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Improvement of Ayran quality by the selection of autochthonous microbial cultures

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ABSTRACT

Ayran is a traditional Turkish milk drink which is fermented and salted. Inadequate production and storage conditions contribute to its variable organoleptic quality and stability during shelf-life. A thorough physico-chemical, nutritional and microbial characterization of artisanal Ayran was carried out in order to standardize its overall quality without altering its original traits. Ayran microbial ecosystem was largely dominated by *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LDB). High counts of other lactic acid bacteria species, including *Lactobacillus helveticus* (LH), *Lactobacillus fermentum* (LF), and *Lactobacillus paracasei* (LP), were also found. Selected LDB, LP and LH strains grew well in milk displaying fast acidification and high proteolysis, differently from ST and LF strains that did not cause noticeable changes. A selected autochthonous three-strain culture (TSC), composed of one strain of LDB, LP and ST, was applied for the pilot-scale production of traditional Ayran. The Ayran produced with this TSC resulted in the most extensive shelf-life (one month) and in the best terms of its nutritional and sensory quality nevertheless altering its typical pleasant yogurt and cottage cheese notes. This TSC is at disposal of SMEs who need to standardize the overall quality of this traditional fermented milk, preserving its typical traits.

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1. Introduction

Ayran is a popular Turkish yogurt-based drink which has a distinctive salty taste. It is also known as “Dough” in Iran, “Tan” in Armenia, “Laban Ayran” in Syria and Lebanon, “Shenina” in Jordan, “Moru” in South India, “Laban Arbil” in Iraq, and “Ayrani” in Cyprus (Yildiz, 2010). It is estimated that Ayran consumption in Turkey is ca. 1 million ton annually (Koçak and Avşar, 2010). Keeping an ancient practice (Kabak and Dobson, 2011), which is still followed in some Turkish villages, yogurt is diluted with 30–50% of water containing almost 1% of salt to preserve the product and to disguise its sour taste (Koçak and Avşar, 2010). However, the inadequate hygienic conditions dramatically affect the final quality of this fermented milk resulting in an unpleasant sour and bitter taste. In addition, these conditions cause whey separation in few days, also under low temperature storage (Koksoy and Kılıç, 2003).

Nowadays, artisanal Ayran is started by using part of the previous day Ayran production. This practice, usually known as “back-slopping”, allows to preserve a complex microbial community from which autochthonous bacteria can be isolated and selected for improving hygienic characteristics and the production process of this original food. In addition, “back-slopping” can be a source of strains endowed with functional properties (Alegría et al., 2010; Baruzzi et al., 2002, 2011; Morea et al., 2007; Smid et al., 2014).

Grishina et al. (2011) suggested that some Ayran samples can be endowed with functional and healthy traits such as antioxidant and protective activities on intestinal cells, presumably related to its high amount of natural mesophilic and thermophilic lactic acid bacteria (LAB).

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Proteolytic LAB, thanks to their peptidases, could promote flavour enhancement during fermentation and storage of fermented milk, even though casein hydrolysis also contributes to the syneresis in the final products (Tamime and Robinson, 2007). Besides, the occurrence of microorganisms such as yeasts, *Enterobacteriaceae* or heterofermentative LAB can be responsible for reducing Ayran shelf-life (Koksoy and Kılıç, 2003).

An alternative to the "back-slopping" procedure is the industrial production of Ayran performed by applying commercial yogurt starter cultures. This, however, cause a progressive loss in both microbial diversity and original sensory profile of this traditional fermented milk (Koçak and Avşar, 2010).

An in-depth characterization of traditional Ayran as well as the development of selected autochthonous starter cultures are essential prerequisites to promote the production of Ayran on an industrial-scale, preserving its traditional taste and flavour.

The present study aimed at improving and standardizing the shelf-life of Ayran by a thorough physico-chemical, nutritional and microbiological characterization of this traditional beverage. The bacterial community of this fermented milk was analysed; LAB isolates were characterized for their promising technological features. Selected autochthonous starter cultures were successfully applied at lab- and pilot-scale for manufacturing Ayran, following the traditional production protocol.

2. Materials and methods

The experimental flowchart carried out in the present work is reported in Fig. 1.

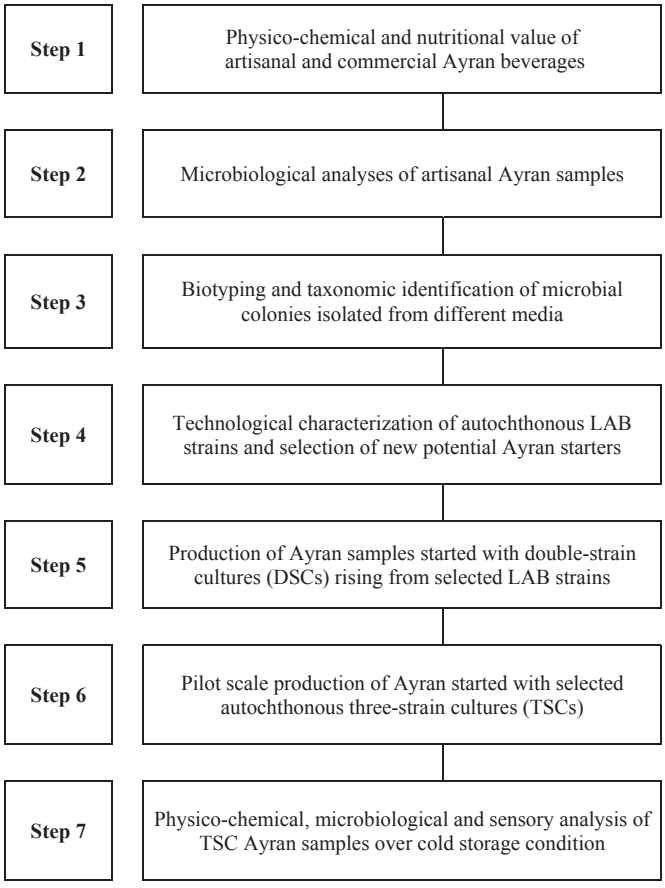

Fig. 1. Graphical scheme of the experimental plan carried out in the present work.

2.1. Ayran sampling

Seven batches of artisanal Ayran productions (samples A1-A7) were collected (from February 2008 to May 2009) from farmhouses of two different Turkish villages located near Konya, Turkey.

All artisanal Ayran drinks were manufactured by using the "back-slopping" procedure. In brief, 8–10 L of full fat cow milk was heated to about 85 °C for a few minutes and cooled to 40–43 °C. The milk was mixed with ca. 1.0% (v/v) from the previous day Ayran production and incubated at ca. 40 °C until a gel formed (4–6 h, pH 4.2–4.6). The yogurt obtained was added to cold tap water which had previously been salted and boiled (ca. 1.25–2.50% of NaCl), as described by Koçak et al. (2006). Commercial Ayran samples were manufactured by the four most widespread commercial Turkish brands, including those manufactured by Aygin Süt A.S. (Konya, Turkey). This company also provided the pilot-plant which was subsequently used to carry out new Ayran productions. Three replicates from these Turkish brands (12 in total) were transported under refrigerated condition to the laboratories and immediately analysed as described below. Both artisanal and commercial samples were collected within 24 h after fermentation.

2.2. Physico-chemical and nutritional value of artisanal and commercial Ayran beverages

The pH of Ayran samples was measured with the Φ 340 pH/Temp Meter system (Beckman Coulter, Fullerton, CA, USA) equipped with a liquid food drill at 20 °C. Total titratable acidity (TTA), total fat, protein content, solid, and ash contents were calculated following the AOAC methods 947.05/2000b, 989.05/2000a, 991.20/1995b, 925.23/1995a and 945.46/1990, respectively. Water activity (a_w) was determined with the DECAGON AquaLab Serie 3 TE system (Aqualab, Pulman, WA, USA), following the manufacturer' instructions. Ayran samples were also evaluated for their content in glucose and galactose by HPAE-HPLC (Dionex) with pulsed amperometric detection after acid hydrolysis of the original samples (Cefola et al., 2014). The amount of acetic acid, lactic acid and D (−) and L (+) lactate were determined monitoring the changes in NADH absorption at 340 nm (Roche, R-Biopharm, Darmstadt, Germany). Ayran energy values were estimated on the average conversion factors for proteins, fat and carbohydrates. All parameters were measured three times per sample.

2.3. Microbiological and molecular analyses

2.3.1. Microbiological analyses of artisanal Ayran samples

Ten mL of each Ayran was homogenized in 90 ml of autoclaved saline peptone water (0.85% NaCl, 0.1% peptone, pH 7.0) for 2 min in a stomacher apparatus (400 Circulator, PBI International, Milan, Italy) and serially diluted in sterile 0.1% buffered peptone water. The appropriate dilutions were plated in triplicate on M17 supplemented with 0.5% lactose (LM17, Oxoid S.p.A., Milan, Italy) and on MRS agar (Oxoid S.p.A.) containing 0.1 g/L of cycloheximide (Sigma-Aldrich, Milan, Italy) for enumerating mesophilic and thermophilic cocci- and rod-shaped LAB; the plates were incubated under anaerobiosis for 48 h at 30 °C and 42 °C, respectively. Total coliforms, presumptive enterococci, yeasts and moulds, coagulase positive and negative staphylococci were enumerated as previously reported (Baruzzi et al., 2000, 2002, 2006; Borcakli et al., 2013; Caputo et al., 2012). The ISO standards ISO 6785:2001, ISO 11290-1:1996, ISO 6888-1:1999 and their amendments were applied to ascertain the presence of *Salmonella* spp., *Listeria monocytogenes* and coagulase-positive staphylococci. All microbial analyses were performed in triplicate.

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