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Exploitation of Albanian wheat cultivars: Characterization of the flours and lactic acid bacteria microbiota, and selection of starters for sourdough fermentation

Luana Nionelli [a], Nertila Curri [b], José Antonio Curiel [a], Raffaella Di Cagno [a], Erica Pontonio [a], Ivana Cavoski [b], Marco Gobbetti [a], Carlo Giuseppe Rizzello [a, *]

[a] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, 70126 Bari, Italy
[b] CIHEAM-MAIB, Mediterranean Agronomic Institute of Bari, 70010 Valenzano, Bari, Italy

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ABSTRACT

Six Albanian soft and durum wheat cultivars were characterized based on chemical and technological features, showing different attitudes for bread making. Gliadin and glutenin fractions were selectively extracted from flours, and subjected to two-dimensional electrophoresis. Linja 7 and LVS flours showed the best characteristics, and abundance of high molecular weight (HMW)-glutenins. Type I sourdoughs were prepared through back slopping procedure, and the lactic acid bacteria were typed and identified. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were the predominant species. Thirty-eight representative isolates were singly used for sourdough fermentation of soft and durum wheat Albanian flours and their selection was carried out based on growth and acidification, quotient of fermentation, and proteolytic activity. Two different pools of lactic acid bacteria were designed to ferment soft or durum wheat flours. Sourdough fermentation with mixed and selected starters positively affected the quotient of fermentation, concentration of free amino acids, profile of phenolic acids, and antioxidant and phytase activities. This study provided the basis to exploit the potential of wheat Albanian flours based on an integrated approach, which considered the characterization of the flours and the processing conditions.

1. Introduction

Agriculture represents one of the main resources for the Albanian economy. It provides ca. 25% of the total gross domestic product. This primate also reflects on the high proportion (58%) of the active population operating in this sector (Arcotrass, 2006). Important changes occurred in the Albanian agriculture during the last years, which mainly consist on the increases of productivity and export (Arcotrass, 2006). Nevertheless, the agriculture sector still suffers for the abundant fragmentation of the cultivable land, and the trend of the younger generations is to leave agricultural activities (Arcotrass, 2006).

Similarly to other Mediterranean countries like Greece, Italy, Malta and Croatia, the consumption of cereals is also elevated in Albania (Arcotrass, 2006). Wheat was cultivated for centuries in Albania and, currently, almost 30% of the cultivable soil is designated to such crop (Arcotrass, 2006). The total production of cereals is estimated to be ca. 300,000 t per year (FAOSTAT, http://faostat.fao.org/). The reduction of the area under cultivation and the concomitant increase of the crop yield are also characterizing the production of cereals in Albania. Altogether these changes did not significantly affect the massive trade deficit for cereals (Arcotrass, 2006). Albanian wheat cultivars are the fall ones, having average low body, and showing a marked adaptation to the local environmental conditions (Arcotrass, 2006).

Research and development on local, ethnic, and ancient grains have a worldwide renewed interest (Coda et al., 2014). The use of local cultivars of wheat, rice and maize as well as minor cereals and pseudo-cereals has a nutritional interest, mainly due to their chemical composition (e.g., dietary fiber, resistant starch, minerals,

Abbreviations: 2-DE, two-dimensional electrophoresis; D, dough; DY, dough yield; FAA, free amino acids; HMW, high molecular weight; L, extensibility; LAB, lactic acid bacteria; LMW, low molecular weight; ME, methanolic extract; P, tenacity; PCA, Principal Component Analysis; QF, quotient of fermentation; SLS, selected sourdough starters; SS, spontaneous sourdough; TTA, total titratable acidity; W, flour strength; WSE, water/salt-soluble extract.

* Corresponding author. Tel.: +39 0805442948; fax: +39 0805442911.
E-mail addresses: carlogiuseppe.rizzello@uniba.it, rizzello@agr.uniba.it (C.G. Rizzello).

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vitamins and phenolic compounds) which reflects on baked goods features. For these reasons, local or indigenous varieties of cereal and pseudo-cereals attract bakery industries and consumers all over the world as niche products, more than the other modern wheat counterparts (Coda et al., 2014).

Besides, local or indigenous varieties are usually low input crops, suitable for growing without the use of pesticide in harsh ecological conditions and marginal areas of cultivation (Coda et al., 2014).

Cereal fermentation processes depend on specific determinants, which have to be strictly controlled to get standardized and agreeable products (Hammes and Gänzle, 1998). Among these determinants, the type of flour is one of the most important. It affects the technological features and the nutritional value of the baked goods and, more in general, the microbial fermentation through the level and type of fermentable carbohydrates, nitrogen sources and growth factors (Hammes et al., 2005). The use of industrial starter cultures for cereal fermentations is limited, and when used starter cultures often lack of biochemical properties to differentiate the products and to exploit the potential of the various flour matrices (Coda et al., 2014). Mainly based on the above considerations, the manufacture of bakery products with local flours and tailor made starter cultures for specific raw ingredients and products is deserving marked interest to get new niche products for the market (Coda et al., 2014). Nowadays, the characterization of the Albanian wheat cultivars, based on the main chemical and technological properties, would represent a useful tool to focus some of the national agriculture choices. Furthermore, no information are available in the literature regarding the microbiota of Albanian sourdoughs and the selection of lactic acid bacteria suitable for industrial or artisanal baking.

This study aimed at characterizing the technological and chemical features, and the lactic acid bacteria microbiota of six Albanian wheat cultivars. Suitable lactic acid bacteria strains were selected for sourdough fermentation. A comparison between spontaneous and started (autochthonous lactic acid bacteria) sourdoughs was also done based on the main technological, nutritional, and functional features.

2. Materials and methods

2.1. Wheat grains

Six Albanian wheat cultivars, belonging to *Triticum aestivum* (Dajti, Progresi, Linja 7, LVS and Shumzim 21) and *Triticum turgidum* ssp. *durum* (STF 24) species, were used in this study. Wheat grains were provided by the Agriculture Technologies Transfer Center of Lushnje, Albania. The cultivation of wheat was carried out into experimental fields, under low input agricultural practices, and the harvesting was made during the season 2011–2012. Milling was carried out at the pilot plant of Tandoi S.p.a. (Corato, BA, Italy). Moisture, ash, proteins, falling number, and the rheology properties were determined according to AACC standard methods (AACC, 2003). In particular, the water absorption capacity, development time and stability were determined using the Brabender Farinograph (S300H Brabender mixer type, Duisburg, Germany). Flour strength (W), tenacity (P) and extensibility (L) were determined through the Chopin Alveograph (MA82, Villeneuve-la-Garenne, France).

2.2. Two-dimensional electrophoresis (2-DE)

Proteins were selectively extracted from wheat flour, according to the method of Osborne (1907), further modified by Weiss et al. (1993). The concentration of proteins was determined by the Bradford method (Bradford, 1976). Two-dimensional electrophoresis (2-DE) was carried out with the immobiline–polyacrylamide system, as described by Di Cagno et al. (2002). Aliquots of proteins (30 μg) were used for the electrophoretic run. Isoelectric focusing (IEF) was carried out on immobiline strips. A non-linear pH gradient from 3.0 to 10.0 (IPG strips; Amersham Pharmacia Biotech, Uppsala, Sweden) for glutenin fraction, and a linear pH gradient 6–11 for gliadin fraction, were carried out using the IPG-phore at 20 °C. The second dimension was carried out in a Laemmli system on 12% polyacrylamide gels (13 cm by 20 cm by 1.5 mm) at a constant current of 40 mA/gel and at 15 °C for approximately 5 h, until the dye front reached the bottom of the gel. 2-D protein standards (Bio-Rad Laboratories, USA) were used for isoelectric point (IP) and molecular mass estimation. Gels were silver stained (Bini et al., 1997). Image analysis of the gels, acquired by a gel scanner (Amersham Pharmacia Biotech, Uppsala, Sweden), was carried out with the UTHSCSA ImageTool software (Version 2.0, University of Texas Health Science Centre, San Antonio, Texas, available by anonymous FTP from maxrad6.uthscsa.edu). Gray scale (0–255) images of the gels, at 600 dots per inch, were obtained and processed. The intensity of the spots was calculated as the black pixel area using a threshold method (Gámbaro et al., 2004).

2.3. Type I sourdoughs

Sourdoughs were made and propagated through traditional protocol (sourdough type I), without use of starter cultures or baker's yeast. Flours were mixed with tap water at 60 × *g* for 5 min with an IM 5e8 high-speed mixer (Mecnosud, Flumeri, Italy) and the doughs (D), having dough yield (DY) 160, were incubated at 25 °C for 24 h. DY was calculated with the formula: dough weight × 100/flour weight. After this first fermentation, six further back-slopping (refreshment) were carried out, mixing 25% of the previously fermented dough with flour and water (final dough yield of 160), and incubating for 8 h at 25 °C. After each fermentation, doughs were stored at 4 °C until the next refreshment. After refreshments and when the acidification rate was stable, spontaneous sourdoughs (SS) were characterized. All the analyses were carried out in triplicate.

2.4. Determination of pH, titratable acidity, organic acids, quotient of fermentation, and free amino acids

The values of pH were determined on-line by a pHmeter (Model 507, Crison, Milan, Italy) with a food penetration probe. Total titratable acidity (TTA) was determined after homogenization of 10 g of dough with 90 ml of distilled water, and expressed as the amount (ml) of 0.1 M NaOH needed to reach the value of pH of 8.3.

The water/salt-soluble extract (WSE) of wheat flour, was prepared according to Weiss et al. (1993) and used to analyze organic acid, peptides, and free amino acids. Organic acids were determined by High Performance Liquid Chromatography (HPLC), using an ÄKTA Purifier system (GE Healthcare, Buckinghamshire, UK) equipped with an Aminex HPX-87H column (ion exclusion, Biorad, Richmond, CA), and a 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 mM as mobile phase (Coda et al., 2011). The quotient of fermentation (QF) was determined as the molar ratio between lactic and acetic acids. The peptide concentration was determined by the *o*-phthaldialdehyde (OPA) method (Church et al., 1983). A standard curve prepared using tryptone (0.25–1.5 mg/ml) was used as the reference. Free amino acids were analyzed by a Biochrom 30 series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Na-cation-exchange column (20 by 0.46 cm internal diameter), as described by Rizzello et al. (2010).