Improvement of the protein quality of wheat bread through faba bean sourdough addition

Rossana Coda[1], Jutta Varis[1], Michela Verni[2], Carlo G. Rizzello[2]*, Kati Katina[1]

[1] Department of Food and Environmental Science, University of Helsinki, 00014, Helsinki, Finland

[2] Department of Soil, Plant, and Food Science, University of Bari "Aldo Moro", 70126 Bari - Italy

*Corresponding author. Tel.: +39 0805442950; Fax: +390805442911.

E-mail address: carlogiuseppe.rizzello@uniba.it

Abstract

The effects of the substitution of wheat flour with faba bean flour and faba bean sourdough on the properties of composite bread were investigated. Bread was prepared by replacing wheat flour with 30% of faba bean flour, native or after sourdough fermentation. The addition of faba bean flour influenced the structure of the breads, causing a slight decrease of volume and higher hardness compared to wheat bread. However, when fermented faba bean flour was added, the crumb porosity of the bread was not affected. The addition of 30% of faba bean flour increased wheat bread protein content from 11.6 up to 16.5 % of dry matter. The addition of native faba bean flour did not affect the *in* vitro protein digestibility, resulting similar to wheat bread (64%). On the contrary, faba bean sourdough bread showed higher protein digestibility (73%). Generally, the addition of native faba bean flour caused an improvement of the nutritional indexes of the composite bread, further enhanced when fermentation was carried out. The free amino acid profile, protein chemical score, and biological value index were the highest in faba bean sourdough bread. In addition, the predicted glycemic index was the lowest in faba bean sourdough bread.

Keywords: faba bean, sourdough, wheat bread

Abbreviations: BV, Biological Value; CS, Chemical Score; EAAI, Essential Amino Acid Index; FAA, Free Amino Acids; FSB, Faba bean Sourdough Bread; GI, Glycemic Index; HI, Hydrolysis Index; IVPD, i*n vitro* Protein Digestibility; NFB, Native Faba bean Bread; NI, Nutritional Index; OPA, o-phtaldialdehyde; PER, Protein Efficiency Ratio; RH, Relative Humidity; TPA, Texture Profile Analysis; TTA, Total Titratable Acidity; WCB, Wheat Control Bread; WSE, Water/salt-soluble extracts.

1. Introduction

Grain legumes have been intensely studied in recent years due to their richness in protein, fibres, minerals and other bioactive compounds, with the aim to develop novel foods with improved nutritional profile (Angioloni & Collar, 2012; Vaz Patto *et al.*, 2015). Particularly, the fortification of cereals with legume flour has been recognized as a good strategy to complement cereal-based food nutritional quality, setting new technological and marketing possibilities for staples like bread, bakery products, and pasta (Angioloni & Collar, 2012).

With its high protein content (about 30%), protein quality, and other health benefits, faba bean has been widely used as food and feed in many parts of the world (Crépon *et al*., 2009). The nutritional and agronomic properties of faba bean make this legume a good alternative to other protein-rich sources of animal origin. Considering the good economical, ecological and nutritional properties, the use of legumes and faba bean should be promoted more among food industry and consumers.
Notwithstanding the positive impact on nutrition, the use of legume flours as ingredients in food manufacturing is not so straightforward. For instance, the presence of anti-nutritional factors such as trypsin inhibitors, phytic acid, raffinose family oligosaccharides, tannins, and glucopyranosides in faba beans, are a recognized cause of problems (Youssef & Bushuk, 1986). For this reason, pre-processing treatments of faba bean flour have been developed, obtaining a reduction of the anti-nutrients concentration (as reviewed by Multari, Stewart, & Russel, 2015). Particularly, fermentation with lactic acid bacteria has been shown as a simple, low cost and successful biotechnology to achieve a nutritionally enhanced ingredient (Coda *et al.*, 2015; Rizzello *et al.*, 2016).

The use of faba bean flour in breadmaking has been previously reported in literature (Angioloni & Collar, 2012; Morad, Leung, Hsu, & Finney, 1980; Youssef & Bushuk, 1986), however, still very little is known about the use of faba bean sourdough. On the contrary, legume-based sourdough has been

used before for bread fortification obtaining an overall improvement of the nutritional quality of the bread (Chinma *et al.*, 2016; Coda, Rizzello, & Gobbetti, 2010; Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014).

In this work faba bean flour, native or fermented with lactic acid bacteria, was added to wheat bread at a high percentage (30%), aiming at the improvement of the protein content and quality. Together with the reduction of anti-nutritional factors, sourdough technology can be used to modify the protein quality of a vegetable food matrix, due to the effects of acidification on the activation of endogenous proteolytic enzymes, and to the proteolytic activity of the microorganisms (Ganzle et al., 2008). The technological and nutritional characteristics of wheat bread enriched with faba bean sourdough were compared to wheat bread containing native faba bean and to common wheat bread in order to assess the effects imputable to faba bean addition and its fermentation.

2. Materials and Methods

2.1 Material and microorganism

Faba bean (*Vicia faba* major, harvest year 2014) flours, obtained from stone-milling of the dehulled seeds, was purchased from CerealVeneta (San Martino di Lupari, PD, Italy). The flour had the following chemical composition: moisture content of 9.45 ± 0.72g/100g; carbohydrates 51.2 ± 2.1g/100g of dry matter (d.m.); protein 35.7 ± 1.2g/100g of d.m; lipids were 1.63 ± 0.25g/100g of d.m; dietary fibers and ash were 7.23 ± 0.75g/100g and 3.87 ± 0.12g/100g of d.m., respectively. Wheat flour purchased from Fazer (Fazer Mills, Lahti, Finland) had the following composition: moisture, 14 ± 1g/100g; protein, 14 ± 2g/100g of d.m.; fat, 1.7g/100g of d.m.; ash, 0.6g/100g ± 0.05 of. d.m.; and total carbohydrates, 65g/100g of d.m.

Pediococcus pentosaceus I02, previously isolated from faba bean flour (Coda *et al*., 2017) was used as starter for sourdough fermentation. The strain was routinely propagated at 30°C in MRS broth (Oxoid, Basingstoke, Hampshire, England).

2.2 Sourdough fermentation

Prior fermentation, *P. pentosaceus* I02, was cultivated at 30°C until the late exponential phase of growth was reached (ca. 12 h). Cells were harvested by centrifugation (10,000 x g, 10 min, 4°C), washed twice in 50 mmol/L sterile potassium phosphate buffer (pH 7.0) and re-suspended in the tap water used for dough preparation. Faba bean flour and tap water were mixed to obtaining a dough with a yield (DY, dough weight x 100/flour weight) of 250, corresponding to 40 and 60% wt/wt flour and water, respectively. The cells of the lactic acid bacterium were inoculated at an initial cell density of ca. log 6.0 cfu/g of dough. The faba bean dough was fermented at 20°C for 24 h and used as ingredient for bread making as described in 2.4. The pH value of faba bean dough was determined by a pH meter with a food penetration probe (Hanna Instruments). Total titratable acidity (TTA) was determined on 10 g of dough with the official AACC method (AACC, 2003). Presumptive lactic acid bacteria were enumerated using MRS agar medium (Oxoid, Basingstoke, Hampshire, United Kingdom) supplemented with cycloheximide (0.1 g liter). Plates were incubated at 30°C for 48 h, under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid).

2.3 Chemical characterization

Water/salt-soluble extracts (WSE) of the uncooked doughs were prepared according to Weiss, Vogemeier, & Görg (1993) and used to analyze organic acids, ethanol, peptides, and free amino acids (FAA). Organic acids were determined by High Performance Liquid Chromatography (HPLC), using an ÄKTA Purifier system (GE Healthcare, UK) equipped with an Aminex HPX-87H column (ion

exclusion, Biorad, Richmond, CA), and an UV detector operating at 210 nm. Elution was at 60°C, with a flow rate of 0.6 ml/min, using H_2SO_4 10 mmol/L as mobile phase (Coda, Rizzello, Trani, & Gobbetti, 2011).

To evaluate the degree of proteolysis of the uncooked doughs, the concentration of peptides and free amino acids was determined on the WSE. The concentration of peptides was determined by the *o*-phtaldialdehyde (OPA) method as described by Church, Swaisgood, Porter, & Catignani (1983). Free amino acids were analyzed by a Biochrom30 series Amino Acid Analyzer (Biochrom Ltd, Cambridge Science Park, UK) with a Na-cation exchange column (20 cm x 0.46 cm internal diameter as reported in Rizzello, Nionelli, Coda, De Angelis, & Gobbetti (2010a).

2.4 Bread making

Three breads were prepared: wheat control bread (WCB), native faba bean bread (NFB) and faba bean sourdough bread (FSB). In faba bean containing bread, wheat flour was replaced with 30% of faba bean flour. The formulas used for bread making are reported in Table 1.

Breads were prepared by mixing ingredients for 3 min slow + 4 min fast with a Diosna spiral mixer (SP 12 F, Dierks & Söhne, Osnabrück, Germany). After a floor time of 15 min at 26°C and 75% RH, the doughs were divided into 250 g loaves and modelled manually. The loaves were proofed in pans (45 min, 35°C, 75 RH%) and baked at 200°C, with 15 s of steam. Breads were cooled for 2 h before further analyses.

2.5 Structural and image analysis of bread

Loaf volume was determined using the rapeseed displacement method as in AACC (2003). Specific loaf volume was calculated dividing the loaf volume by the corresponding loaf weight (AACC 2003).

Bread structure was evaluated by Texture profile analysis (TPA) by using a TPA analyzer (Stable

Micro Systems, UK). Breads (6 parallel samples) were sliced into 25-mm thick slices and cut with round mould on the centre of bread. TPA were performed by using a 35-mm diameter probe SMS P/36, 5-kg load cell, 40% penetration depth, at a compression rate of 5 mm/s and a 30-s gap between compressions. Pre-test and test speed were 1.7 mm/s and post-test speed was 10 mm/s. Hardness values were expressed as g.

The crumb grain of breads was evaluated after 24 h of storage using image analysis technology. Images of the sliced breads were captured using an Image Scanner (Amersham Pharmacia Biotech, Uppsala, Sweden). Images were scanned full-scale at 300 dots per inch and analyzed in gray scale (0–255). Image analysis was performed using the UTHSCSA ImageTool program (Version 2.0, University of Texas Health Science Centre, San Antonio, Texas, available by anonymous FTP from maxrad6.uthscsa.edu). A threshold method was used for differentiating gas cells and non-cells (Crowley, Grau, & Arendt, 2000). Analysis was carried out on two sub-images of 500×500 pixels (field of view) selected from within the bread slice. Two slices were analyzed per treatment. The crumb cell features recovered were: number, area, perimeter, and gas cell to total area ratio.

2.6 Nutritional characterization

Energy value was calculated as reported by USDA method (IOM, 2002). The *in vitro* protein digestibility (IVPD) of breads was determined by the method of Akeson & Stahmann (1964) modified by Rizzello, Nionelli, Coda, Di Cagno, & Gobbetti (2010b). The IVPD was expressed as the percentage of the total protein, which was solubilized after enzyme hydrolysis. The supernatant, which contained the digested protein, was freeze-dried and used for further analysis. The modified method of AOAC (2005) was used to determine the total amino acid profile. The digested protein fraction, which derived from 1 g of sample, was added to 5.7 mol/L HCl (1 mL/10 mg of proteins), under a nitrogen steam and incubated at 110°C for 24 h. Hydrolysis was carried out under anaerobic

conditions to prevent the oxidative degradation of amino acids. After freeze-drying, the hydrolyzate was re-suspended (20 mg/mL) in sodium citrate buffer, pH 2.2, and filtered through a Millex-HA 0.22 µm pore size filter (Millipore Co.). Amino acids were analyzed by a Biochrom 30 series Amino Acid Analyzer as described above. Since the above procedure of hydrolysis does not allow the determination of tryptophan, it was estimated by the method of Pinter-Szakács & Molnán-Perl (1990). Chemical Score (CS) estimates the amount of protein required to provide the minimal essential amino acids (EAA) pattern, which is present in the reference protein (hen's egg). It was calculated using the equation of Block & Mitchel (1946). The sequence of limiting essential amino acids corresponds to the list of EAA, having the lowest chemical score Block & Mitchel (1946). The protein score indicates the chemical score of the most limiting EAA present in the test protein Block & Mitchel (1946). Essential Amino Acid Index (EAAI) estimates the quality of the test protein, using its EAA content as the criterion. EAAI was calculated according to the equation (1):

$$EAAI = \sqrt{\frac{(EAA_1*100)(EAA_2*100)(\dots)(EAA_n*100)[\text{sample}]}{(EAA_1*100)(EAA_2*100)(\dots)(EAA_n*100)[\text{reference}]}} \qquad (1)$$

The Biological Value (BV) indicates the utilizable fraction of the test protein. BV was calculated using the equation of Oser (1959) (2):

BV = ([1.09*EAAI]-11.70) (2)

The Protein Efficiency Ratio (PER) estimates the protein nutritional quality based on the amino acid profile after hydrolysis. PER was determined using the equation (3) developed by Ihekoronye (1981):

PER = -0.468 + (0.454*[Leucine]) – (0.105*[Tyrosine]) (3)

The Nutritional Index (NI) normalizes the qualitative and quantitative variations of the test protein compared to its nutritional status. NI was calculated using the equation (4) of Crisan & Sands (1978), which considers all the factors with an equal importance:

NI = (EAA*Protein (%)/100) (4)

2.6 Starch hydrolysis index and predicted glycemic index

The analysis of starch hydrolysis was carried out on breads with a procedure mimicking the *in vivo* digestion of starch (De Angelis *et al*., 2009). Portion of bread, containing 1 g of starch, were given in randomized order to 10 healthy volunteers (aged 21-45 years), who rinsed their mouths with tap water and chewed the samples for 15 s. The samples were then expectorated and subjected to a multi-step enzymatic digestion, according to the protocol previously proposed by De Angelis *et al*., 2009. The glucose content of the dialysates obtained from digested samples was measured with D-Fructose and D-Glucose kit (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). The degree of starch digestion was expressed as percentage of potentially available starch hydrolyzed at different times (30, 60, 90, 120 and 180 min). A non-linear model (De Angelis *et al*., 2009) was applied to determine the kinetics of starch hydrolysis, using the software Statistica 8.0. Wheat flour bread was used as the control to estimate the hydrolysis index (HI = 100). The predicted glycemic index (GI) was calculated using the equation (5) with wheat bread as the reference (GI wheat bread = 100):

$$GI = 0.549*HI + 39.71 \quad (5)$$

2.7 Statistical analyses

The results of the microbiological, chemical and bread properties analyses are presented as an average of two parallel measurements (two repetitions). The statistical difference was measured with one-way analysis of variance (ANOVA). The effect of treatments was measured with Tukey's test with significance level of $P < 0.05$ (Statistica for Windows).

3. Results and discussion

3.1 Sourdough and bread dough properties

P. pentosaceus was the most represented species isolated during faba bean flour fermentation and thus was selected as autochthonous starter for fermentation (Coda *et al*., 2017). In particular, the starter was inoculated at the initial cell density of 5.9 ± 0.03 log cfu/g. After fermentation, lactic acid bacteria cell density increased of ca. 3.3 log cycles, reaching a value of 9.3 ± 0.1 log cfu/g of sourdough. The pH and TTA values of the faba bean flour dough before fermentation were 6.2 and 3.3 mL of NaOH 1 N, respectively. In the sourdough, pH decreased to 5.3, while TTA increased to 8.6± 0.5 mL of NaOH 1 N. The results obtained can be related to an efficient growth of the lactic acid bacteria, as compared with previous faba bean fermentation process (Coda *et al.*, 2015), and to a mild acidification, achieved by the low fermentation temperature (20° C). These conditions were selected in order to avoid a potential negative impact of strong acidity on the technological quality of sourdough, as previously shown for other food matrices (Coda *et al.*, 2014; Katina *et al.*, 2005).

The chemical composition of the doughs before baking is reported in Table 2. Due to the fermentation, the amount of lactic acid in the dough containing 30% of faba bean sourdough, reached 25 mmol/kg of dough, while acetic acid amount was similar for both the doughs containing faba bean flour. Ethanol in the bread doughs before cooking was mostly the result of the baker's yeast activity and its content was the highest in NFB dough. Peptides and free amino acid concentration clearly distinguished the three doughs (Table 2). Generally, the simple addition of faba bean flour caused an increase of the peptides and free amino acids, markedly when fermentation was carried out. The dough containing 30% of faba bean sourdough was in fact characterized by a content of peptides ca. 15 and 30% higher than NFB and WCB, respectively (Table 2). Similarly, the total free amino acid amount of FSB dough reached 2007 mg/kg of dough, corresponding to an increase of ca. 20 and 80% in comparison with NFB and WCB doughs (Table 2).

The effects of fermentation on protein modification also reflected on the single amino acids profile (Fig. 1). The concentration of almost all the essential amino acids increased in FSB dough compared to NFB and WCB doughs, and particularly Thr, Val, Phe, and Lys, which reached concentrations from 3 up to 8 times higher than the value in WCB dough, corresponding to 55, 98, 75, and 81 mg/kg respectively. Additionally, the content of γ-Aminobutyric acid (GABA) increased in both NFB and FSB doughs to values of 89 and 315 mg/kg of dough. GABA is the major inhibitory neurotransmitter of the central nervous system and has several physiological functions with positive effects (Coda *et al.*, 2010). It has been shown that daily intake of 10 mg of GABA (contained in fermented milk) for 12 weeks decreased blood pressure by 17.4 Hg in hypertensive human patients (Inoue *et al.*, 2003). Therefore, in theory, the consumption of 50 g of bread made with 30% of faba bean sourdough, containing ca 15 mg of GABA could potentially have a beneficial effect on the diet.

The extent of the protein degradation together with the proportion of sourdough in the bread are important parameters to consider, since they will impact on the final bread properties. Generally, a moderate proteolysis does not cause adverse effects on texture and volume and beneficially improves bread flavor (Thiele, Gänzle, & Vogel, 2002).

3.2 Structure of faba bean enriched breads

The pH values of the three breads ranged from 5.50 ± 0.03 to 4.92 ± 0.02, while TTA values ranged from 2.6 ± 0.2 to 7.4 ± 0.1, with lower pH and higher TTA value belonging to FSB. Faba bean flour sourdough addition slightly but significantly decreased the specific volume of bread as compared to WCB and NFB (Table 3). The high percentage of faba bean flour substitution induced an effect on the textural properties of bread (Table 3). Compared to WCB, hardness was ca. 30 and 50% higher in NFB and FSB, respectively. Cohesiveness, springiness and resilience decreased significantly in faba bean

flour enriched breads, independently of fermentation, and no significant differences were found between native or fermented faba bean flour additions for these parameters. On the contrary, chewiness of WCB and FSB was comparable, whereas it had a lower value for NFB (Table 3). In these conditions, the high replacement of wheat flour with faba bean markedly affected the texture of bread due to a decrease in gluten content, and weakening of the gluten network.

The crumb grain of the breads was determined by image analysis technology. Digital images were pre-processed to detect crumb cell total area by a binary conversion (black/white pixels) (Table 3 and Fig. 2). The gas cell-total area (corresponding to the black pixel ratio) of the breads containing faba bean were lower than WCB, however, fermentation improved the porosity of the bread, in comparison with the addition of native faba bean. Crumb cell detection of bread slice portions showed that no significant difference in the mean area of gas cells could be observed between WCB and FSB, having values of 48.53 and 41.44 pixels, respectively. In general, WCB showed rounder and more uniform crumb cells while more uneven crumb cells distribution was found for NFB and FSB (Fig. 2).

The incorporation of high amount of legumes has been successfully obtained in biscuits, cake, and pasta (Gómez, Oliete, Rosell, Pando, & Fernández, 2008; Rizzello *et al.*, 2017). On the contrary, it has been challenging in bread making, thus limiting the addition of legumes below 15% of wheat flour (Angioloni & Collar, 2012; Gómez *et al.*,2008; Jayasena, Leung, Nasar-Abbas, Palta, & Berger, 2008; Rizzello *et al.*, 2014). Comparably to the results of this study, the addition of legume flour was previously shown to increase crumb firmness (Angioloni & Collar, 2012; Yamsaengsung, Schoenlechner, & Berghofer, 2010). A similar behavior was observed when fermented cowpea flour was used at 20% for wheat bread making, leading to high dough resistance (Hallén, İbanoğlu, & Ainsworth, 2004). However, in the presence of fermented faba bean flour, the bread porosity was less affected, resulting in higher mean area of gas cells. This phenomenon could be attributed to the

intrinsic structure of faba bean starch and protein and to their modification occurring during fermentation, as consequence of the activity of flour enzymes and microorganisms. More in depth analysis of crumb structure is although required in order to clarify the impact of faba bean addition on the physical structure of bread.

3.3 Nutritional properties of the breads

The characterization of the nutritional properties of breads was mostly focused on the protein component, to assess the impact of native and fermented faba bean flour on its quality. Based on calculation, the sole addition of 30% on flour weight (f.w.) of faba bean flour increased wheat bread protein content from 11.6 up to 16.5 % of dry matter. This ratio was chosen to obtain a "high protein bread". According to EC regulation, in order to receive the "high protein claim", 20% of the energy value of food must be provided by protein. In this study, the "protein dependent" energy value of faba bean bread was 24% of the total value of bread, calculated according to USDA method (IOM 2002). For the determination of the protein quality indexes, the digestible protein fraction was used. The addition of native faba bean flour did not affect the IVPD of NFB compared to WCB (Table 4). On the contrary, when faba bean flour was fermented, the IVPD value reached 74%, showing an increase of ca. 13%, compared to the other breads. The IVPD gives information on the stability of protein hydrolysates, and on how they withstand to digestive processes. The increase of IVPD in FSB can be attributed to the proteolysis occurring during fermentation, as already reported for other cereal-legume processing (Chinma *et al*., 2016; Rizzello *et al.*, 2014) and protein-rich sources (Arte *et al.*, 2015). Protein quality is one of the most important attribute for defining the nutritional characteristics of a food matrix. The protein digestibility value in combination with amino acid composition therefore gives a better prediction of the nutritive value.

297 It was previously observed that the total protein content analysis should hide the effect of the
298 proteolysis degree, which results otherwise in similar values for samples that are instead characterized
299 by different bioavailability and nutritional features of the protein (Rizzello *et al*., 2014). Based on CS,
300 the sequence of limiting amino acids for all the bread samples were found to be Lys, Thr, and Met
301 (Table 4). Nevertheless, compared to WCB (CS of 19, 33, and 40% for Lys, Thr, and Met,
302 respectively), the addition of native faba bean flour caused significant ($P<0.05$) increase of the CS in
303 NFB (34, 55, and 45% for Lys, Thr, and Met, respectively), and particularly after fermentation, leading
304 to the highest CS of FSB for almost all the EAA (data not shown).
305 Overall, EAAI values ranged from 68 to 75.4, showing a slightly higher value for FSB, even though
306 not significant (Table 4). On the contrary, BV index was highest for FSB (71.8) while no significant
307 difference was observed between WSB and NFB. EAAI indicates the ratio of essential amino acids of
308 the sample compared to the reference, while BV estimates the nitrogen potentially retained by human
309 body after consumption. The PER index, which reflects the capacity of a protein to support the body
310 weight gain, was not significantly different for the three breads, even though, also in this case, slightly
311 higher values were found when faba bean flour was added. Within the indexes that are used to evaluate
312 the nutritional value of foods, NI combines qualitative and quantitative factors and it is considered a
313 global predictor of the protein quality (Curiel *et al*., 2014). In the conditions here applied, the value of
314 NI, varying from 16.9 to 23.6, did not show any significant difference between the breads (Table 4).
315 Starch hydrolysis, determined mimicking the *in vivo* digestion, represents a presumptive measure of the
316 glycemic index (GI) in healthy subjects (De Angelis *et al*., 2009). In this analysis, white bread is the
317 control, corresponding to a HI = 100. The HI value of NFB was 94% and a significantly lower value of
318 81 % was found for FSB. Consequently, the predicted GI value of NFB and FSB was 91.4 and 84.2%
319 (Table 4). The lowest value of HI (and predicted GI) of bread containing fermented faba bean flour,
320 could be attributed to biological acidification, which is one of the main factors that decreases starch

hydrolysis rate and HI (De Angelis *et al*., 2009). Generally, GI depends on the food texture and particle size, type of starch, degree of starch gelatinization, and physical entrapment of starch molecules within food, food processing and other ingredients (Petitot, Boyer, Minier, & Micard, 2010). A decrease of starch digestibility after fermentation was previously observed for faba bean fermented matrices attributed also to the strict interactions between protein and starch (Coda *et al*., 2015). Faba bean flour was shown to have a compact protein structure surrounding the starch granules, suggesting strong interactions between protein and starch which appeared markedly changed after fermentation (Coda *et al*., 2015).

4. Conclusions

The addition of faba bean sourdough had a positive impact on the nutritional properties of the wheat bread. Notwithstanding the high level (30%) of faba bean flour substitution, the technological performance of faba bean sourdough bread was not severely affected, resulting in slightly smaller volume than the white bread, but with comparable chewiness and porosity. Although sensory analysis is ongoing to complete the assessment of the impact of native and fermented faba bean on bread attributes, the acceptability of the organoleptic profile of other cereal foods fortified with fermented faba bean flour was already reported (Rizzello *et al*., 2017). In comparison with white bread and with the bread containing native faba bean, the better amino acids profile, higher protein digestibility, protein biological value, and lower glycemic index of faba bean sourdough bread, indicate the important role of fermentation technology in the effective modification of protein and nutritional quality of the legume-wheat bread.

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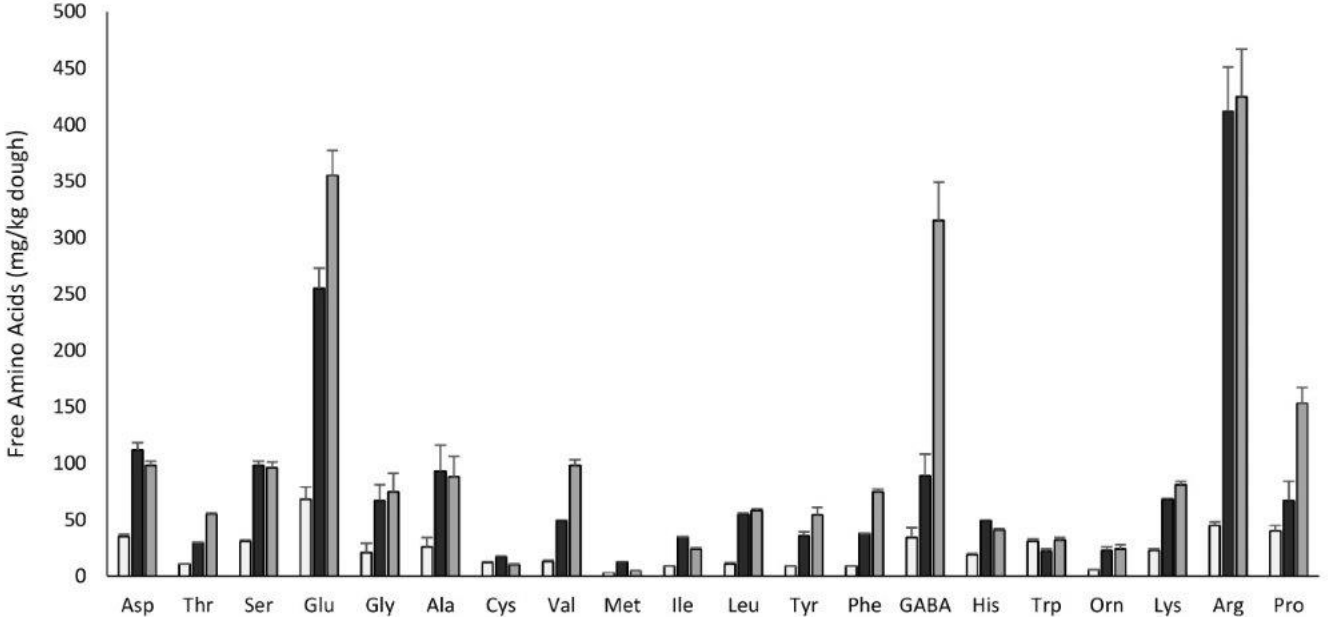
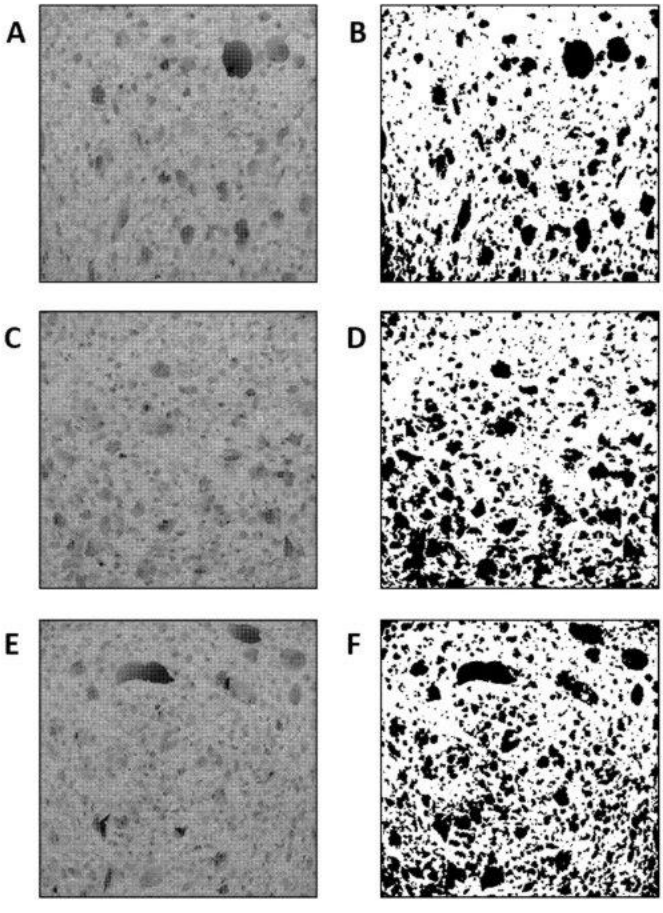
Figure caption

Figure 1. Concentration of free amino acids and their derivatives (mg/kg) of the experimental breads: wheat flour (WCB, □), non-fermented faba (NFB, ■) and faba sourdough (FSB, ■) breads. Data are the means of three independent analyses. Three-letters amino acid code (IUPAC) is used.

Figure 2. Representative images of wheat flour (WCB) (panels A and B), non-fermented faba (NFB) (panels C and D) and faba sourdough (FSB) (panels E and F) breads. Digital images of bread showing the original gray level images (A, C, and E) and computed binary results from gray level thresholding at the two-cluster (B, D, and F).


Free Amino Acids (mg/kg dough)
500
450
400
350
300
250
200
150
100
50
0
Asp
Thr
Ser
Glu
Gly
Ala
Cys
Val
Met
Ile
Leu
Tyr
Phe
GABA
His
Trp
Orn
Lys
Arg
Pro


A
B
C
D
E
F