International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 1768-1772

Received: 19-07-2019
Accepted: 21-08-2019

Sonali Parekh
Department of Dairy Chemistry, SMC College of Dairy Science, AAU, Anand, Gujarat, India

AI Shaikh
Department of Dairy Chemistry, SMC College of Dairy Science, AAU, Anand, Gujarat, India

KD Aparnathi
Department of Dairy Chemistry, SMC College of Dairy Science, AAU, Anand, Gujarat, India

Correspondence
Sonali Parekh
Department of Dairy Chemistry, SMC College of Dairy Science, AAU, Anand, Gujarat, India

Detection of physical properties and solubility characteristics of Khoa prepared from adulterated milk

Sonali Parekh, AI Shaikh and KD Aparnathi

Abstract
Sweets are mainly prepared from intermediate products as base material, such as khoa, chhana and chakka. Khoa, a base material of sweets, is soft target for adulteration. Synthetic milk, an artificial fluid prepared from blend of several chemicals, has emerged as an economically motivated adulterant in milk. Use of such adulterated milk in manufacture of khoa carries the adulterants to khoa and subsequently to sweets when prepared from such khoa. The information on methods available for detection of adulteration in khoa is very scanty. Therefore, the present study was planned with detection of physical properties and solubility characteristics of khoa prepared from adulterated milk.

Keywords: Khoa, adulteration, physical properties, solubility index

Introduction
Indian sweets have been developed to preserve the nutritional goodness of milk and to extend its shelf life under high ambient temperature. Sweets prepared from milk are integral part of Indian food heritage. Much of the processing of sweets is done on a small-scale by the halwaies. Indian dairy sweets are ubiquitous part of every festival, wedding, religious ritual and are symbol of joy and pride. The appeal and satiety they offer makes them ambrosia for consumers (Kumari *et al*., 2012) [12]. A major market for Indian milk-based sweets is developing overseas. The estimated market size of khoa based sweets is 520 billion INR (Rasane *et al*., 2015) [16].

Khoa is a heat desiccated traditional indigenous milk product. It is a major intermediate product used as a base material for a variety of sweets like gulabjamun, kalajamun, burfi, kalakand, milk cake, peda, khurchan, kulfi, etc. Khoa production is the easiest way of preserving rurally produced milk by its conversion into semi-solid form i.e. khoa which can be utilized for the production of variety of sweets (Choudhary *et al*., 2017) [4].

The demand of market khoa is enhanced markedly during festive seasons (Bajaj *et al*., 2013) [2]. Deliberate addition of high levels of edible and inedible non-milk fats in khoa appears to be more rampant. There are no literature and surveillance reports available on the quality analysis of khoa to know the presence of non-milk fat adulterants (Amruthakala, 2012) [1]. The gap between the demand and availability of milk has led to some unscrupulous activities of adulteration (Chaudhary and Singh, 2015) [5]. Despite its high economic and nutritional importance of khoa, it has not received adequate attention for monitoring the quality. As there is no strict quality parameters prescribed to khoa, adulteration is a common practice and occurs in every step of manufacture and marketing of khoa and khoa-based products (Amruthakala, 2012) [1]. When khoa is prepared from the adulterated milk containing chemical substances, these adulterants are carried forward in to khoa.

Adulterants in milk can be generally classified in to three major groups of substances. The first group of adulterants in milk comprises those substances whose purpose is economically motivated with view to increase volume of milk. In economically motivated adulteration of milk when mixing of water in milk is the most common. While, in order to conceal the watering, co-mixing of other substances also necessitated. These substances constitute second group of adulterants. These selections for substances from second group of adulterant is planned out in such a way that visual appearance, common physical properties (e.g. density and viscosity) and gross chemical composition are simulated to that of genuine milk. However, now artificially prepared fluid (termed as synthetic milk) is percolating as an economically motivated adulterant in milk. This artificial fluid is prepared so meticulously by mixing several chemicals in such a way that its appearance, common physical properties and gross compositional parameters resembles to genuine milk. Among various adulterants in milk, those of the most concerned are detergent, urea, ammonium compound, sucrose, glucose, maltodextrin, starch, common salt (NaCl), nitrate and sulphate. Similarly, the most commonly reported preservatives in milk are hydrogen peroxide, formaldehyde and neutralizers (sodium hydroxide, sodium carbonate and sodium bicarbonate) (Nascimento *et al*., 2017) [15].

A large number of published papers are available on methods for the detection of various adulterants in liquid milk. These methods are based upon the physicochemical properties of the adulterants as well as that of milk. During the manufacture of milk products like khoa, significant changes occur in the physicochemical properties. Hence, the methods available for detecting adulterants in milk may not be either efficient or applicable at all for the khoa. This is because milk undergoes drastic heat treatment for the removal of water during preparation of khoa. Such treatments may change the original state of the adulterant and/or milk constituents. From survey of literature it clearly appears that there is a paucity of work carried out on methods for detection of adulterants in khoa.

Materials and Methods

Preparation of artificial fluid

The artificial fluid was prepared from a blend of adulterants and water. In preparation of the artificial fluid urea was used as a source of nitrogen. Other details of the preparation are not given for obvious reason.

Adulteration of milk

Milk was adulterated with artificial fluid at the rate of 20, 30 and 40 per cent (v/v).

Preparation of khoa

The khoa was prepared from the adulterated samples of milk by using traditional open pan desiccation method (De, 2004) [7]. The khoa was also prepared from pure milk to act as control.

Preparation of khoa samples

To study the effect of adulteration on viscosity, electrical conductivity, and surface tension, slurry from the khoa was prepared in water. In preparation of slurry from khoa TS content of the slurry was adjusted to that of the TS content of the milk used in preparation of khoa. For study of refractive index serum was prepared from the respective slurry of the khoa by coagulation with the help of copper sulphate solution.

Detection of Physical Properties

Density

The density of khoa sample was determined by Gravimetric method as described in Ueda, (1999) [20] with some modification.

Viscosity

Viscosity of khoa (slurry) was determined using ‘Brookfield’ viscometer (DV II + Pro Viscometer, Model- LVDV- II + P, USA) as described in Morison, (2013) [13] with some modification. Spindle no 61 was used for analysis. Temperature of samples during analysis was 30 °C.

Electrical conductivity

Electrical conductivity of khoa slurry was determined by using ‘Conductivity meter’ (Systronics, Gujarat) as described in Fernando *et al*. (1985) [9] with some modification. For the calibration of cell, standard solution of 0.01 N KCl was used. Temperature of samples during analysis was 30 °C.

Refractive index

Refractive index of khoa slurry was determined by using ‘Refractometer’ as described in Jaaskelainen *et al*. (2001) [11] with some modification. Serum of khoa was prepared using copper sulphate method. Obtained serum was used for the reading. Temperature of samples during analysis was 20 °C.

Surface tension

Surface tension of khoa slurry was determined using ‘capillary rise method’ (Mukherjee *et al*., 2005) [14]. Temperature of water and sample were adjusted to 30 °C.

Detection of solubility Characteristics

Solubility index

Solubility index of khoa suggested as characteristics for skim milk and whole milk powder was measured by following method as prescribed by BIS, (1981) [3] with some modification.

Solubility percentage

Solubility per cent of khoa suggested as characteristics for skim milk and whole milk powder was measured by following method as prescribed by BIS, (1981) [3] with some modification.

Results and Discussion

Effect on density

The density of khoa was determined by measuring the volume of given amount of khoa and dividing the weight of the khoa taken by its volume. Results of density of khoa along with their statistical analysis are presented in Table 1.

Table 1: Effect on density of khoa

Rate of adulteration (%)	Density (g/ml) at 30 °C	
	Urea	**Melamine**
0	0.87	0.87
20	1.26	1.40
30	1.45	1.64
40	2.17	2.36
SEm	0.02	0.02
CD (P<0.05)	0.08	0.06
CV %	3.82	2.55

The examination of data showed that the average value of density of khoa prepared from unadulterated milk was 0.87 g per ml at 30 °C. The adulteration of milk had significant effect on density of the resultant khoa. Urea or melamine, had significant (P<0.05) effect on density of the resultant khoa, when used singly as a source of nitrogen.

The density of milk is approximately 1030 kg per m^3 at 20 °C. The density of a given milk sample is influenced by its total solids content. It increases with increase in total solids content of milk (Walstra and Jennes, 1984; Sherbon, 1988; Singh *et al*., 1997) [21, 17, 18]. Similarly, the density of khoa also depends on total solids content of the khoa. As indicated in research carried out by Parekh *et al*. (2019) [19], the moisture content of the khoa decreased on adulteration of the milk with concomitant increased in its total solids content. Therefore, increase in density of the khoa with adulteration might be attributed to increase in its total solids content.

However, densities of unadulterated and adulterated samples of khoa are not reported in the literature. Therefore, density of the khoa prepared from the unadulterated as well as from adulterated milk could not be compared with those reported in the literature.

Effect on viscosity

The viscosity of khoa slurry was determined by 'Brookfield' viscometer. Results of viscosity of the slurry along with its statistical analysis are presented in Table 2.

Table 2: Effect on viscosity of slurry from khoa

Rate of adulteration (%)	Viscosity (cP) at 30 °C	
	Urea	Melamine
0	4.14	4.14
20	4.90	5.12
30	5.15	5.25
40	5.74	5.27
SEm	0.08	0.05
CD (P<0.05)	0.26	0.16
CV %	3.37	2.15

The examination of data showed that the average value of viscosity of khoa slurry prepared from unadulterated milk was 4.14 cP at 30 °C. The adulteration of milk had significant effect on viscosity of the resultant khoa slurry. Urea or melamine, had significant (P<0.05) effect on viscosity of the resultant khoa, when used singly as a source of nitrogen.

The viscosity of milk is approximately 2.127 cP at 20 °C (Walstra and Jenness, 1984; Sherbon, 1988; Singh *et al*., 1997) [21, 17, 18]. Casein, and to a lesser extent fat, are the principal contributors to the viscosity of milk whereas, whey proteins and low molecular mass species have less influence. So, the viscosity of milk and Newtonian milk products is influenced by composition, concentration, pH, temperature, thermal history and processing operations. The concentration of individual components of concentrated milk products mainly contributes to changes in viscosity. Heating skim milk to an extent that denatures most of the whey proteins increases its viscosity by about 10 per cent (Fox and McSweeney, 1998) [10]. Khoa being a heat desiccated product involves intensive heat treatment for a period of about 20-25 min. Such intensive heat treatment leads to denaturation of whey proteins and its complex formation with casein via disulphide linkage. Therefore, increase in viscosity of slurry from khoa compare to milk might be attributed to such phenomenon. The increase in viscosity of slurry from khoa upon adulteration might be attributed to increase in total solids content of the khoa due to decrease in its moisture content.

The viscosity of unadulterated and adulterated samples of khoa slurry is not reported in the literature. Therefore, viscosity of the khoa prepared from the unadulterated as well as from adulterated milk could not be compared with those reported in the literature.

Effect on electrical conductivity

The electrical conductivity of khoa slurry was determined by 'Conductivity meter' using 0.01N KCl solution. Results of electrical conductivity of khoa slurry along with their statistical analysis are presented in Table 3.

Table 3: Effect on electrical conductivity of khoa slurry

Rate of adulteration (%)	Electrical conductivity (mS) at 30 °C	
	Urea	Melamine
0	3.63	3.63
20	3.65	3.67
30	3.69	3.76
40	3.72	3.79
SEm	0.07	0.06
CD (P<0.05)	NS	NS
CV %	4.06	3.33

The examination of data showed that the average value of electrical conductivity of khoa slurry prepared from unadulterated milk was 3.63 mS at 30 °C. However, electrical conductivity of unadulterated and adulterated samples of khoa slurry is not reported in the literature. Therefore, electrical conductivity of the khoa prepared from the unadulterated as well as from adulterated milk could not be compared with those reported in the literature. Use of urea or melamine, used singly as a source of nitrogen had non significant effect on electrical conductivity of the resultant khoa slurry.

Effect on refractive index

The refractive index of khoa slurry was determined by 'Abbe refractrometer' using copper sulphate method. Results of refractive index of khoa slurry along with their statistical analysis are presented in Table 4.

Table 4: Effect on refractive index of khoa slurry

Rate of adulteration (%)	Refractive index at 20 °C	
	Urea	Melamine
0	1.340	1.340
20	1.341	1.342
30	1.343	1.351
40	1.343	1.356
SEm	0.02	0.02
CD (P<0.05)	NS	0.10
CV %	0.33	0.59

The examination of data showed that the average value of refractive index of khoa serum prepared from unadulterated milk was 1.340 at 20 °C using the D-line of the sodium spectrum (589 nm). Refractive index of slurry from khoa increased upon adulteration. Melamine had significant (P<0.05) effect on refractive index of the resultant khoa, when used singly as a source of nitrogen.

The refractive index of milk at 20 °C using the D-line of the sodium spectrum (589 nm), is normally in the range 1.3440-1.3485. However, there is a linear relationship between the solids content (weight per unit volume) and refractive index. The relationship between the refractive index of milk and its total solids content varies with changes in the concentration and composition of the solutes in milk (Sherbon, 1988) [17]. Therefore, increase in refractive index of slurry from adulterated khoa might be attributed to increase in total solids content of khoa.

The refractive index of unadulterated and adulterated samples of khoa slurry is not reported in the literature. Therefore, refractive index of the khoa prepared from the unadulterated as well as from adulterated milk could not be compared with those reported in the literature.

Effect on surface tension

The surface tension of khoa slurry was determined by 'Capillary rise method'. Results of surface tension of khoa slurry along with their statistical analysis are presented in Table 5.

Table 5: Effect on surface tension of khoa slurry

Rate of adulteration (%)	Surface tension (dynes/cm) at 30 °C	
	Urea	Melamine
0	64.15	64.15
20	65.22	65.39
30	65.38	65.76
40	65.55	66.91
SEm	0.03	0.40
CD (P<0.05)	0.08	1.26
CV %	0.08	1.24

The examination of data showed that the average value of surface tension of khoa slurry prepared from unadulterated milk was 64.15 dynes per cm at 30 °C. Use of urea or melamine as a source of nitrogen had significant effect on surface tension of the resultant khoa slurry. When the artificial fluid containing urea or melamine, used singly was mixed at the rate of 20 per cent or above, the surface tension of the khoa slurry increased significantly (P<0.05) compared to the khoa prepared from unadulterated milk.

Reported values for the interfacial tension between milk and air vary from 40 to 60 dynes per cm, with an average of about 52 dynes per cm at 20 °C (Singh *et al*., 1997) [18]. Khoa being a heat desiccated product involved intensive heat treatment for a period of about 20-25 min. Such intensive heat treatment leads to denaturation of whey proteins and loss of their ability to decrease the surface tension (Fox and McSweeney, 1998) [10]. Therefore, increase in surface tension of slurry from khoa compared to milk might be attributed to such phenomenon. Increase in surface tension of slurry from adulterated khoa compared to that of the khoa from unadulterated milk might be attributed to decrease in concentration of surface active substances of milk, their dilution caused by artificial fluid.

However, surface tension of unadulterated and adulterated samples of khoa slurry is not reported in the literature. Therefore, surface tension of the khoa prepared from the unadulterated as well as from adulterated milk could not be compared with those reported in the literature.

From amongst the different physical properties studied viz. density, viscosity, electrical conductivity, refractive index and surface tension of khoa; the density of khoa was found to be useful for the purpose of detection of adulteration in khoa.

Solubility Characteristics

Solubility percentage and solubility index of khoa was measured by same methods suggested for skimmed milk and whole milk powder.

Effect on solubility percentage

Results of solubility percentage of khoa along with their statistical analysis are presented in Table 6.

Table 6: Effect on solubility percent of khoa

Rate of adulteration (%)	Solubility (%)	
	Urea	Melamine
0	94.50	94.50
20	96.23	93.97
30	98.43	93.28
40	98.60	91.57
SEm	0.07	0.48
CD (P<0.05)	0.22	1.48
CV %	0.15	1.03

The examination of data showed that the average value of solubility percentage of khoa prepared from unadulterated milk was 94.50 per cent. Use of urea or melamine as a source of nitrogen had significant effect on solubility percentage of the resultant khoa. When the artificial fluid containing urea was mixed at the rate of 20 per cent or above, the solubility percentage of the khoa increased significantly (P<0.05) compared to the khoa prepared from unadulterated milk. While in case of melamine, the artificial fluid was mixed at the rate of 20 per cent or above, the solubility percentage of the khoa decreased significantly (P<0.05) compare to the khoa prepared from unadulterated milk.

Dalgleish *et al.* (1989) [6] found that urea addition modified the behavior of the milk. Diameters of the casein particles were considerably altered and the extent of covalent cross-linking was diminished. These changes resulted into more protein solublization from the casein micelles when urea was present. Therefore, increase in solubility percentage in slurry from adulterated khoa might be attributed to such possible changes in casein micelles when urea was present as a nitrogen source in artificial fluid used in the adulteration.

On the other hand, decrease in solubility percentage in slurry from adulterated khoa, when melamine was present as a source of nitrogen in artificial fluid, might be attributed to low solubility of melamine. The solubility of melamine in water is only about 0.31 per cent at 20 °C (EFSA, 2010) [8].

However, solubility percentage of unadulterated and adulterated samples of khoa is not reported in the literature. Therefore, solubility percentage of the khoa prepared from the unadulterated as well as from adulterated milk could not be compared with those reported in the literature.

Effect on solubility index

Results of solubility index of khoa along with their statistical analysis are presented in Table 7.

Table 7: Effect on solubility index of khoa

Rate of adulteration (%)	Solubility index (ml)	
	Urea	Melamine
0	12.30	12.30
20	10.38	12.90
30	9.63	13.65
40	8.65	14.35
SEm	0.09	0.18
CD (P<0.05)	0.28	0.58
CV %	1.79	2.84

The examination of data showed that the average value of solubility index of khoa prepared from unadulterated milk was 12.30 ml. Urea or melamine, used singly as a source of nitrogen had significant effect on solubility index of the resultant khoa. When the artificial fluid containing urea was mixed at the rate of 20 per cent or above, the solubility index of the khoa decreased significantly ($P<0.05$) compared to the khoa prepared from unadulterated milk. While in case of melamine, the artificial fluid was mixed at the rate of 20 per cent or above, the solubility index of the khoa increased significantly ($P<0.05$) compare to the khoa prepared from unadulterated milk.

Dalgleish *et al*. (1989) [6] found that urea addition modified the behavior of the milk. Diameters of the casein particles were considerably altered and the extent of covalent cross-linking was diminished. These changes resulted in to more protein solubilzation from the casein micelles when urea was present. Therefore, decrease in solubility index in slurry from adulterated khoa might be attributed to such possible changes in casein micelles when urea was present as a nitrogen source in artificial fluid used in the adulteration.

On the other hand, increase in solubility index in slurry from adulterated khoa, when melamine was present as a source of nitrogen in artificial fluid, might be attributed to low solubility of melamine. The solubility of melamine in water is only about 0.31 per cent at 20 °C (EFSA, 2010) [8].

However, solubility index of unadulterated and adulterated samples of khoa is not reported in the literature. Therefore, solubility index of the khoa prepared from the unadulterated as well as from adulterated milk could not be compared with those reported in the literature. Solubility percentage and solubility index of khoa were found useful for detection of adulteration in khoa along with other physical parameters.

Conclusion

Adulteration of milk with 20 per cent artificial fluid fluids, containing urea or melamine as nitrogen source, had significant effect on density, viscosity, and surface tension of the resultant khoa. Since urea and melamine had opposite effect on solubility percentage and solubility index of khoa, use these two compounds in combination might eliminate the effect of each other on these parameters. Therefore, these parameters did appeared much promising.

References

1. Amruthakala AL. A survey of lipid composition of khoa samples in relation to possible adulteration. Int. J Dairy Technol. 2012; 65(3):444-450.
2. Bajaj SJ, Deshmukh YD, Shirfule AL, Deshmukh KP, Satav PD. Quality assessment of khoa marketed in Nanded city. Int. J Green and Herbal Chem. 2013; 2(3):660-664.
3. BIS. Handbook of food analysis. Dairy Products. Bureau of Indian Standards, Manak Bhavan, 9, Bahadur Shah Zafar Marg, New Delhi-110002, 1981.
4. Choudhary S, Arora S, Kumari A, Narwal V, Tomar SK, Singh AK. Effect of developed acidity and neutralization of milk on sensory, micro structural and textural changes in khoa prepared from cow and buffalo milk. J Food Sci. and Technol. 2017; 52(2):349-358.
5. Chaudhary A, Singh R. Physical and Microbial quality of khoa available in different Markets of Kanpur city. Indian Res. J Gene. and Biotechnol. 2015; 7(3):389-392.
6. Dalgleish DG, Law AJR. pH-Induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. J Dairy Res. 1989; 56:727-735.
7. De S. Outlines of dairy technology (19th edn). Oxford publishing Company New Delhi, 2004.
8. EFSA. Scientific Opinion on Melamine in Food and Feed. EFSA Panel on Contaminants in the Food Chain (CONTAM) and EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). European Food Safety Authority. 2010; 8:1573.
9. Fernando RS, Spahr SL, Jaster EH. Comparison of Electrical Conductivity of Milk with Other Indirect Methods for Detection of Subclinical Mastitis. J Dairy Sci. 1985; 68:449-456.
10. Fox PF, McSweeney PLH. Dairy Chemistry and Biochemistry (First edition). Thomson Science, New York, 1998.
11. Jaaskelainen AJ, Peiponen KE, Raty JA. On Reflectometric Measurement of a Refractive Index of Milk. J Dairy Sci. 2001; 84:38-43.
12. Kumari R, Shrivastava SL, Mishra HN. Optimization of khoa and tikhur mix preparation of khoa-jalebi sweet. Open Access Scientific Reports. 2012; 1(10):1-4.
13. Morison KR, Phelan JP, Bloore CG. Viscosity and non-newtonian behavior of concentrated milk and cream. Int. J.Food Properties. 2013; 16:882-894.
14. Mukherjee N, Bansal B, Chen X. Measurement of surface tension of homogenized milk. Int. J Food Eng. 2005; 1(2):1-6.
15. Nascimento CF, Santos PM, Pereira-Filho ER, Rocha FRP. Recent advances on determination of milk adulterants. Food Chem. 2017; 221:1232-1244.
16. Rasane P, Tanwar B, Dey A. Khoa: A heat desiccated indigenous Indian dairy product. Res. J Pharma. Biol. Chem. Sci. 2015; 6(5):39-48.
17. Sherbon JW. Physical properties of milk, in Fundamentals of Dairy Chemistry, 3rd edn (eds.). N.P. Wong, R. Jenness, M. Keeney and E.H. Marth), Van Nostrand Reinhold, New York, 1988, 409-60.
18. Singh H, McCarthy OJ, Lucey JA. Physico-chemical properties of milk, in Advanced Dairy Chemistry, Lactose, Water, Salts and Vitamins, (ed. P.F. Fox), Chapman & Hall, London. 1997; 3:469-518.
19. Parekh S, Shaikh AI, Aparnathi KD. Effect of milk adulteration on chemical composition of Khoa, Int. J Chem. Studies. 2019; 7(2):1652-1655.
20. Ueda A. Relationship among milk density, composition, and temperature. M.Sc. Thesis, the Faculty of Graduate Studies of the University of Guelph, Canada, 1999.
21. Walstra P, Jenness R. Dairy Chemistry and Physics. John Wiley & Sons, New York, 1984.