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PHYSICO-CHEMICAL, SENSORY AND ANTI-OXIDANT ACTIVITY OF HERBAL EXTRACT ON KHOA PREPARED FROM COW MILK

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Abstract

A study was undertaken to assess the effect of betel leaves (Piper betel Linn) extract on the physico-chemical, sensory and antioxidant properties of khoa made from cow's milk and stored under room temperature. The physico-chemical parameters viz., pH, titratable acidity, fat, free fatty acids, sensory and antioxidant properties were evaluated periodically at an interval of 3 days upto 9 days. The result revealed that sensory evaluation of khoa was not influenced by the presence of 0.5 per cent aqueous extract of betel leaves up to 9 days of storage period with an overall acceptability score of 6.83. The physico- chemical properties of khoa showed an increasing trend in acidity and decrease of pH as the storage period progresses. The free fatty acids levels were well within the prescribed limit because of antioxidant properties exhibited by the aqueous extract of betel leaves. From the study, it was concluded that khoa with 0.5 aqueous extract of betel leaves restricted the free fatty acid compared to control due to antioxidant property of betel leaves.

Key words: *Khoa, betel extract, physico-chemical, sensory and antioxidant properties*

Introduction

India rank first in the world with record production of 132.4 million tons of milk per annum with per capita availability 299 gms/day (Anonymous, 2013). The consumption of traditional dairy products is likely to grow at annual growth rate of more than 20 per cent, but for Western dairy products the growth rate is relatively much lower, varying from 5-10 per cent. Thus, the expanding business prospects provided by the traditional Indian dairy products to the organized dairy sector triggers a thorough facelifts of these products (Bandyopadhyay et al., 2006). Khoa is a heat desiccated Indian dairy product used as a base material for a variety of sweetmeats like burfi, peda, gulabjamun etc. It has been estimated that about 50-55 % of milk produced is being converted into variety of traditional Indian dairy products of which 6.5% of milk is used for manufacture of khoa mostly in private and unorganized sector (Jadhav *et al*. 2011). Khoa has a limited shelf life of less than a week under ambient condition. Khoa is more prone to chemical and microbial spoilage irrespective of the storage conditions due to its high moisture content (Kumar *et al*. 2010). So, attempts have been made to select certain packaging materials for preventing the growth of microbes and oxidative rancidity under refrigerated and ambient storage of khoa. Bashir *et al*. (2003) used potassium sorbate a chemical preservative to extend the shelf life of khoa. The major cause of khoa spoilage is not only by microbial action but also oxidation of fat which is responsible for producing rancid flavor. Currently there is a

growing interest to use natural antimicrobial and antioxidant compounds like extracts of herbs and spices for preservation of food (Smid and Gorris, 1999). These natural preservatives are gaining importance in recent years because of little or no harmful effects. Betel leaves (*Piper betel*) are also known to contain significant amount of antioxidants like hydroxyl chavicol, eugenol, ascorbic acid and beta carotene (Chakraborty and Shah, 2011). The present study has been formulated to assess the efficacy of aqueous betel leaves extract on the physic-chemical, sensory and antioxidant activity of khoa.

Materials and Methods

Pasteurized milk samples have been procured from the Model Dairy Plant, Department of Dairy science, Madras Veterinary College, Chennai. Betel leaves (*Piper betel*) has been purchased from the local market in Chennai which has been brought from Kumbakonam, Tamil nadu.

Preparation of betel leaves extract

Collected fresh betel leaves (*Piper betel*) were washed properly with sterile water and dried under shade. The dried leaves were powdered and stored in airtight bottles for further studies at room temperature. Ten gram of betel leaf powder was mixed in 100 ml of sterile distilled water, and allowed to soak for 24 hours, then filtered through Whatman No.4 filter paper to obtain a clear extract. The extract was diluted to a concentration of 100 mg/ml (Preethi *et al*. 2010) and then stored in air tight containers at refrigerated temperature.

Khoa was prepared as per method of De (1980) using milk added with 0.5 per cent aqueous extract of betel leaves.

Physico-chemical analysis of khoa

Physico-chemical analysis of khoa viz., pH (Rajorhia *et al*., 1990), titratable acidity (BIS, SP: 18 (Part IX)-1981) and fat estimation (BIS, SP: 18(Part IX)-1981) were carried out. Estimation of free fatty acids (Deeth *et al*. 1975) was done in control and treatment (0.5%aqueous betel leaves extract) samples of khoa stored at room temperature at three days interval upto 9 days.

Sensory evaluation of khoa was carried out during the storage period using 9 - point hedonic scale (Larmond, 1977).

Estimation of antioxidant activity of betel leaves extract on khoa

The antioxidant activity of khoa prepared from the cow milk with 0.5 per cent aqueous betel leaf extract was determined as per the method of Himaja *et al*. (2010). 0.1µl of extract was mixed with 2.9 µl of 0.1mM Diphenyl Picryl Hydrazyl(DPPH) solution. Negative control was prepared by mixing 0.1 µl of methanol with 2.9 µl of DPPH solution. The samples were kept in the dark room for 30 minutes after which the absorbance was measured at 517 nm. The DPPH free radical scavenging activity was calculated using the following formula.

$$\% \text{ Scavenging} = \frac{\text{Absorbance of control - Absorbance of the test sample}}{\text{Absorbance of Control}} \text{ x } 100$$

Absorbance of control – Absorbance of the test sample

Statistical analysis was carried out as per Snedecor and Cochran (1994).

Results and Discussion

Physico-chemical properties of khoa viz., pH, titrable acidity, fat and FFAs are presented in table 1. As the storage period progresses the pH and acidity of khoa showed a raising trend. This might be due to the action of microbes on khoa which contain essential nutrients along with moisture in appropriate level and thereby it favors the growth of microbes. The pH of control and treatment differed significantly ($p<0.05$). Similar, results were observed by Acharya and Agarwal (2010) in Khoa packed at LDPE and stored under room temperature who found that the product remained acceptable upto 9 days of storage. The titratable acidity of the control khoa samples increased from an initial value of 0.22 ± 0.006 to 0.48 ± 0.014 at the end of the 9th day of storage, whereas khoa with aqueous betel leaves extract showed a titratable acidity of 0.30 ± 0.009 at the end of the 9 days of storage. The study of Patel *et al*. (1985) supports the present study, who stated that titratable acidity of khoa stored with the addition of sodium and potassium metabisulphite was much lower when compared to control.

Fat per cent of the khoa stored at 37°C showed a decrease in values for control and treated samples at the end of the 9th day of storage period. Free fatty acids (per cent oleic acid) in khoa stored at 37 °C showed an increase in the control samples from 0.247 ± 0.004 to 0.424 ± 0.006 on the 9th day of storage. The results of the present study coincide with the observations of Joshi and Thakur (1994) who reported that hydrolysis of milk fat was catalysed by lipase resulted in the formation of glycosides and FFA. The FFA was increased when khoa was stored at 25 ± 1°C. The aqueous betel leaves extract and BHA treated samples showed a controlled increase from 0.247 ± 0.002 to 0.285 ± 0.003 on the 9thday of storage. Lower rate of free fatty acid development was noticed in khoa samples treated with 0.5 per cent aqueous betel leaves extract during 9 days of storage as stated by Ray *et al*. (2000). Statistical analysis showed a highly significant difference ($P \leq 0.01$) between treatments during 3rd, 6th and 9th day of storage.

Sensory evaluation of khoa with 0.5 per cent aqueous extract of betel leaves at 37 °C of storage is shown in Table -2. The overall acceptability score for control and khoa prepared using milk with 0.5 per cent aqueous extract of betel leaves when stored at 37 °C for 9 days were 6.34 ± 0.35 and 6.83 ± 0.34 which is in accordance with the report of Acharya and Agarwal (2010). Statistical analysis showed no significant difference between the control and treated samples during 3, 6 and 9 days of storage, which indicates that the addition of aqueous extract of betel leaves did not influence the sensory quality of khoa.

Antioxidant activity of aqueous extract of betel leaves on khoa

Antioxidant activity of khoa containing 0.5 per cent aqueous extract of betel leaves by DPPH method is presented in the fig-1. The free radical scavenging activity of the control and khoa with 0.5 per cent aqueous extract of betel leaves was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The khoa with 0.5 per cent aqueous betel leaves extract showed an increased percentage of scavenging activity than the control. These observations were in accordance with the findings of Banerjee and Bonde (2011) who reported that the phenolic compounds in herbs acted as antioxidants because of their redox properties and free radical quenchers. Chakraborthy and Shah (2011) also reported that antioxidative property of *Piper betel* leaf extracts was due to the high concentrations of flavonoids and polyphenols, which support the findings of the present study.

Table 1: Physico-chemical properties of khoa with 0.5 per cent aqueous extract of betel leaves at storage at 37 °C (Mean±SE)@

Storage (Days)	pH			Titratable acidity#			Fat			Free fatty acids		
	C	TAB_2-K	t value	C	TAB_2-K	t value	C	TAB_2-K	t value	C	TAB_2-K	t value
0	5.95± 0.085	5.95± 0.076	0.00 NS	0.22± 0.006	0.22± 0.006	0.00 NS	22.50± 0.189	22.50± 0.189	0.00 NS	0.247± 0.004	0.247± 0.002	0.00 NS
3	5.35± 0.009^{a}	5.94± 0.045^{b}	18.5 **	0.28± 0.008^{a}	0.23± 0.004^{b}	20.60 **	21.20± 0.166^{a}	22.20± 0.309^{b}	2.97*	0.291± 0.001^{b}	0.252± 0.001^{a}	181.6**
6	5.00±.052^{a}	5.86± 0.009^{b}	31.8 **	0.35± 0.010^{a}	0.25± 0.006^{b}	47.50**	20.40± 0.512^{a}	22.33± 0.422^{b}	3.12*	0.362± 0.003^{a}	0.266± 0.004^{c}	248.6**
9	4.80±0.007^{a}	5.65±0.003^{c}	40.51**	0.48±0.014^{a}	0.30±0.009^{c}	73.15**	20.10± 0.498^{a}	22.25± 0.250^{b}	3.49*	0.424± 0.006^{b}	0.285± 0.003^{a}	304.6**

@Average of six trials

Different superscripts in a row (lowercase letters) differ significantly

NS – Non significant (P>0.05),

* Significant (P≤0.05),

** Highly significant (P≤0.01),

Titratable acidity expressed as percentage of lactic acid,

C - Control (khoa),

TAB_2-K - Khoa with 0.5 per cent aqueous extract of betel leaves

Table 2: Sensory evaluation of khoa with 0.5 per cent aqueous extract of betel leaves stored at 37°C (Mean±SE)@

Sensory Parameters	Storage period (Days)											
	0			3			6			9		
	C	TAB_2-K	t value	C	TAB_2-K	t value	C	TAB_2-K	t value	C	TAB_2-K	t value
Colour and Appearance	8.33± 0.40	8.42± 0.24	0.18 NS	8.17± 0.21	8.33± 0.31	0.45 NS	7.33± 0.33	7.67± 0.42	0.62 NS	6.42± 0.42	7.10± 0.27	1.34 NS
Flavour	8.33± 0.03	8.33± 0.25	0.00 NS	8.10± 0.27	8.25± 0.31	0.41 NS	7.50± 0.22	7.67± 0.49	0.31 NS	6.17± 0.31	6.33± 0.49	0.28 NS
Body and Texture	8.50± 0.03	8.58± 0.33	0.18 NS	8.25± 0.31	8.33± 0.33	0.18 NS	7.75± 0.36	8.10± 0.33	0.68 NS	6.42± 0.42	7.10± 0.37	1.19 NS
Overall acceptability	8.40± 0.20	8.48± 0.21	0.59 NS	8.25± 0.07	8.35± 0.22	0.73 NS	7.50± 0.43	7.81± 0.21	0.70 NS	6.34± 0.35	6.83± 0.34	0.85 NS

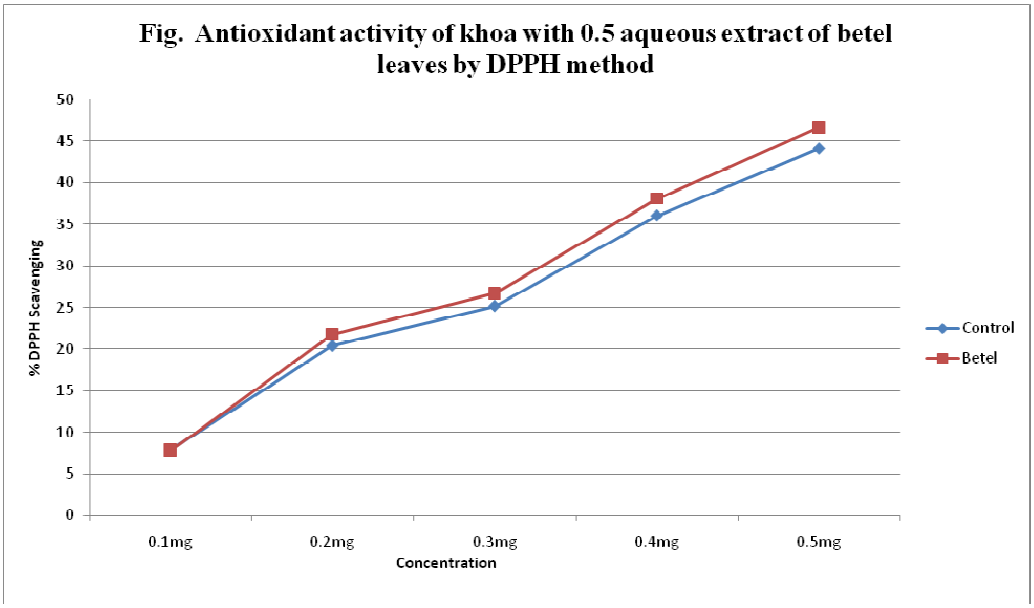
@ Average of six trials

NS - Non significant (P>0.05),

C - Control (khoa)

TAB_2-K - Khoa with 0.5 per cent aqueous extract of betel leaves

Figure 1: Antioxidant activity of khoa with 0.5 per cent aqueous extract of betel leaves by DPPH method



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