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Microbiological, physico-chemical, nutritional and sensory characterization of traditional Matsoni: Selection and use of autochthonous multiple strain cultures to extend its shelf-life

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ABSTRACT

Matsoni, a traditional Georgian fermented milk, has variable quality and stability besides a short shelf-life (72–120 h at 6 °C) due to inadequate production and storage conditions. To individuate its typical traits as well as select and exploit autochthonous starter cultures to standardize its overall quality without altering its typicality, we carried out a thorough physico-chemical, sensorial and microbial characterization of traditional Matsoni. A polyphasic approach, including a culture-independent (PCR-DGGE) and two PCR culture-dependent methods, was employed to study the ecology of Matsoni. Overall, the microbial ecosystem of Matsoni resulted largely dominated by *Streptococcus* (*S.*) *thermophilus* and *Lactobacillus* (*Lb.*) *delbrueckii* subsp. *bulgaricus*. High loads of other lactic acid bacteria species, including *Lb. helveticus*, *Lb. paracasei* and *Leuconostoc* (*Leuc.*) *lactis* were found to occur as well. A selected autochthonous multiple strain culture (AMSC) composed of one *Lb. bulgaricus*, one *Lb. paracasei* and one *S. thermophilus* strain, applied for the pilot-scale production of traditional Matsoni, resulted the best in terms of enhanced shelf-life (one month), sensorial and nutritional quality without altering its overall typical quality. This AMSC is at disposal of SMEs who need to exploit and standardize the overall quality of this traditional fermented milk, preserving its typicality.

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1. Introduction

Matsoni (also known as matzoon, matsoon, matsoun, matzoun, madzoon, madzounmacun), is a traditional fermented milk produced in Georgia, a region listed among the cradles of fermented milks; this product, popular throughout the whole Caucasus region, is consumed not only as such but also as ingredient for making cakes and dough (e.g. for khachapuri) typical of the Caucasian cuisine (http://georgiaabout.com/2012/08/31/about-food-potato-rice-and-herb-soup-with-matsoni). It is so much appreciated by the Georgians that it was declared complementary food for babies aged over 6 months by the Georgian Ministry of Labour's Health and Social Affairs Department (Nemsadze, 2004).

Matsoni is usually manufactured in farmhouses following a traditional protocol foreseeing the use of cow's milk (and seldom ewe's, goat's, buffalo milk or their mixtures) and whose production process, usually carried out in glass bottles, is based on spontaneous fermentation and back-slopping, i.e. "inoculation of milk with a small quantity of the previous performed successful fermentation" (Leroy and De Vuyst, 2004). This ancient but obsolete practise makes Matsoni quality and stability variable. Moreover, inadequate hygienic conditions as well as occasional interruptions of the cold-chain distribution may affect the final quality of this fermented milk resulting in a high sour and bitter taste product with a short shelf-life of max 70–120 h. To overcome these drawbacks, commercial starter cultures could be used,

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resulting in a loss of the typical traits that make unique each traditional fermented milk (Leroy and De Vuyst, 2004).

Use of preservatives and stabilizers is becoming subject to public concern and ever-tighter legislative control. Thus, manufacturers need procedures suitable to control the fermentation process, stabilizing microbial loads and extending Matsoni shelf-life without altering its traditional taste and flavour.

An in-depth characterization of traditional Matsoni as well as the development of autochthonous starter cultures to be used by dairy factories seem to be essential pre-requisites to reach these goals and favour the production of Matsoni at industrial-scale. Indeed, although Matsoni has been manufactured since more than 100 years, only few studies have been performed to characterize it and mainly from the microbiological point of view (Erzinkjan, 1971; Merabishvili and Chanishvili, 2001; Reddy et al., 1986; Uchida et al., 2007).

The present study aimed at performing a thorough physico-chemical, nutritional, sensory and microbiological characterization of Matsoni and develop an autochthonous starter culture to improve and standardize the shelf-life of this product maintaining its typical traits. The bacterial community of this fermented milk was analysed using culture-dependent and -independent techniques. Isolates of the dominant species were characterized for promising technological features. Selected autochthonous starter cultures were successfully applied at lab- and pilot-scale for manufacturing Matsoni, following the traditional production protocol.

2. Materials and methods

2.1. Sampling and fermented milks preparation

Sixteen batches of Matsoni, named M1-M16, were collected in farmhouses located in Georgia. These fermented milks were manufactured following the traditional protocol (Chanishvili et al., 2001) foreseeing raw cow's milk (60 L) pasteurized at 90 °C for 10 min, cooled up to 42 °C and started with a 3% (vol/vol) backslopping obtained from the batch production of the previous day. Matsoni is produced in glass bottles, each containing 250 mL of milk, which is fermented at 42 °C until the pH value of 4.6 is reached (after ca. 5–7 h). Thereafter, Matsoni bottles are placed in a cooling cell and stored at 6 °C for 72–120 h.

Samples (raw milk, pasteurized milk, natural starter, artisanal and commercial Matsoni) were transported under refrigeration condition to the laboratories and immediately analysed.

The analyses were carried out in triplicate.

2.2. Physico-chemical analyses

Matsoni samples were analysed for pH, total titratable acidity (TTA), fat, sugar, total proteins, dry matter and short chain fatty acid (SCFA) content.

The pH value and TTA were measured in fresh Matsoni and after its storage at 6 °C for 72 h.

The pH value was determined by direct insertion of a pH meter (Oakton Benchtop pH 510 Meters, Cole-Parmer, Vernon Hills, Illinois, USA).

TTA was measured as follows: 10 g of each fermented milk were weighted in a 250 mL Erlenmeyer flask and distilled water was added up to 50 mL including few drops of phenolphthalein. The mixture was titrated with 0.1 N NaOH, according to the AOAC method n. 947.05 (AOAC, 2000) and expressed as percentage of lactic acid in 100 g of sample.

Fats were first methylated and then extracted with hexane. Methylated fatty acids were analysed by gas chromatography using a Varian Wcot fused silica column, as reported in the European Commission Regulation no. 2568 (1991) and European Commission Regulation no. 1429 (1992).

As concerns the calculation of total protein content, the total N occurring in the fermented milks was determined with the Kjeldahl method, as described for Protein in Beer (AOAC 920.53 method, AOAC, 1998). Briefly, fermented milks (0.7–2.2 mL) were digested boiling them with H_2SO_4 for at least 30 min and the developing NH_3 was titrated with NaOH.

Dry matter (d.m.) content was determined applying the gravimetric method AOAC 925.23 (AOAC, 1999). In accordance with this method, 3 mL of sample were desiccated for 3 h at 130 °C; the remaining amount of the fermented milk was weighted and expressed as dry matter in percentage of the fresh sample.

The ash content percentage, based on the initial weight of 105 °C dried materials, was determined in accordance with the following formula:

$$\text{Ash\%} = (W1/W2) \times 100$$

where: W1 = weight of ash, and W2 = initial weight of 105 °C dried sample. (Method provided by the National Renewable Energy Laboratory, Midwest Research Institute, Kansas City, Mo 64110, United States).

The available saccharide content was calculated as difference between the fermented milk dry matter value and the sum of the contents of fermented milk ash and two determined nutrients.

The Matsoni samples were evaluated for their content in simple sugars (mono- and disaccharides). A rough assessment of the content of these saccharides was obtained by the determination of glucose and galactose originated after acid hydrolysis of the original sample. The analyses were performed by HPAE-HPLC (Dionex) with pulsed amperometric detection.

The amount of lactic acid and D(−) and L(+) lactate were determined by the UV-method (Roche, R-Biopharm, Darmstadt, Germany) on spectrophotometer absorption of NADH at 340 nm.

The above mentioned analyses were carried out in triplicate.

2.3. Nutritional value

The method used to estimate the Matsoni energy value was based on the average conversion factors for proteins, fat and carbohydrates (Kunachowicz et al., 1998). These factors are: i) 1 g of protein, 17 kJ/4 kcal; ii) 1 g of fat, 37 kJ/9 kcal; iii) 1 g of carbohydrates, 17 kJ/4 kcal. Energy value of the fresh Matsoni was calculated as the sum of the energy values of its basic nutrients contained in 100 g of fresh Matsoni (means ± standard deviations of the 16 Matsoni analysed).

2.4. Sensory analyses

Sensory profiles of four Matsoni samples (M1–M4), randomly chosen among the 16 fresh Matsoni, were evaluated in laboratory, meeting the requirements of ISO 8589:1988 (Anonymous, 1988), by the trained panel of six assessors (ISO 8586-1:1993; Anonymous, 1993) using the Quantitative Descriptive Analysis (QDA) method (Stone et al., 1974) to establish descriptors of sensory notes perceived as the most essential within the attributes of odour, taste and texture. The note descriptors were proposed, discussed and agreed by the panel members during the training sessions. Note intensities and the values of the overall Sensory Quality (SQ) factors for the samples were delimitated using scales (ISO 4121:1987, Anonymous, 1987): non-structured scales with the border restraints denominated as "slight/none"–"high" and the 10-unit ratio scales. The evaluation of segments, marked by assessors on

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