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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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.

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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 in 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).
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–Gilan 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 (Dalmás & 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, Stanislić, 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. 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 a 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, Wirral, 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 (CO2) 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’-AGAGTTTGTATCTTGCCAG-3’) and 1525R (5’-AAGAGGTTATCCTACGAAC-3’) used to obtain 1500 bp PCR amplicons (Erko & Michael, 1991). The PCR was carried out in a thermal cycler Mestercycler (Eppendorf, Hamburg, Germany) following: 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.
(Invitrogen, Cergy-Pontoise, France) and the DNA was visualised under UV light after staining with ethidium bromide (0.5 μg/ml). The 16S rDNA fragments were purified with a gel DNA GF-1 recovery kit (Vivantis, Malaysia), sequenced and compared to data in GenBank using Basic Local Alignment Search Tool (BLAST).

2.4. Sucuk manufacturing

Sausages were manufactured in the Food Pilot Plant of the University of Salamanca according to the following basic formula for 100 kg: 80 kg beef meat (with 18% fat), 20 kg beef fat, 2.5 kg NaCl, 0.4 kg sucrose, 1 kg garlic, 0.9 kg cumin, 0.7 kg red pepper, 0.5 kg black pepper, 0.25 kg pimento, and 0.015 kg NaN03. The meat was minced in a meat grinder to about 1.3–2.5 cm, and all other ingredients except the fat were added and mixed with the minced meat in a mixer for 20 min at 4 °C. Four different types of sucuk batches (10 kg each) were manufactured, three types of them with the addition of different starter isolates, which had been previously isolated and identified as described in Section 2.2. Briefly, the selected strains were propagated in 100 ml MRS broth and incubated at 30 °C for 24 h. Cells were harvested by centrifugation (10,000 rpm, 10 min), washed twice in 0.9% (w/v) saline solution, and finally resuspended in 10 ml sterile saline solution. The cell density was determined by total viable count after plating the serial dilutions in different containing peptone water on MRS agar followed by incubation at 30 °C for 2 days. Batches were named according to the starter culture added: (i) Control (non-inoculated) batch; (ii) LBP7 batch; (iii) LBP10 batch; and (iv) LBP14 batch, inoculated with Lactobacillus plantarum in an amount of 10⁶ CFU/g of sausage dough. The mixtures were held at 4 °C for 12 h, then re-minced using a 3 mm plate while refrigerated fat was added slowly. After mixing, the meat batter was stuffed into 38 mm collagen casings (Fibran, SA, Girona, Spain) using a vacuum filler machine at 4 °C. The ripening process was programmed as follows: first day 22 °C, second and third days 20 °C and the following days 18 °C. The first three days, relative humidity (RH) was 90 ± 2%; this was lowered by 5% after each day to obtain 85 ± 2% at the end of the seventh day, and finally stabilized at 80 ± 2% until the end of ripening.

2.5. Sampling procedure

The sucuk manufacture was conducted in 3 different replications performed on the same day for each of the 4 batches (LBP14, LAB10, LB7 and control). For the microbiological, textural and physicochemical assays, three samples were taken from each batch at days 1, 3, 7, 11 and 16 of ripening; for the sensory evaluation and volatile compounds determination, the samples were taken at the end of ripening. In other words, at each sampling time, three sausages from each of the three replicated batches were withdrawn which meant that the analysis was carried out nine times in all. Each sample was stored in a single, sealed, polyethylene stomacher bag and placed in a refrigerator until its analysis. All the analyses were conducted within 30 min of sampling.

2.6. Water activity, and pH determination

The pH was measured in the samples using a Crison penetration 52–32 electrode connected to a Crison Basic 20 pH-metre (Crison Instruments S.A., Alella, Spain) by inserting the electrode directly into the sausage samples. The results reported are the means of the values from the core and the edge of the sausage. Water activity (aw) was measured at 25 °C using a Novasina TH-500 electrolytic hygrometer (Novasina, Axarli Ltd., Pfäffikon, Switzerland).

2.7. Instrumental colour analysis

Colour measurements were carried out using a Hunter Lab colourimeter (ColorFlex 45/0-D Model Colourimeter, HunterLab, Reston, VA, USA) with D65 illuminant. The colourimeter was calibrated using standard white and black plates (L0 = 93.01, a0 = −1.11, b0 = 1.30). The samples were sliced and wrapped with a single layer of stretch film by slight pressure application to obtain a uniform, bubble-free surface. It was ensured that there was no gap between the sample surface and the film, and the colourimeter readings were carried out on the surface of the wrapped samples. Six readings were taken and averaged for each replication. Total colour difference (ΔE), was calculated using Hunter L, a, and b values (Homco-Ryan et al., 2004).

2.8. Texture profile analysis (TPA)

Texture profile analysis (TPA) of the samples was performed at room temperature, using a TAXTA21 texture analyser (Texture Technologies Corp., Scarsdale, NY/Stable MicroSystems, Godalming, UK) equipped with a cylindrical probe of 50 mm diameter. After discarding the external layer of the sausage, the samples (six cylinders), approximately 1 cm thick and 3 cm in diameter, were equilibrated to room temperature and compressed twice to 50% of their original thickness. Force–time curves were recorded at a crosshead speed of 1 mm s⁻¹. Hardness (g), springiness (mm), cohesiveness, gumminess (g), chewiness (g·mm) and resilience were evaluated at the end of the ripening process. The Texture Expert software version 1.0 (Stable Micro System, Surrey, UK) was used to collect and process the data.

2.9. Microbiological analysis

For each sampling time, after aseptically removing the casing, 10 g from the core of each sample was 10-fold diluted with 90 ml of 0.9% (w/v) NaCl and homogenised in a Lab blender stomacher (BagMixer®400, Interscience, Saint Nom, France) for 2 min. Appropriate dilutions of the samples were prepared using the same diluent, and one aliquot of each dilution was plated in triplicate on selective agar plates. Total aerobic bacteria were determined by plating samples on Brain Heart Infusion agar (BHI, Merck) at 35 °C for 24–48 h. LAB were enumerated by plating on MRS agar incubated for 48 h at 30 °C in anaerobiosis. Anaerobic conditions were achieved using anaerobic jars (GasPak, BBL, Cockeysville, MD) equipped with a GasPak envelope (GasPak, BBL, Cockeysville, MD). Gram-positive Catalase-positive Coccic (GCC) counts were determined by plate counting in Mannitol salt agar (MSA, Merck) after incubation at 35 °C for 24 h. Enterobacteriaceae were cultured on Violet Red Bile Glucose agar (VRBGA, Merck) after incubation at 37 °C for 24 h. The results were expressed as colony-forming units per gramme (log CFU/g).

2.10. Volatile compound analysis

Analysis was performed using an SPME device (Supelco, Bellefonte, PA, USA) with a 10 mm long, 100 μm thick fibre coated with a 50/ 30 μm thickness of DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethyloxilane) as described by Fonseca, Cachaldora, Gómez, Franco, and Carballo (2013). For headspace SPME (HS-SPME) extraction, 5 g of sausages was minced, placed into 10 ml headspace vials and subsequently screw-capped with a PTFE-faced silicone septum (Supelco, Bellefonte, PA). The vials were left at 50 °C for 1 h in a thermo-block to equilibrate their headspace. Then, the SPME fibre, previously preconditioned at 220 °C for 50 min in a gas chromatograph (GC) injection port, was inserted into the sample vial through the septum and exposed to the headspace while maintaining the sample at 40 °C for 30 min. Once sampling was finished, the SPME fibre was withdrawn into the needle and compounds absorbed by the fibre were desorbed in the GC injection port for 5 min at 280 °C with the purge valve off (split-less mode). A Shimadzu GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a Shimadzu GC–MS QP-5050A mass selective detector and a TRACSIIL Meta.XS (30 m × 0.25 mm i.d., × 0.25 μm film thickness) capillary column was used for peak detection. Helium was used as a carrier gas.
with a linear velocity of 1.0 ml/min. The injection port was in a splitless mode. The temperature program was isothermal for 5 min at 40 °C, raised to 200 °C at 3 °C/min, and then raised to 280 °C at 15 °C/min, and held for 10 min. Compounds were identified by comparing their mass spectra with those contained in the NIST05 (National Institute of Standards and Technology, Gaithersburg, MD, USA) library database and/or by calculation of a retention index relative to a series of standard alkanes (C5–C14) for calculating Kovats indexes and matching them with data reported in the literature. Results for each volatile compound were the mean value of three replicates; expressed as AU (arbitrary units) × 10^6/g.

2.11. Sensory analysis
A sensory evaluation was performed on the four batches at the end of the ripening process. A total of 30 experienced panelists, including graduate students and staff members of Miguel Hernández University who had experience in consuming fermented meat products were chosen to perform a sensory characterization of the batches. A preparatory session was held prior to testing, so that each panelist could thoroughly discuss and understand each attribute of the sausages to be evaluated. The sausage slices (two samples per session) were 3 mm thick, cut with a sharp knife, numerically identified and blind-coded and were immediately served on a white plastic dish covered with a plastic film. Each sample was evaluated 3 times. Water and unsalted crackers were provided to cleanse the palate between samples. To reduce fatigue, they conducted no more than three sessions per day lasting a maximum of 1 h. The panel of judges used quantitative descriptive analysis to evaluate differences in appearance (colour intensity), texture (firmness, gumminess, chewiness, cohesiveness, fattiness and fibrousness), flavour (saltiness, sweetness, bitterness, sourness and pepper flavour), and aroma (odour intensity, cured-meat aroma, mold, rancidity and pepper aroma). A 7-point structured hedonic scale (7 = like extremely; 6 = like moderately; 5 = like slightly; 4 = neither like nor dislike; 3 = dislike slightly; 2 = dislike moderately; 1 = dislike extremely) was used to score the sensorial attributes of various sucuk batches.

2.12. Statistical methods
The data were presented as the mean ± standard deviation of each treatment. The experiments were factorial with a completely randomized design using analysis of variance (ANOVA) in the Statgraphics 5.0 Plus System (Statistical Graphics Corporation, Herndon, VA, USA). Differences between the mean values of the measured properties were compared using Duncan’s multiple range tests; a probability value of p < 0.05 was considered significant.

3. Results and discussion
3.1. Screening of well-adapted strains for application in fermented sausage
In this study, 71 LAB strains isolated from Siahmazgi cheese, which were Gram-positive and catalase negative, were screened to select eligible strains as starter cultures for fermented-sausage manufacture. Rod-shaped cells were observed in 52 isolates (73.2%) and classified as lactobacilli. Heterofermentative LAB are not suitable for sausage production because the formation of large amounts of carbon dioxide leads to holes of different sizes in the product (Ammor & Mayo, 2007). In addition, the most important characteristic for potential starter strains is their ability to acidify their environment rapidly, as the acid production and the accompanying decrease in pH give a specific aroma and extend the lag phase of sensitive organisms including foodborne pathogens (Kostinek et al., 2007). Therefore, to reduce the number of strains for further examinations, heterofermentative strains and strains with weak acidifying capacity were excluded (data not shown). All remaining strains were able to grow in MRS broth containing 3 and 6.5% (w/v) NaCl, but exposure to 10% (w/v) NaCl was found to be a highly discriminating factor, with only three strains resisting adequately after 48 h of exposure (data not published yet). Identification of LAB species based on biochemical tests has proven to be difficult and time-consuming. These phenotypic methods are limited in terms of both discriminating ability and accuracy. Similarly, Benito et al. (2008) reported that in LAB strains isolated from Iberian dry-fermented sausages, biochemical identification showed some errors at genus and species level. For this reason, to ensure definitive identification, these three strains were subjected to genetic analysis; interestingly, all strains were identified as L. plantarum and coded as LBP7, LBP14 and LBP10. Their sequences were deposited in GenBank under the accession numbers of NR_075041.1, NR_075041.1 and KC166237 (99% identity with L. plantarum WCFS1 and L. plantarum B23, respectively). These strains were used to make up the experimental starter culture.

3.2 Physicochemical analysis
Fig. 1 shows the evolution of pH during the ripening of different fermented sausages. The initial mean pH values of various sausages were in the range of 5.16 to 5.35. A rapid decline in pH was noticed during the first three days of ripening in all batches; pH then remained almost the same up to the seventh day of ripening except for the batches inoculated with LBP10, which presented higher values (P < 0.05) than the other treatments. Therefore, LBP10 showed lower acidifying ability than either LBP7 or LBP14. The lowering of pH at the beginning of fermentation is an essential requirement, since it contributes to the inhibition of undesirable microorganisms, accelerates the development of a red colour, affects taste and reduces the water-binding capacity of proteins (Baka, Papavergou, Pragalaki, Bloukas, & Kotzekidou, 2011). Many other studies reported that sausages inoculated with selective lactic acid bacteria starters gave lower pH values than control sausages (Casaburi et al., 2007; Essid & Hassouna, 2013). After seven days of ripening, the pH progressively increased until the end of ripening. Bacterial proteases induce proteolytic degradation, generating peptides, amino acids and amines; these have a buffering effect on the organic acids produced by LAB during fermentation (Ruíz-Moyano et al., 2011). The pH values increased slightly in LBP14, which was the most acidic after fermentation, they remained lower than those of other sausages. Fig. 1 also shows the aw values and moisture content of the sausages throughout fermentation (Ruiz-Moyano et al., 2011). The pH values in Fig. 1 increased slightly in LBP14, which was the most acidic after fermentation, they remained lower than those of other sausages. Fig. 1 also shows the aw values and moisture content of the sausages throughout fermentation and ripening. Initially, there were no significant differences (P > 0.05) in aw of all batches, which fell in the range of 0.940 to 0.950, but after 11 days of ripening, decreases in aw became significant (P < 0.05) in the control and inoculated samples. Lower aw helps control microbial growth in fermented products. In the present work, low aw values (ranging between 0.800 and 0.820) were detected at the end of ripening, which ensured safety, and suppressed bacterial growth and over-fermentation.

3.3. Colour properties
Fig. 2 shows the results of lightness (L), redness (a), yellowness (b) and ΔE during the ripening of sucks. Lightness increased in the first seven days of ripening in all batches, probably due to moisture absorption, because the RH in the fermentation room was relatively high (85–90%). Then, it gradually declined throughout ripening. This could be attributed to the rapid reduction of pH on the seventh day, which accelerated the weight loss of fermented sausages, thus darkening their colour (Baka et al., 2011). A decrease in the L values of sucks was also reported by Bozkurt and Bayram (2006). The L values of sausages in batches LBP7 and LBP14 were significantly (P < 0.05) higher than those of the control and LBP10 throughout ripening, although the difference was not remarkable (P > 0.05) at the end of the ripening period. After fermentation, the a values of all batches remained constant up to 11 days of ripening, then decreased up to the end of the ripening. Some authors have reported that the decrease in a values during the
ripening of fermented sausages was probably due to the production of lactic acid, which partially or totally denatured the different states of myoglobin, such as myoglobin, oxymyoglobin and nitrosomyoglobin (García-Marcos et al., 1996). The b value decreased during ripening with the exception of day 7, which showed a slight increase. The decrease in yellowness of dry-fermented sausages was in agreement with the results of Bozkurt and Bayram (2006), and has been attributed to oxygen consumption by microorganisms during their exponential growth.

**Fig. 1.** Evolution of pH (a) and aw (b) of different fermented sucuks during ripening. Control: non-inoculated sausages; LBP7, LBP10 and LBP14: inoculated with different strains of *Lactobacillus plantarum*.

**Fig. 2.** Evolution of lightness (a), redness (b), yellowness (c), and total colour difference (d) of different sucuks during ripening. Control: non-inoculated sausages; LBP7, LBP10 and LBP14: inoculated with different strains of *Lactobacillus plantarum*. 
leading to the reduction in oxymyoglobin. Regarding $\Delta E$, it was found that LBP14 had lower values than other batches during ripening. In the same way, Casaburi et al. (2007) and Lorenzo and Franco (2012), found that L, a and b decreased with ripening time of dry-fermented sausages. The present results were in close agreement with those of Casaburi et al. (2008) who found that the colour of sausages was only affected by the ripening time and not by the starter addition.

3.4. Microbiological analysis

Fig. 3 shows the viable counts of total mesophilic aerobic bacteria on BHI, GCC on MSA, LAB on MRS and Enterobacteriaceae on VRBGA. The evolution of total mesophilic aerobic bacteria was similar to that found in other traditional fermented products (Ensoy, Kolsarici, Candoğan, & Karslioğlu, 2010). A gradual increase in the count of aerobic bacteria ($P < 0.05$) was observed until the seventh day of ripening, after which the count maintained the same levels until the end of ripening ($P > 0.05$). Among all batches, LBP14 showed significant adaptability to the prevailing conditions with 7.89 log CFU/g after 24 h ripening, possibly due to the short lag phase of the inoculated $L$. plantarum used in the sausage mixture. As expected, the counts of LAB at the beginning of the process were lower in the control batches than in those inoculated with starter cultures. The LAB count of the LBP10, LBP7 and control batches increased significantly ($P < 0.05$) during the first seven days, reaching values of 8.86, 9.29 and 9.08 log CFU/g, respectively; these values were then maintained until the end of the ripening period. However, the LAB count of the samples produced with LBP14, although higher in the initial samples, increased slowly ($P < 0.05$) during the ripening period and reached 8.96 log CFU/g at the end of ripening. It is interesting to note that the LAB count of batches inoculated with LBP10 was even lower than that of the control batches; this low growth rate was consistent with the pH profile. This result confirms the good adaptation of isolate LBP7 to the meat environment and its fast growth rate during fermentation and ripening of sausages. These findings confirmed those of Essid and Hassouna (2013), who reported that LAB become predominant during the first two weeks of ripening and remain stable or show slight decreases. Initial counts of GCC were around 6.35 log CFU/g in the inoculated batches, whereas those in the control ones were 6.23 CFU/g ($P < 0.05$). Except for control batches, which showed an increase up to the third day of ripening, the counts of the rest of the batches decreased significantly ($P < 0.05$) throughout the ripening period. Decrease in GCC counts during ripening of the inoculated and control sausages suggests their poor competitiveness; this finding has been reported by other authors (Bonomo, Ricciardi, & Salzano, 2011; Zdolec et al., 2008). The initial counts of Enterobacteriaceae in all the batches ranged between 4.24 and 5.32 log CFU/g. The two batches of LBP14 and LBP7 showed a strong decrease and reached levels of 0.20 and 1.17 log CFU/g, respectively, whereas the control sausages showed a slight decrease, reaching 2.11 log CFU/g at the end of ripening ($P < 0.05$). VRBGA counts of the batches inoculated with LBP10 maintained after day 7, and were greater even than that of the control, probably due to the low acidification observed during the manufacture of this sausage (discussed above). In

![Fig. 3. Evolution of total aerobic counts (a), lactic acid bacteria (b), Gram-positive catalase-positive cocci (c) and Enterobacteriaceae (d) of different sucus during ripening. Control: non-inoculated sausages; LBP7, LBP10 and LBP14: inoculated with different strains of Lactobacillus plantarum.](image-url)
this experiment, the striking reduction of *Enterobacteriaceae* below the detection limit at the end of ripening was a good indicator of the sausages’ microbiological safety and quality and of their preservation conditions; the acidifying activity of *L. plantarum* starters was responsible for this reduction. These results were in agreement with those of Lu et al. (2010), who had found that *Enterobacteriaceae* counts significantly decreased by the end of ripening in fermented sausage made with starter culture. These results were also similar to those of Essid and Hassouna (2013) in a study on Tunisian dry-fermented sausage with selected *Staphylococcus xylosus* and *L. plantarum* strains.

3.5. Texture profile analysis (TPA)

Fig. 4 shows the hardness, gumminess, chewiness, springiness, cohesiveness and resilience of the all samples. The first measurement was taken on the first day, after 24 h fermentation, because the initial texture
Table 1
Volatile compounds (AU (arbitrary units) × 10^6/g dry matter) of sucuk at the end of the ripening in the control batch and in batches manufactured with different starter cultures (means of three replicates).

<table>
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<tr>
<th>Peak no.</th>
<th>Compound</th>
<th>KI</th>
<th>RI</th>
<th>Dry fermented sausages</th>
<th>Significance</th>
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<td></td>
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</table>

Results are expressed in Arbitrary Area Units (×10^6) as means of 3 replicates of each sausage. Means with different lowercase letters in the same row indicate significant differences (P < 0.05) between batches.

KI: Kovats index calculated for TRACSL Meta.X5 capillary column (Teknokroma S. Coop. C. Ltd: 30 m × 0.25 mm id, 0.25 mm film thickness).
RI: Reliability of identification: k: Kovats index in agreement with literature. m: mass spectrum and retention time identical with mass database (NIST05).
ND: not detected.
IS: Internal standard
n.s.: not significant; *(P < 0.05); **(P < 0.01); *** (P < 0.001).

Samples: Control: non-inoculated sausages; LBP7, LBP10 and LBP14: inoculated with different strains of Lactobacillus plantarum.
development mainly takes place during that time. As fermentation continues, the meat mixture, which is crumbly and not particularly cohesive, is gradually transformed into a semisolid product with a higher consistency (day 3) due to loss of solubility of sarcoplasmic and myofibrillar proteins. A continuous and more significant increase in hardness was observed in batches of LBP14 and LBP7, whereas the hardness of LBP10 and control remained stable until day 7, and then increased until the end of ripening. Similar patterns were observed in chewiness and gumminess; this suggests that the sauscs became tougher, pasty and gummy during the ripening period. Initially, cohesiveness values gradually increased during fermentation and then decreased significantly (p < 0.05) by the end of ripening, irrespective of the sausage type. Generally, sauscs with starter cultures had higher springiness values than those without starter; these two textural parameters (cohesiveness and springiness) had similar values, and there were hardly any significant differences among all batches, especially in the final products. Resilience values did not significantly change (p > 0.05) during ripening. During fermentation, a rapid decline in pH induces conformational changes of muscle proteins, and might have caused acid-induced gelation (Zeng, Xia, Jiang, & Yang, 2013). Additionally, the firmness of sauscs is also associated with the extent that water is removed during fermentation and ripening. Therefore, it was most likely that acid-induced gelation of these proteins and the amount of released water dominated the textural formation. Due to a higher acid production rate, the textural development of sucuk inoculated with LBP14 was more pronounced.

3.6. Volatile compound profile

A total of 50 volatile compounds were identified and quantified after 16 days of ripening in the four different batches of dry-fermented sausages (Table 1). Most compounds identified have been previously described in different types of dry-fermented sausages. Nearly 32–65% of the desorbed volatiles came from spices such as red pepper, black pepper and pimento used in sucuk preparation. Among the pepper terpenes identified, the most abundant were sabine, β-pinene, d-3-carene, p-cymene and γ-terpinene. The identified sulphur compounds were diallyl disulphide, diallyl trisulphide, methyl allyl disulphide and methyl allyl disulphide. Eugenol essentially originated from pimento. This spice is known to have eugenol as its main active component (Kikuzaki, Hara, Kawai, & Nakatani, 1999). These results were in agreement with those found by Kaban (2010), who reported that 56.11–76.32% of the total volatile compounds in their sucuscs were terpenes, due to the use of spices as an ingredient. Volatile compounds originating from lipid oxidation are considered to be important contributors to the characteristic taste and odour of dry-fermented sausages. They generated approximately 2–10% of the total volatiles, which is very low compared to other fermented sausages (Meynier, Novelli, Chizzolini, Zanardi, & Gandemer, 1999; Viallon et al., 1996). The low recovery of lipid oxidation products could be attributed to the anti-oxidative activity of garlic and pimento. Another explanation may be the short ripening period of 16 days used in the present study, compared to 90 days in the two mentioned studies. The values of heptanal, octanal, nonanal and piperonal did not show significant differences between batches, while the amount of two amino-acid products (benzaldehyde and benzeneacetaldehyde) appeared to be related to starter-culture inoculations, since they were found in batches inoculated with the starter culture. The higher amounts of hexanal in the LBP7 and LBP14 batches may also be the result of the influence of the starter culture on lipolysis and autoxidation rates. However, compounds that typically originate from microbial amino-acid catabolism and esterification were missing, with the exception of ethyl acetate and ethyl hexanoate. Consequently, the flavour profiles of four batches were not markedly different. In the present study – similar to normal procedure in industrial scale – spices were added to all sausages. Edwards, Ordoñez, Dainty, Hierro, and de la Hoz (1999) concluded that spices, because of their characteristic low odour thresholds, could determine the aroma of the products, overriding the aromatic notes of other volatile compounds. For this reason, it could be supposed that the commercial spice mixture might have overruled the slight differences in flavour profile caused by the different starters used. As noted by numerous researchers (Erkkilä et al., 2001; Hammes & Hertel, 1998), biological activity varies between strains, resulting in different flavour profiles in dry-fermented sausages. But it is also possible that the strains in the present study do not result in different flavour profiles. The genetic fingerprints of the L. plantarum strains are very similar (Huang, Lee, & Liou, 2010), which gives reason to expect similar flavour profiles for the dry-fermented sausages produced using these strains.

3.7. Sensory evaluation

Fig. 5 shows the results of the descriptive sensory analysis of the different batches at the end of the ripening period. There was no significant difference (p > 0.05) between the colour intensity of the fermented sausages in the different batches. Starter cultures noticeably influenced the texture of the sausages compared with the control batches. Sausage development mainly takes place during that time. As fermentation continues, the meat mixture, which is crumbly and not particularly cohesive, is gradually transformed into a semisolid product with a higher consistency (day 3) due to loss of solubility of sarcoplasmic and myofibrillar proteins. A continuous and more significant increase in hardness was observed in batches of LBP14 and LBP7, whereas the hardness of LBP10 and control remained stable until day 7, and then increased until the end of ripening. Similar patterns were observed in chewiness and gumminess; this suggests that the sauscs became tougher, pasty and gummy during the ripening period. Initially, cohesiveness values gradually increased during fermentation and then decreased significantly (p < 0.05) by the end of ripening, irrespective of the sausage type. Generally, sauscs with starter cultures had higher springiness values than those without starter; these two textural parameters (cohesiveness and springiness) had similar values, and there were hardly any significant differences among all batches, especially in the final products. Resilience values did not significantly change (p > 0.05) during ripening. During fermentation, a rapid decline in pH induces conformational changes of muscle proteins, and might have caused acid-induced gelation (Zeng, Xia, Jiang, & Yang, 2013). Additionally, the firmness of sauscs is also associated with the extent that water is removed during fermentation and ripening. Therefore, it was most likely that acid-induced gelation of these proteins and the amount of released water dominated the textural formation. Due to a higher acid production rate, the textural development of sucuk inoculated with LBP14 was more pronounced.

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slices from the batches LBP7 and LBP14 were less brittle and had a better texture, which agrees with the instrumental textural analysis. Moreover, the fat perception of the sausages of batches LBP10 was more intense in the mouth. No difference was found between all batches in cohesiveness and fibrousness. The saltiness of the samples with starter culture scored higher than the control ones. The mean score for sourness was considerably lower in LBP10 and the control than in LBP7 and LBP14, coinciding with the pH and lactic-acid values found. However, bitterness and sweetness did not show significant difference (P > 0.05). This observation reinforced the consideration expressed by Zeng et al. (2013) that the higher flavour score of batches with autochthonous starter cultures were related with higher concentrations of peptides, amino acids, aldehydes, organic acids, etc. Odour intensity and cured-meat aroma reached higher scores in batches LBP7 and LBP14; these values were significantly (P < 0.05) different from batch LBP10 but not from the control batches (P > 0.05). It was observed that LBP10 and control samples were found to be the worst (P < 0.05) for mould odour. Rancidity did not appear to have a marked influence on sensory acceptance. However, the LBP10 sausages scored higher values than the other samples. The aroma and taste of pepper were analysed to detect possible interference, although no differences were found between batches in this respect.

4. Conclusion

The present work, to our knowledge, is the first study investigating the feasibility of using autochthonous starter cultures isolated from Siahmazgi cheese as a promising substitute in the dry-fermented sausage sucuk. The results established that the cultures affected the psychochemical and microbiological properties of sucuk through specific and important activities that contribute to the quality and safety of this product. The results clearly demonstrated that the lactobacillus strains of Siahmazgi cheese were well adapted to the new habitat of fermented sausage, reaching maximum counts of 8.67–9.29 log CFU/g after seven days of ripening. In particular, they exhibited good souring properties, with pH values ranging from 4.87 to 5.20 at the end of ripening. However, a rather different behaviour was observed between the different isolates, even though they belonged to the same species and originated from the same product. The results of the sensory analysis confirmed the positive effect of the autochthonous starter cultures of Siahmazgi cheese in controlling the sucuk manufacturing process.

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References


