Original research article

Nutrient composition and *in vitro* digestibility of fresh pasta enriched with *Vicia faba*

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Abstract

Nutritionally enriched fresh pasta was prepared from semolina fortified with *Vicia faba* flour. Three addition levels were tested (10, 30 and 50%) and plain pasta (100% semolina) was used as a control. Enriched pasta showed lower cooking time, and higher dry matter loss, but with a similar water uptake. The shape of the pasta was not significantly affected by the cooking process. Color parameters indicated comparable brightness between samples and higher redness values for enriched pasta. The incorporation of broad-bean flour resulted in a significant increase in protein levels

(21% against 13.7% in 50% enriched pasta and the control, respectively), fiber, resistant starch (from 1.4% in the control to 2.5% in 50% pasta), ash and minerals (calcium, iron and zinc). The mineral dietary reference intake contributions were higher in fortified pasta, and the enrichment percentage of 30% was the highest level, allowing improved iron availability. *In vitro* percent protein digestibility increased proportionally with the broad-bean substitution level. The rate of starch hydrolysis was reduced upon broad-bean enrichment, resulting in lower glycemic index (GI) for enriched pasta (91.9, 83.4 and 71.3 in 10%, 30% and 50% pasta, respectively) compared to traditional pasta (95.9) and white bread (100).

Keywords: Fresh pasta; Broad-bean flour; Food composition; Food analysis; Cooking properties *In vitro* digestibility; Mineral availability; Food fortification

Chemical compounds studied in this article

Myo-inositol hexakisphosphate (PubChem CID: 890)

1 Introduction

Pasta has been consumed in the Mediterranean countries for many centuries and takes the second place after bread in world consumption (Mariani-Constantini, 1988; Torres et al., 2007). However, in the last few decades the demands for wheat-based products with added value have been growing rapidly (Gandhi and Zhou, 2014). Pasta may represent an excellent model food vehicle for the addition of specific nutrients through incorporation of various products (eggs, milk powder, vegetables, fiber, legumes, and so on) in a targeted food product to enhance nutritional quality, improve health condition and reduce the risk of diseases (Miceli et al., 2015).

Broad beans (*Vicia faba*), belonging to the family of leguminosae, are largely consumed in the Middle East, North Africa and South America. They represent a source of energy, protein, folic acid, niacin, vitamin C, magnesium, potassium, iron and dietary fiber (Azasa et al., 2009; Gimenez et al., 2013). Legume proteins are known to contain high levels of lysine and threonine, two essential amino acids that are deficient in cereal proteins (Abdel-Aal and Hucl, 2002). Hence, they represent an adequate complement to cereal proteins.

Pasta is classified into two major classes: fresh and dried. There are more than 400 unique types of filled and non-filled pasta, all with different forms and shapes. Non-filled fresh pasta is a widespread and appreciated type of pasta in Italy. This category includes various kinds such as *fettuccine, tagliatelle, penne, maccheroni, fusilli, pappardelle, rigatoni, capellini, conchiglie, Cicatelli*, etc. (Zanini De Vita, 2010). Fresh pasta can be produced with either soft wheat (*Triticum aestivum*) or hard wheat (*Triticum durum*) (Miceli et al., 2015). Some of these pastas are commonly produced artisinally as *Cicatelli*, which is a typical southern Italian fresh pasta, made exclusively with durum semolina wheat and water, and having an elongated, hollow shape. Starch, an important part of a balanced diet, presents up to 70% of wheat semolina pasta. While highly refined grains have a high glycemic index (GI), pasta, due to its compact structure, is considered as a source of slowly released carbohydrates; therefore processing a low GI (Hager et al., 2013). Fresh pasta fortification with legumes has not received so much attention from the scientific community. Hence, it is interesting to focus on this topic and investigate and bring out more information about the ability to produce this kind of food and evaluate their nutritional quality, along with technological and sensory aspects.

The purpose of the present work was to substitute durum wheat semolina in fresh *Cicatelli* pasta production with broad-bean (*Vicia faba*) flour at different percentages (10, 30 and 50%) in order to assess quality attributes, cooking behavior, starch and protein digestibility and mineral availability.

2 Material and methods

2.1 Raw materials

Durum wheat semolina is the common type of wheat used for pasta production, and it was kindly supplied by Manfredonia Fattoria (Foggia, Italy). The broad beans were cultivated and harvested in Kabylia region (Feraoun, Bejaia, Algeria). The dehulled grains were ground with a traditional mill and then sieved to pass through a 500 µm mesh screen.

2.2 Pasta production

Pasta produced was of the *Cicatelli* type, on a pilot scale according to the specifications and procedure of the Manfredonia Fattoria. Formulations included durum wheat semolina mixed with water in the case of traditional pasta, and durum wheat semolina and *Vicia faba* flour with water in the case of composite pasta. Four types of pasta were produced: a control made of 100% durum wheat semolina and three fortified pastas with 10%, 30% and 50% of the semolina, respectively, was replaced with *Vicia faba* flour. Fresh pasta was made on a moving belt by a robot that simulates the work of the human hand forming the *Cicatelli* shape. After that, a pasteurization treatment was performed by conveying the pasta through a chamber where steam was circulated both over and under the product. This step was subsequently followed by transporting the pasta to another chamber, where the product was dried with hot air to a final moisture content of 30-32%. Pasteurization was applied on fresh pasta in order to eliminate mould spores

and avoid the proliferation of spoilage microorganisms. After pasteurization and packaging in vacuum packages, the *Cicatelli* were freeze-dried and the obtained powders stored at 4±2°C in polyethylene tubes.

2.3 Chemical composition

Freeze-dried raw pasta samples were analyzed for moisture (AOAC 1998 Official Method 925.09), starch (AOAC Official Method 996.11), proteins (Kjeldahl, AACC 46-13), lipids (AOAC 1998 Official Method 945-16) and ash (AACC 1999 Official Method 08-03.01). Measurements were done in triplicate. The resistant starch content in cooked pasta was determined by quadruplicate according to the AOAC Method 2002.02. Briefly, samples were incubated with an enzymatic solution of pancreatic α-amylase (10 mg/mL) containing amyloglucosidase (3 U/mL) for 16 h in a shaking water bath at 37°C. After centrifugation, the non-digested material was solubilized in 2mol/L KOH, and then hydrolyzed with amyloglucosidase (EC 3.2.1.3) into glucose. The free glucose was finally quantified using glucose oxidase/peroxidase and measured spectrophotometrically at 510 nm.

2.4 Determination of total, soluble and insoluble dietary fiber

Total, soluble and insoluble dietary fiber contents were determined using AOAC Method 991.43, based on an enzymatic and gravimetric method. Fresh pasta samples were heated and gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove proteins and starch present in the sample. Ethanol was added to precipitate the soluble dietary fiber. The residues were then filtered and washed with ethanol and acetone. After drying, the residues were weighed. Half of the samples were analyzed for crude protein and the others were ashed. Analyses were performed in triplicate.

2.5 Determination of minerals

The total iron, calcium and zinc concentrations in uncooked and cooked fresh pasta were estimated using a Flame Atomic Absorption Spectrometer (Unicam 939 spectrometer, Burladingen, Germany). Previously, samples (0.5g) were placed in a Teflon perfluoroalkoxy (PFA) vessels and treated with 4 mL HNO_3 14M (Merck, Germany) and 1 mL of H_2O_2 30% v/v (Pancreac Quimica, Spain). The Teflon PFA vessels were irradiated at 800 W (15 min at 180°C) in a microwave accelerated reaction system (MARS) from CEM (Vertex, Spain). At the end of the digestion program, the digests were placed in polypropylene tubes and made up to final volume with 5% HCl. Measurements were done in triplicate.

2.6 Determination of phytate

Phytate content of fresh pasta was measured in triplicate using a commercially available kit (K-Phyt 07/11 Megazyme, Ireland 2011). As per the manufacturer's instructions, phytates were extracted from 1 g of sample using 10 mL of HCl and then subjected to a dephosphorylation step with phytase and alkaline phosphatase. The total phosphate realized was measured using a colourimetric method using a color reagent of ammonium molybdate and ascorbic acid. The amount of molybdenum blue formed is proportional to the amount of inorganic phosphate present in the sample and was measured by the increase in the absorbance at 655 nm. Total phosphates was quantified as phosphorous from a calibration curve generated using phosphorus standard solutions using a spectrophotometer (Spectronic Unicam, Helios gamma, Birmingham, UK).

2.7 *In vitro* starch digestion and glycemic index

In vitro starch digestion and GI evaluation were estimated following the method described by Goñi et al. (1997). Briefly, the digestion procedure included a cooked fresh

pasta sample (100 mg) in 10 mL HCl-KCl buffer (pH 1.5) with 400 μL pepsin 0.1 g/mL (Sigma P7000) and constant stirring for 1 h in a water bath at 40 °C. The volume was adjusted to 20 mL with Tris-Maleate buffer (pH 6.9). Then, 10 mL of a solution containing α-amylase (Sigma A6255), equivalent to 48 IU of enzyme activity per gram of sample in Tris-Maleate buffer (pH 6.9) was added. The samples were incubated at 37 °C in a shaking water bath (Ultrasonic Raypa UCL-200, Barcelona, Spain). Aliquots of 1 mL each at 0, 20, 40, 60, 90, 120 and 180 min were obtained and incubated at 100 °C for 5 min to inactivate the enzyme. Each test was cooled at the end of the incubation time. After centrifugation (10,000 g at 4 °C) 500μL of each supernatant was taken to a volume of 2 mL with sodium acetate buffer (pH 4.75). Then, 60 μL amyloglucosidase, 82 mg/mL, equivalent to 330 units (Sigma 10115) were added and incubated at 60 °C for 45 min with constant stirring. Subsequently, released glucose was determined in quadruplicate spectrophotometrically according to a commercially available enzymatic kit (D-Glucose Assay Procedure, K-GLUC 07/11, Megazyme). The rate of starch digestion was expressed as the percentage of total starch hydrolyzed at 0, 20, 40, 60, 90, 120 and 180 min. The area under the curve (AUC) from 0 to 120 min, and total digestible starch was used to calculate an *in vitro* GI value normalized against white bread (SigmaPlot software, Version 12.0) expressed as a percentage.

2.8 *In vitro* protein digestion

The *in vitro* protein digestibility of cooked fresh pasta samples was determined using the method described by Hsu et al. (1977). Then 50 mL suspensions of aqueous protein based on crude protein content (6.25 mg protein/mL) were allowed to rehydrate for 60 min at 5°C with intermittent mixing. After rehydration, samples were placed in a 37°C water bath and the pH was adjusted to 8 using 0.1 N HCl (Merck, Germany) or 0.1 N NaOH (Pancreac Quimica, Spain). Five milliliters of enzyme solution were then added

166 to the protein suspension, which was stirred at 37°C. The trypsin (≥10,000 BAEE
167 units/mg protein, Sigma T1426) had an activity of 13766 BAEE units/mg proteins. A
168 rapid decline of pH occurred immediately. The pH drop was recorded 15 s after enzyme
169 addition and at 1 min intervals for 10 min. Sample analysis was carried out in triplicate.
170 The enzyme solution was freshly prepared before each series of tests. The percent
171 protein digestibility (*Y*) was calculated using the following equation:

172 $Y = 210.464 - 18.1\,x$,

173 where *x* is the change in pH after 10 min.

174 ## 2.9 Cooking properties

175 ### *2.9.1 Cooking procedure*

176 The method described by Gelencsér et al. (2008) was followed, with some
177 modifications. Fresh pasta (25 g) was boiled in distilled water (250 mL) for the optimal
178 cooking time, considered as the time necessary to obtain complete gelatinization of
179 starch, and was estimated by removing a piece of pasta from the water at 30 s intervals
180 and pressing it between fingers. After cooking, pasta was removed from the water,
181 rinsed with distilled water and drained for 2 min. The cooking procedure was repeated
182 twice for each formulation.

183 ### *2.9.2 Cooking behavior*

184 Cooking loss: the matter loss of *Cicatelli* during cooking was evaluated by combining
185 the cooking and rinse waters in a beaker and transferring triplicate aliquots (1 mL) to a
186 pre-weighed 1.5 mL microfuge tube (Vaudaux-Eppendorf, Hamburg, Germany). The
187 tubes were then subjected to a concentration of their content in a concentrator
188 (Eppendorf Concentrator 5301 AG 22331, Hamburg, Germany) for water evaporation.

After that, the tubes were dried overnight at 105 °C. The resulting weighed residue was reported as a percentage of the original pasta sample.

Ash loss: to evaluate the loss of inorganic material in cooking water, 10 mL of cooking water were transferred to a previously heated and weighed porcelain vessels (Porcelaine d'Avignon, France) and left to dehydrate over a sand bath overnight at 40°C. The vessels were then ashed in a muffle furnace (Nabertherm controller B170, Germany) at 600°C for 2 hours. The resulting residue was weighed and reported as mg of ash in 100g of pasta sample.

Water absorption: the amount of water in drained pasta was evaluated by weighing the cooked pasta and expressed as a percentage following the equation:

$$WA = [(W_1 - W_2)/ W_2] \times 100,$$

Where WA = water absorption, W_1 (g) = weight of cooked pasta, and W_2 (g) = weight of raw pasta.

2.10 Color of pasta

Samples color was measured using a Chromameter (Model Konika Minolta, Sensing INC, Japan). The color differences were recorded as CIELab, L^*(lightness), a^*(redness-greeness) and b^*(yellowness-blueness) values. The colorimeter was standardized with a white plate, supplied with the equipment. Three readings were made for each sample. ΔE^* was calculated to estimate how far apart visually the samples and control are in color for uncooked and cooked pasta, following equation:

$$\Delta E^* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

2.11 Statistical analysis

Multiple sample comparison of the means (ANOVA) and Fisher's least significant differences (LSD) were applied to establish statistical significant differences between samples. All statistical analyses were carried out with the software Statgraphics Plus 7.1 (Bitstream, Cambridge, MN, USA) and differences were considered significant at $p<0.05$.

3 Results and discussion

Raw materials have been analyzed in the laboratory for their chemical composition and the results (in dry matter) were as follows: 11.73±0.04% moisture, 78.6±0.1% starch, 13.53±0.07% proteins, 2.02±0.04% lipids and 0.95±0.01% ash, for durum wheat semolina; 10.7±0.2% moisture, 46.6±0.1% starch, 30.9±0.5 proteins, 2.7±0.1 lipids and 2.97±0.01% ash, for broad-bean flour.

3.1 Composition of fresh pasta and technological parameters

Table 1 shows the chemical composition, color parameters and cooking properties of the fresh pasta that was artisinally produced for this study. Moisture content decreased significantly ($p<0.05$) as the substitution level increased. Similar results were reported by Giuberti et al. (2015) for spaghetti enriched with bean (*Phaseolus vulgaris L.*) flour. The higher nutrient content of broad-bean flour (proteins, ash and minerals, dietary fibre and resistant starch) as compared to semolina allowed their incorporation into pasta formulations and resulted in composite pasta with improved nutritional value. Starch is the main component in cereal grains and pasta products. It tends to decrease significantly as the level of substituted semolina increases (69.2% to 56.7% in the control and 50% enriched pasta, respectively). As expected from the high protein content of broad-bean flour, the amount of this macromolecule increased significantly

(*p<0.05*) as the percentage of added bean flour increased (from 13.6 to 20.9% in the control and 50% enriched pasta, respectively). Ash follows the same behavior as proteins (from 0.7% in traditional pasta to 1.9% in 50% composite pasta, respectively). Fortification of pasta with broad-bean flour gradually and significantly (*p<0.05*) increased the concentrations of Ca, Fe and Zn. While the amount of calcium almost doubled from traditional pasta to 50% composite pasta (from 125 to 243 μg/g, respectively), iron concentration was tripled and quadrupled in 30% and 50% enriched pasta, respectively. Zinc values varied from 6.8 µg/g in control pasta to 17.1 µg/g in pasta substituted with 50% broad-bean flour. These results are in agreement with those reported by Petitot et al. (2010) who noticed an increase of protein, fiber, ash and mineral contents in pasta containing broad-bean flour and by Carini et al. (2012) for fresh soy-enriched pasta. Substitution of 10% durum wheat semolina by broad-bean flour in pasta production was not able to significantly improve the contents of soluble, insoluble and total dietary fiber. However, 30% and 50% fortification levels resulted in a significant increase in these components. Insoluble dietary fiber was higher than soluble dietary fiber in all pasta produced.

The replacement of durum wheat semolina with broad-bean flour in *Cicatelli* production significantly (*p<0.05*) reduced its optimal cooking time (Table 1). This reduction is probably due to the higher dietary fiber contribution that may have facilitated the penetration of water to the core of pasta (Chillo et al., 2008). Water uptake is similar in all tested fresh pasta. This result does not support the results reported by Alamprese et al. (2007) or Brennan and Tudorica (2008), who noticed a higher water uptake in fresh pasta enriched with buckwheat and non-starch polysaccharides, respectively, but is similar to the values found by Akillioglu and Yalcin (2010). This may indicate a different fiber composition of broad beans compared to the materials used by the

authors cited above, or that fiber content in composite pasta is lower than the level required to induce an increase in water absorption during cooking. Cooking losses increased proportionally with the substitution level as shown by Carini et al. (2012) in pasta-soy formulation. Durum wheat proteins are mainly composed of insoluble proteins (glutenins and gliadins) that are responsible of the formation of intra and inter-molecular disulphide bonds during processing of pasta or bread, which leads to the formation of a strong tridimensional gluten network having the ability to entrap starch granules. On the other hand, legumes are essentially composed of soluble proteins (globulins and albumins) (Mahe et al., 1994; Duc, 1997). Thus, the increase of cooking loss in enriched fresh pasta may be due to the introduction of non-gluten proteins that diluted and weakened the gluten network strength. The weakening of pasta structure results in leaching of more dry material in cooking water as shown in Table 1. The values of cooking losses of our pasta are higher than those obtained by Zardetto and Dalla Rosa (2009) but lower than those reported by Brennan and Tudorica (2008) for fresh fiber-enriched pasta. Ash content in cooking water is generally higher in enriched fresh pasta compared to the control (from 4.71 mg/100g in control pasta to 7.28 mg/100g in 50% substituted pasta).

Fresh pasta color is a very important quality attribute that greatly influences consumer acceptance, and it is the only property that the consumer can evaluate when selecting a product in the market (Carini et al., 2009). L* values did not greatly change between samples ($p>0.05$). The a* values were negative for all tested pasta, indicating a tendency towards red, and enriched pasta was redder than the control regardless of the substitution level. Pasta yellowness decreased in enriched pasta compared to the control; this is probably due to the lower carotenoid pigments present in beans compared to durum wheat semolina and the existence of other pigments, particularly the

proanthocyanidins (Baginsky et al., 2013). The 30% and 50% enriched pasta were closer to the control in yellowness with regard to pasta substituted with 10% broad-bean flour.

Our results did not support those obtained by Carini et al. (2009) who reported significantly different parameters (L^*, a^* and b^*) in soy composite fresh pasta compared to the control. ΔE^* values indicated that both enriched raw and cooked pasta are significantly different from control raw and cooked pasta, respectively, regardless of the substitution level. These results are supported by the images in Fig. 1, showing *Cicatelli* aspect before and after cooking. Indeed, there were slight but perceptible differences in color between samples. The size of pasta has expanded as a result of broad-bean flour addition and cooking process, with control pasta having the best quality aspect with regard to enriched pasta.

3.2 *In vitro* starch digestion and glycemic index

White bread showed the highest levels of starch hydrolysis compared to traditional and enriched fresh pasta (Table 2). This result is supported by Jenkins et al. (1983), who reported that the blood glucose response of diabetic subjects was reduced upon consumption of spaghetti pasta compared to white wheat bread. There were significantly ($p<0.05$) more reducing sugars released from pasta made of exclusively durum wheat semolina than from broad-bean composite pasta samples. This may be due to the higher fiber content of enriched pasta compared to the fresh pasta control (Table 1). Brennan and Tudorica (2008) observed the same behavior in fresh pasta samples with increased fiber content.

Resistant starch is the proportion of starch that is not hydrolyzed in the small intestine and partially or totally fermented in the large intestine. Its amount is relatively higher in

legume seeds compared to cereals; hence, it increased significantly as the percentage of fortification increased (from 1.4% in the control to 2.5% in 50% enriched pasta) (Table 2). The GI is a ranking parameter for carbohydrate-containing foods, from 0 to 100 based on the ratio of area under the curve (0-180 min) compared to that of a reference white wheat bread. In this study, the hydrolysis index decreased significantly ($p<0.05$) as the percentage of added broad-bean flour increased (from 95.9 in control pasta to 71.3 in 50% enriched pasta, respectively); this latter value recorded for 50% enriched *Cicatelli* is close to the range of "Medium GI foods" in the GI ranking system (Kumar and Prabhansankar, 2014).

According to Cavallero et al. (2002), bread exhibits a sponge structure which is highly accessible to α-amylase and illicit high glycemic responses. On the other hand, the structure of pasta has been described as a compact matrix with starch granules entrapped in a protein network (Pagani et al., 1986). This feature is thought to be largely responsible for the slow digestibility of the starch in pasta (Monge et al., 1990). Legume seeds, due to their poor digestibility related to the inherent physical and structural properties of starch, generally exhibit higher resistant starch content (Table 2) and lower GI when compared to cereal grains (Sandhu and Lim, 2008).

3.3 *In vitro* protein digestibility

Incubation of samples in the presence of trypsin results in protein hydrolysis into amino acids. The release of amino acids leads to a decrease in the pH of the medium (Dahlin and Lorenz, 1993). As illustrated in Table 2, a slight but significant increase ($p<0.05$) in the percent *in vitro* protein digestibility was observed as the level of substitution with broad-bean flour increased (from 69.8% in traditional fresh pasta to 71.5% in pasta substituted with 50% bean flour, respectively). The reason for this improvement is

probably that broad-bean flour addition increased the content of more digestible proteins (globulins) accompanied by a decrease in the poorly digestible durum wheat semolina proteins (glutelins and gliadins). Our results are supported by those reported by Rathi et al. (2004) and Anyango et al. (2011), who found an improvement of the protein digestibility of pearl millet and cowpea enriched foods, respectively.

3.4 Contribution of minerals to the dietary reference intakes (DRIs)

We caluculated the contribution of minerals to the DRIs for the consumption of a daily average portion of 200 g of Cicatelli pasta in terms of the minerals remaining in cooked pasta and prediction of their bioavailability. The data in Table 3 show the contribution of minerals from traditional pasta and enriched pasta to the DRIs given by the Food and Nutrition Board of the Institute of Medicine, National Academy Science (NAS, 2001; NAS, 2011), minerals remaining after cooking of *Cicatelli* and prediction of their availability. When expressed in terms of DRIs, both traditional and broad-bean enriched *Cicatelli* contribute less than 5% of the daily recommendation for Ca, which represents a poor intake and requires an additional dietary calcium source to offset the deficit of pasta based meal or its fortification. The NAS recommendation for Fe is based on the presumption that 75% of Fe is supplied under the form of hemic iron, and they specify that for vegetarian diet the recommendations can be doubled. Iron contribution quadruplicates when consuming a 200g portion of *Cicatelli* pasta fortified with 50% beans for adult males between 14 and 18 years old. On the other hand, 30% enriched pasta could provide three times more iron than pasta made exclusively of semolina in the case of middle-aged females (Table 3). The same tendency was observed with zinc, where increasing broad-bean flour substitution levels in pasta resulted in higher zinc contribution to dietary requirements, from 16.2 to 37.1% (0 and 50% enriched pasta, respectively) for young male and female adults, and for females aged 70 or more.

Finally, 30% bean-enriched *Cicatelli* provides twice as much zinc as does traditional *Cicatelli* for both males and females.

Phytic acid, also called *myo*-inositol hexakisphosphate, is a form of phosphorus storage in plants. Phytic acid is an antinutritional factor that has a negative effect on the bioavailability of positively charged minerals and proteins. This compound is strongly negatively charged and has therefore a great potential for complexing positively charged multivalent cations such as Ca, Zn and Fe (Graf et al., 1987; Oatway et al., 2001). This linkage affects mineral bioavailability owing to the formation of insoluble complexes which are not absorbable in the human gastrointestinal tract (Lopez et al., 2001). It is highly present in legume seeds and is therefore more pronounced in bean-enriched pasta (0.44% in 50% broad-bean fortified pasta compared to 0.13% in traditional fresh semolina pasta). Phytic acid concentrations in the produced pasta ranged from 0.17 g/100g in traditional fresh pasta to 0.44 g/100g in 50% fortified pasta. Therefore, the human body would most likely not be able to use in the increased minerals resulting from broad-bean flour addition, since this increase in minerals is accompanied by an increase in phytic acid content.

In order to evaluate the effect of the resulted higher phytic acid presence in enriched pasta on the bioavailability of the tested minerals (Ca, Fe and Zn), the phytate/mineral molar ratios have been investigated (Ma et al., 2005). The phytate/calcium molar ratio could induce less calcium bioavailability in humans at values higher than 0.24 (Morris and Ellis, 1985). Phytate begins to lose its inhibitory effect on iron when phytate/Fe molar ratios are less than 1 (Hallberg et al., 1989); whereas if the phytate/Zn molar ratio is higher than 5, the bioavailability of Zn could be reduced by 50% (Turnlund et al., 1984). InsP_6/Ca showed values ranging from 0.08 to 0.15 in tested pasta (Table 3), which indicates that calcium availability is not reduced whatever is the substitution

382 percentage. The same tendency is observed for zinc since molar ratios are inferior to 5
383 for all the samples.

384 On the other hand, InsP_6 content compromises iron absorption in both traditional and
385 enriched fresh pasta, but its adverse effect is more obvious in the control (1.83)
386 compared to broad-bean fortified pasta. Elsewhere, iron contribution is greater in the
387 case of 30% enriched pasta (1.11 against 1.44 and 1.52 in 10 and 50% composite pasta,
388 respectively). The inhibitory effect of phytate on iron bioavailability has already been
389 demonstrated in fortified bread by several authors (Sanz-Penella et al., 2012; Garcia-
390 Mantrana et al., 2015; Iglesias-Puig et al., 2015).

391 ## 4 Conclusion

392 In summary, this study has shown that production of legume-fortified pasta with
393 satisfactory visual aspect and cooking characteristics, even with relatively high
394 substitution levels (50%), is possible. The nutritional value of the *Cicatelli* that was
395 produced improved. The progressive enrichment of the pasta formula with broad-bean
396 flour resulted in significantly higher protein, ash, dietary fiber, resistant starch and
397 mineral contents. Increasing the fortification levels significantly raised phytate content
398 in pasta. The high fiber and resistant starch contribution induced a slower rate of starch
399 hydrolysis of enriched pasta and consequently lowered its GI. The *in vitro* digestibility
400 of proteins was slightly enhanced as a result of the legume-flour addition. The solubility
401 of broad-bean proteins reduced the cooking time of enriched pasta, but weakened the
402 gluten matrix and thus increased cooking losses of legume-enriched fresh pasta. Color
403 parameters indicated that composite *Cicatelli* were slightly darker, redder and less
404 yellow when compared to the control pasta. Finally, substituting 30% of semolina with

405 broad-bean flour made it possible to obtain the best mineral availability (Ca, Fe, Zn)
406 with regard to phytate content.

407 **Conflict of interest**

408 The authors declare no conflict of interest.

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417 ## References

418 Abdel-Aal, E.S.M., Hucl, P. (2002). Amino acid composition and *In vitro* protein
419 digestibility of selected ancient wheats and their end products. *Journal of Food*
420 *Composition and Analysis, 15,* 737-747.

421 Akillioglu, H.G., Yalcin, E. (2010). Some quality characteristics and nutritional
422 Properties of traditional egg pasta (Erişte). *Food Science and Biotechnology, 19*
423 *(2),* 417-424.

424 Alamprese, C., Casiraghi, E., Pagani, M.A. (2007). Development of gluten-free fresh
425 egg pasta analogues containing buckwheat. *European Food Research and*
426 *Technology, 225,* 205-213.

Anyango, J.O., de Koch, H.L., Taylor, J.R.N. (2011). Impact of cowpea addition on the protein digestibility corrected amino acid score and other protein quality parameters of traditional African foods made from non-tannin and tannin sorghum. *Food Chemistry, 124,* 775-780.

Azasa, M. S., Wassim, K., Mensi, F., Abdelmouleh, A., Brini, B., Kraïem, M. M. (2009). Evaluation of faba beans (*Vicia fabal L.* var. minuta) as a replacement for soybean meal in practical diets of juvenile Nile tilapia Oreochromis miloticus. *Aquaculture, 287,* 174–179.

Baginsky, C., Peña-Neira, I., Caceres, A., Hernandez, T., Estrella, I., Morales, H., Pertuze, R. (2013). Phenolic compound composition in immature seeds of fava bean (*Vicia faba L.*) varieties cultivated in Chile. *Journal of Food Composition and Analysis, 31,* 1-6.

Brennan, C.S., Tudorica C.M. (2008). Fresh pasta quality as affected by enrichment of nonstarch polysaccharides. *Journal of Food Science, 72(9),* 659-665.

Carini, E., Curti, E., Spotti, E., Vittadini, E. (2012). Effect of formulation on physicochemical properties and water status of nutritionally enriched fresh pasta. *Food Bioprocess and Technology, 5,* 1642-1652.

Carini, E., Vittadini, E., Curti, E., Antoniazzi, F. (2009). Effect of different shaping modes on physico-chemical properties and water status of fresh pasta. *Journal of Food Engineering, 93,* 400-406.

Cavallero, A., Empilli, S., Brighenti, F., Stanca, A. M. (2002). High (1-3, 1-4)–β-glucan barley fractions in bread making and their effects on human glycaemic response. *Journal of Cereal Science, 36,* 59–66.

Chillo, S., Laverse, J., Falcone, P. M., Del Nobile, M. A. (2008). Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. *Journal of Food Engineering, 84,* 101–107.

Dahlin, K., Lorenz, K. (2009). Protein digestibility of extruded cereal grains. *Food Chemistry, 48,* 13-18.

Duc, G. (1997). Faba bean (*Vicia faba L.*). *Field Crop Research, 53,* 99-109.

Gandhi, V.P., Zhou, Z. (2014). Food demand and the food security challenge with rapid economic growth in the emerging economies of India and China. *Food Research International, 63,* 108-124.

Garcia-Mantrana, I.; Monedero, V.; Haros, M. (2015). *Myo*-inositol hexakisphosphate degradation by *bifidobacterium pseudocatenulatum* ATCC 27919 improves mineral availability of high fibre rye-wheat sour bread. *Food Chemistry,* doi: http://dx.doi.org/10.1016/j.foodchem.2015.01.099.

Gelencser, T., Gal V., Hodsagi, M., Salgo, A. (2008). Evaluation of quality and digestibility characteristics of resistant starch-enriched pasta. *Food Bioprocess and Technology, 1,* 171-179.

Gimenez, M. A., Gonzalez, R. J., Wagnerc, J., Torres, R., lobo, M. O., Samman, N. C. (2013). Effect of extrusion conditions on physicochemical and sensorial properties of corn broad beans (*Vicia faba*) spaghetti type pasta. *Food Chemistry, 136,* 538–545.

Giuberti, G.,Gallo, A., Cerioli, C., Fortunati , P., Masoero, F. (2015). Cooking quality and starch digestibility of gluten free pasta using new bean flour. *Food Chemistry, 175,* 43–49.

Goñi, I., Garcia-Alonso, A., Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17,* 427-437.

Graf, E., Empson, K. L., Eaton, J. W. (1987). Phytic acid. A natural antioxidant. *Journal of Biological Chemistry,* 262*(24),* 11647-11650.

Hager, A.S., Czerny, M., Bez, J., Zannini, E., Arendt, E.K. (2013). Starch properties, *in vitro* digestibility and sensory evaluation of fresh egg pasta produced from oat, teff and wheat flour. *Journal of Cereal Science, 58,* 156-163.

Hallberg, L., Brune, M., Rossander, L. (1989). Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *American Journal of Clinical Nutrition, 49,* 140-144.

Hsu, H. S., Vavak, D.L., Satterlee, L.D., Miller, G.A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science, 42,* 1269-1273.

Iglesias-Puig, E., Monedero, V., Haros, M. (2015). Bread with whole quinoa flour and bifidobacterial phytases increases dietary mineral intake and bioavailability. *LWT-Food Science and Technology, 60,* 71-77.

Jenkins, D.J.A., Wolever, T.M.S., Jenkins, A.L., Lee, K., Wong, G.S., Josse, R. (1983). Glycaemic response to wheat products-reduced response to pasta but no effect of fibre. *Diabetes Care,* 6*(2),* 155-159.

491 Kumar, S.B., Prabhansankar P. (2014). Low glycemic index ingredients and modified
492 starches in wheat based food processing: A review. *Trends in Food Science &*
493 *Technology,* 35 *(1),* 32-41.

494 Lopez, H. W., Krespine, V., Guy, C., Messager, A., Demigne, C., Remesy, C. (2001).
495 Prolonged fermentation of whole wheat sourdough reduces phytate level and
496 increases soluble magnesium. *Journal of Agricultural and Food Chemistry, 49,*
497 2657-2662.

498 Ma, G., Jin, Y., Plao, J., Kok, F., Guusie, B., Jacobsen, E. (2005). Phytate, calcium, iron
499 and zinc contents and their molar ratios in foods commonly consumed in China.
500 *Journal of Agricultural and Food Chemistry, 53,* 10285-10290.

501 Mahe, S., Gausseres, N., Tome, D. (1994). Legume proteins for human requirements.
502 *Grain Legumes, 7,* 15–17.

503 Mariani-Constantini, A. (1988). Image and nutritional role of pasta-changing food
504 patterns. In: Fabriani G., Lintas C. (eds.), *Durum Chemistry and Technology*.
505 AACC, ST.Paul, MN.

506 Miceli, A., Francesca, N., Moschetti, G., Settani, L. (2015). The influence of addition of
507 *Borago officinalis* with antibacterial activity on the sensory quality of fresh pasta.
508 *International Journal of Gastronomy and Food Science, 2,* 93-97.

509 Monge, L., Cortassa, G., Fiocchi, F., Mussino, G., Carta, Q. (1990). Glyco-insulinaemic
510 response, digestion, and intestinal absorption of the starch contained in two types
511 of spaghetti. *Diabetes Nutrition and Metabolism, 3,* 239–246.

Morris, E.R, Ellis, R. (1985). Bioavailability of dietary calcium-effect of phytate on adult men consuming nonvegetarian diets. In: Kies C (eds). *ACS Symposium Series 275: Nutritional Bioavailability of Calcium*. Amerian Chemical Society: Washington, DC, USA, pp. 63

NAS. (2001). National Academy of Sciences, Institute of Medicine Food and Nutrition Board. In: Dietary Reference Intakes: *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.* URL https://www.nap.edu/login.php?record_id=10026&page=http%3A%2F%2Fwww.nap.edu%2Fdownload.php%3Frecord_id%3D10026. Accessed 15.09.15

NAS. (2011). National Academy of Sciences, Institute of Medicine Food and Nutrition Board. In: Dietary Reference Intakes: *Dietary Reference Intakes for Calcium and Vitamin D.* URL https://www.nap.edu/login.php?record_id=13050&page=http%3A%2F%2Fwww.nap.edu%2Fdownload.php%3Frecord_id%3D13050. Accessed 15.09.2015

Oatway, L., Vasanthan, T., Helm, J. (2001). Phytic acid. *Food Research International, 17*, 419-431.

Pagani, M. A., Gallant, D., Bouchet, B., Resmini, P. (1986). Ultrastructure of cooked spaghetti. *Food Microstructure, 5*, 111–129.

Petitot, M., Boyer, L., Minier, Ch., Micard, V. (2010). Fortification of pasta with split pea and faba bean flours: Pasta processing and quality evaluation. *Food Research International, 43,* 634–641.

534 Rathi, A., Kawatra, A., Sehgal, S. (2004). Influence of depigmentation of pearl millet
535 (*Pennisetum glaucum L.*) on sensory attributes, nutrient composition, in vitro
536 protein and starch digestibility of pasta. *Food Chemistry, 85,* 275-280.

537 Sandhu, K.S., Lim, S.T. (2008). Digestibility of legume starches as influenced by their
538 physical and structural properties. *Carbohydrate Polymers, 71,* 245-252.

539 Sanz Penella, J.M., Laparra, J.M., Sanz, Y., Haros, M. (2012). Assessment of iron
540 bioavailability in whole wheat bread by addition of phytase-producing
541 bifidobacteria. *Journal of Agricultural and Food Chemistry, 60,* 3190-3195.

542 Torres, A., Frias, J., Granito, M., Guerra, M., Vidal-Valverde, C. (2007). Chemical,
543 biological and sensory evaluation of pasta products supplemented with α-
544 galactoside-free lupin flours. *Journal of Science and Food Agriculture, 87,* 74-81.

545 Turnlund, J.R., King, J.C., Keyes, W.R., Gong, B., Michel, M.C (1984). A stable
546 isotope study of zinc absorption in young men: effects on phytate and α–
547 cellulose. *American Journal of Clinical Nutrition, 40,* 1071-1077.

548 Zanini De Vita, O. (2009). Encyclopedia of pasta. In: *California Studies in Food and*
549 *Culture* (eds). University of California Press, Ltd. London, England, pp. 16-19.

550 Zardetto, S., Dalla Rosa, M. (2009). Effect of extrusion process on properties of cooked,
551 fresh egg pasta. *Journal of Food Engineering, 92,* 70-77.

552

Figure captions

Figure 1. Aspect of *Cicatelli* before and after cooking. A and A', control pasta; B and B', 10% broad-bean pasta; C and C', 30% broad-bean pasta, D and D', 50% broad-bean pasta, uncooked and cooked pasta, respectively.

Figure 2.

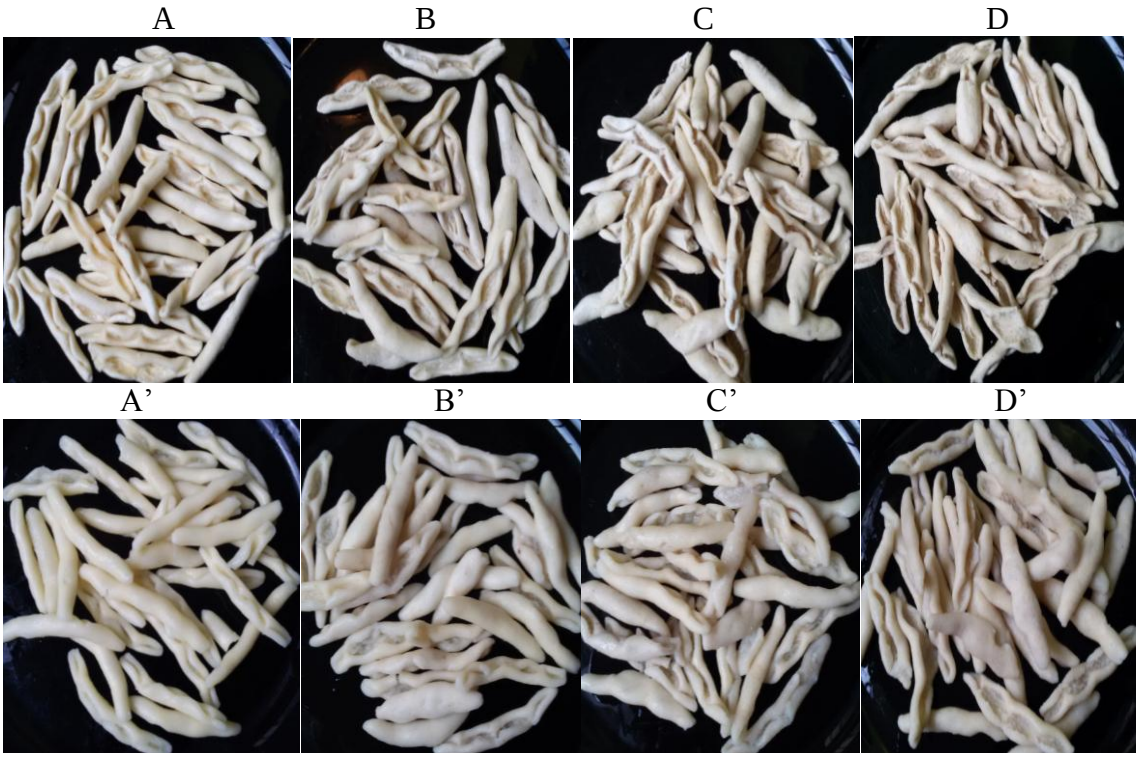

Table 1. Composition of fresh pasta and technological parameters

Parameter	Units	g of bean/100g of fresh pasta			
		0	**10**	**30**	**50**
Chemical Composition[A]					
Moisture	g/100g	11.2±0.0[d]	10.6±0.0[c]	10.4±0.0[b]	10.1±0.0[a]
Starch[B]	g/100g d.m.	69.3±0.0[d]	62.2±0.0[c]	60.1±0.0[b]	56.7±0.0[a]
Proteins	g/100g d.m.	13.7±0.4[a]	15.5±0.6[b]	18.2±0.1[c]	21.0±0.1[d]
Lipids	g/100g d.m.	1.47±0.09[a]	1.48±0.03[a]	1.45±0.05[a]	1.44±0.17[a]
Ash	g/100g d.m.	0.7±0.01[a]	0.96±0.01[b]	1.4±0.00[c]	1.88±0.02[d]
Insoluble dietary fiber	g/100g d.m.	4.1±0.3[a]	4.2±0.2[a]	6.1±0.2[b]	6.3±0.4[b]
Soluble dietary fiber	g/100g d.m.	1.0±0.2[a]	1.1±0.1[a]	1.3±0.1[b]	1.3±0.2[b]
Total dietary fiber	g/100g d.m.	5.1±0.4[a]	5.8±0.3[a]	7.2±0.1[b]	8.4±0.4[b]
Ca	mg/100g d.m.	12.5±0.2[a]	15.7±0.7[b]	21.6±1.4[c]	24.3±1.7[d]
Fe	mg/100g d.m.	0.60±0.01[a]	1.01±0.01[b]	1.81±0.03[c]	2.55±0.02[d]
Zn	mg/100g d.m.	0-68±0.01[a]	0.94±0.02[b]	1.45±0.03[c]	1.71±0.02[d]
Color Parameters[A]					
L*	uncooked	85.5±1.8[bc]	84.6±0.7[ab]	85.7±0.6[bc]	84.9±1.2[ab]
a*	uncooked	-1.94±0.08[a]	-1.19±0.01[c]	-1.49±0.05[b]	-1.84±0.07[a]
b*	uncooked	19.8±0.7[c]	14.2±0.2[a]	15.3±0.5[b]	17.7±0.4[c]
$\Delta E_{uncooked}$*	uncooked	-	9.73±0.05[c]	5.54±0.03[a]	6.14±0.02[b]
ΔE_{cooked}*	Cooked	-	8.74±0.04[g]	8.45±0.07[f]	7.77±0.05[e]
Cooking Properties[B]					
Optimal cooking time	Min	4.20±0.07[d]	3.70±0.14[c]	3.32±0.02[b]	3.00±0.14[a]
Water uptake	g/100g	50.9±1.4[a]	50.8±1.9[a]	50.3±1.1[a]	49.9±0.1[a]
Cooking loss[C]	g/100g	4.1±0.1[a]	4.9±0.2[b]	5.2±0.3[c]	5.5±0.4[d]
Ash loss in cooking water	mg/100g	4.7±0.2[a]	5.9±0.2[ab]	7.0±0.6[bc]	7.3±0.6[c]

Mean±SD, [A]n=3; [B]n=4, [C]n=6. Values followed by the same letter in the same line are not significantly different at 95% confidence level. d.m. dry matter

Table 2. Estimation of glycemic index and protein digestibility of fresh pasta

Parameter	Units	Food				
		Bread	Fresh Pasta, g of bean/100g			
			0	10	30	50
Glycemic Index Estimation						
Starch[A]	g/100g d.m.	77.1±0.5 [e]	69.1±0.1 [d]	61.9±0.2 [c]	59.9±0.2 [b]	56.2±0.1 [a]
Resistant Starch[A]	g/100g d.m.	n.d.	1.44±0.01 [a]	1.86±0.08 [b]	2.25±0.08 [c]	2.47±0.07 [d]
AUC[A]		59.9±0.1 [e]	57.5±0.2 [d]	55.1±0.5 [c]	50.0±0.2 [b]	42.7±0.2 [a]
TSH_{90}	%	69.8±1.2 [d]	58.7±0.8 [c]	51.3±0.6 [b]	48.8±0.5 [b]	45.6±0.4 [a]
Glycemic index[A]		100.0±0.2 [e]	95.9±0.3 [d]	91.9±0.9 [c]	83.4±0.4 [b]	71.3±0.4 [a]
Protein digestibility						
Proteins[B]	g/100g d.m.	n.d.	13.8±0.0 [a]	14.5±0.0 [b]	18.4±0.1 [c]	21.6±0.0 [d]
Drop of pH after 10 min[A]		n.d.	7.77±0.01 [d]	7.74±0.01 [c]	7.69±0.00 [b]	7.67±0.00 [a]
In vitro digested protein[B]	%	n.d.	69.8±0.1 [a]	70.4±0.1 [b]	71.2±0.1 [c]	71.5±0.1 [d]

Mean±SD, [A]n=4, [B]n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level. AUC, area under the curve of starch digestion; TSH_{90}, total starch hydrolyzed at 90 min; d.m. dry matter; n.d. no determined.

Table 3. Estimation of the contribution of minerals from an average portion (200g) of *Cicatelli* to the dietary reference intakes (DRIs), minerals remaining after cooking of pasta and prediction of their availability.

	DRIs	Units	% of bean flour in fresh pasta formulation			
			0	10	30	50
Contribution to DRI, %						
Ca[AB]		mg/100 g d.m.	8.7±0.6[a]	12.7±0.5[b]	17.8±0.1[c]	17.5±0.6[d]
Adult male and female (years)						
9-18	1300	mg/day	1.3	2.0	2.7	2.7
19-70[C]	1000	mg/day	1.8	2.6	3.6	3.5
≥70	1200	mg/day	1.5	2.1	3.0	2.9
Fe[AB]		mg/100 g d.m.	0.58±0.01[a]	0.99±0.02[b]	1.82±0.02[c]	2.45±0.08[d]
Adult male (years)						
14-18	11	mg/day	10.5	18.0	33.08	44.46
9-13, 31-70, ≥70	8	mg/day	14.5	24.8	45.49	61.13
Adult female (years)						
14-18	15	mg/day	7.7	13.2	24.3	32.6
19-50	18	mg/day	6.4	11.0	20.2	27.3
9-13, 51-70, ≥70	8	mg/day	14.5	24.8	45.5	61.1
Zn[AB]		mg/100 g d.m.	0.65±0.01[a]	0.90±0.02[b]	1.35±0.03[c]	1.48±0.07[d]
Adult male (years)						
9-13	8	mg/day	16.3	22.4	33.7	37.1
31-50	11	mg/day	11.8	16.28	24.53	27.0
Adult female (years)						
14-18	9	mg/day	14.5	19.9	30.0	33.0
9-13, 19-70, ≥70	8	mg/day	16.3	22.4	33.7	37.1
Phytates		g/100g d.m	0.15±0.01[a]	0.23±0.01[b]	0.40±0.01[c]	0.64±0.01[d]
Mineral Ratios	**Inhibitory threshold values**					
InsP_6/Ca	**>0.24**	mol/mol	0.08	0.08	0.08	0.15
InsP_6/Fe	**>1.0**	mol/mol	1.8	1.4	1.1	1.5
InsP_6/Zn	**>5.0**	mol/mol	1.9	1.9	1.8	2.9

[A]Mean±SD, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level. [B]Amount of minerals after cooking. [C]Except female 51-70 years 1200 mg/day.

InsP_6, phytates; d.m., dry matter; DRIs, dietary reference intakes; DRI contribution (%) for a daily average intake of 200 g of pasta if the protein and mineral absorption inhibitors are absent, NAS (2001; 2011).