Original article

The effects of beeswax coating on quality of Kashar cheese during ripening

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(Received 6 January 2012; Accepted in revised form 2 June 2012)

Summary The aim of this study was to evaluate the effects of the application of beeswax coating on the microbiological, physicochemical and sensory properties of Kashar cheese during ripening (120 day). Kashar cheeses were coated with two different thickness of beeswax (single-layer coating, BW1, and double-layer coating, BW2). For comparison, vacuum packaged (VP) and without packaging material (control) were also studied. Generally, no differences were found in total aerobic mesophilic bacteria, LAB on M-17 agar, coliform bacteria and *S. aureus* counts among cheeses. Microbiological analyses also showed the beeswax-coated cheeses presented a decrease of 2.5 logarithmic units on mould counts compared to control at 120th day. The control cheese had significantly ($P < 0.05$) higher dry matter, fat and protein contents, followed by BW1. However, the coating reduced formation of a thick crust layer by delaying moisture loss. At the end of 120-day storage period, no significant differences in pH and acidity values were observed among the cheeses studied. Compared to other cheeses, control and BW1 cheeses had higher levels of WSN and ripening index in the end of storage. In the result of sensory analysis, while cheese BW1 and control were more preferred by the panellists, cheese VP received the lowest scores.

Keywords Beeswax, coating, Kashar cheese, ripening.

Introduction

Kashar cheese is a semi-hard cheese, which is the most commercially produced cheese in Turkey after Beyaz cheese. According to 2005 data, it is known the total cheese production is about 420 000 tonnes in Turkey, and it is estimated that Kashar cheese production is 80 000 tones (Anonymous, 2005). Kashar cheese is a pasta filata-type cheese and is also known by different names in other countries: Kashkaval (Bulgari), Kasseri (Greece) and Caciocavallo (Italy) (Hayaloglu, 2009). Traditionally, Kashar cheese is made from raw sheep or cows' milk or their mixtures, and the natural flora of milk is effective on maturation. According to Turkish Standard (3272) (TSE, 2006), this cheese is classified as 'fresh Kashar cheese' and 'old or matured Kashar'. This cheese must be ripened at least 90 days according to Kashar cheese standard (TSE, 2006). It is generally ripened for 3–10 months, and after ripening period, it has a unique flavour, taste and aroma.

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Several types of spoilage can be seen in cheese during processing and ripening. The main cause is the development of microorganisms, mainly fungi, yeasts and bacteria, which not only can damage the appearance of the product but also can affect the consumers' health badly. One of the most common problems that affects the quality and shelf-life of Kashar cheese during ripening is the growing of the mould on the surface. Mould growth is not only causes economical losses but also results in health problems because of mycotoxin production. Mechanical processes such as brushing and washing are not sufficient for the complete removal of mould layer (Sarıoglu & Oner, 2006). Also antifungal agents can be used for controlling unfavourable mould growth. It is reported that using of these applications is laborious and has limited benefits (Min *et al.*, 2005; Ture *et al.*, 2011). A recent methodology proposed to prevent mould growth is the incorporation of natural antimicrobial agents into packaging and coating materials. The main function of packaging is to protect maximum product quality, creating conditions that minimise chemical, biochemical and microbiological alterations (de Oliveira *et al.*, 2007). Recently, several researchers have reported that

doi:10.1111/j.1365-2621.2012.03137.x

antimicrobial films and coatings including organic acids, enzymes, fungicides and natural antimicrobial compounds such as spices are used for various types of cheeses (Seydim & Sarikus, 2006; Duan *et al*., 2007; Di-Pierro *et al*., 2011).

Beeswax (white and yellow) is obtained from the honeycombs after removal of the honey. The wax is a secretion from bees of the genus *Apis*, for example *Apism dorsata, A. indica, A. florea* and the domesticated *A. mellifera*. After removal of insoluble impurities, the liquid wax is cast into cakes for further purification to obtain food-grade yellow beeswax. Beeswax is a complex mixture of several chemical compounds, predominantly compounds based on straight-chain monohydric alcohol with even-numbered carbon chains from C_{24} to C_{36} and straight-chain acids also having even numbers of carbon atoms up to C_{36} (including some C_{18} hydroxy acids), for example esters, diester and triesters. Beeswax is used for some food applications such as a glazing and coating agent, as a texturiser for chewing gum base and as a carrier for food additives (Cakmakcı *et al*., 2008). Bourtoom (2008) reported that lipid compounds utilised as protective coating materials, and the most effective lipid substances were paraffin wax and beeswax.

Although several studies on the application of various antimicrobial films in Kashar cheese were carried out, only one study was published on the use of beeswax as coating material (Seydim & Sarikus, 2006; Yıldırım *et al*., 2006; Ture *et al*., 2011). In this previous study, authors investigated only the effect on sensory characteristics of fresh Kashar cheese coated with beeswax (Cetinkaya *et al*., 2005). To the best of the knowledge, there is no information related to effect of beeswax on quality characteristics of Kashar cheese during ripening. Therefore, the aim of this research was to determine the effectiveness of using beeswax to control mould growth and also to investigate effects on microbiological, physicochemical properties and proteolysis levels of Kashar cheese during ripening.

Materials and methods

Materials

Raw cow milk was supplied by the Research and Application Farm of Atatürk University. Commercial microbial rennet at the strength of 1/15 000 was obtained from Mayasan Company, Istanbul, Turkey. Beeswax used for coating Kashar cheeses was obtained from beekeepers located in Erzurum, Turkey. Beeswax was used for coating of cheeses after melted at 65 °C. Also plastic vacuum bags (Ak Sedef plastic, Izmir, Turkey), had low oxygen permeability and suitable for food packaging, were used for vacuum packaging of cheeses.

Methods

Cheesemaking

Cheesemaking was performed in the Pilot Plant of Food Engineering Department, Agriculture Faculty, Atatürk University. Kashar cheese was produced traditionally from raw cow's milk without starter culture. The raw cow milk (400 kg) was heated to 35 °C, and rennet diluted in water (1/10) was added for coagulation. After 45 min, the coagulum was cut into 1-cm^3 cubes and allowed to rest for 10 min. The coagulum was cooked by increasing the temperature (until 41 °C). Then, the coagulum was pressed for 2 h to drain out. The pressed curd was cut into blocks (25 × 35 cm) and kept at room temperature for 16 h. At the end this time, the curd gained an elastic form and pH reached to 5.10. The curd was cut into 0.5 cm thick and then stretched at 75 °C in 8% NaCl solution for 3 min. Plasticised fresh cheese was then put into stainless steel cylindrical moulds. After 24 h, the cheeses were removed from moulds and left to preripening for 5 day at 15 °C. Following the preripening, cheese was divided into four parts. The first part of the cheese (BW1) was covered with wax by immersing into the melted beeswax only once. Same method was used for the second part (BW2), with the exception that this time cheeses were immersed into melted beeswax twice. The residual beeswaxes were allowed to drip off, and then, the cheeses were left during 1 h at 20 °C until coating would be cake. The third part of cheeses (VP) was packaged with plastic bags under vacuum. The fourth part of cheeses (C) accepted as a control. All samples were left to ripen for 4 months at 4 °C and a relative humidity of%85. The initial analysis of cheese samples was made at the end of preripening before coating, and later, the cheeses were analysed in 30-day intervals. All determinations were performed in duplicate.

Microbiological analyses

Beeswax was separated from cheese wheels with sterile gloves before microbiological analyses. For each cheese sample, 11 g was weighted and diluted in 99 mL of 0.85% (wt/vol) sterile saline solution. Then, samples were homogenised in a sterile polyethylene bag using a Stomacher (Seward Laboratory Blender Stomacher 400 Lab Blender, London, UK) for 1.5 min. Serial dilutions were prepared in sterile 9 mL 0.85% (wt/vol) NaCl and plated on selective media to enumerate the following microorganisms: total aerobic mesophilic bacteria on plate count agar (TAMB) (Merck, Darmstadt, Germany) at 30 ± 1 °C for 72 h; lactic acid bacteria (LAB) on MRS agar (Merck) at 30 °C for 48 h in anaerobic conditions; LAB on M-17 agar at 30 °C for 48 h; coagulase-positive *Staphylococci* on Baird–Parker agar with egg yolk tellurite

enrichment (Merck) at 37 °C for 24 h; coliform bacteria on violet red bile agar (Merck) at 37 °C for 24 h and moulds on potato dextrose agar acidified with 10% tartaric acid (Merck) at 25 °C for 4 days.

Chemical analyses

Beeswax was removed from cheeses, and all cheese samples were fully shredded. Cheeses were analysed in duplicate for dry matter by the gravimetric method (International Dairy Federation, 1982), for salt by titration with $AgNO_3$ (Bradley *et al*., 1993), for fat by the method of Van Gulik (Ardo & Polychroniadou, 1999) and for total nitrogen (TN) by the Kjeldahl method (International Dairy Federation, 1993). pH measurement was carried out on a sample (10 g) of cheese dispersed in 20 mL of deionised water using a pH meter (WTW 340–1) (Savello *et al*., 1989). Titratable acidity was determined by titration method with NaOH using phenolphthalein as an indicator (Kurt *et al*.,2007).

Nitrogen fractions

The water-soluble nitrogen (WSN) and nitrogen soluble in the 12% trichloroacetic acid (TCA-SN) were determined in aliquots of the same cheese extract prepared and described by Kuchroo & Fox (1982). Grated cheese (20 g) was homogenised for 2 min with 40 mL of H_2O using an Ultra turrax blender (IKA, Wilmington, NC, USA). The homogenate was held at 40 °C for 1 h and then centrifuged at 3000 g for 30 min at 4 °C. After centrifugation, the fatty layer was removed and the supernatant filtered with filter paper (Whatman 113). Twenty-five millilitres from extract prepared for WSN was taken at an equal volume of 24% (w/v) TCA was added for further fractionation of the nitrogenous compounds. The mixtures were incubated for 2 h at room temperature. Precipitates were filtered through filter paper (Polychroniadou *et al*., 1999). Contents of TN, WSN and TCA-SN were determined by Kjeldahl method. The ripening index (RI) was determined using the formula WSN/TN × 100.

Preliminary sensory acceptance

The cheeses were assessed organoleptically at 5, 30, 60, 90 and 120 days by a panel of eight laboratory staff members, well experienced and familiar with Kashar cheese. The cheeses were evaluated according to the method of Bodyfelt *et al*. (1988), and the sensory criteria were modified considering the characteristics of Kashar cheese. Coded cheese samples were removed from the storage room about 1 h prior to evaluation and kept at room temperature. The cheeses were graded for five sensory attributes including colour and appearance, texture, taste, odour and general acceptability. All these attributes were recorded on a 1 (poor)- to 9 (excellent)-point scales. Water and bread were also provided to the panel members to cleanse their palates between samples.

Experimental design and statistical analyses

The experimental design consisted of completely randomised design in a factorial arrangement: four treatments (cheese C, BW1, BW2 or VP), five ripening periods (5, 30, 60, 90 and 120 days) and two replicates. All statistical calculations were performed using SPSS Statistical Software, version 13. Mean values with a significant difference were compared by Duncan's multiple range tests. All analyses were performed in duplicate.

Results and discussion

The gross chemical composition of the cow milk used in the production of the Kashar cheeses was as follows: dry matter 12.25 ± 0.08%, fat 3.80 ± 0.00%, protein 3.46 ± 0.18%, acidity 0.17 ± 0.04% and pH 6.61 ± 0.01. The counts of TAMB, LAB on MRS, LAB on M-17, coliform bacteria and yeast–moulds of the milk used in the production of Kashar cheeses were 7.58 ± 0.15, 7.12 ± 0.12, 7.28 ± 0.04, 4.48 ± 0.35 and 3.36 ± 0.11 log cfu g^{-1}, respectively.

The logarithmic counts of the microorganisms enumerated throughout ripening time for Kashar cheese samples are presented in Table 1. There were no statistically significant differences in respect of TAMB among cheese samples during ripening, except for 90th day. Generally, TAMB counts showed an increase for all samples until 90th day, being a slight decrease observed in the end of ripening. This result was in agreement with that of Sarıoglu and Oner who indicated that the coating with Na-caseinate did not affect in significant level TAMB counts of Kashar cheeses.

During ripening, degradation of lactose, proteins and fat is carried out by ripening agents. One of the ripening agents in cheese is LAB. They contribute to cheese texture, flavour and keeping quality. Therefore, LAB counts were important to determine whether a negative impact of beeswax coating on growth of these microorganisms. The LAB counts on MRS were significantly lower ($P < 0.05$) in control samples at 5 and 30 days of ripening and then reached the similar numbers as measured for the BW-coated and vacuum-packed cheeses. LAB in the BW1 cheese exhibited the highest logarithmic value in the end of ripening period (Table 1). Generally, the using of beeswax did not appear to have significant effect on LAB counts growth in M-17 agar. These results showed coating with beeswax had not a negative influence on the growth of microorganisms, which is necessary for the maturation of cheese.

Table 1 The changes in microbiological groups of Kashar cheeses during ripening

Microbial groups (log cfu g^{-1})	Ripening time (day)	Cheese samples			
		C	BW1	BW2	VP
Total mesophilic bacteria	5	$7.00 \pm 0.00^{A,a}$	$7.12 \pm 0.09^{A,a}$	$7.13 \pm 0.15^{A,a}$	$7.19 \pm 0.07^{A,a}$
	30	$7.36 \pm 0.92^{AB,a}$	$7.30 \pm 0.08^{AB,a}$	$7.65 \pm 0.12^{B,a}$	$7.41 \pm 0.46^{AB,a}$
	60	$7.92 \pm 0.40^{B,a}$	$7.75 \pm 0.45^{BC,a}$	$7.95 \pm 0.22^{BC,a}$	$7.84 \pm 0.20^{B,a}$
	90	$7.95 \pm 0.16^{B,ab}$	$7.64 \pm 0.36^{BC,a}$	$8.25 \pm 0.11^{C,b}$	$7.79 \pm 0.29^{B,a}$
	120	$7.46 \pm 0.37^{AB,a}$	$7.87 \pm 0.24^{C,a}$	$7.89 \pm 0.55^{BC,a}$	$7.65 \pm 0.16^{B,a}$
LAB growth in MRS agar	5	$6.77 \pm 0.40^{A,a}$	$7.11 \pm 0.10^{A,b}$	$7.06 \pm 0.33^{A,ab}$	$7.20 \pm 0.19^{A,b}$
	30	$6.94 \pm 0.62^{A,a}$	$7.26 \pm 0.24^{A,a}$	$7.55 \pm 0.07^{B,a}$	$7.30 \pm 0.68^{AB,a}$
	60	$7.80 \pm 0.34^{B,a}$	$7.51 \pm 0.64^{AB,a}$	$7.77 \pm 0.23^{BC,a}$	$7.81 \pm 0.08^{B,a}$
	90	$7.94 \pm 0.16^{B,bc}$	$7.30 \pm 0.37^{A,a}$	$8.14 \pm 0.23^{C,c}$	$7.56 \pm 0.37^{AB,ab}$
	120	$7.52 \pm 0.18^{B,a}$	$7.98 \pm 0.08^{B,b}$	$7.76 \pm 0.29^{BC,ab}$	$7.62 \pm 0.12^{AB,a}$
LAB growth in M-17 agar	5	$6.84 \pm 0.09^{A,a}$	$6.93 \pm 0.39^{A,a}$	$6.73 \pm 0.45^{A,a}$	$7.09 \pm 0.38^{A,a}$
	30	$7.22 \pm 0.26^{AB,a}$	$7.23 \pm 0.39^{AB,a}$	$7.27 \pm 0.46^{B,a}$	$7.29 \pm 0.64^{AB,a}$
	60	$7.58 \pm 0.52^{BC,a}$	$7.59 \pm 0.62^{AB,a}$	$7.82 \pm 0.30^{BC,a}$	$7.87 \pm 0.11^{B,a}$
	90	$7.91 \pm 0.28^{C,bc}$	$7.45 \pm 0.35^{AB,a}$	$8.13 \pm 0.19^{C,c}$	$7.62 \pm 0.25^{AB,ab}$
	120	$7.45 \pm 0.17^{BC,a}$	$7.85 \pm 0.11^{B,b}$	$7.46 \pm 0.34^{B,a}$	$7.63 \pm 0.19^{AB,ab}$
Moulds	5	$2.75 \pm 0.19^{A,b}$	$2.13 \pm 0.16^{AB,a}$	$2.60 \pm 0.54^{AB,ab}$	$2.45 \pm 0.44^{AB,ab}$
	30	$4.09 \pm 0.47^{B,c}$	$1.94 \pm 0.07^{AB,a}$	$2.83 \pm 0.66^{B,b}$	$2.30 \pm 0.13^{A,ab}$
	60	$4.48 \pm 0.45^{BC,c}$	$1.70 \pm 0.20^{A,a}$	$2.15 \pm 0.31^{AB,a}$	$3.31 \pm 0.21^{D,b}$
	90	$4.85 \pm 0.13^{C,d}$	$2.47 \pm 0.50^{B,b}$	$1.80 \pm 0.64^{A,a}$	$3.18 \pm 0.21^{CD,c}$
	120	$4.60 \pm 0.39^{BC,c}$	$2.05 \pm 0.70^{AB,a}$	$1.89 \pm 0.29^{A,a}$	$2.82 \pm 0.19^{BC,b}$

Samples showing capital letters (during storage days) and lower letters (between cheeses at the same storage day) do not differ significantly ($P > 0.05$).

The treatment and storage were significantly affected mould counts ($P < 0.05$). The initial mould counts were 10^2 cfu g^{-1} levels for all samples (Table 1). However, the mould count of control sample increased drastically from 2.75 to 4.60 log cfu g^{-1} during storage period, and also visible mould growth was observed in control sample beginning from 15th day of ripening. The BW-coated and vacuum-packed cheeses had statistically lower mould counts when compared to the uncoated samples (control). The beeswax was able to inhibit mould growth by reduction approximately 2.5 logarithmic units compared with that of control at 120th day. This condition could be attributed to that BW-coated and vacuum-packed samples had lower O_2 concentration with respect to control sample. Also thickness of both beeswax (BW1 and BW2) was more effective on mould growth than on vacuum packaging. Ture *et al.* (2011) stated that approximately 0.6 log reduction in fungal population of cheese samples wrapped with methyl cellulose film which did not contain any antimicrobial was observed compared to unwrapped samples after 30 days of storage.

The coliform bacteria was detected in vacuum-packaged Kashar cheese at initial stage of ripening (1.15 log cfu g^{-1}) and then reduced to <1 log cfu g^{-1} level. The count of coliform bacteria was <1 log cfu g^{-1} level for other samples during storage. These findings were in agreement with those of Aydemir (2010) in which coliforms were detected in only one Kashar cheese sample on first day of ripening. *Staphylococcus aureus* count was under detectable level (2 log cfu g^{-1}) in all samples during ripening. This condition is attributed to that the scalding process applied in the production of Kashar cheese is effective in eliminating unwanted microorganism such as coliforms, *S. aureus*. Similar results were reported by Var *et al.* (2006) and Sengül *et al.* (2010) in Kashar cheeses.

Changes in chemical characteristics of the Kashar cheese samples are given in Table 2. The dry mater content of control samples was significantly higher than BW-coated and vacuum-packaged cheeses in all stages of ripening. As expected, this situation was owing to being not coated with the any packaging material of control samples, and this also caused the higher loss of moisture. In all cases, dry matter values increased during the ripening time. This increase was higher for the cheeses without coating ($P < 0.05$). These results showed the coating with beeswax retarded moisture loss when compared to control. Also sample BW1 had higher dry matter than that of sample BW2 in the end of ripening period. The packaging material has a significant influence on wastage formed by the loss of moisture in cheese. Generally, the crust layer is comprised on the surface of Kashar cheese during ripening depending on storage conditions and period. This layer is mostly removed before consumed, and therefore, being a very thick crust is not desirable as it will cause economic loses. Because of this reason,

Table 2 The changes in chemical characteristics of Kashar cheeses during ripening

Variables	Ripening time (day)	Cheese samples			
		C	BW1	BW2	VP
Dry matter, %	5	$58.19 \pm 0.06^{A,b}$	$57.62 \pm 0.33^{A,ab}$	$57.89 \pm 0.02^{AB,ab}$	$57.31 \pm 0.83^{A,a}$
	30	$61.10 \pm 0.74^{B,b}$	$57.90 \pm 0.52^{AB,a}$	$57.28 \pm 1.14^{A,a}$	$57.10 \pm 0.61^{A,a}$
	60	$62.05 \pm 0.51^{C,b}$	$58.68 \pm 0.29^{BC,a}$	$58.55 \pm 0.19^{B,a}$	$57.91 \pm 0.90^{A,a}$
	90	$64.23 \pm 0.61^{D,b}$	$59.56 \pm 0.29^{C,a}$	$60.02 \pm 0.27^{C,a}$	$60.20 \pm 0.49^{B,a}$
	120	$67.85 \pm 0.88^{E,c}$	$62.65 \pm 1.09^{D,b}$	$61.36 \pm 0.61^{D,a}$	$61.84 \pm 0.12^{C,ab}$
Fat, %	5	$25.00 \pm 0.00^{A,b}$	$24.50 \pm 0.58^{A,ab}$	$24.50 \pm 0.00^{A,ab}$	$24.25 \pm 0.29^{A,a}$
	30	$26.00 \pm 0.00^{A,c}$	$25.50 \pm 0.41^{B,b}$	$24.63 \pm 0.25^{A,a}$	$24.50 \pm 0.41^{A,a}$
	60	$27.80 \pm 2.00^{B,b}$	$26.88 \pm 0.25^{C,ab}$	$25.63 \pm 0.48^{B,a}$	$25.75 \pm 0.65^{B,a}$
	90	$29.00 \pm 1.41^{B,b}$	$27.25 \pm 0.29^{C,a}$	$26.75 \pm 0.50^{C,a}$	$27.25 \pm 0.29^{C,a}$
	120	$30.88 \pm 0.75^{C,b}$	$28.55 \pm 0.64^{D,a}$	$27.75 \pm 0.29^{D,a}$	$28.50 \pm 0.58^{D,a}$
Salt, %	5	$3.04 \pm 0.00^{A,a}$	$2.89 \pm 0.11^{A,a}$	$2.98 \pm 0.06^{A,a}$	$3.10 \pm 0.33^{A,a}$
	30	$4.65 \pm 0.54^{B,b}$	$3.13 \pm 0.08^{B,a}$	$4.21 \pm 0.19^{B,b}$	$3.60 \pm 0.26^{B,a}$
	60	$4.68 \pm 0.00^{B,b}$	$4.15 \pm 0.11^{C,a}$	$4.62 \pm 0.11^{C,b}$	$4.27 \pm 0.12^{C,a}$
	90	$4.91 \pm 0.00^{BC,a}$	$4.79 \pm 0.13^{D,a}$	$4.79 \pm 0.13^{C,a}$	$4.62 \pm 0.30^{C,a}$
	120	$5.27 \pm 0.13^{C,b}$	$5.03 \pm 0.14^{E,a}$	$5.10 \pm 0.12^{D,ab}$	$5.02 \pm 0.14^{D,a}$
Protein, %	5	$26.63 \pm 0.62^{BC,a}$	$27.20 \pm 1.80^{B,a}$	$26.04 \pm 0.59^{A,a}$	$26.29 \pm 0.17^{AB,a}$
	30	$25.92 \pm 0.14^{A,a}$	$26.12 \pm 0.72^{AB,a}$	$25.49 \pm 0.49^{A,a}$	$25.77 \pm 0.59^{AB,a}$
	60	$26.34 \pm 0.44^{AB,c}$	$25.32 \pm 0.31^{A,a}$	$26.05 \pm 0.30^{A,bc}$	$25.54 \pm 0.65^{A,ab}$
	90	$27.06 \pm 0.14^{CD,c}$	$25.83 \pm 0.30^{AB,a}$	$26.93 \pm 0.15^{B,c}$	$26.56 \pm 0.07^{B,b}$
	120	$27.40 \pm 0.36^{D,b}$	$26.40 \pm 0.37^{AB,a}$	$26.06 \pm 0.56^{A,a}$	$26.25 \pm 0.83^{AB,a}$

Samples showing capital letters (during storage days) and lower letters (between cheeses at the same storage day) do not differ significantly ($P > 0.05$).

we think that wastage formation because of moisture loss may be reduced by coating beeswax.

The highest fat content was determined in control samples during storage, followed by BW1, VP and BW2 samples. This might be explained that control sample had high dry matter content. The fat contents increased in all samples during storage in parallel with the increment of dry matter. The initial salt contents were statistically similar for all samples (Table 2). The salt concentrations increased constantly in all cheeses during storage. This increase was higher in control samples compared to others. The salt concentration of BW1 samples was statistically lower than BW2 on day 120.

The titration acidity increased linearly during the first 2 months in all samples, probably due to accumulation of lactose degradation products such as lactic acid and other volatile acids (Fig. 1). This increase was higher in sample BW2. While acidity of vacuum-packaged samples increased on day 90, the slight decrease was determined in BW-coated samples. The acidity remained stable in the control samples. In the end of ripening period, the acidity values of all samples reached approximately the same level. Similarly Di-Pierro *et al.* (2011) reported that titratable acidity of Ricotta cheese, coated with a chitosan/whey protein film, reached the same level as measured for the control sample at the end of storage. The change in pH values of Kashar cheeses during storage is shown in

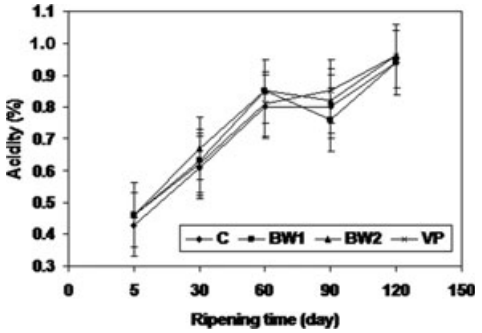

Figure 1 The changes in acidity values of Kashar cheese during ripening C, control(uncoated); BWI, coated with single layer of beeswax; BW2, coated with double layer of beeswax; VP, vacuum packaged.

Fig. 2. The coating with beeswax and ripening period significantly affected pH values. The pH values of cheese samples slightly increased on day 30, except for sample BW2. The decreases and increases were seen until the 90th day in cheeses C, BW1 and VP, whereas pH values constantly decreased in cheese BW2. At 120 days of ripening, pH values were the same level for all samples. These fluctuations are probably related to alkaline components forming in result of proteolytic degradation. Fox *et al.* (1999) reported that reduction in pH was expected at the initial stages of ripening

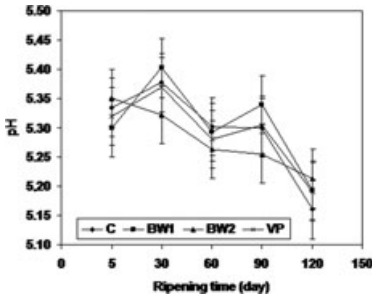

Figure 2 The changes in pH values of Kashar cheeses during ripening C, control (uncoated); BW1, coated with single layer of beeswax; BW2, coated with double layer of beeswax; VP, vacuum packaged.

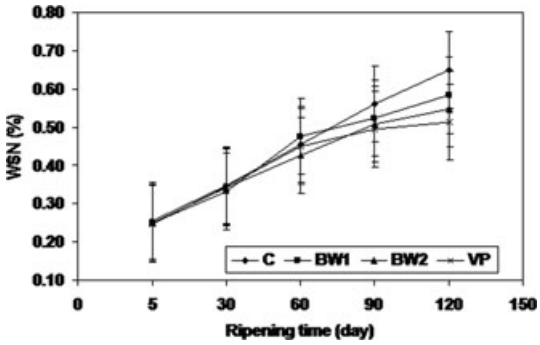

Figure 3 The changes in WSN contents of Kashar cheeses ripening ripening C, control (uncoated); BW1, coated with single layer of beeswax; BW2, coated with double of beeswax; VP, vacuum packaged.

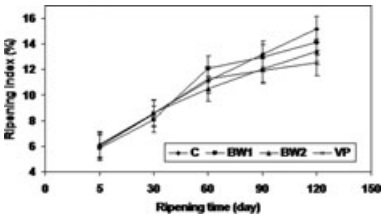

Figure 4 The changes in ripening index values of Kashar cheeses during ripening C, control (uncoated); BWl, coated with single layer of beeswax; BW2, coated with double layer of beeswax; VP, vacuum packaged.

because of metabolism of residual lactose to lactic acid, followed by an increase in the pH depending on the type of cheese. Fluctuations in pH during the ripening were determined by Yıldırım *et al.* (2006) in Kashar cheese coated with casein solution.

For the development of texture, taste and aroma characteristics of ripened cheeses, such as Kashar cheese, a balanced degradation of proteins into peptides and amino acids is necessary. Also the fatty acids and other volatile compounds play an important role in the formation of characteristics of Kashar cheese. Proteolysis in cheese is often measured by means of quantification of WSN fraction of cheese, which consists of whey proteins, medium- and small-sized peptides from the degradation of caseins and free amino acids (Christensen *et al.*, 1991). In many cheese varieties, the initial hydrolysis of caseins is caused by the coagulant and plasmin. The effect of the coating with beeswax on rate and extent of proteolysis was estimated quantitatively by the level of soluble nitrogen components in cheese during ripening. The levels of WSN of Kashar cheeses are shown in Fig. 3. The WSN levels of all samples were similar at day 30 (Fig. 3). However, WSN was significantly higher in control cheese until day 120, followed by cheese BW1. The WSN contents increased in all samples during ripening. The rate of increase was higher in control sample. Degradation of casein to low molecular weight of peptides and amino acids by enzymes from the milk, residual coagulant and starter bacteria can lead to an increase in WSN during the ripening of control and experimental cheeses (Azarnia *et al.*, 2011). Figure 4 shows the changes in the ripening index values (WSN %TN) during ripening of Kashar cheeses. In parallel with the changes in WSN values, the ripening index levels continuously increased in all cheeses throughout ripening. The ripening index ranged from 6.12 to 15.15% for cheese control, 5.87–14.14% for cheese BW1, 6.13–13.40% for cheese BW2 and 5.97–12.51% for cheese VP. While the lowest percentage of WSN% TN was determined in VP and BW2 cheeses, C and BW1 cheeses showed the highest percentages. Also the ripening index values obtained at 120 day were different statistically. A decreasing trend was observed in ripening index levels of Kashar cheeses with increasing thickness of beeswax. These results were in similar to those of Guven *et al.* (2003). Generally the proteolysis developed rather slowly in all samples during the ripening period. We think that this condition may be originated because of storing of cheeses at 4 ± 1 °C. Similarly, Aydemir (2010) reported that the increase in WSN%TN and TCA%TN values was low when the Kashar cheeses were ripened at 4 ± 1 °C following the preripening.

The TCA-SN fraction in cheese contains small peptides (2–20 residues) and amino acids, resulting mainly from the proteolytic activity of bacteria (Christensen *et al.*, 1991) and, to a lesser extent, rennet (McSweeney & Fox, 1997). The values for 12% TCA-soluble N, expressed as%, showed a significant increase ($P < 0.05$) over the whole ripening process (Fig. 5). The highest increase was determined in cheese VP up to 60 day due to probably high LAB count. The cheeses BW1 and VP showed similar trend after 60 days. The TCA-SN evolution profile was similar for all cheeses at the end of ripening period. The

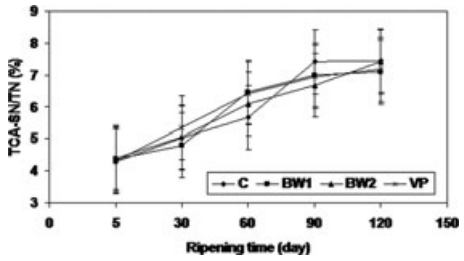

Figure 5 The changes in TCA-SN/TN values of Kashar cheeses during ripening C, control (uncoated); BW1, coated with single layer of beeswax; BW2, coated with double layer of beeswax; VP, vacuum packaged.

Table 3 Mean sensory characteristics of 120-day-old Kashar cheeses

	Cheese samples			
Variables	**C**	**BW1**	**BW2**	**VP**
Colour and appearance	7.85 ± 0.25^{a}	7.79 ± 0.16^{a}	7.81 ± 0.29^{a}	7.70 ± 0.24^{a}
Texture	7.78 ± 0.31^{b}	7.62 ± 0.21^{b}	7.65 ± 0.38^{b}	7.23 ± 0.37^{a}
Taste	7.54 ± 0.17^{bc}	7.61 ± 0.20^{c}	7.38 ± 0.24^{ab}	7.34 ± 0.32^{a}
Odour	7.66 ± 0.27^{b}	7.62 ± 0.34^{b}	7.33 ± 0.23^{a}	7.46 ± 0.40^{ab}
General acceptability	7.61 ± 0.23^{b}	7.62 ± 0.19^{b}	7.46 ± 0.22^{ab}	7.40 ± 0.23^{a}

Lower letters do not differ significantly ($P > 0.05$).

results obtained by soluble nitrogen fractions showed that coating with beeswax had no negative impact on proteolysis when compared to control and vacuum-packaged cheeses.

The sensory characteristics of 120-day-old Kashar cheeses are shown in Table 3. There was no significant difference ($P > 0.05$) existed for the acceptance level of colour and appearance among all the cheese samples. However, texture, taste, odour and general acceptability scores of cheeses were significantly different. While vacuum-packaged cheeses took the lowest texture and taste scores compared to other cheeses, cheese BW1 had the highest taste scores. Yıldırım *et al.* (2006) reported that texture and taste scores of vacuum-packaged Kashar cheeses were lower than control and cheeses coated with casein. The odour scores were statistically lower for cheese BW2, due to probably characteristic odour of beeswax. Also the some of panellists indicated that the characteristic odour of beeswax was intensely perceived in cheeses coated with double-layer beeswax. The control and BW1 cheeses took higher general acceptability scores when compared to others.

Conclusion

The results obtained indicated that the coating with beeswax did not affect microbial groups such as the total aerobic mesophilic bacteria, LAB on M-17 agar, coliform bacteria and *S. aureus* counts. On the other hand, thickness of both beeswax has significantly reduced mould growth during ripening process and also extended the shelf-life when compared to control. The coating retarded moisture loss, better maintained the texture. The beeswax had no negative effect on pH and acidity development. Although the proteolysis developed rather slowly in all samples during the ripening period, the soluble nitrogen fractions for cheeses coated with beeswax (especially cheese BW1) were close to uncoated cheeses. The vacuum packaging is mostly used for packaging of Kashar cheese. However, the results of this study indicated that vacuum packaging reduced sensory characteristics of Kashar cheeses compared to control and BW1. However, we think that a larger consumer test should be performed in terms of increasing the sensitivity of the sensory results. Based on all of these results, beeswax coating (especially in single-immerged beeswax) can be used to improve cheese shelf-life and sensory characteristics. Also, this work points that beeswax may be new alternative to vacuum packaging for the cheese industry. In addition, the further studies need to be done related to the determination of effect of beeswax on volatile compounds and lipolysis.

Acknowledgments

The researchers are grateful to the Atatürk University Research Fund for financial support (Project No: 2009/08).

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