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Effect of autochthonous starter cultures isolated from Siahmazgi cheese on physicochemical, microbiological and volatile compound profiles and sensorial attributes of sucuk, a Turkish dry-fermented sausage

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ABSTRACT

The effect of adding autochthonous starter cultures isolated from Siahmazgi cheese, on the physicochemical parameters and microbial counts of sucuk was investigated during the ripening period. SPME–GC/MS was used in volatile compound analysis and a trained group of panelists carried out sensory analysis of the final product. After preliminary screening, three strains of *Lactobacillus plantarum*, which possess desirable technological properties, were used to prepare three starter cultures: LBP7, LBP10 and LBP14. The addition of LBP7 and LBP14 starter cultures had a significant effect ($P < 0.05$) on lightness, leading to higher L values compared to control sausages during the ripening period. Both LBP7 and LBP14 sausages showed higher counts of lactic acid bacteria, lower growth of *Enterobacteriaceae* and Gram-positive catalase-positive cocci and greatly lowered the pH value compared to control sausages throughout the ripening process. At the end of the ripening process, lactic acid bacteria counts were affected ($P < 0.05$) by the addition of starter culture since higher counts were observed in sausages prepared with LBP7 (9.14 log CFU/g) and LBP14 (8.96 log CFU/g) batches. The decrease of water activity during the ripening of sausages was not affected by the various starters. The texture profiles of all sausages were similar except for LBP10, which showed lower hardness and gumminess during ripening. Under the conditions of the study, volatile compounds were mainly from spices, and no marked differences were found among inoculated sausages. However, sensory evaluation revealed that most of the sensory attributes were scored higher for inoculated sausages than for the control ones. Therefore, LBP7 and LBP14 could be promising candidates for inclusion as starter cultures for the manufacture of sucuk.

1. Introduction

Traditional fermented meat products are produced and consumed in many countries. The safety of fermented sausages, the most popular of such meat products, is essentially gained by a fall in pH and a decrease of water activity (a_w) below the growth limit of most pathogens, thus enabling efficient bacterial control using the "hurdle technology" concept (Barbuti & Parolari, 2002). Among the most important factors determining the characteristics and quality of fermented sausages is the choice of starter cultures. Due to changes in shopping and consumption habits, together with the growing importance of consumer demand for products with high quality and long shelf life, the problem of safe preservation in the meat industry has become more complex: today's products require a longer shelf life and greater assurance of protection from microbial spoilage (Zhao et al., 2011). It is thus not surprising that in recent years the research has shifted towards the selection of starter cultures, due to the scientific progress in understanding their desirable role in meat fermentation. Commercial starters, because of quick acidification, cannot always compete well with natural fermentation, and their use could have a negative impact on the product's sensory properties (Casquete et al., 2011). The most promising microorganisms for starter cultures are those that are well adapted to the meat environment and to the specific manufacturing process, and are capable of dominating the microbiota of the product due to their specific composition and metabolic activity (Babić et al., 2011).

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Lactic-acid bacteria (LAB) play an important role in meat preservation and can decrease the pH by lactic-acid production; produce bacteriocins, which prevent growth of some pathogenic and spoilage organisms; provide diversity of sensory properties by modification of raw material; and contribute to the development of flavour, colour and texture, thereby improving the overall quality and shelf life of meat products (Holko, Hrabě, Šalaková, & Rada, 2013; Leroy & De Vuyst, 2004). Therefore, it is worthwhile to continue searching for optimal LAB starter cultures. Since there are no fermented meat products in Iran, from which to isolate a proper LAB, strains from a dairy product were tested. Dairy products such as fermented milk and cheese are often carriers for LAB cultures. While much recent attention has been directed to LAB isolation from different kinds of fermented sausages (Kaban & Kaya, 2008; Kaban & Kaya, 2009), there is little research on their isolation from dairy products for use in the manufacture of fermented sausages.

Siahmazgi cheese is a semi-hard artisanal cheese originating in the mountainous area of Talesh–Guilan in Iran. It is manufactured traditionally from raw whole goats' milk from both the evening and morning milkings in a one-month period when goats' milk becomes plentiful. Ripening takes approximately six months. Due to the cheese's hard texture and low pH value, the existing LAB flora may be able to adapt to the environment and produce optimum acid levels in fermented sausages. However, no surveys reporting the commercial application of LAB isolated from Siahmazgi cheese in fermented sausage products have been published.

Sucuk is a traditional dry-fermented sausage in Turkey that has a broad acceptance by consumers and widespread distribution in local markets. The typical sucuk consists of a mixture of beef, beef fat, salt, sugar, nitrite and various spices in which the mixture subsequently undergoes bacterial fermentation followed by a ripening period (Kilic, 2009). This process favours the growth of autochthonous microflora, which influences the flavour, texture, nutritional qualities, safety, and other characteristics of this type of sausage. The commercial starter cultures commonly used in sucuk production are selected according to their technological activities such as fermentative, proteolytic or lipolytic characteristics. However, in the last decades, they are not preferred for sucuk production as they may result in losses of some desirable sensory characteristics (Dalmış & Soyer, 2008) For this reason, traditional dry-fermented sausages are often of superior sensory quality to those inoculated with commercial starters. Although a number of studies about different aspects of sucuk have been published, such as the effect of replacing beef fat with hazelnut oil (Yıldız-Turp & Serdaroğlu, 2008), the effect of different levels of orange fibre and fat (Yalınkılıç, Kaban, & Kaya, 2012), the effect of recipe formulation and inoculation of starter cultures (Stajić, Perunović, Stanišić, Žujović, & Živković, 2012) or the effect of ripening period, nitrite level or heat treatment on sensory evaluation (Kurt & Zorba, 2012), to our knowledge this is the first study of the effect of non-meat originated starter cultures on the properties of sucuk. In view of the fact that traditional starters such as LAB are included in the QPS (Qualified Presumption of Safety of Micro-organisms in Food and Feed) list and provide a more natural means of food preservation, which can allay consumer concerns over possible adverse health effects, the aim of this study was to evaluate the LAB strains isolated from Siahmazgi cheese to determine their suitability for use as starter cultures in sucuk production. Furthermore, in an attempt to investigate their effect on various properties of sucuk, physicochemical, microbiological and volatile compound profiles and sensory characteristics were assessed.

2. Materials and methods

2.1. Cheese samples

Siahmazgi cheese was produced from raw, full-cream milk from goats by traditional methods. Coagulation was induced without the addition of a starter culture by adding 1 g of commercially available Rennet of fungal origin (Enzymaks, Iran Industrial Enzymes Company) to each 25 L of milk. The milk was then allowed to curdle at room temperature (20–25 °C) for about 30–35 min. The curd was cut into pieces while the whole batch was heated up to about 80 °C. Thereafter, the curd was kneaded by hand for 15 min to remove whey and to homogenise the whole mass. Simultaneously, salting was carried out by adding dry salt (5% w/w). Finally, the salted curd was piled up into goats' skin and then ripened at about 15 °C for up to six months. Three different types of Siahmazgi cheese were collected after 180 days of ripening, in both their original fermentation vessels and sterile sample bottles, from throughout the Northwestern region of the province of Guilan, Iran. They were purchased from small-scale facilities producing traditional cheese. The samples were purchased when the fermentation process was considered complete based on labels or verbal information from the producers. The samples' pH was checked at the sampling site using a Corning pH metre (model no. 220, Corning Science Products, Corning, NY, USA). Samples were kept at 4 °C after collection and analysed in a laboratory immediately upon arrival.

2.2. Isolation of lactic acid bacteria (LAB)

Fifty grammes of each cheese sample was removed aseptically from the centre of each sample, then ground together to produce a composite sample, of which 25 g was aseptically transferred to a sterile stomacher bag containing 225 ml sterile buffered peptone water and homogenised in a Lab blender stomacher (BagMixer®400, Interscience, Saint Nom, France) for 2 min. Serial dilutions of the homogenates were prepared in the same diluent, and appropriate dilutions were spread-plated on de Man, Rogosa and Sharpe (MRS, Merck, Darmstadt, Germany) agar and incubated anaerobically at 30 °C for 48 h. Following incubation, 20–30 single colonies of each sample were randomly selected from MRS agar media based on colour, shape and colony size, and purified by streak plating at least three times on the same medium. The isolates were stored in Microbank™ vials (Pro-labo Diagnostics, Neston, Wirrall, UK) at −80 °C in MRS broth plus 20% (v/v) sterile glycerol until further analysis. Isolates from stocks were subcultured in MRS broth for daily use.

2.3. Identification and technological properties of LAB strains

Plates with pure cultures were initially screened for cell morphology by phase-contrast microscopy, Gram reaction and catalase formation. Gram-positive and catalase-negative rod-shaped LAB were further investigated for gas (CO_2) production from glucose and sodium gluconate in phenol red broth (Merck, Germany) containing inverted Durham tubes. The *Lactobacillus* colonies were then sub-cultured on MRS medium using the streak plate technique. They were then classified into obligate homo-fermentative, facultatively hetero-fermentative and obligate hetero-fermentative lactobacilli. The homo-fermentative and facultatively hetero-fermentative lactobacilli were subsequently checked for their acidifying capacity. Those strains that were able to rapidly produce acid at higher levels, were then tested for the capacity to grow in MRS broth in the presence of 3, 6.5 and 10% (w/v) of NaCl. Prior to identification, the isolates were grown overnight at 37 °C in 10 ml of MRS broth and DNA was extracted using the Microlysis kit (Labogen, Rho, Italy) according to the manufacturer's instructions. The 16S ribosomal DNA (16S rDNA) was amplified using standard PCR protocol, and the universal primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGATTCCAAGCC-3′) used to obtain 1500 bp PCR amplicons (Erko & Michael, 1991). The PCR was carried out in a thermal cycler Mastercycler (Eppendorf, Hamburg, Germany) as follows: one cycle of initial heating at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 90 s, annealing at 62 °C for 90 s and extension at 72 °C for 120 s. PCR products were separated by electrophoresis (1 h at 85 V) on 1% (w/v) agarose gel electrophoresis