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NOTE

Investigation of Sodium Benzoate and Potassium Sorbate Content and Evaluation of Microbiological Parameters of Fresh Kashar Cheeses

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Abstract

Food safety is important issue for consumers and recently the usage of food ingredients especially food preservatives are limited by regulations. However, some manufacturers use food preservatives instead of improving their hygienic production. Therefore, the levels of sodium benzoate and potassium sorbate of 147 vacuum packaged Kashar (fresh) cheese samples produced in Black Sea Region, Turkey were investigated and some microbiological properties were determined. Research results demonstrated that the production of vacuumed Kashar cheese in Black Sea Region was not standardized for all production periods depending on the microbiological properties. Coliform and *E. coli* counts detected in the cheese samples showed that necessary hygienic conditions were not provided for Kashar cheese production. *Staphylococcus aureus* was not determined in the cheese samples. The sodium benzoate and potassium sorbate were analyzed by HPLC-DAD. Potassium sorbate levels (69.39 mg/kg) of Kashar cheese samples were determined to be lower than the maximum permitted concentration of Turkish Food Codex. Although the utilization of sodium benzoate is prohibited by the Codex, the average level of sodium benzoate of cheese samples was detected to be 68.63 mg/kg. Sodium benzoate can be naturally occurred in fresh cheese at concentrations of up to 50 mg/kg.

Key words: food safety, Kashar cheese, potassium sorbate, sodium benzoate

Introduction

Food additives are generally added to processed foods for prolong the shelf-life by protecting them from deterioration caused by microorganisms (Mpountoukas *et al.,* 2008). For this purpose widely used chemical preservatives are benzoic and sorbic acids and their salts (sodium benzoate and potassium sorbate) (Tfouni and Toledo, 2002). These compounds show their effect in acidic conditions (pH 4.5) as preservatives. They are generally used to inhibit mold and yeast growth and also effective against many bacteria (Can *et al.,* 2011). Sodium benzoate which is the sodium salt of benzoic acid is preferred compared to benzoic acid since its solubility in water is higher than that of benzoic acid (Pylypiw and Grether, 2000). Although sodium benzoate is known as generally recognized as safe (GRAS), some adverse effects, such as asthma, urticaria, metabolic acidosis and convulsions, have been observed at low doses in sensitive persons (Gi *et al.,* 2009). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an Acceptable Daily Intake (ADI) of sodium benzoate to be 0-5 mg/kg of the body weight (WHO, 1996). Potassium sorbate is the potassium salt of sorbic acid and it is more soluble in water than sorbic acid (Deeb and Ahmed, 2010). Sorbic acid and its salts have less harmful effect than benzoic acid and its salts since they are metabolized rapidly like some fatty acids (as butyric, caproic acid) in human and animals (Koyuncu and Uylaser, 2009). The ADI value of sorbic acid and its salts is 0-25 mg/kg body weight as established by the JECFA (WHO, 1974). Through limits of food additives in foodstuff are important for consumer health, their usage have been established by regulatory authorities in different countries. Therefore, sodium benzoate and potassium sorbate have also limitations of usage to protect human health (Can *et al.,* 2011). According to Turkish Food Codex maximum legally allowable limit for potassium sorbate in cheese is set at 1,000 mg/kg and the usage of sodium benzoate in dairy products is prohibited. However, the sodium benzoate is available as a maximum 5 mg/kg in cheese produce with rennet (Anon, 2013).

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Kashar is a semi-hard Turkish traditional cheese which is the second popular one among cheeses in Turkey, after White cheese (Koca and Metin, 2004). This cheese shows some similarities with other kinds of cheeses, such as Kashkaval in Bulgaria, Kasseri in Greece, and Caciocavallo and Ragusona in Italy (Guler, 2005). It is classified as "fresh Kashar cheese" and "ripened Kashar cheese" depending on the ripening by the Turkish Standards (TS-3272). Ripened Kashar cheese is ripened at least 90 d to improve characteristic properties while fresh cheese is produced from pasteurized milk without a ripening process and sold as fresh (Anon, 2006). Ripened Kashar cheese has a hard texture, but the texture of fresh Kashar cheese is semi hard (Hayaloglu, 2009).

Although there have been many studies related to microbiological quality of Kashar cheese (Gulmez *et al*., 2004; Gunsen and Buyukyoruk, 2003; Oksuztepe *et al*., 2009), number of the studies highlighting the quality of Kashar cheese produced in Black Sea Region which takes place in north of Turkey is limited. The aim of the present study was to determine the content of sodium benzoate and potassium sorbate and some microbiological characteristics of vacuum packaged Kashar cheeses manufactured at October-November, February-March and May-June time periods in the Black Sea Region of Turkey.

Materials and Methods

Materials

Kashar cheese is produced using raw ewe or cows' milk or their mixture and fermentation is performed with the native microflora of milk (lactic acid bacteria). The traditional method involves renneting, curd forming, curd fermentation (about pH 5.1-5.4), scalding and texturing of curd in hot water (65-80°C) containing 6-8% NaCl, shaping of the scalded curd, pre-ripening at 15-20°C and ripening at 2-4°C for at least 3 mon (Hayaloglu, 2009). Maturated Kashar cheese is produced in dimensions of 27-30 cm diameter and 10-13 height and 6-10 kg weight. It is maturated before packaging at 2-3°C for 3-10 mon (Cetinkaya *et al*., 2003). Therefore, the cheese is gained its characteristic flavor after storage period (Hayaloglu, 2009).

In our study, a total of 147 vacuum packaged Kashar cheese samples produced by different 49 plants in the period of October-November 2007 (49 cheese samples), February-March 2008 (49 cheese samples) and May-June 2008 (49 cheese samples) were purchased randomly from shops in (the city of Amasya, Bartın, Bayburt, Bolu, Çorum, Düzce, Giresun, Gümüşhane, Karabük, Kastamonu, Ordu, Samsun, Sinop, Tokat, Trabzon and Zonguldak) Black Sea Region of Turkey. Cheese samples (0.5-1 kg) were kept in insulated containers at 4±1°C until analysis.

Microbiological analysis

Cheese samples (10 g) were homogenized in 90 mL of a 0.85% (w/v) sterile NaCl solution using a stomacher Lab-Blender (Smasher, AES Chemunex, France). Decimals dilutions ranged from 10^{-1} to 10^{-6} were prepared and petri plated on specific media. The following media and incubation conditions were used to enumerate microbial counts of samples: Baird Parker agar (incorporating 5% Egg Yolk Tellurite Emulsion) for *Staphylococcus aureus* at 37°C for 24 h (Anon, 2001), Violet-Red Bile agar for coliform bacteria at 37°C for 24 h (Mc Brearty *et al.,* 2001), Chromocult TBX Agar for *Escherichia coli* at 44°C for 24 h (Anon, 1999) and Wort agar for yeasts and molds at 30°C for 72 h (Gobbetti *et al.,* 1999).

Analysis of sodium benzoate and potassium sorbate

Reagents and standards

Acetonitrile (Lichrosol), glacial acetic acid and ammonium acetate were purchased from Merck (Germany) and commercial standards of sodium benzoate and potassium sorbate were obtained from Supelco (USA). Ultra pure water used for chromatography processing was produced using Millipore Direct-Q UV system from Millipore SAS (France).

Sample preparation

The liquid chromatography technique was used to determine the concentrations of sodium benzoate and potassium sorbate in the samples by following the procedures described by Pylypiw and Grether (2000). Briefly, 10 g of homogenized cheese sample was thoroughly mixed with 50 mL of mobile phase and blended for 2 min. The sample blend was then allowed to settle for 5 min and 1 mL of the supernatant liquid was diluted 1:10 with mobile phase. After dilution, all samples were filtered through a 25 mm×0.45 μm nylon Acrodisk filter to remove particulate matter from the samples. The filtrate was used in the analysis that was done duplicate.

Chromatographic conditions

Sodium benzoate and potassium sorbate analysis were carried out using HPLC system (Shimadzu, Japan) equipped with a pump (Shimadzu LC-20AT, Japan), photo-

diode array detector (Shimadzu SPD-M20A, Japan), column oven (Shimadzu CTO-10AS VP, Japan) set 20°C, auto sampler (Shimadzu SIL-10A, Japan) and data station (Shimadzu LC-20AT, Japan). The chromatography column was a Supelcosil LC-18, 5 μm, 250 mm×4.6 mm (Supelco, USA). The mobile phase was 90:10, acetate buffer (pH 4.2) and acetonitrile. The analysis was carried out isocratically at a flow rate of 0.8 mL/min. Injection volume was assayed as 10 μL. The detection of sodium benzoate and potassium sorbate was carried out at the wavelengths of maximum absorption of the compounds to be 225 and 255 nm, respectively.

Preparation of standard curves

Firstly, sodium benzoate and potassium sorbate stock solutions (buffer stock) were prepared in HPLC-grade water at 500 mg/L concentration. Then, final concentrations (1, 5, 10, 20 and 50 mg/L) for calibration curve were injected into mobile phase from buffer stock. The solutions were found to be stable at least 30 d when they kept at room temperature.

Statistical analyses

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc. USA). ANOVA was used to determine the effect of region and period on preservative content and quality parameters and Duncan test was applied for multiple comparison ($p = 0.05$).

Results and Discussion

Microbiological characteristics

The microbiological analysis of vacuumed Kashar cheese samples were given in Table 1. The mean yeast and mold counts of the samples was found to be 6.5 Log CFU/g. This was higher than those found by Oksuztepe *et al.* (2009) and Gunsen and Buyukyoruk (2003). Yeasts and molds which may result from different sources contaminate the product during production (Var *et al.,* 2006). Yeast and molds growth on dairy products is one of the most important problems. Through the post process contamination during handling and packaging of the product, the quality and shelf-life of cheese is affected negatively (Ture *et al*., 2011). Especially, when yeast and mold counts reached 7-8 Log CFU/g, they cause organoleptic change by hydrolyzing fats (Var *et al*., 2006).

Presence of coliform bacteria and *E. coli* was detected in 21 (14.29%) and 11 (4.48%) of cheese samples, respectively. Oksuztepe *et al.* (2009) found that all vacuum cheese samples produced in Elazig were contaminated with coliform the mean of 1.71 Log CFU/g and were contaminated with *E. coli* (8% of total samples) the mean of 1.3 Log CFU/g. The coliform bacteria are fecal origin and their presence in cheese indicates the evidence of poor manufacturing conditions and lack of hygiene (Hayaloglu and Kirbag, 2007). *E. coli* species is often used as indicator microorganism of which presence implies a risk that other enteric pathogens may be also present (Chye, 2004). The presence of *E. coli* in the cheese may be due to use of raw milk and/or contamination of the product during manufacture of the product.

S. aureus was not detected in any samples. Oksuztepe *et al.* (2009) and Gulmez *et al.* (2004) also reported similar results in cheeses. On contrary to these studies, *S. aureus* was found in 4 (8%) of total vacuum cheese samples by Gunsen and Buyukyoruk (2003). Normally, this pathogen may be present in cheese at first stage of maturation, and then it becomes inactivated as ripening proceeds (Hayaloglu and Kirbag, 2007). But, fresh Kashar cheese is produced from pasteurized milk, without a ripening process and sold as fresh. Therefore, the presence of this pathogen suggests that environmental conditions were not controlled during manufacturing or inadequately pasteurized milk was used in the cheese production.

Levels of sodium benzoate and potassium sorbate

The standard curves were obtained from sodium benzoate and potassium sorbate concentration between 1-50

Table 1. Microbiological counts (Log CFU/g) of vacuumed Kashar cheese obtained from Black Sea region*

Period	Yeasts and Molds		Coliform		E. coli	
	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max
October-November	6.17[a]	4.57–7.34	1.31[a]	<0.01–1.9	1.43[a]	<0.01–1.9
February-March	6.68[b]	4.76–8.01	1.79[a]	<0.01–3.45	1.11[a]	<0.01–2.08
May-June	6.58[b]	4.48–7.97	2.76[b]	2.18–3.29	2.4[b]	1.95–2.69
Overall Average	6.5	4.48–8.01	1.77	<0.01–3.45	1.65	<0.01–2.69

**S. aureus* was not detected in any sample.

[1]a–b: Means with different letters in a row within the category are significantly different ($p<0.05$).

Means were calculated with samples where the Coliform and *E. coli* were detected.

mg/L (r^2 = 0.9993 and 0.9998, respectively). The detection limit, calculated as the concentration corresponding to the background noise, was 0.2 mg/kg for both preservatives. For mean recoveries, 5, 20 and 50 mg/L of both preservatives were added to cheese and afterwards the levels of both preservatives were measured in these samples. The mean recoveries were calculated to be 92.8% and 96.7 for sodium benzoate and potassium sorbate, respectively.

Table 2 shows mean concentrations of sodium benzoate and potassium sorbate in vacuumed Kashar cheese samples. From 147 samples analyzed, sodium benzoate and potassium sorbate were detected in 83 (56.46%) and 14 (9.52%) samples, respectively. Mean concentrations of sodium benzoate and potassium sorbate in cheese samples were 68.63 and 69.39 mg/kg and at the levels ranged between 25.46-310.84 and 22.47-191.64 mg/kg. Only 6 (4.08%) cheese samples contained both of sodium benzoate and potassium sorbate. The potassium sorbate levels of Kashar cheese samples were determined lower than the maximum permitted concentration by Turkish Food Codex. Although, the usage of sodium benzoate in cheese is prohibited by the Codex, cheese produce with rennet must contain maximum of 5 mg/kg sodium benzoate (Anon, 2013). Additionally, benzoic acid is naturally produced from hippuric acid in fermented dairy products at concentrations of up to 50 mg/kg. It is also present in fresh cheese and its concentration is normally below 50 mg/kg (Sieber *et al*., 1995). Fresh Kashar cheese is produced from pasteurized milk without a ripening process and sold as fresh, and there is no information about the occurrence of sodium benzoate in the cheese. Therefore, it is unknown that the sodium benzoate levels detected in cheese samples which was added or occurred naturally. On the other hand, if these additives are used in cheese, they must be declared on the label (Anon, 2006). On the labels of cheese samples produced in Black Sea Region were not stated any information about these additives.

Kucukcetin *et al.* (2008) examined sodium benzoate and potassium sorbate levels of 20 Kashar and 20 Tulum cheese samples collected from Antalya, and reported that Kashar and Tulum cheese samples contained the means of 280.4 and 187.5 mg/kg sodium benzoate and 49.7 and 45.6 mg/kg potassium sorbate, respectively. In another study, Kucukcetin *et al.* (2004) reported that sodium benzoate levels were between 42.4-441.9 mg/kg in 7 of 15 white pickled cheese samples and only one of 15 samples contained potassium sorbate (946.5 mg/kg). Tfouni and Toledo (2002) found that cheese samples contained sorbic acid in the range of 376-1,371 mg/kg. The minimum concentrations of benzoic and sorbic acids required to inhibit microbial growth may vary due to species, pH of the substrate and other factors (El-Ziney, 2009). Most yeasts and molds are inhibited by 0.001-0.1% sorbic acid and by 0.002-0.07% benzoic acid (Gi *et al.,* 2009). According to the results of the present study, the uses of both preservatives were the range of antimicrobial activity.

The highest mean concentrations of sodium benzoate and potassium sorbate were determined in cheese samples produced in May-June, respectively (Table 2). Correlation statistical analysis showed that sodium benzoate was affected to inhibition of yeast and mold counts in fresh Kashar cheese samples collected in May-June ($R_{sodium\ benzoate/yeast\ and\ molds}$ = -0.574; p<0.01).

Conclusions

Our findings demonstrated that there was no hygienic production of vacuumed Kashar cheese in Black Sea Region for all periods. These problems could probably be due to type of milk, manufacturing techniques, storage and marketing conditions, and poor hygienic conditions during manufacturing, storage and marketing. To improve the microbiological quality of cheese, milk should be adequately pasteurized and the processing and storage should be carried out under good hygienic conditions.

Table 2. Sodium benzoate and potassium sorbate contents (mg/kg) of vacuum Kashar cheese samples obtained from Black Sea region

Period	N	Sodium benzoate			Potassium sorbate		
		n	Mean	Min–Max	n	Mean	Min–Max
October-November	49	34	61.76[a]	37.34–247.69	4	66.45[a]	25.34–176.02
February-March	49	18	68.82[a]	25.46–168.32	5	56.35[a]	22.47–144.93
May-June	49	31	74.81[a]	39.50–310.84	5	84.79[b]	29.09–191.64
Overall Average	147	83	68.63	25.46–310.84	14	69.39	22.47–191.64

N = Number of the samples analyzed, n = Number of the samples detected.
Means were calculated with samples where the preservatives were detected.
a–b: Means with different letters in a column within the category are significantly different (p<0.05).

The research results about preservatives showed that the levels of potassium sorbate in cheeses were mostly lower than the legal limitations. However, although the usage of sodium benzoate in cheese is prohibited by the legislations, it is being used by most dairy manufacturers in Black Sea Region. Addition to, on the labels of cheese samples were not stated any information about the including these additives. Overall, those problems are mostly resulted from the lack of knowledge of cheese manufacturers about Turkish Food Codex. It may also concluded that new training and monitoring programs are necessary for most dairy producers and the certain legislations should be revised.

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