defatted sample to successive enzymatic digestions: first with a α-amylase, second with a protease and finally with an amyloglucosidase enzyme.

2.3.3. Squalene analysis
Squalene contents in extracted and purified oil samples were determined using a HPLC system (Shimadzu, Japan), controlled by the LC software, solution V.1.22 SP1 (Shimadzu, Japan). Lipid extraction from freeze dried cooked pasta was purified through a solid phase extraction (SPE), using silica cartridges (SPE Si 1000 mg/6 mL, Teknokroma Finisterre, Spain), conditioned with 6 mL of n-hexane (SIN-088003-01, Sintorgan, Argentina) before the application of oil solution. Squalene was eluted with 10 mL of n-hexane. The fraction collected was evaporated under reduced pressure (R110 Büchi, Switzerland), at room temperature. The dry residues were dissolved in the appropriate solvent for HPLC analysis. Squalene determination was carried out on a reversed phase Microsorb MV100 C18 (particle size 5 μ, 150 × 4.6 mm i.d., Varian®, USA), maintained at 26°C. The elution solvent (HPLC grade) was acetonitrile-2-propanol-hexane (72:17:11, v/v), according to Vidal-Escales and Borrós (Vidal-Escales & Borrós, 2004). The flow rate was 1 mL/min and the injection volume was 20 μL. Detection was achieved with a photodiode array detector (PDA SPD-M20A Shimadzu®, Japan) at 205 nm. Identification was made by spectral peak homogeneity and peak purity evaluation, whereas quantification was accomplished with the use of standard curves (Squalene standard S3626 Sigma-Aldrich, USA) calculated by linear regression analysis.

2.3.4. In vitro starch digestibility and GI

In vitro starch digestion was measured according to Brennan, Kuri, and Tudorica (2004), with some modifications. The cooked sample (1.7 ± 0.05 g) was disrupted for 1 min with a plunger of a manual homogenizer (Cole Parmer, 50 mL, EW-44468-18, USA) into a 50-mL Falcon tube to simulate the chewing process, previous addition of 5 mL of phosphate buffer pH 6.9. Once the homogenizer plunger was rinsed with 20 mL of the same buffer and removed, the pH was adjusted to 1.5 (using 8 M HCl) and 5 mL of pepsin solution (pepsin 115 U/mL, Sigma Aldrich P7000, 077K0661, dissolved in sodium potassium buffer pH 1.5) were added to the samples, followed by incubation at 37°C for 30 min in a shaking water bath (200 strokes/min) (Vikinf Dufnoff 5002, Argentina). After the pH of the sample was readjusted to 6.9 with 10% NaOH, the sample volume was brought to 50 mL with sodium potassium phosphate buffer (pH 6.9). Then 1 mL of porcine pancreatic α-amylase 110 U/mL solution (α-amylase type VI B from porcine pancreas A3176—500 KU, Sigma, dissolved in sodium phosphate buffer pH 6.9) was added. At zero time, duplicate 1-mL aliquots were taken from sample tubes and considered as blank samples. Samples, with a remaining volume of 49 mL, were incubated in a shaking water bath for 3 h at 37°C. Every 30 min (30, 60, 90, 120 and 180 min), 1-mL aliquot of each sample, in duplicate, were withdrawn, for analysis of reducing sugar content. To stop enzymatic reaction, immediately after removal, aliquots were immersed in a boiling water bath for 5 min and kept at −20°C until sugar reducing analysis, performed using 3,5-dinitrosalicylic acid (DNS) method, was made. For sugar analysis, the aliquots were centrifuged at 5000×g for 10 min, at 25°C, and 0.1 mL of supernatant was put into a glass test tube containing 0.4 mL of sodium buffer phosphate pH 6.9 and 0.5 mL of DNS (Sigma D0550). After the test tubes were heated for 5 min at 100°C, 5 mL of distilled water were added prior to measuring the absorbance at 540 nm (Spectrum SP 2000, China). A standard curve, using maltose (D-(+)-maltose monohydrate 63,418 ≥99.0%, Sigma), covering a concentration range of 0.8–4.0 mg/mL was used. Maltose values were expressed as starch, multiplying the first one by 0.9. The rate of starch digestion was expressed as the percentage of total hydrolysed starch at different times (30, 60, 90, 120 and 180 min).

From the areas under hydrolysis curves (AUC, 0–180 min), the hydrolysis index (HI) was calculated, as the relationship between the AUC values of pasta and the AUC values of a reference food (in this case, white bread, prepared with the same wheat flour of pasta), and the GI = 39.71 + (0.549 × IH), according to Goñi, Garcia-Alonso, and Saura-Calixto (1997).

2.3.5. In vitro protein digestibility analysis

Protein digestibility was determined according to Hamaker, Kirleis, Butler, Axtell, and Mertz (1987), with some modifications. On 0.5 ± 0.1 g of cooked samples, a chewing process was simulated into a Falcon tube as starch digestibility test, previous addition of 2 mL of distilled water. Homogenizer plunger was rinsed with 25 mL of phosphate buffer 0.1 M, pH 2.0 (prepared from 0.1 M orthophosphoric acid, Cicarelli Argentina, and brought to pH 2.0 with 4 M NaOH); and with the same buffer, sample volume was brought to 30 mL. For protein digestion, 9 mL of pepsin (Sigma P-7000, 975 units/mg protein, E1%/280) solution (5.8 mg of pepsin/mL in 0.1 M phosphate buffer, pH 2.0) was added. Samples were incubated for 2 h at 37°C in a shaking bath (200 strokes/min) (Vikinf Dufnoff 5002, Argentina). Digestion reaction was stopped by the addition of 2 mL of 2 N NaOH. Duplicate 1-mL aliquots were taken from digested samples, and centrifuged at 14,000×g, 25°C, for 10 min. Digested protein was determined on 200 µL of supernatants by the Lowry method (Waterborg, 2009), measuring the absorbance at 550 nm (Spectrum SP 2000, China). A standard curve prepared with bovine serum albumin (Sigma 85040C), covering the concentration range of 0.1–2.0 mg/mL, was used.

2.4. Statistical analysis

Unless otherwise specified, determinations of every sample in each of the three batches were performed at least in duplicate at all substitution levels.

Data were statistically analysed using InfoStat Statistical Software (Facultad de Ciencias Agropecuarias, UNC, Argentina) to calculate Fisher least significant difference and DGC, both tests of multiple comparisons, Pearson's correlation coefficients and analysis of variance (ANOVA).

3. Results and discussion

3.1. Cooking quality

Optimum cooking time (OCT), cooking loss and firmness of cooked pasta are shown in Table 1. Samples containing WMAF showed shorter OCT than control sample. Although cooking loss values were higher than the control sample, all of them were within 7–8% the values suggested for semolina spaghetti by Dick and Youngs (1988). Overall, firmness values decreased with the addition of WMAF to pasta formulation; but it was only at substitution level of 40% (w/w) or over, that significant differences with regard to Co were found. Indeed WMAF had a negative effect on pasta quality for being a nongluten flour. In fact, in a previous study (Martinez, Ribotta, Leon, & Añón, 2013), where the effect of different substitution levels of WMAF on the physicochemical, technological and sensory quality of pasta was evaluated, it was determined that the quality of the resulting paste remained acceptable up to 30% w/w substitution level.

3.2. Protein and dietary fibre contents in cooked pasta

Pasta made with WMAF showed protein and dietary fibre values significantly higher than the control sample (Table 2); results were consistent with the higher contents of those components measured in WMAF compared to CBWF. The protein content of the sample replaced with 30% WMAF or over, showed an increase of 23% compared to the control sample. The weakening in pasta structure produced by the addition of WMAF was responsible for the lack of higher protein values at substitution levels higher than 30%, since part of this compound was lost as cooking loss during pasta cooking. According to previous research, Rayas-Duarte et al. (1996), the addition of nongluten flours diluted the gluten strength and interrupted and weakened the overall structure of the pasta.

In the case of dietary fibre, A30 showed a 50% higher content than the control sample. The FDA has approved a health claim about "fiber-containing grain products and cancer risk", since the scientific evidence establishes that diets low in fat and high in fibre-containing grain products, fruits and vegetables are associated with a reduced risk of some types of cancer (Code of Federal Regulations [CFR], 2015a). Thereby a grain product, as is the case of the pasta studied in this work, must contain between 10 and 19% of the reference daily value of fibre content per serving (CFR, 2015b)—which for dietary fibre is 25 g (CFR, 2015c), i.e. between 2.5 and 4.75 g of dietary fibre per serving—to include this health claim on its label. Therefore, the health claim requirements could be obtained from pasta made from WMAF at a substitution level of 30% or higher, since a raw pasta portion of 55 g—according to 21 CFR 101.12 (CFR, 2015d)—with 30, 40 and 50% of WMAF presented 3.23, 3.65 and 3.62 g of dietary fibre, respectively (Table 2).

3.3. Squalene content

From Soxhlet lipid extraction of lyophilized WMAF, CBWF and pasta samples, the squalene was recovered by SPE. The identification and quantification of this compound were obtained using RP-HPLC equipped with PDA detector and a squalene reference standard, through linear regression analysis ($y = 7 \times 10^7 + 57509$, $R^2 = 0.995$; where y = area, and x = mg/mL). A typical HPLC profile of squalene and PDA UV spectrum between 190 and 400 nm is shown in Figure 1(A) and (B).

The amount of oil found in WMAF was 7.8 ± 0.1 g/100 g (dry basis), while the squalene content was 4.56 ± 0.14 g/100 g of oil present in the flour, which is equivalent to 0.35 ± 0.01 g/100 g WMAF. Results were consistent with previous works where squalene content was determined in *A. mantegazzianus* (Berganza et al., 2003), and also in other amaranth species (He et al., 2002; León-Camacho, García-González, & Aparicio, 2001).

Cooked pasta presented a squalene content ranging from 15 to 68 mg/100 g for A15 and A50, respectively; while all substitution levels presented significant differences among them (Figure 1C). The importance of enriching pasta with squalene derives from the many health benefits attributed to this compound, such as anticancer activity, hypocholesterolemic effects, antihypertensive action as well as cardioprotective and antioxidant properties, as described above. The average dietary

intake of squalene in the United States has been estimated to be only about 30 mg/day, while in Mediterranean countries it has been calculated to be in the range of 200–400 mg/day. This large difference in average dietary intake of squalene between Mediterranean countries and the United States (7–13-fold) may be related to lower cancer mortality rate in the Mediterranean basin countries (Newmark, 1997). In this regard, the amount of squalene in a serving of A30 (55 g of raw pasta, according to 21 CFR 101.12, (CFR, 2015d), resulted in 60 ± 5 mg; therefore, this portion could contribute to double the amount of squalene consumed in United States, and to increase between 15 and 30% of that consumed in Mediterranean countries per person, per day.

3.4. In vitro starch hydrolysis and GI

The course of *in vitro* starch hydrolysis of cooked pasta samples and the bread sample, considered as the reference food, are shown in Figure 2. Quantification was performed by linear regression analysis ($y = 0.84613x − 0.00080$, $R^2 = 0.99950$; where $y = abs$, $x =$ mg of maltose/0.5 mL). It was found that the amount of hydrolysed starch of bread sample was much higher than that measured in pasta samples, at all testing times. The degree of hydrolysis increased rapidly in all samples from time 0 to time 30 min, but then the measured amount of hydrolysed starch vs. time was much lower. In the case of bread sample, the rate of hydrolysis was so small after 30 min that upon reaching 90 min, the curve resembled a plateau. Although the rate of hydrolysis in pasta samples was much less pronounced after the first 30 min, it was sustained until the 180 min evaluated. This marked difference between the hydrolysis curve of bread and those observed for pasta samples, confirmed that enzyme accessibility to a compact structure like pasta delayed hydrolysis rate (Kim et al., 2008; Sung & Stone, 2005). According to Giacco et al. (2001), bread has a highly porous structure because of the entrapment of gas bubbles that expand during cooking, resulting in an increase in the surface exposed to enzyme activity.

The HI and the GI of all samples, calculated from the relationship between the areas under hydrolysis curves, are shown in Table 3. To assess the degree of fit of the equation $C = C_\infty (1−e^{−kt})$ calculated, and thus,

Table 1. Optimum cooking time (OCT), cooking loss and firmness of cooked pasta			
Samples	OCT (min)	Cooking loss (g/100 g)[1]	Firmness (N)
Co	8	6.1 ± 0.1a	1.42 ± 0.11c
A15	7	6.6 ± 0.1b	1.38 ± 0.04bc
A30	5	6.7 ± 0.1b	1.39 ± 0.08bc
A40	5	7.3 ± 0.2c	1.26 ± 0.08b
A50	4	7.9 ± 0.1d	1.08 ± 0.09a

Notes: A15, A30, A40 and A50: pasta prepared with 15, 30, 40 and 50% (w/w) of wholemeal amaranth flour, respectively. Co: pasta prepared with 100% of bread wheat flour.

[1]g/100 g of dry pasta. Values followed by different letters in the same column are significantly different ($p ≤ 0.05$), according to Fisher test.

Table 2. Protein and dietary fibre contents of cooked pasta		
Samples	Protein[1] (g/100 g)	Dietary fibre[1] (g/100 g)
Co	4.3 ± 0.0a	1.4 ± 0.0a
A15	4.7 ± 0.1b	1.7 ± 0.1b
A30	5.3 ± 0.1c	2.1 ± 0.1c
A40	5.3 ± 0.0c	2.2 ± 0.1d
A50	5.3 ± 0.0c	2.3 ± 0.0e

Notes: A15, A30, A40 and A50: pasta prepared with 15, 30, 40 and 50% (w/w) of wholemeal amaranth flour, respectively. Co: pasta prepared with 100% of bread wheat flour.

[1]Grams of protein or dietary fibre/100 g of cooked pasta. Values followed by a different letter are significantly different ($p ≤ 0.05$), according to Fisher test.
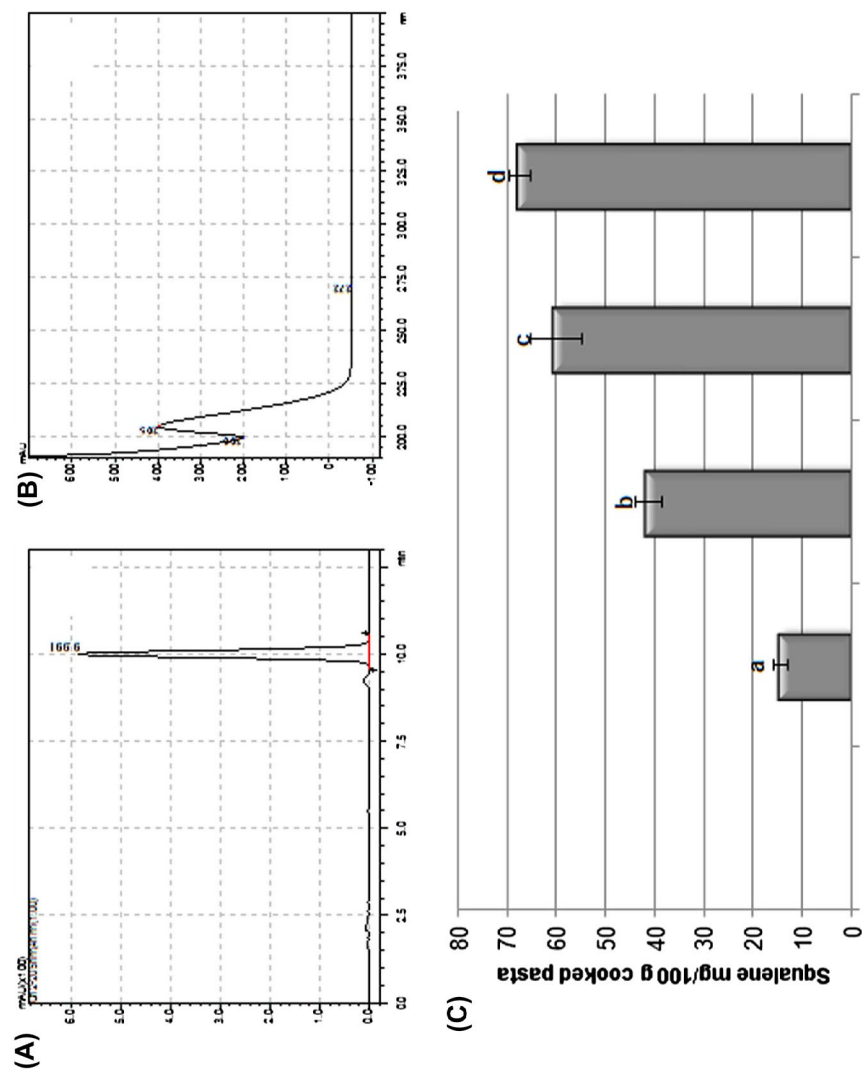
Figure 1. (A) HPLC profile of squalene; (B) PDA UV spectrum between 190 and 400 nm; (C) Squalene content in cooked pasta made with amaranth flour. A15, A30, A40 and A50: pasta made with 15, 30, 40 and 50% (w/w) of WMAF, respectively. Co: control sample made with 100% of CBWF. Values followed by a different letter are significantly different ($p \leq 0.05$). LOQ: 0.1 µg/mL under experimental conditions.

Figure 2. Hydrolysed starch percentage vs. total starch of cooked pasta made with 15, 30, 40 and 50 (w/w) of WMAF, respectively. Co: control sample made with 100% of CBWF.
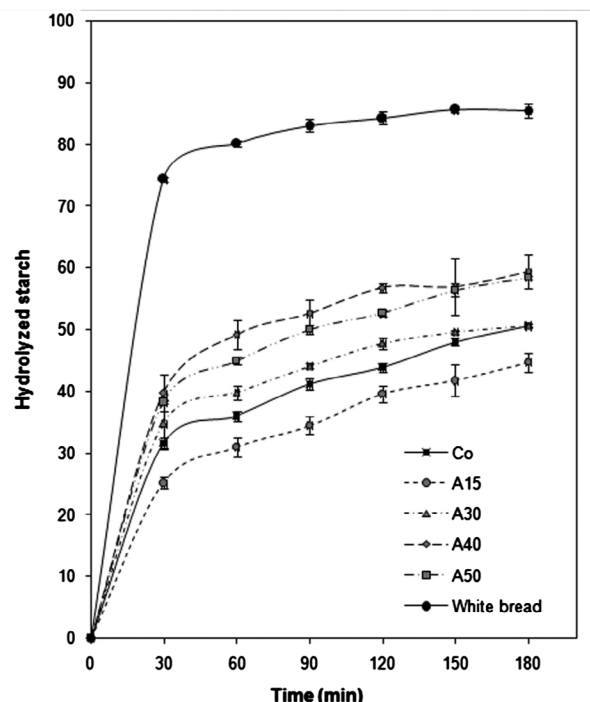
Table 3. Hydrolysis index (HI), estimated glycemic index (GI) and GI classification			
Sample	HI[1]	GI[2]	GI classification (vs. bread)[3]
Co	50.2 ± 0.8b	67 ± 1b	Low GI
A15	43.1 ± 1.8a	64 ± 1a	Low GI
A30	53.6 ± 1.5c	69 ± 1b	Low GI
A40	63.3 ± 2.6d	74 ± 2c	Low GI
A50	60.3 ± 0.5d	73 ± 0c	Low GI
White bread	100	95 ± 1d	Medium GI

[1]HI = area under hydrolysis curve (AUC) of cooked pasta/AUC of bread, taken between 0 and 180 min, using the equation $C = C_\infty (1-e^{-kt})$ to plot and integrate the curves.

[2]GI: calculate as GI = 39.71 + (0,549 × HI) according to Goñi et al. (1997).

[3]According to Schakel et al. (2008). Values followed by a different letter are significantly different ($p \leq 0.05$), based on Fisher test.

validate the HI obtained; a Pearson correlation analysis was performed between experimental and estimated values at each time of the test, for all samples (data not shown). Significant correlation coefficients ($r \geq 0.997$; $p < 0.001$) at all times studied were found, corroborating the validity of the HI calculated.

The highest values of HI corresponded to A40 and A50, while A15, followed by Co, showed the lower ones. The different HI values may be explained by the competition of two factors: the TOC and the weakening of the pasta structure given by the addition of WMAF. In the case of A15, the lower exposure time to cooking water, with respect to the control sample, weighed more than the weakening of its structure; so cooking water took less time to diffuse into the pasta, water absorption was lower (data not shown), starch was less likely to gelatinize and therefore, hydrolytic enzymes were less able to digest it. But as substitution levels increased, the damage of pasta structure due to WMAF addition prevailed over the shorter cooking times. Since pasta structure was becoming weaker and more porous, it was easier to hydrate, promoting starch gelatinization and thus the activity of the enzymes; hydrolysed starch produced similar values for A30 with respect to Co, and higher for A40 and A50.

According to Schakel, Schauer, Himes, Harnack, and Van Heel (2008), food can be classified as low, medium and high GI. When the reference food is bread, these values are: low GI ≤ 79; medium GI between 80 and 99; and high GI ≥ 100.

In this context, all pasta samples were classified as low GI with the health benefits involved. Indeed, the consumption of low-GI diets has been associated with higher HDL cholesterol concentrations (Ford & Liu, 2001), and with a lower risk of developing diabetes and cardiovascular disease (Jenkins et al., 2002). In addition, studies have shown positive associations between GI and colorectal and endometrial cancer risk (Gnagnarella, Gandini, La Vecchi, & Maisonneuve, 2008).

3.5. In vitro protein digestibility

One of the first factors that must be evaluated to estimate food protein quality is its digestibility. In principle, *in vitro* digestion provides a faster and easier alternative to animal and human models (Hur, Lim, Decker, & McClements, 2011). Indeed, in this work, *in vitro*-digested proteins were quantified through Lowry method, by linear regression analysis ($y = 2.739x + 0.0364$, $R^2 = 0.9928$; where $y = abs$, $x = $ mg/41 mL), using bovine serum albumin as reference standard. Protein digestibility of pasta made with WMAF was higher than that of pasta made with CBWF only, at all substitution levels (Table 4). Thus, Co showed a protein digestibility value of 76.1% w/w of the total protein presented in cooked pasta, while samples made with WMAF showed values between 84.7 and 89.7% w/w, comparable to those obtained by Nur Herken, İbanoğlu, Öner, and İbanoğlu (2006) and Rayas-Duarte et al. (1996), in *durum* wheat macaroni and in spaghetti made with amaranth flour, respectively.

The higher values of protein digestibility found in pasta prepared with WMAF could be due to the greater degree of breaking up of the samples produced during the chewing simulation performed in the test. In addition, the use of non-gluten flour and the presence of large tegument plaques from amaranth seed pericarp, which cause a marked discontinuity in the protein network, were responsible for the weakening of pasta structure. As a result, enzyme activity was facilitated, thus allowing protease enzyme to hydrolyse the proteins present in the pasta.

Apart from the structure and conformation of proteins, antinutritional factors such as phenolics, and trypsin inhibitors, cytoagglutinin in amaranth grains have been identified (Silva-Sanchez, Gonzalez-Castaneda, De Leon-Rodriguez, & Barba De La Rosa, 2004). Given that the amaranth flour used in this work was prepared from raw grains, a negative effect on digestibility could be expected. However, according to the results, it is considered that the cooking process of the pasta was enough to inactivate those anti-nutritional factors that might be present in the samples, which were, in fact, described as thermolabile by Gamel, Linssen, Mesallam, Damir, and Shekib (2006).

Additionally, since pasta was dried at low temperature (30°C), the presence of protein aggregates in pasta dried at high temperatures after Maillard reaction which may affect protein digestibility by up to 10% (Petitot et al., 2009), was also dismissed.

Table 4. *In vitro* protein digestibility of cooked pasta	
Sample	Protein digestibility[1] (g/100 g)
Co	76.1 ± 3.7a
A15	89.7 ± 6.9b
A30	89.7 ± 1.8b
A40	84.7 ± 4.2b
A50	84.9 ± 5.7b

[1]Grams of digested protein/100 g total protein content of cooked pasta. Values followed by a different letter are significantly different ($p \leq 0.05$), based on DGC test.

4. Conclusions

The substitution of bread wheat flour for WMAF up to a level of 30% (w/w), significantly improved protein and dietary fibre contents, reaching an increase of 23 and 50% with regard to control sample, respectively. With regard to dietary fibre content achieved in pasta made with WMAF at a substitution level equal or higher than 30%, it is understood that these samples could meet the requirements of FDA heath claim about fibre-containing grain products and cancer risk.

Squalene content attained in a single portion of dry pasta made with 30% (w/w) of WMAF could provide twice as much the squalene consumed per person in the United States, and between 15 and 30% of that consumed in Mediterranean countries per day.

From *in vitro* starch hydrolysis curves, it was found that the amount of hydrolysed starch of pasta samples was much lower than the one measured in bread sample during all testing time. According to the GI estimated, all samples were classified as low GI with all the health benefits involved.

Besides, *in vitro* protein digestibility of pasta made with WMAF was significantly higher at all substitution levels than control sample, thus ensuring that the enhanced protein content will be assimilated and utilized by the body.

In conclusion, it is possible to assert that the substitution of bread wheat flour with wholemeal flour from *A. mantegazzianus* significantly improves the nutritional quality and functional properties of the resulting pasta. So, the pasta from this work are presented as part of a healthy diet.

Competing interests
The authors declare no competing interests.

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