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Effects of fermentation and rye flour on microstructure and volatile compounds of chestnut flour based sourdoughs

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ABSTRACT

The study is aimed at developing a new cereal-based product, with increased nutritional quality, by using natural fermentation of blends of chestnut and rye flour. In spite of the remarkable similarity, the technological potential of combinations of both flours has never been explored before. Three spontaneous chestnut/rye sourdough fermentations were performed over a period of twelve days with daily back-slopping. Samples taken at all refreshment steps were used for culture-dependent and culture-independent evaluation of the microbiota present. Dominant species basically overlapped to those associated to sourdoughs strengthened with chestnut flour, such as *Pediococcus pentosaceus* or *Weissella paramesenteroides*. Microstructures, evaluated by means of Scanning Electron Microscopy, revealed the presence in chestnut sourdoughs of a distinguishable network surrounding starch granules, while rye flour-added sourdoughs showed a less structured matrix. By gas chromatography coupled to mass spectrometry, 51 volatile organic compounds were identified at 24 h and after prolonged fermentation. Within volatile organic compounds, alcohols, esters, acids, aldehydes and ketones, all well-known flavour compounds in sourdough fermentation, appeared as dominant. The PCA discriminated the sourdoughs into three distinct clusters and highlighted a clear influence of fermentation time on the volatile composition of sourdoughs.

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1. Introduction

The use of the sourdough process as a means of leavening is one of the oldest biotechnological processes in cereal food production. Sourdough is a mixture of mainly cereal flour and water, which is made metabolically active by a heterogeneous population of lactic acid bacteria (LAB) and yeasts, either by spontaneous fermentation or by fermentation initiated through the addition of a sourdough starter culture, whether or not involving backslopping (De Vuyst, Vrancken, Ravyts, Rimaux, & Weckx, 2009). Sourdough fermentation has proved to be essential in rye (*Secale cereale* L.) bread making by ensuring the dough acidification and inhibition of amylases activity, as well as by improving the water binding capacity of starch (Weckx et al., 2010). Furthermore, rye sourdough fermentation improves the nutritional value of rye bread by increasing the levels of bioactive (Weckx et al., 2010) and odour active compounds (Kirchoff & Schieberle, 2002).

Rye flour (RF) is known to contain many essential and nonessential dietary components and B-complex vitamins (Mihhalevski, Nisamedtinov, Hälvin, Ošeka, & Paalme, 2013). As rye does not contain enough gluten, the structure of rye bread depends on the starch in the rye flour, as well as other carbohydrates known as pentosans (Wing & Scott, 1999 , p. 255).

In recent times, there has been a growing interest in the use of chestnut (*Castanea sativa* Mill) flour for the production of leavened bakery. Chemical composition of chestnut flour (CF) is close to that of many cereals, with starch (50–60%) as main component. The high quality proteins with essential amino acids (4–7%), as well as the content in mineral salts and vitamins (B1, E and C) make CF a worthy ingredient for healthy bakery products.

Moreover, CF is a rich source of phytochemicals and polyphenolics, with gallic and ellagic acid as predominant among hydrolysable and condensed tannins (De Vasconcelos, Bennett, Rosa, & Ferreira-Cardoso, 2010; Durazzo, Turfani, Azzini, Maiani,

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& Carcea, 2013). CF contains interesting levels of lignans, compounds known to exert significant positive effects on human health (Durazzo et al., 2013). On the other hand, phenolic compounds, particularly phenolic acids and tannins, exhibit antimicrobial activity (De Vasconcelos et al., 2010). As matter of fact, in sourdoughs realized with the sole chestnut flour, the achievement of the microbial equilibrium may require a longer time (Aponte et al., 2013). CF needs to be mixed with other flours and the definition of the exact content of CF in the blend becomes crucial. Encouraging results were achieved when moderate levels of CF are added to wheat (Dall'Asta et al., 2013) or to rice flour (Demirkesen, Mert, Sumnu, & Sahin, 2010). Indeed, in several traditional recipes to prepare breads, other than cakes, and pancakes, the percentage of CF in the dough is generally around 40%. A further confirmation about this chestnut ratio may be retrieved in surveys of Demirkesen et al. (2010); Demirkesen, Sumnu, Sahin, and Uysal (2011): according to authors, a ratio 40/60 chestnut/rice flour, appeared to be a good compromise to obtain bread with fair firmness, density and colour, but still characterized by a good fibre content.

With respect to the increasing demand for functional cereal-based products with high nutritional quality, this study focuses on the potential use of blends of CF and RF, since both flours are characterized by a fair content in dietary fibre, polyphenolic compounds and essential amino acids. Moreover, the cultivations of chestnut and rye, share the same mountain environment, because of their ability to withstand cold. In detail, the impact of natural fermentation on the volatile organic compounds (VOCs) formation, by means of gas chromatography coupled to mass spectrometry (GC/MS), and on the microstructure, as revealed by Scanning Electron Microscopy (SEM) analysis, was evaluated during sourdoughs maturation of blends of RF and CF. Microbial dynamics were followed by means of a culture-independent PCR-DGGE-based method.

2. Materials and methods

2.1. Samples

RF and CF, used in the preparation of laboratory sourdoughs, were kindly provided by Ipafood (Avellino, Italy). Nutritional characteristics (g/100 g) can be reassumed as follows: CF – 10.7 moisture, 69.3 carbohydrates, 4.6 proteins, 9.5 fibre, 3.8 fat, 1.99 minerals; RF – 12.5 moisture, 73.5 carbohydrates, 9.5 proteins, 2.5 fibre, 1.5 fat, 0.5 minerals.

2.2. Laboratory mature sourdough preparation

Sourdoughs from chestnut flour (100% wt/wt) (A) and chestnut and rye flours B (60/40% wt/wt) or C (40/60% wt/wt) were prepared according to a procedure previously described (Aponte et al., 2013). Briefly, sourdoughs were prepared from 100 g of flour and about 100 g of sterile water (yield dough about 200), in sterile 500 mL containers. Bakery yeast isolated in pure culture was added (about 10^7 CFU/g) at the sole first step. After 24 h of incubation, back-slopping was performed with 10% of the ripe sourdough and repeated every 24 h. Fermentations were propagated, in asepsis and at room temperature, until a stable microbiota was established. Each trial was performed in triplicate. At each refreshment step, samples, taken from the ripe sourdough, were submitted to pH and total titratable acids (TTA) determination according to a standard method (Aponte et al., 2013). LAB and yeast loads were evaluated by counting on MRS modified and YGC according to Aponte et al. (2013), respectively. 10-g samples were taken from the sourdoughs C at seven selected times (24, 48, 72, 96, 120, 168 and 288 h) and submitted to DNA extraction according to a protocol previously described (Aponte et al., 2013). Amplicons of hypervariable region V3 within 16S rDNA were obtained by nested PCR according to a routinely procedure previously detailed (Aponte et al., 2013). PCR products were, then, analysed by DGGE using a Bio-Rad D-code apparatus. Parallel electrophoresis experiments were performed at 60 °C in 1× TAE buffer, by using polyacrylamide gels (8% wt/vol) containing 30–60% urea-formamide denaturing gradient (100% corresponded to 7 mol/L urea and 40%, wt/vol, formamide) increasing in the direction of the electrophoresis. The gels were run for 10 min at 50 V and 4 h at 200 V, stained with ethidium bromide for 5 min and rinsed for 20 min in distilled water. DGGE bands to be sequenced were purified and sequenced according to Aponte et al. (2013). Research for DNA similarity was performed with the National Centre of Biotechnology Information GenBank.

2.3. Scanning Electron Microscopy (SEM) analysis

Small portions of each sample were cut, fixed in 10% glutaraldehyde and sequentially embedded in acetone solutions of increasing concentration to ensure full dehydration. Samples were dried at the critical point and coated with gold particles. Microstructure was examinated by means of Scanning Electron Microscopy (LEO EVO 40 SEM, Zeiss, Germany) with a 20 kV acceleration voltage and a magnification of × 1.000.

2.4. Characterization of volatile organic compounds (VOCs)

2.4.1. Head space solid phase microextraction (HS-SPME) analysis

The volatile fraction of samples was analysed by headspace sampling, using the solid phase microextraction technique (HS-SPME). For each SPME analysis, 2 g of sourdough (24 and 288 h) were placed into a 20 mL headspace vial, and added of 2 mL of distilled water and 5 μL of 4-methyl-2 pentanol (internal standard, 100 mg/L standard solution). The vial was placed in a thermostatic block (40 °C) on a stirrer and the fibre was inserted and maintained in the sample head space for 30 min, than it was removed and immediately inserted into the GC–MS injector for the desorption of compounds. For the analyses, a silica fibre, coated with 85 μm of Carboxen–Polydimethylsiloxane (Carboxen/PDMS) according to Aponte et al. (2013) was used (Supelco, Bellefonte, PA, USA).

2.4.2. Gas chromatography–mass spectrometry (GC–MS) analysis

For VOCs evaluation, an Agilent Technologies (Agilent Technologies, USA) 7890A gas-chromatograph coupled to an Agilent Technologies 5975 mass spectrometer equipped with a 30 m × 0.25 mm ID, film thickness 0.25 μm capillary column (HP-INNOWAX, Agilent Technologies, USA) was used. Gas carrier was Helium (flow 1.5 mL/min) and SPME injections were splitless (straight glass line, 0.75 mm I.D.) at 240 °C for 20 min during which time thermal desorption of analytes from the fibre occurred. The oven parameters were as follows: initial temperature was 40 °C held for 3 min, followed by an increase to 240 °C at a rate of 5 °C/min, then held for 10 min. Injector temperature was 240 °C. Mass spectrometer operated in scan mode over mass range from 33 to 300 amu (2 s/scan) at an ionization potential of 70 eV. Mass spectral matches were made by comparison of mass spectra and retention time with those of MS database (Wiley7, Nist 05). A semi-quantitive analysis was obtained by comparison of the VOCs peak areas with that of internal standard (4-methyl-2 pentanol), obtained from the total ion chromatograms, using a response factor of 1. Blank experiments were conducted in two different modalities: blank of the fibre and blank of the empty vial. These types of control were carried out every 20 analyses. All analyses were performed in triplicate.

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