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Original Research Article

Chemical and fatty acid composition of cow and sheep milk cheeses in a lamb skin sack

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ABSTRACT

In this study, the basic chemical and fatty acid compositions of autochthonous Croatian cow and sheep cheeses in a lamb skin sack (local name: "sir iz mišine") were determined. Also, the influences of ripening period and different starter cultures on chemical and fatty acid composition of these cheeses were investigated. Samples of cow ($n = 20$) and sheep ($n = 20$) milk cheeses were produced in three different ways: from raw milk without the addition of a starter culture, from pasteurized milk with commercial starter cultures and with previously isolated autochthonous starter cultures (*Lactococcus lactis* S1 or *Lactobacillus plantarum* B or a mixture of both). Samples were taken during a 45-day ripening period (on days 0, 15, 30 and 45). The ripening time significantly affected all basic chemical parameters, while different starter cultures significantly ($p < 0.05$) influenced protein, fat and ash content. Ripening time had no significant effect on the representation of the investigated fatty acid groups ($p > 0.05$), but a significant difference was found depending on the starter cultures used and the type of cheese analysed, in terms of statistically higher proportion ($p < 0.05$) of polyunsaturated fatty acids in the finished sheep's milk cheese (2.58–2.97%) in comparison to the cow's milk cheese (1.93–2.14%). Fatty acids most represented in the analysed cheeses were palmitic, oleic and stearic acid.

1. Introduction

Dietary milk fats have been long associated with various diseases, due to the high saturated fatty acids content. However, recent studies have focused on their healthy components, such as conjugated linoleic acid (CLA, isomer *cis*-9 *trans*-11) (Bergamo et al., 2003). CLA has been identified as a potential anti-carcinogen primarily present in the human diet due to the consumption of dairy products (Belury, 2002; Parodi, 1999). It has also been reported that medium-chain fatty acids (MCFAs) can be beneficial for human health because of their unique metabolic ability to provide energy directly, instead of being deposited in adipose tissues, affecting the reduction of serum cholesterol therein (Bach et al., 1996; Haenlein, 2004; Kasai et al., 2003). A considerable effort has been made to enhance the nutritional quality of milk and its products based on the fact that nutritional composition of dairy products varies owing to several factors, such as milk-producing animal breed, genetics, physiology, feeding regimen, the environment and the production technology, the aforementioned being especially true when it comes to fat and fatty acid composition (Raynal-Ljutovac et al., 2008; Woods and Fearon, 2009; Vargas-Bello-Pérez et al., 2013).

Autochthonous cheeses are valued as any other cheese because of their high nutritional value, due to the high amounts of protein, calcium, phosphorus, and vitamins A and D (Lavasani et al., 2012). Autochthonous cheeses are additionally appreciated by consumers who care about the nature, tradition and the origin of their food. One Croatian autochthonous dairy product is a cheese in a lamb skin sack, manufactured at family farms in the Dinara Mountain area. Ripening in a lamb skin sack for a period of 2–3 months is responsible for the characteristic sensory properties of this cheese (Tudor Kalit et al., 2010). Ripening is the most complex phase of

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cheese production that includes three main processes: glycolysis, proteolysis, and lipolysis (Rodrigues et al., 2012; Collins et al., 2003). Ripening time affects the taste, smell, appearance and consistency of a cheese that come as a result of microbial and enzymatic activity, as well as free fatty acid formation (Tratnik, 1998).

All cheeses contain casein, milk fat, carbohydrates, vitamins and minerals, although represented in different quantities. The amount of these components in a cheese, that is to say, the nutritional value of that cheese, depends on the type of cheese and the type of milk it was made from (Lukač Havranek et al., 2000). Activity of microorganisms during the ripening process can also influence the nutritive value of cheeses. For example, research results have shown that the fermentation activity of lactic acid bacteria (LAB) present in milk increases the share of short-chain fatty acids (SCFAs) and MCFAs found in the final product (Slačanac et al., 2005). Microorganisms involved in cheese-making and ripening can be divided into those added into the cheese milk as starter and adjunct cultures, carefully selected so as to improve the overall quality of the cheese, and into non-starter LAB (Awad et al., 2007). Traditionally, cheese in a lamb skin sack is produced from raw sheep milk without the addition of any commercial starter culture. However, cheese can be produced from cow, goat and buffalo milk and their mixtures, as well as from pasteurized rather than raw milk (Kaić et al., 2012; Sert et al., 2014). The composition of sheep's milk significantly differs from that of cow's milk in terms of its greater amounts of fat, protein, milk ash and dry matter (Antunac and Lukač Havranek, 1999).

A few types of cheese ripened in an animal skin sack are produced in certain geographic areas and can have different local and regional names such as "sir iz mišine" (Croatia), "sir iz mjeha" (Bosnia and Herzegovina), or "Tulum" (Turkey) (Tudor Kalit et al., 2010; Yilmaz et al., 2005; Kivanc, 1989). Microbiological, chemical and physical properties of these cheeses depend on the milk quality, production conditions and procedures, the experience of the producers and storage conditions. In view of the foregoing, these products can hardly be expected to be standardised in quality (Sert et al., 2014). However, in order to preserve the tradition, earn a market position and protect the designation-of-origin (PDO) status of these products, production technology standards should be observed by the producing family farms, so as to raise the quality of the final product (Tudor et al., 2009).

Due to the rarity and specificity of the cheeses detailed above, there are only limited data on their fatty acid composition (e.g., Tudor Kalit et al., 2013, 2014). Therefore, the aim of this study was to characterize cow and sheep cheeses in a lamb skin sack by analysing their basic chemical and fatty acids composition. Also, the aim was to investigate the influence of different starter cultures and ripening times on cheese composition.

2. Materials and methods

2.1. Cheese production

For the production of the cheeses, Dalmatian "Pramenka" sheep milk and Holstein-Friesian and Montafon brown cattle cow milk mix were used. The cheeses were produced in three different variants, as follows: from raw milk without starter cultures (NO), from pasteurized milk with commercial starter culture DI-PROX® MT 1001 (Bioprox, Levallois-Perret, France) (17 g of lyophilisate culture/100 L milk) and with autochthonous starter cultures consisting of *L. lactis* S1 or *L. plantarum* B or a mixture of both (1.5 g of wet biomass/100 L milk), which were isolated and selected from traditional cow's milk cheese in a lamb skin sack (Frece et al., 2014; Babić et al., 2011; Kaić et al., 2012). As for the cheese into which no starter cultures were added, the curd was obtained at 33 °C using commercial rennet. The curd was then sliced into regularly shaped cubes having an average size of 2 cm using a knife. Curd grains were heated up to 38–39 °C and stirred by hand. After drying, the curd grains were the size of hazelnuts/peas. Rough curd was shaped by hand and squeezed into plastic vats using a self-moulding process.

As for the cheese in a lamb skin sack into which the previously isolated starter cultures and the commercial culture were added, pasteurized milk was used in its production. The milk was heated to 65 °C, then allowed to rest for 30 min and then cooled to 32–33 °C. Half an hour before the rennet was put in place, the starter culture was added. After resting for half an hour, commercial rennet was introduced at 32–33 °C. The curd grains were heated to 38–39 °C. After whey draining the cheese was cut into pieces measuring approximately 10 cm × 10 cm × 5 cm, salted with large-grain sea salt (0.8 kg salt/20 kg cheese) and then put into a lamb skin sack ("mišina"). Depending on the size of the lamb skin, the average weight of the cheese in the sack was about 20 kg.

The cheese was ripened in a lamb skin sack for 45 days at 16–18 °C and relative humidity of 65–80%.

2.2. Sample collection

The research was conducted on two family farms near Knin, Croatia. The samples of cow and sheep milk cheese in a lamb skin sack were produced in five variants: without starter culture, with the use of commercial culture and with the use of autochthonous starter cultures *L. lactis* S1 or *L. plantarum* B or a mixture of both. Each of the five cheese variants were sampled four times during the 45-day ripening period (on days 0, 15, 30 and 45), meaning that 20 samples of cow and 20 of sheep cheese were finally obtained.

Before opening, the lamb skin sack was accommodated by a specially designed sealed container from which the air was drawn out and replaced by nitrogen (so as to create a nitrogen atmosphere). Cheese samples (200 g) were taken through a larger container opening using sterile gloves, thus preventing the possibility of aerobic microorganism development.

2.3. Preparation of samples intended for analysis

Before analysis, samples were homogenized with a Grindomix GM 200 homogenizer (Retsch, Haan, Germany) and stored in a plastic container filled to the top so as to reduce the air contact and delay the spoilage process. After determining their moisture contents, the samples were stored at 4 °C pending determination of other chemical properties and fatty acid composition. Each (of the 20 cow and 20 sheep) cheese sample was prepared and analysed in triplicate.

2.4. Standards and reagents

A standard solution composed of 37 fatty acid methyl esters each at 10 mg/mL was prepared by virtue of dissolving the standard Supelco™ 37 Component FAME Mix (Bellefonte, Pennsylvania, SAD) in hexane. The solution was stored at −20 °C and used for the identification of fatty acid methyl esters on the occasion of each analysis.

Hexane and methanol used with fatty acid analysis were of HPLC grade (JT Baker, Derventer, the Netherlands), while all other chemicals were of analytical grade (Kemika, Zagreb, Croatia). Ultra-pure water having an electrolytic conductivity of ≤0.05 S/cm was obtained using a Millipore Direct-Q 3UV (Merck, Darmstadt, Germany).