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Manufacture and characterization of a yogurt-like beverage made with oat flakes fermented by selected lactic acid bacteria

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ABSTRACT

This study aimed at investigating the suitability of oat flakes for making functional beverages. Different technological options were assayed, including the amount of flakes, the inoculum of the starter and the addition of enzyme preparations. The beverage containing 25% (wt/wt) of oat flakes and fermented with *L. plantarum* LP09 was considered optimal on the basis of sensory and technological properties. The enzyme addition favored the growth of the starter, shortened the time needed to reach pH 4.2 to ca. 8 h, and favored a decrease of the quotient of fermentation. Fermentation increased the polyphenols availability and the antioxidant activity (25 and 70% higher, respectively) and decreased the hydrolysis index *in vitro*. Sensory analyses showed that fermented oat flakes beverage had the typical features of a yogurt-like beverage, enhancing the overall intensity of odor and flavor compared to the non-fermented control. Selection of proper processing and fermentation condition allowed the obtainment of a beverage with better nutritional and sensory properties.

1. Introduction

The demand of consumers for non-dairy milk substitutes with high acceptance and functionality is increasing (Mårtensson et al., 2000). Cereal-based beverages have a huge potential either to fulfill this expectation and to act as potential vehicles for functional compounds such as antioxidants, dietary fiber, minerals, prebiotics and vitamins (Kreisz et al., 2008).

A variety of technologies (e.g., cooking, sprouting and milling) are routinely used to process cereals, but fermentation still remains one of the best choice to improve nutritional and sensory properties, and shelf-life (Mattila-Sandholm, 1998). A large proportion of cereals is traditionally and currently processed into foods and beverages through fermentation (Nout, 2009). Although several preparations remain like a house art, especially in African countries, the raw grain materials and/or the type of fermentation are the main criteria to classify cereal-based fermented beverages. Lactic acid bacteria (LAB, *Lactobacillus* and *Pediococcus spp.*), *Enterobacter* spp., yeasts (*Candida*, *Debaryomyces*, *Endomycopsis*, *Hansenula*, *Pichia*, *Saccharomyces* and *Trichosporon* spp.) and filamentous fungi (*Amylomyces*, *Aspergillus*, *Mucor* and *Rhizopus* spp.) are mainly used for the manufacture of cereal-based alcoholic beverages (e.g., tchoukoutou, jnard), non-alcoholic beverages (e.g., uji, ben-saalga), porridges (e.g., mawè), and cooked gels (e.g., kenkey, idli, and mifen) (Nout, 2009).

The selection of appropriate starter cultures for each variant of cereal beverage is an industrial need to drive, accelerate and standardize the fermentation (Coda et al., 2014). Selected starters, through their complex enzyme systems, generate metabolites (volatile and non-volatile) that provide peculiar flavor attributes to fermented cereal-based foods (Salmerón et al., 2009, 2014). Moreover, the mechanisms by which LAB fulfill the role of efficient cell factory for the production of functional biomolecules and food ingredients to enhance the quality of cereal based beverages, were largely demonstrated (Waters et al., 2013). The failure of the fermentation may lead to spoilage and/or to survival and contamination of pathogens, thereby creating unexpected healthy risks (Coda et al., 2014). Fermentation by LAB is an effective tool to prevent microbial contamination and it may positively affect the nutritional and functional features of cereal-based beverages (Katina et al., 2005). This also allows to develop market strategies to build up nutritional claims that respond to consumer awareness towards healthy diet (Coda et al., 2014).

Oat, mostly as flakes, is included in the human diet because of the healthy status, which is mainly related to the high concentration of β-glucans. Oat is an excellent source of energy and unsaturated fatty acids (Klensporf and Jeleń, 2008), and it contains dietary fibers, high quality proteins and volatile compounds (Heiniö et al., 2001, 2002). Different classes of natural antioxidants, like tocols, phenolic compounds

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and avenanthramides are also largely found in oat (Peterson, 2001). Although still debated in some countries, clinical data showed that oat may be included in the gluten-free diet (Sontag-Strohm et al., 2008), as recommended by Food and Drug Administration in the USA (Sontag-Strohm et al., 2008).

When collected from the field, oat lacks flavor, and the development of the aroma inevitably requires heat treatments (Heydanek and McGorrin, 1981). Without suitable heating, oat products retain flat, green, raw and bitter taste (Klensporf and Jeleń, 2008). The distinctive flavor results from lipid oxidation and n-heterocyclic compounds, which are synthesized during thermal processing of groats (Heydanek and McGorrin, 1986). Simultaneously, the thermal treatment inactivates lipolytic enzymes (Moltenberg et al., 1986), which are significantly more active in oat than in other cereals like barley or wheat (O'Connor et al., 1992). The high lipolytic activity causes the rapid release of free fatty acids, which are further subjected to oxidation, leading to an increase of rancidity (Moltenberg et al., 1986). Flaking is the typical processing for oat. It includes steam stabilization to inactivate enzymes, followed by kiln- or drum-drying to generate flavor compounds (Klensporf and Jeleń, 2008). The use of cereals as ingredient for beverage has been largely proposed in literature, but the selection of suitable fermentation conditions and starters, were only partially investigated.

This study aimed at manufacturing and characterizing the physical, chemical, functional and sensory properties of a non-alcoholic yogurt-like beverage made with oat flakes, which were subjected to fermentation by LAB. Different starters, technology options and enzyme preparations were assessed.

2. Materials and methods

2.1. Bacterial strains, culture media and enzymes

Lactobacillus plantarum LP01, LP06, LP09, LP32, LP39, LP40, LP48 and LP51; *Lactobacillus casei* LC10, LC11 and LC03; and *Lactobacillus paracasei* LPC02 and LPC16 (Sacco Srl, Cadorago, CO, Italy) were singly used as starters for fermentation. LAB were cultivated in modified MRS (mMRS), prepared with 1% (wt/v) maltose and 5% (v/v) fresh yeast extract, final pH of 5.6 (Oxoid Ltd, Basingstoke, Hampshire, England). Fresh yeast extract was prepared by re-suspending baker's yeast (60 g) in deionized water (300 ml). After sterilization (120 °C for 20 min), the suspension was centrifuged at 6,000 ×*g* for 20 min, and the supernatant was recovered and added to mMRS prior to sterilization (Coda et al., 2010).

Preliminarily, different options to inoculate LAB were considered: (i) lyophilized preparation (ca.5×10^{11} cfu/g) (100 mg/L of beverage); (ii) cell suspension in the tap water used for making beverages, after cultivation in mMRS (late exponential phase of growth, ca. 10 h), harvesting (centrifugation at 9,000 ×*g* for 10 min at 4 °C) and washing (twice in 50 mM pH 7.0 phosphate buffer, 4 °C); and (iii) pre-culture (24 h of incubation at 30 °C) in oat flakes (25% wt/wt in tap water) to be inoculated (5% wt/wt) into beverage. For all these conditions, the cell density after the inoculum was 5×10^7 cfu/ml of beverage. The enzyme preparations Depol 740 L (xylanase activity, 55 μkat/g; endoglucanase activity, 3.6 μkat/g; ferulic acid esterase activity, 0.12 μkat/g; β-glucanase activity, 27.7 μkat/g; Biocatalyst Ltd., Great Britain) and Grindamyl 1000 (amylase activity, 120 μkat/g; Danisco, Denmark) were used (Anson et al., 2009).

2.2. Manufacture of cereal beverages

Oat flakes (OF) were supplied by Barilla Spa (Parma, Italy). The characteristics of OF were as follows: moisture, 11.3%; protein (N × 5.70), 9.8% of dry matter (d.m.); fat, 5.5% of d.m.; ash, 2.9% of d.m; and carbohydrates, 70.4% of d.m, respectively.

Oat flakes were milled by Ika M20 Universal Mill (Sigma Chemical Co., Milan, Italy) to obtain oat flakes flour (OFF). The amount of OFF used for making oat flakes beverages (OFB) was set up based on sensory analysis. LAB were used alone or in combination with Depol 740 L (dosage 50 nkat xylanase/g of OFF) and Grindamyl (dosage 75 nkat α-amylase /g of OFF). OFB, without microbial inoculum, was incubated under the same conditions and used as the control (Ct-OFB). All beverages were incubated at 30 °C under stirring conditions (100 rpm). After fermentation beverages were pasteurized at 63 °C for 30 min and stored at 4 °C for 30 days.

2.3. Determination of pH, total titratable acidity (TTA) and kinetics of acidification

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 on 10 g of beverage, which were homogenized with 90 ml of distilled water, and expressed as the amount (ml) of 0.1 M NaOH to get pH of 8.3.

Kinetics of growth and acidification were determined and modelled in agreement with the Gompertz equation, as modified by Zwietering et al. (1990),: $y = k + A \exp\{-\exp[(\mu_{max} \text{ or } V_{max}\ e/A)(\lambda - t) + 1]\}$; where y is the growth expressed as log cfu/g or the acidification rate expressed as dpH/dt (units of pH) at the time t; k is the initial level of the dependent variable to be modelled (log cfu/g or pH units); A is the cell density or pH (units) variation (between inoculation and the stationary phase); μ_{max} or V_{max} is the maximum growth rate expressed as Δlog cfu/g/h or the maximum acidification rate expressed as dpH/h, respectively; λ is the length of the lag phase measured in hours. The experimental data were modelled by the non-linear regression procedure of the Statistica 8.0 software (Statsoft, Tulsa, USA).

2.4. Microbiological analysis

The number of presumptive LAB was estimated by plating on mMRS agar (Oxoid) at 30 °C for 48 h. Total bacteria were determined on Plate Count Agar (PCA, Oxoid) at 30 °C for 48 h, yeasts were counted on Yeast extract-Peptone-Dextrose agar (YPD, Oxoid), supplemented with 150 ppm chloramphenicol, at 30 °C for 72 h, and total enterobacteria were determined on Violet Red Bile Glucose Agar (VRBGA, Oxoid) at 37 °C for 24 h.

2.5. Water holding capacity, water activity, viscosity, total dry matter, and color

Water holding capacity (WHC) was measured according to the method described by Remeuf et al. (2003). After fermentation and storage, 10 g of OFB were centrifuged (5,000 rpm for 40 min at 7 °C). The expelled water was removed and weighed. The percentage of WHC was defined according to the equation: WHC = [(Sample weight − Expelled water) / Sample weight] * 100. Water activity (a_w) was determined at 25 ºC by the Aqualab Dew Point 4TE water activity meter (Decagon Devices Inc., USA).

An aliquot (100 ml) of OFB held at 30 °C was used for viscosity measurements. A rotary viscometer Myr VR3000 (Viscotech, El Vendrell, Spain) model V1-L at disc spindle speeds of 30, 40 and 60 rpm was used. Readings were taken after 3 min of revolution. The appropriate disc spindle was selected so that the torque readings were not below 10% of the total scale. Total dry matter was determined on 100 ml of beverage at 105 °C for 24 h (AOAC, 1985).

The chromaticity co-ordinates of the beverages (obtained by a Minolta CR-10 camera) were reported in the form of a color difference, dE^*_{ab}, as follows: $dE^*_{ab} = \sqrt{(dL)^2 + (da)^2 + (db)^2}$