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Effect of respirative cultures of *Lactobacillus casei* on model sourdough fermentation

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ABSTRACT

Aim of the study was to evaluate the effect of respiratory metabolism of *Lactobacillus casei* species on qualitative characteristics of sourdough. For this purpose, the respiration-competent strain *L. casei* N87, grown both in anaerobic (AN) and respiratory (RS) condition, was used as starter culture, in combination with the yeast (Y), in model wheat dough fermentation. Three mixed starter cultures were designed for dough fermentation, namely, *L. casei* RS + Y, *L. casei* AN + Y, and Y; a control sample C was produced without starter. At time zero and after 6 and 24 h of fermentation the doughs were analyzed for pH, total titratable acidity, lactic acid bacteria and yeast concentration, volatile organic compounds production by SPME-GC/MS and the changes in albumin/globulin, gliadin and glutenin fractions by SDS-PAGE.

The respirative strain *L. casei* N87 affected significantly the characteristics of the dough showing major acidification, increased biomass, and different volatile (higher production of diacetyl, acetic acid, acetoin, ethyl acetate) and proteolytic profiles compared to the anaerobic culture *L. casei* N87. The findings proved the significant role for respiratory cells of *L. casei* N87 in the definition of acidification, aroma and proteolysis during dough fermentation suggesting a potential role in influencing flavor and structure of baked goods.

1. Introduction

Lactobacillus casei is a lactic acid bacterium (LAB) used in the production of many fermented foods and feed products, i.e. as acid-producing starter cultures for milk fermentations, and as "adjunct cultures" to improve flavour development in several cheeses (Hosseini-Nezhad, Hussain & Britz, 2015). This species is often used as starter or adjunct cultures in the production of sourdoughs and bakery products for its ability to influence the sourdough acidification, degradation of gluten proteins, production of amino acids and aroma compounds, improving sensorial and nutritional properties of bakery foods (Gaggiano et al. 2007, Denkova, Ilieva, Denkova, Georgieva, & Krastanov, 2014), Furthermore, this specie comprises strains commercially exploited as probiotic cultures having a beneficial effect on gut physiology and human health. Different strains of the *Lactobacillus casei* group have been widely studied with respect to their health-promoting properties. Several beneficial functions for the human organism have been attributed to regular consumption of food products containing these strains (Buriti & Saad, 2007).

However, when LAB are added to food formulations, several factors may affect the physiological status of microbial cells, influencing their survival, growth as well as their ability to enhance the final product quality.

The adaptive response of *Lactobacillus casei* group to adverse conditions, including low pH, bile salts, high osmotic pressure, high and low temperature (Reale et al., 2015) and oxidative stress (Zotta et al., 2014) has been recently investigated. The understanding and manipulation of stress response mechanisms may be interesting from both scientific and technological point of view. Thus, the development of novel strategies to promote stress adaptation in LAB could enhance strain survival and improve safety, functional and technological properties of fermented foods (Lee, Tachon, Eigenheer, Phinney, & Marco, 2015).

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Recently, it has been demonstrated that respiratory growth results, in some LAB species, in the expression of phenotypes with enhanced technological and stress response properties (increase in biomass, synthesis of antioxidant enzymes, robustness to stress conditions; Lechardeur et al. 2011; Pedersen, Gaudu, Lechardeur, Petit, & Gruss, 2012; Zotta, Guidone, Ianniello, Parente, & Ricciardi, 2013; Ianniello et al., 2015). Several LAB species have a double metabolic life: they can switch from fermentation to aerobic respiration metabolism when provided with heme, and for some bacteria, heme and menaquinone (Pedersen et al., 2012).

Respiratory metabolism of LAB, however, has been generally investigated in synthetic media. Respiration alters the central metabolism of LAB, rerouting pyruvate away from lactate accumulation, leading to a higher final pH and to the production of secondary metabolites (i.e. acetic acid, acetoin, diacetyl) (Lechardeur et al. 2011; Pedersen et al., 2012; Ricciardi, Castiglione Morelli, Ianniello, Parente, & Zotta, 2015a). The changes in metabolic pathways, therefore, may significantly affect the aromatic pattern and flavour development of fermented foods. Despite the complete documentation of the respiration capacity of several LAB in *in vitro* experiment, to our knowledge, no studies have considered the effect of respirative cultures in a model food fermentation.

For this reason, in this work we studied and discussed the effect of respirative cultures of respiration-competent strains *L. casei* N87 (Ianniello et al. 2015; Zotta et al., 2014) during model wheat sourdough fermentations. Specifically, the evolution of microbial population, the production of volatile organic compounds (VOCs; by SPME-GC/MS) and the changes in albumin/globulin, gliadin and glutenin fractions (by SDS-PAGE) were evaluated. Multivariate statistical analysis, additionally, was used to find correlations between volatilome of sourdoughs and respirative or anaerobic cultures of *L. casei* N87. The findings obtained could open new perspectives on respirative metabolism of LAB for the exploitation in future food applications.

2. Materials and methods

2.1. Bacterial strain and culture conditions

Lactobacillus casei N87 (selected for its capability to tolerate oxygen and different stress conditions; Zotta et al. 2014; Ianniello et al. 2015; Reale et al., 2015) was used in this study. The strain was maintained as freeze-dried stock in reconstituted 11% (w/v) skim milk containing 0.1% (w/v) ascorbic acid in the Culture Collection of Institute of Food Sciences, National Research Council of Italy, and routinely propagated in Weissella Medium Broth, pH 6.8 (WMB; Zotta et al. 2012), for 16 h at 37 °C.

2.2. Anaerobic and respiratory cultivation and production of freeze-dried cultures

L. casei N87 was cultivated (1% v/v inoculum) in WMB containing 10 g/L glucose (initial pH 6.8) in anaerobic (AN; static growth in screw-cap bottles) and respiratory (RS; supplementation of WMB with 2.5 μg/mL hemin and 1 μg/mL menaquinone; baffled shaken flasks on a rotary shaker at 150 rpm) conditions, for 16 h at 37 °C. The optical density at 650 nm (OD650; Bio- Rad SmartSpec™Plus, Bio-Rad Laboratories Inc.) and pH values (Double Pore Slim electrode, Hamilton Company, Reno, Nevada, USA) were measured at the end of incubation (16 h). Anaerobic and respirative growing cells (1.5 mL) were recovered by centrifugation (12,000 g, 5 min, 4 °C), washed twice in 20 mM potassium phosphate buffer pH 7.0 (PB7) and re-suspended in Skim Milk containing 0.1% v/v ascorbic acid. Cultures were frozen at −80 °C for 24 h and then freeze-dried with a Heto Drywinner 3 Benchtop freeze-drier, at −50 °C, 0.1 hPa for 24 h. The freeze-dried cultures were stored 30 days at −20 °C before the use.

2.3. Dough formulation

Wheat doughs were prepared in sterile beakers mixing 60 mL of sterilized water and 40 g of flour, adding microbial starter when necessary. Anaerobic and respiratory freeze-dried cells of *L. casei* N87 were used as starter (8 log cfu/g) in wheat dough in combination with a commercial baker's yeast culture (7 log cfu/g). A dough inoculated just with yeast cultures was also prepared, while an un-inoculated dough was used as control. Therefore, four different types of dough were prepared (C, control without microbial starter; Y, yeast; Y + AN, yeast + *L. casei* N87 grown in anaerobic condition; Y + RS, yeast + *L. casei* N87 grown in respirative condition) and incubated at 37 °C for 24 h. Two biological replicates of each dough formulation were performed on separated days. At time zero and after 6 and 24 h of fermentation, differential counts for LAB and yeast, pH, Total Titratable Acidity values (TTA), production of volatile organic compounds (VOCs; by SPME-GC/MS), and changes in albumin/globulin, gliadin and glutenin fractions (by SDS-PAGE) were evaluated and described below.

2.4. Determination of pH, TTA, LAB and Yeast count

The pH values were determined with a pHmeter Medidor PH Basic 20 (CRISON, Spain). Total titratable acidity was measured on 10 g dough samples, which were homogenized with 90 mL of distilled water for 2 min in a Stomacher laboratory blender (BAG MIXER 400, Interscience, France) and was expressed as the amount (in mL) of 0.1 N NaOH to achieve pH 8.3.

Lactic acid bacteria were counted using modified MRS (Ricciardi et al., 2015b), supplemented with 40 mg/L actidione. The plates were incubated under anaerobiosis (AnaeroGen and AnaeroJar; Oxoid; Basingstoke, Hampshire, United kingdom) at 37 °C for 48 h. Yeasts were estimated after incubation at 28 °C for 72 h on YPD (20 g/L glucose, 20 g/L bacteriological peptone, 10 g/L yeast extract, 20 g/L agar) supplemented with chloramphenicol (0.1 g/L).

2.5. Characterization of volatile organic compounds (VOCs)

The volatile fraction of samples was analyzed by headspace sampling, using the solid phase microextraction technique (HS-SPME) according to Aponte et al. (2014). In detail, for each SPME analysis, 2 g of samples were placed into a 20 mL headspace vial, and added 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 mm of CarboxenePolydimethylsiloxane (Carboxen/PDMS) was used (Supelco, Bellefonte, PA, USA).

2.6. 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 mmI.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