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Effect of synthetic antioxidants on storage stability of Khoa – a semi-solid concentrated milk product

Zia-ur Rehman, A.M. Salariya *

Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Ferozepur Road, Lahore 54600, Pakistan

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Abstract

Effect of synthetic antioxidants on storage stability of freshly prepared khoa was evaluated. Free fatty acids (FFA), peroxide value (POV) and iodine values (IV) were used to assess the development of rancidity during 30 days of storage of khoa at 25 and 45 °C. Butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) were added to freshly prepared khoa to extend the storage life. After 30 days of storage at 45 °C, freshly prepared khoa containing 200 ppm of BHA and BHT showed lower values of FFA (0.066%, 0.058%) and POVs (23.0, 21.0 meq/kg) than the control samples (FFA 0.320%, POV 127 meq/kg). Iodine values of khoa sample containing 200 ppm of BHA and BHT were 67 and 69 after 30 days storage at 45 °C. However, iodine value of a khoa sample without antioxidant (control) after 30 days of storage at 45 °C was 30. Similarly, khoa samples treated with 100 ppm of BHA, along with 100 ppm of BHT, showed FFA value (19.0%), POV (0.049 meq/kg) and iodine value (72) after storage for 30 days at 45 °C. These results illustrate that synthetic antioxidants inhibited the development of rancidity during storage of khoa. Therefore, storage life of khoa can easily be extended for 30 days by the addition of BHA and BHT.

Keywords: Khoa; Synthetic antioxidants; Storage; Stability

1. Introduction

Khoa is a popular indigenous semi-solid milk product which is prepared by thermal evaporation of milk to 65–70% solids in an open pan. The food value of khoa is very high as it contains fairly large quantities of protein, fat, lactose and bone-forming minerals, especially calcium. It is usually used for direct consumption or as a base material for sweets preparation in the Indo-Pak sub-continent (Musaiger, Al-Hooti, Khunji, & Zakaria, 1998). Storage life of khoa is only two to three days, under ambient conditions, and 15–20 days under refrigerated conditions (Ramzan & Rahman, 1973). There are many factors which affect the nutritional quality and storage life of khoa. It has been observed that the quality of khoa generally depends on the initial composition of milk and the method of manufacturing (Byron, Arnold, & Johnson, 1987). Storage temperature and time also affect the quality of khoa (Ramzan & Rahman, 1973). Goyal and Srinivasan (1989) found that packing in cellophane paper or aluminium foil affects the nutritional quality of khoa during storage. The effect of storage on the physicochemical characteristics of khoa at ambient and high temperature has been studied by Rehman, Ali, Malik, and Shah (1995). Quratulain, Bashir, Rehman, and Kashmiri (2002) reported the effect of different storage conditions on quality of khoa and found marked deterioration in khoa after three months of storage at 25 and 45 °C. Bashir, Rehman, Syed, and Kashmiri (2003) also found quality deterioration of khoa due to development of rancidity which adversely affected its storage life. These studies also showed that addition of potassium sorbate effectively improved the storage life of khoa at

* Corresponding author.
E-mail address: pcsir@brain.net.pk (A.M. Salariya).

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higher temperatures. The findings of Jha and Verma (1988) also revealed that potassium sorbate increased the storage stability of khoa for 40 days. It has also been observed, by other workers, that storage life of khoa can be enhanced by using different types of food preservatives and antimicrobial agents (Wadhawa, Gandhi, & Goyal, 1993; Yanping, Mourning, Yuhang, & Zhying, 1999). Synthetic antioxidants such as bulylated hydroxyanisole (BHA) and bulylated hydroxytoluene (BHT) are also known to increase the storage life of fatty food materials (Allen & Hamilton, 1984). However, there is scarce information in the literature regarding improvement in storage life of khoa by using these synthetic antioxidants.

Though khoa is prepared in great abundance in the Indo-Pak sub-continent from cow and buffalo milk, no measures have been made to increase its storage life. Since the temperature during summer is very high in this part of the world, methods for increasing the storage life of khoa will be very beneficial. The present study was, therefore, undertaken to improve the storage life of khoa using synthetic antioxidants.

2. Materials and methods

Freshly prepared khoa was purchased from a local market which was kept in a refrigerator at low temperature (10 °C) to carry out this study. Synthetic antioxidants, namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), were purchased from Sigma Chemical Co., USA.

Khoa samples, containing 200 ppm of BHA and BHT, were separately prepared, whereas 100 ppm of BHA, alongwith 100 ppm of BHT, was also thoroughly mixed in khoa samples to carry out the comparative study. Control samples of khoa without antioxidant were also placed under identical conditions. All khoa samples, with each treatment, were prepared in triplicate and were kept in air-tight containers at 25 and 45 °C for 30 days. The khoa samples of each treatment were withdrawn periodically after 10 day intervals to assess the development of rancidity.

Free fatty acid (FFA), peroxide value (POV) and iodine values (IV) of khoa samples were determined to assess the development of rancidity. Free fatty acids, as oleic acid percentage, in khoa samples were determined using an alkali titration method, whereas peroxide value (meq/kg) was measured by titration with 0.1 N sodium thiosulphate, using starch as indicator (A.O.A.C., 1990). Iodine value in khoa samples was determined by Wij's method, as described in A.O.A.C. (1990). Sensory quality evaluation of khoa samples was carried out by a trained taste panel of 10 persons using a nine point hedonic scale ranging from 1 for dislike extremely to nine like extremely (Larmond, 1987). All determinations were carried out in triplicate and mean values were calculated. Significant differences ($p < 0.05$) were calculated using Dumcan's multiple range test, as described by Steel and Torrie (1980).

3. Results and discussion

The freshly prepared khoa sample contained 17.7% protein, 23.5% lactose and 3.8% minerals (ash). However, the moisture and fat contents in freshly prepared khoa were found to be 22.9% and 32.1% respectively. Development of off-flavours and rancidity was observed during storage of khoa. The range of temperature included in this study i.e., 25–45 °C covered the atmospheric temperature that the processed khoa would encounter during storage, in Pakistan and many other parts of the world. Development of rancidity was assessed by measuring free fatty acids, peroxide value and iodine value during storage of khoa at different temperatures.

Table 1 summarizes the effect of storage conditions on the free fatty acids (FFA), peroxide value (POV) and iodine value (IV) of freshly prepared khoa. A gradual increase in FFA contents and POV, alongwith decrease in iodine value, was observed during storage of khoa at 25 and 45 °C for 30 days. The changes were more pronounced at 45 °C than at 25 °C. Initially, the FFA content, POV and IV of freshly prepared khoa without antioxidant (control) were 0.025%, 0.38 meq/kg and 80, respectively. After 30 days of storage, FFA contents were 0.275% and 0.320% and POVs were 60.0 and 127 meq/kg at 25 and 45 °C, respectively. On the other hand, IVs of freshly prepared (control) decreased from 80 to 38 at

Table 1
Effect of storage conditions on peroxide value (POV), free fatty acids (FFA) and iodine value (IV) of freshly prepared khoa without antioxidants

Storage time (days)	25 °C			45 °C		
	POV (meq/kg)	FFA (%) (oleic acid)	IV	POV (meq/kg)	FFA (oleic acid)	IV
0	$0.38^{a} \pm 0.15$	$0.025^{a} \pm 0.01$	$80^{a} \pm 1.44$	$0.38^{a} \pm 0.15$	$0.025^{a} \pm 0.01$	$80^{a} \pm 1.44$
10	$17.8^{b} \pm 0.26$	$0.095^{b} \pm 0.02$	$65^{b} \pm 1.29$	$31.0^{b} \pm 0.22$	$0.118^{b} \pm 0.03$	$57^{b} \pm 1.27$
20	$36.0^{c} \pm 0.33$	$0.132^{c} \pm 0.04$	$49^{c} \pm 1.37$	$70.0^{c} \pm 0.22$	$0.215^{c} \pm 0.02$	$39^{c} \pm 1.22$
30	$60.0^{d} \pm 0.26$	$0.275^{d} \pm 0.06$	$38^{d} \pm 1.07$	$127^{d} \pm 0.28$	$0.32^{d} \pm 0.05$	$30^{d} \pm 1.32$

Mean values ± SD of triplicate determinations.
Mean values within a column with different superscripts are significantly different at ($p \leqslant 0.05$).

25 °C and 30 at 45 °C after storage for 30 days. The decrease in iodine value could be attributed to breaking of double bonds of unsaturated fatty acids of lipid during storage of khoa at elevated temperature, as reported by earlier workers (Noor & Augustin, 1984). Generally, the principal route of the deterioration of fat is through oxidative rancidity, which takes place at the double bond in the triglyceride molecule (Akhtar, Asghar, & sheikh, 1985). In fat deterioration, the first initiating step is the formation of free fatty acids which are susceptible to oxygen attack in the presence of light, resulting in the formation of many organic compounds and free fatty acids which are responsible for development of rancidity and off-flavours in fatty food materials (Sattar & Demen, 1973). Production of free fatty acids and increase in peroxide values are the best predictors of fat deterioration which could be used to monitor the extent of oil spoilage. However, decrease in iodine value is another factor by which deterioration of fat can also be examined. Statistical analysis of the data revealed that FFA, POV and IV of khoa were significantly ($p < 0.05$) changed during 30 days storage at 25 and 45 °C.

The changes in FFA, POVs and IVs during storage of khoa at 25 and 45 °C after the addition of BHA and BHT are given in Table 2. It is apparent from these results that addition of BHA and BHT significantly ($p < 0.05$) retarded the development of rancidity in khoa but BHT resulted in better protection than BHA. The FFA values were reduced from 0.320 (control) to 0.066% and 0.058% by the addition of BHA and BHT; POVs decreased from 127 meq/kg (control) to 23.0 and 21.0 meq/kg, respectively, after 30 days of storage at 45 °C. At 25 °C, the addition of BHA and BHT caused reduction in FFA from 0.275% (control) to 0.056% and 0.050%, respectively, during storage for 30 days. Similarly, POVs were also reduced from 60.0 meq/kg (control) to 21.8 and 19.0 meq/kg by the addition of BHA and BHT, respectively, during 30 days of storage at 25 °C. Besides increase in free fatty acids and peroxide values, remarkable decrease in iodine value was observed during storage of khoa at 25 and 45 °C (Table 1). In fact, a decreasing trend in iodine values indicate the development of rancidity due to formation of secondary oxidation products in fatty foods during storage. Addition of synthetic antioxidants retarded the decreasing trend of iodine value during storage of khoa. Addition of BHA and BHT in khoa, showed iodine values 69 and 71 at 25 °C, and 67 and 69, at 45 °C, respectively, after 30 days of storage, whereas iodine values of freshly prepared khoa were 38 at 25 °C and 30 at 45 °C on storage for 30 days. Similarly, addition of BHA along with BHT, showed iodine values of 74 and 70, respectively, during storage of khoa at 25 and 45 °C. Therefore, iodine values of stored khoa samples treated with synthetic antioxidants were significantly ($p < 0.05$) higher than control samples of khoa. These results clearly illustrate that auto-oxidation of, khoa, due to the presence of high contents of fat, was greatly reduced in the presence of both synthetic antioxidants. However, this behaviour was more pronounced when BHA, along with BHT, was added to khoa before storage. Kiyomi and Yasuko (1995) and Yanping et al. (1999) reported that lipid peroxides were significantly reduced by the addition of synthetic antioxidants to processed foods. Kathy, Randel, Peter, and George (1994) suggested that addition of BHA, along with another antioxidant, inhibited food deterioration during storage at both high and ambient temperatures.

Table 2
Effect of synthetic antioxidants on free fatty acids (FFA), peroxide value (POV) and iodine value during storage of khoa

Storage time (days)	25 °C			45 °C		
	POV (meq/kg)	FFA (%) (oleic acid)	IV	POV (meq/kg)	FFA (%) (oleic acid)	IV
BHA (200 ppm)						
0	$0.38^{a} \pm 0.15$	$0.025^{a} \pm 0.01$	$80^{a} \pm 1.44$	$0.38^{a} \pm 0.15$	$0.025^{a} \pm 0.01$	$80^{a} \pm 1.44$
10	$6.50^{b} \pm 0.17$	$0.036^{a} \pm 0.02$	$77^{a} \pm 1.66$	$8.0^{b} \pm 0.14$	$0.038^{a} \pm 0.02$	$75^{a} \pm 1.27$
20	$15.0^{c} \pm 0.29$	$0.040^{b} \pm 0.01$	$72^{b} \pm 1.50$	$19.0^{c} \pm 0.11$	$0.048^{b} \pm 0.03$	$71^{b} \pm 1.29$
30	$21.8^{d} \pm 0.16$	$0.056^{c} \pm 0.03$	$69^{b} \pm 1.32$	$23.0^{d} \pm 0.19$	$0.066^{c} \pm 0.03$	$67^{b} \pm 1.09$
BHT (200 ppm)						
0						
10	$5.0^{a} \pm 0.11$	$0.030^{a} \pm 0.01$	$78^{a} \pm 1.40$	$7.50^{a} \pm 0.22$	$0.033^{a} \pm 0.04$	$76^{a} \pm 1.66$
20	$12.0^{b} \pm 0.20$	$0.038^{a} \pm 0.02$	$75^{a} \pm 1.09$	$17.0^{b} \pm 0.20$	$0.040^{a} \pm 0.03$	$73^{a} \pm 2.01$
30	$19.0^{c} \pm 0.19$	$0.050^{b} \pm 0.01$	$71^{b} \pm 2.00$	$21.0^{c} \pm 0.20$	$0.058^{b} \pm 0.02$	$69^{b} \pm 2.00$
BHA + BHT (100 ppm each)						
0						
10	$4.50^{a} \pm 0.14$	$0.028^{a} \pm 0.02$	$79^{a} \pm 1.77$	$6.0^{a} \pm 0.15$	$0.030^{a} \pm 0.03$	$78^{a} \pm 1.56$
20	$9.8^{b} \pm 0.17$	$0.035^{b} \pm 0.02$	$76^{a} \pm 1.08$	$15.0^{b} \pm 0.21$	$0.037^{a} \pm 0.02$	$75^{a} \pm 1.07$
30	$17.0^{c} \pm 0.18$	$0.048^{c} \pm 0.03$	$74^{b} \pm 1.70$	$19.0^{c} \pm 0.20$	$0.049^{b} \pm 0.023$	$72^{b} \pm 1.00$

Mean values ± SD of triplicate determinations.
Mean values within a column with different superscripts are significantly different at ($p \leqslant 0.05$).

Table 3
Sensory quality evaluation of khoa with and without antioxidants after storage

	Before storage			After one month of storage					
	Without antioxidant (control)	BHA	BHT	Without antioxidant (control)		BHA		BHT	
				25 °C	45 °C	25 °C	45 °C	25°C	45 °C
Colour	8.7 ± 0.17	8.7 ± 0.22	8.8 ± 0.18	6.5 ± 0.25	5.0 ± 0.16	8.0 ± 0.16	7.7 ± 0.22	8.5 ± 0.27	8.0 ± 0.19
Taste	8.8 ± 0.20	8.5 ± 0.25	8.6 ± 0.20	6.0 ± 0.19	5.0 ± 0.20	7.8 ± 0.15	7.3 ± 0.27	8.0 ± 0.11	7.7 ± 0.25
Odour	8.6 ± 0.22	8.8 ± 0.27	8.7 ± 0.22	5.5 ± 0.22	4.8 ± 0.21	8.2 ± 0.27	8.0 ± 0.20	8.7 ± 0.15	8.3 ± 0.21
Texture	8.8 ± 0.21	8.8 ± 0.25	8.8 ± 0.22	5.25 ± 0.20	4.5 ± 0.22	7.5 ± 0.22	7.0 ± 0.20	8.0 ± 0.17	7.5 ± 0.20
Overall acceptability	8.8 ± 0.20	8.8 ± 0.23	8.7 ± 0.23	5.0 ± 0.18	4.5 ± 0.19	8.2 ± 0.20	8.0 ± 0.18	8.5 ± 0.20	8.0 ± 0.23

Mean values SD triplicate determinations.

Sensory quality evaluation of khoa, with and without synthetic antioxidants, was carried out by a trained taste panel of 10 persons after three months of storage at 25 and 45 °C. It is apparent from Table 3 that addition of BHT and BHA to khoa improved sensory quality characteristics of the khoa during storage. After three months of storage at 25 and 45 °C, colour, flavour, taste and overall acceptability scores were significantly ($p < 0.05$) higher for khoa containing antioxidants than for the control. However, score ratings for sensory qualities of the khoa containing BHT were slightly better than BHA. Therefore, it is concluded that synthetic antioxidants (BHT & BHA) can be used for increasing the storage life of khoa at higher temperatures.

4. Conclusions

It is apparent from this study that storage stability of freshly prepared khoa was adversely affected on storage at elevated temperature. Storage stability of khoa was assessed by measuring free fatty acids, peroxide value and iodine value. Initially, free fatty acid, peroxide and iodine values for freshly prepared khoa were 0.025%, 0.38 meq/kg and 80, respectively. Significant increase in free fatty acid and peroxide value and decrease in iodine value indicate the development of rancidity in khoa during three months of storage at elevated temperature. Addition of BHA and BHT retarded the development of rancidity in khoa on storage. However, addition of BHT showed comparatively better results than BHA. Therefore, it is suggested that storage stability of khoa could be increased by the addition of synthetic antioxidants at elevated temperatures.

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