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Exploitation of the nutritional and functional characteristics of traditional Italian legumes: The potential of sourdough fermentation

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ABSTRACT

This study aimed at evaluating the composition of nineteen traditional Italian legumes and at investigating the potential of the sourdough fermentation with selected lactic acid bacteria to improve the nutritional and functional features. Traditional Italian legumes, all with product certifications and belonging to *Phaseolus vulgaris*, *Cicer arietinum*, *Lathyrus sativus*, *Lens culinaris* and *Pisum sativum* species, were used in this study. Seeds were milled, and flours were analyzed for proximate composition and subjected to sourdough fermentation at 30 °C for 24 h. *Lactobacillus plantarum* C48 and *Lactobacillus brevis* AM7 were used as selected starters. Compared to control doughs, without bacterial inoculum, the concentrations of free amino acids (FAA), soluble fibres, and total phenols increased for all legume sourdoughs. Raffinose decreased of up to ca. 64%. During sourdough fermentation, the level of GABA markedly increased and reached values up to 624 mg/kg. Condensed tannins decreased. At the same time, almost all legume sourdoughs showed increases of the antioxidant and phytase activities. As shown by PCA analysis based on data of total FAA, GABA, raffinose, soluble/insoluble dietary fibre, condensed tannins and antioxidant and phytase activities, all legume sourdoughs were clearly differentiated from control doughs. The traditional Italian legumes are bio-diverse, and all showed high levels of nutritional elements and suitability for optimal sourdough fermentation. Legume flours subjected to sourdough fermentation would be suitable to be used alone or better in mixture with cereals, and as gluten-free ingredients for making novel and healthy foods.

1. Introduction

After cereals, *Leguminosae* are the second worldwide food crops. The global value for leguminous crops is thought to be approximately two billion US dollars per year (Duranti, 2006). Although legumes are part of the dietary habit of all countries since thousands of years, only recently their use and nutritional and functional values were rediscovered and deeply investigated with suitable approaches (Coda et al., 2015; Duranti, 2006; Rizzello et al., 2014). The *Leguminosae* family is the most important group of *Dicotyledonae* (Duranti, 2006), being one of the largest families of flowering plants with 18,000 species, which are classified into around 650 genera (Duranti, 2006). The cultivation of legumes for human diet involves a large number of species, varieties and biotypes, which are cultivated extensively or locally (Duranti, 2006; Smartt and Nwokolo, 1996). The Italian production of legumes is characterized by a large number of traditional varieties, which are mainly used for regional recipes and considered to be as niche products. Besides their use for food or feed, legumes are considered for a variety of other purposes, including timber, medicine, tannins and gums. The economic importance of the *Leguminosae* family is increasing also in marginal land, since many species well adapt to grow under restrictive conditions (Duranti, 2006).

Overall, legumes are an excellent source of proteins, carbohydrates and dietary fibres. Besides, they provide many essential amino acids, vitamins, minerals, oligosaccharides and phenolic compounds (Campos-Vega et al., 2010; Roy et al., 2010). The frequent consumption of legumes is considered to be as an effective tool to decrease the risk of cardiovascular disease (Flight and Clifton, 2006), type 2 diabetes (Jenkins et al., 2012), some types of cancer (Feregrino-Perez et al., 2008), overweight and obesity (Mollard et al., 2012). Nevertheless,

Abbreviations: ANF, anti-nutritional factors; BHT, butylated hydroxytoluene; D, dough; DY, dough yield; FAA, free amino acids; GABA, γ-amino butyric acid; IDF, insoluble dietary fibre; ME, methanol extract; OPA, *o*-phthaldialdehyde; PCA, principal component analysis; QF, quotient of fermentation; RFOs, raffinose family of oligosaccharides; S, sourdough; SDF, soluble dietary fibre; TDF, total dietary fibre; TTA, titratable acidity; WSE, water/salt-soluble extract

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legumes also contain several anti-nutritional compounds, namely raffinose, phytic acid, condensed tannins, alkaloids, lectins, pyrimidine glycosides (e.g., vicine and convicine), and protease inhibitors (Coda et al., 2015; Liener, 1990). Most of these anti-nutritional factors (ANF) are heat-labile (e.g., protease inhibitors and lectins), so that thermal treatments would remove potential negative effects during consumption (Muzquiz et al., 2012). On the other hand, phytic acid, raffinose, tannins and saponins are heat-stable, and various methods such as dehulling, soaking, air classification, extrusion, or cooking were used to decrease their negative impact on human consumption (Coda et al., 2015; Jezierny et al., 2010; Rizzello et al., 2014; van der Poel, 1990). Biological methods such as germination, enzyme treatments and fermentation were also proposed (Alonso et al., 2000; Coda et al., 2015; Granito et al., 2002; Luo et al., 2009). The effect of microbial fermentation on ANF was studied for various legumes (Coda et al., 2015; Rizzello et al., 2014). The partial or complete elimination of α-galactosides, tannins, phytic acid and trypsin inhibitory activity was obtained through the spontaneous fermentation of kidney beans (Granito et al., 2002). The lactic fermentation of grass pea with *Lactobacillus plantarum* decreased the levels of phytic acid and trypsin inhibitory activity (Starzyńska-Janiszewska and Stodolak, 2011). Bioprocessing with α-galactosidases eliminated oligosaccharides into pinto bean flour (Song et al., 2009).

Regarding natural and safe biotechnology, sourdough fermentation may have the potential to exploit the nutritional, functional and sensory features of legumes and derived flours. An abundant literature showed how the sourdough may enhance the nutritional and functional features of cereals. In particular, sourdough fermentation improves the content of biogenic compounds, decreases the level of anti-nutritional factors and the value of the glycaemic response, and increases the uptake of minerals (Gobbetti et al., 2014). A careful characterization of minor species and varieties of legumes, which is mainly based on nutritional characteristics and suitability to be processed by sourdough fermentation, is of pivotal importance to select raw ingredients for industrial food processing.

This study aimed at evaluating the proximate composition of nineteen traditional Italian legumes and at investigating the potential of the sourdough fermentation with selected lactic acid bacteria to improve the nutritional and functional features. The effect on dietary fibre, free amino acids and γ-amino butyric acid (GABA), raffinose, condensed tannins, phenols, and phytase and antioxidant activities was mainly addressed.

2. Materials and methods

2.1. Legumes

Nineteen traditional Italian legumes, belonging to *Phaseolus vulgaris* (Fagiolo di Lamon, Fagiolo di Controne, Fagiolo di Cuneo, Fagiolo Stregoni, Fagiolo Vellutina, Fagiolo di Saluggia, Fagiolo Badda di Polizzi — white, and Fagiolo Badda di Polizzi — black), *Cicer arietinum* (Cece di Merella and Cece dell'Alta Valle del Misa), *Lathyrus sativus* (Cicerchia di Serra de Conti and Cicerchia di Campodimele), *Lens culinaris* (Lenticchia di Casteluccio di Norcia, Lenticchia di Ustica, Lenticchia di Santo Stefano di Sessanio, Lenticchia rossa di Pantelleria, Lenticchia di Altamura and Lenticchia di Villalba), and *Pisum sativum* (Pisello riccio di Sannicola) species, were used in this study. All legumes were chosen among Italian pulses having specific product certification. Names, abbreviations, geographical origin and certification are listed in Table 1. Flours were obtained from whole legume seeds by the laboratory mill Ika-Werke M20 (GMBH, and Co. KG, Staufen, Germany). Protein (total nitrogen × 5.7), ash and moisture contents were determined according to the AACC approved methods 46-11A, 08-01 and 44-15A, respectively (AACC, 2003). The etheric extract was analyzed by Soxhlet method to determine lipids. Total carbohydrates were calculated as the difference [100 − (proteins + lipids + ash + moisture)]. Proteins, lipids, carbohydrates and ash were expressed as % of dry matter (d.m.).

2.2. Microbiological analyses

Ten grammes of flour was homogenized with 90 ml of sterile peptone water (1% [wt./vol.] of peptone and 0.9% [wt/vol] of NaCl) solution. Lactic acid bacteria were enumerated using MRS (Oxoid, Basingstoke, Hampshire, UK) agar medium, supplemented with cycloheximide (0.1 g/l). Plates were incubated, under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid), at 30 °C for 48 h. Cell densities of yeasts and moulds were estimated on Yeast extract Peptone Dextrose Agar (YPD) (Oxoid) medium (pour and spread plate enumeration, respectively), supplemented with chloramphenicol (0.15 g/l), at 30 °C for 72 h. The attribution was confirmed by microscope observation. Total mesophilic aerobic bacteria were determined on Plate Count Agar (PCA, Oxoid) at 30 °C for 48 h, and total enterobacteria were determined on Violet Red Bile Glucose Agar (VRBGA, Oxoid) at 37 °C for 24 h.

Table 1
List and abbreviations of traditional Italian legumes used in the study. Product certifications and origin are also included.

Legume	Name	Abbreviation	Product certification[a]	Origin
Kidney bean (*Phaseolus vulgaris*)	Fagiolo di Lamon	FL	IGP	Veneto
	Fagiolo di Controne	FCo	DOP	Campania
	Fagiolo di Cuneo	FCu	PAT	Piedmont
	Fagiolo Stregoni	FSt	PAT	Piedmont
	Fagiolo Vellutina	FV	PAT	Sicily
	Fagiolo di Saluggia	FSa	PAT	Piedmont
	Fagiolo Badda di Polizzi (white)	FBw	SFP	Sicily
	Fagiolo Badda di Polizzi (black)	FBb	SFP	Sicily
Chickpea (*Cicer arietinum*)	Cece di Merella	CM	PAT	Piedmont
	Cece dell'Alta Valle del Misa	CV	SFP	Marche
Grass pea (*Lathyrus sativus*)	Cicerchia di Serra de Conti	CS	SFP	Marche
	Cicerchia di Campodimele	CC	PAT	Lazio
Lentil (*Lens culinaris*)	Lenticchia di Castelluccio di Norcia	LC	IGP	Umbria
	Lenticchia di Ustica	LU	SFP	Sicily
	Lenticchia di Santo Stefano di Sessanio	LS	SFP	Abruzzo
	Lenticchia rossa di Pantelleria	LP	PAT	Sicily
	Lenticchia di Altamura	LA	PAT	Apulia
	Lenticchia di Villalba	LV	PAT	Sicily
Pea (*Pisum sativum*)	Pisello riccio di Sannicola	PS	PAT	Apulia

[a] IGP (Indicazione Geografica Protetta, Protected Geographical Indication) and DOP (Denominazione d'Origine Protetta, Designation of Protected Origin) are regulated by Reg. (CE) N. 510/2006 (20.03.2006); PAT (Prodotti Agroalimentari Tradizionali, Traditional Food Products) are included in the list of the Italian Ministry of Agriculture, Food and Forestry (D.M. 07/06/2012); SFP (Slow Food Presidia) are listed at www.slowfoodfoundation.org.