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ORIGINAL ARTICLE

Effect of developed acidity and neutralization of milk on sensory, microstructural and textural changes in *khoa* prepared from cow and buffalo milk

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Abstract *Khoa* is a heat desiccated milk product which serves as base material for traditional dairy based Indian sweets. Sensory, textural and microstructural changes in *khoa* were studied to assess the effect of developed acidity and neutralization of milk. Noticeable changes were observed in sensory, textural and micro structural quality of *khoa* as affected by quality of milk. Lower hardness, springiness, cohesiveness and accordingly, gumminess and chewiness were observed in *khoa* manufactured using neutralized milk due to higher moisture retention as compared to control and acidic milk *khoa*. It was also evident from the Scanning Electron Microscopy that higher moisture retention in neutralized milk *khoa* resulted in elongation of native microstructure of *khoa* by suppressing the grain formation leading to smooth and plain surface while, developed acidity in milk resulted in greater granule formation in the resulting *khoa* causing shrinkage of native microstructure and acidic flavour in *khoa*. Similarly, acidification and subsequent neutralization of milk adversely affected sensory attributes of resulting *khoa*.

Keywords *Khoa* · Acidification · Neutralization · Texture profile · Microstructure

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Introduction

The textural characteristics of a dairy product is always influenced by its composition, type and quality of raw materials used and manufacturing practices/parameters followed during preparation. There is an interrelationship among the texture, composition and microstructure. Hardness, gumminess and chewiness are negatively correlated with moisture and fat content, but positively with protein, lactose, added carbohydrates, ash and calcium content (Adhikari et al. 1994). The composition of cow and buffalo milks is different with respect to total solids, proteins, lactose and fat. Therefore, the use of these milks as such or their combination produces milk products with different textural properties. Depending on its characteristics, milk is eminently suitable for certain types of region specific indigenous traditional milk products and *Khoa* is one of the most important heat desiccated product in India, Nepal, Bangladesh and Pakistan (Choudhary et al. 2015). Approximately 5.5% of total milk produced in India is utilized for production of *khoa. 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 traditional products (Kumar 2013). Milk with more than 0.17% acidity produces *khoa* with grainy texture. The size and hardness of grains increased with the increase in acidity. As a result of complex interactions occurring among the individual milk components like casein, whey protein, lactose and fat globules in addition to additives such as acidulants, enzymes, and thickening agents added during the production of milk products results in changes in microstructure. These changes in microstructure can be observed using scanning electron microscopy (SEM). The heat treatment employed during *khoa*-making considerably changed the physical state of the milk proteins, fat globules, and lactose (De 2004). These changes include coagulation of the proteins, fat globule coalescence leading to large lumps of fat and super saturation of lactose in the milk serum (Patil et al. 1992). Adhikari (1993) reported protein agglomerates joined together by thick protein bridges in *khoa* and compaction of protein agglomerates with reduction in void spaces and fat globule interspersed in between. Adhikari et al. (1993) also reported coalesced, compact casein micelles with associated fat globules in *chhana* and numerous small voids interspersed throughout the matrix. Rapid development of natural acidity in milk at ambient temperature is a common problem in India. Historically, such type of milk with developed acidity is not suitable for sweets preparation, as heating of milk with developed acidity resulted in coagulation of milk proteins. Therefore, addition of neutralizer to such milk is a common and ancient practice. Manufacture of traditional dairy products adds value to milk as it preserves milk solids for longer time at room temperature and hence increases the shelf life of milk solids. Existing reports on *khoa* are focused on standardization of manufacturing methods, chemical composition, sensory evaluation, preservation and improvement in keeping quality (De and Ray 1952; Kumar and Srinivasan 1982; Patil et al. 1990; Rehman and Salariya 2006; Kulkarni and Hembade 2012; Kumari et al. 2012; Sivakumar et al. 2014). Rajorhia et al. (1990) studied the effect of quality of buffalo milk on *khoa* but the work was limited to chemical, sensory and rheological properties of buffalo milk *khoa* only. However, Patil et al. (1992) studied the texture and microstructure of *khoa* which was limited to buffalo milk *khoa* only. There is hardly any information on the microstructural and textural changes as affected by quality of milk. This was essential, since it largely determines the material and rheological behavior of the product. Therefore, an attempt has been made to determine the effect of developed acidity and subsequent neutralization of milk to recognize the interrelation among sensory, texture and microstructure changes of *khoa*.

Materials and methods

Materials

Ethanol was procured from Jiangsu Huaxi International Trade Co. Ltd., China. Sodium bicarbonate was procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Glutaraldehyde (25%), cacodylate buffer, osmium tetraoxide, from Sigma Aldrich, St. Louis, Missouri, USA.

Methods

Collection and preparation of samples

Good quality pooled cow and buffalo milk was obtained from the experimental dairy of the ICAR-National Dairy Research Institute, Karnal. Fresh milk samples were incubated at 30 °C in an incubator (Narang Scientific Works Pvt. Limited, Delhi, India) for rapid development of acidity up to 0.18% lactic acid (LA). Acidity of milk was evaluated hourly until it reaches up to 0.18% LA. Developed acidity was neutralized with the addition of calculated amount of neutralizer (sodium bicarbonate) at required rate to adjust the acidity to 0.14% LA ($\sim$acidity of fresh milk). The fresh, acidic and neutralized cow and buffalo milk samples were used to prepare *khoa*.

Preparation of khoa

Khoa samples were prepared using the method of De (2004). The concentration of milk was carried out in batches of 5 L each in an open double jacketed stainless steel kettle at a steam pressure of 2 kg/cm^2. Stirring and scrapping with the help of a flat iron ladle was continued to avoid burning of milk solids during boiling. This process was continued until the entire mass reached a pasty consistency and the steam pressure was reduced to 1 kg/cm^2. Vigorous stirring was pursued with the help of an iron ladle with simultaneous scraping of the pan bottom sides, more so at the last stage. As soon as the milk solids started leaving the sides of the kettle, steam was closed and vigorous stirring-cum-scrapping was continued till the viscous mass reached a semi-solid/pasty consistency. The pasty mass was then worked up and down to form a pat of single compact mass i.e. *khoa*. The proximate composition of *khoa* in terms of fat, lactose, ash, total solids pH and acidity of *khoa* was determined by the method described in Indian Standard (IS: SP: 18 part XI 1981). Moisture and protein content was determined by Indian Standard (IS: 16072 2012; AOAC 1970, respectively). The gross composition of three types of *khoa* prepared from buffalo and cow milk is presented in Table 1a, b.

Table 1 Gross composition and physico chemical characteristics of fresh, acidic and neutralized (a) buffalo milk *khoa*, (b) cow milk *khoa* (Choudhary et al. 2016)

Parameters→ Sample↓		Fat	Protein	Lactose	Moisture	Ash	Total solids	pH	Acidity (% LA)
(a)									
Buffalo	FBMK	35.13 ± 1.06^{A}	17.56 ± 0.17^{A}	20.59 ± 0.78^{B}	23.94 ± 0.41^{B}	2.74 ± 0.025^{B}	76.06 ± 0.41^{B}	6.48 ± 0.003^{B}	0.57 ± 0.003^{B}
Milk	ABMK	35.50 ± 1.53^{A}	17.70 ± 0.14^{A}	17.66 ± 1.02^{A}	22.43 ± 0.55^{A}	2.64 ± 0.034^{A}	77.57 ± 0.55^{C}	6.38 ± 0.005^{A}	0.62 ± 0.002^{C}
khoa	NBMK	35.41 ± 1.35^{A}	17.76 ± 0.29^{A}	17.03 ± 1.32^{A}	25.44 ± 0.70^{C}	2.85 ± 0.040^{C}	74.56 ± 0.70^{A}	6.68 ± 0.005^{C}	0.55 ± 0.005^{A}
(b)									
Cow	FCMK	26.46 ± 0.50^{A}	18.30 ± 0.12^{A}	24.87 ± 0.18^{B}	29.65 ± 0.62^{B}	3.24 ± 0.017^{B}	70.35 ± 0.62^{B}	6.40 ± 0.005^{B}	0.63 ± 0.002^{B}
Milk	ACMK	26.13 ± 1.08^{A}	18.43 ± 0.29^{A}	21.53 ± 0.44^{A}	27.07 ± 0.37^{A}	3.17 ± 0.012^{A}	72.92 ± 0.37^{C}	6.22 ± 0.005^{A}	0.68 ± 0.006^{C}
khoa	NCMK	25.19 ± 0.80^{A}	19.03 ± 0.04^{A}	20.80 ± 0.50^{A}	30.75 ± 0.30^{C}	3.46 ± 0.019^{C}	69.25 ± 0.30^{A}	6.51 ± 0.003^{C}	0.58 ± 0.006^{A}

Data are presented as mean ± Standard Error Mean (n = 3)

ABMK acidic buffalo milk *Khoa*, *FBMK* fresh buffalo milk *Khoa*, *NBMK* neutralized buffalo milk *Khoa*, *ACMK* acidic cow milk *Khoa*, *FCMK* fresh cow milk *Khoa*, *NCMK* neutralized cow milk *Khoa*

$^{A–C}$ Means within column with different upper case superscript are significantly different ($p < 0.05$) from each other

Sensory evaluation

Sensory evaluation of fresh, acidic and neutralized cow and buffalo milk *khoa* samples was carried out using a score card developed for *khoa* by Gupta and Pal (1985). This was used for measuring any variation in the sensory attributes due to developed acidity and neutralization of milk by a sensory panel. A sensory panel consisting of 15 selected panelists was drawn from the faculty of Dairy processing departments of ICAR-National Dairy Research Institute, Karnal. The panelists for the present study were dairy professionals having adequate knowledge regarding the sensory evaluation methods and products attributes. All the samples were evaluated for sensory attributes such as flavour (50), body and texture (35), colour and appearance (15) and total score on the basis of 100-point composite score card. This method does not, of course, reflect actual consumer perception, but it does strongly indicate attributes which a good quality product should possess.

Texture profile analysis

The texture profile of *khoa* samples were determined using texture analyzer fitted with 25 kg load cell using cylindrical P25 probe of 25 mm diameter. Cylindrical *khoa* samples (1 × 1 mm) were cut with the help of knife and kept in immersion chamber maintained at 25 °C before texture analysis. The samples were subjected to mono-axial compression of 20 mm distance on the texture analyzer by the pre-test and post-test speed of 2 mm/s and test speed of 1 mm/s with compression of 50% of distance with single TPA (texture profile analysis) i.e. single time penetration. From the resulting force–time graph, obtained with force experienced by probe on Y-axis and time on X-axis various textural characteristics such as hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness were calculated using the Texture Expert Exceed software (v 2.55) supplied by the manufacturer along with the instrument. The firmness of *khoa* sample was estimated as the height of positive peak force up to rupture point. A minimum of three replicates per sample was run.

Scanning electron microscopy of *khoa*

Microstructure of buffalo and cow milk *khoa* prepared using fresh, acidic and neutralized milk was examined using scanning electron microscope (EV018, 18th special edition, Zeiss, Tokyo, Japan by the method of Tomar and Prasad (1989) with slight modification (Instead of one primary fixative, an additional secondary fixative agent was also used). Primary (2% glutaraldehyde) and secondary (1% osmium tetraoxide) fixative agents were prepared in 0.1 M cacodylate buffer (pH 7.2). Small pieces of 1 cm^3 *khoa* samples were fixed using primary and secondary fixatives and examined by SEM at an acceleration voltage of 15 kV under high vacuum (9.0×10^{-5} Torr) and micrographs were recorded at 500 and 1.00 KX (1000X).

Statistical analysis

Means and standard error mean (SEM) were calculated using Microsoft excel, 2007 (Microsoft Corp., Redmond, WA). Significant difference between values was verified by one way or two way analysis of variance and comparison between means was made by critical difference value (Snedecor and Cochran 1994).

Result and discussion

Sensory evaluation

Various sensory score attributes viz. flavour, body and texture, colour and appearance and total score of buffalo and cow milk *khoa* prepared using fresh, acidic and neutralized milk are presented in Table 2a, b. Quality of *khoa* was better when prepared from buffalo milk in comparison to *khoa* prepared from cow milk due to its moist surface, sticky and sandy texture which is not considered suitable for the preparation of sweetmeats (De and Ray 1952). Cow milk gave a product with sticky body leading to lower sensory scores than *khoa* prepared from buffalo milk. Stickiness in *khoa* from cow milk was attributed to insufficient release of free fat (Vogra and Rajorhia 1983). Significant difference ($p < 0.05$) was observed between the flavour, body and texture and total scores of *khoa* from fresh, acidic and neutralized milk (both buffalo and cow milk). *Khoa* prepared from fresh milk possessed the characteristic flavour and secured significantly higher ($p < 0.05$) average scores while, *khoa* samples prepared from acidic milk carried slight acidic smell. In case of *khoa* prepared from neutralized milk, salty taste resulted in lowest flavour scores. *Khoa* prepared from neutralized cow milk exhibited pronounced salty flavour as compared to *khoa* manufactured from neutralized buffalo milk due to higher chloride content in cow milk than buffalo milk. Due to inherent compositional make up of buffalo milk, fresh buffalo milk *khoa* was slightly grainy in texture which is desirable. However, in case of *khoa* prepared from acidic milk, the increase in acidity of milk resulted in increase in size and hardness of grains which resulted in hard body and loose texture hence lower body and texture scores in acidic *khoa* samples (Rajorhia et al. 1990). The abrupt coagulation of casein due to change in pH and acidity of milk made the product grainy which is not desirable for some dairy based sweetmeats such as *peda*. Neutralization of sour milk suppressed the grain formation thereby producing *khoa* with smooth and homogenous texture. The colour and appearance scores of fresh and acidic *khoa* samples prepared from buffalo and cow milk, exhibited non-significant ($p > 0.05$) difference however, neutralized samples had significantly higher ($p < 0.05$) scores. *Khoa* prepared from fresh buffalo milk was white in colour with greenish tint, whereas in case of fresh cow milk *khoa* it was pale yellow in colour due to the presence of carotenoids in cow milk (Fox and Mcsweeney 1998) which imparted yellow colour to *khoa*. *Khoa* prepared from neutralized milk was brownish yellow in colour due to the presence of neutralizers. These neutralizers shifted the pH towards alkaline value, leading to increase in maillard browning (Sakac et al. 2012) and thus resulting in more pronounced colour in *khoa* prepared from neutralized milk (Rajorhia et al. 1990). Significant difference ($p < 0.05$) was observed in total sensory scores of *khoa* prepared from fresh, acidic and neutralized milk (buffalo and cow samples). Total scores

Table 2 Sensory score of fresh, acidic and neutralized buffalo milk *khoa* and, cow milk *khoa*

Sensory → parameters Sample↓		Flavour	Body and texture	Colour and appearance	Total scores
Buffalo	FBMK	48.00 ± 0.41^{C}	34.25 ± 0.25^{C}	13.00 ± 0.57^{A}	95.25 ± 0.25^{C}
Milk	ABMK	47.50 ± 0.29^{B}	32.75 ± 0.25^{A}	12.65 ± 0.33^{A}	92.90 ± 0.25^{B}
Khoa	NBMK	44.50 ± 0.29^{A}	33.50 ± 0.25^{B}	14.00 ± 0.34^{B}	92.00 ± 0.89^{A}
Cow	FCMK	44.75 ± 0.25^{C}	33.25 ± 0.25^{C}	12.75 ± 0.25^{A}	90.75 ± 0.48^{C}
Milk	ACMK	44.25 ± 0.25^{B}	30.75 ± 0.25^{A}	12.50 ± 0.29^{A}	87.50 ± 0.28^{B}
Khoa	NCMK	41.00 ± 0.41^{A}	31.50 ± 0.28^{B}	13.50 ± 0.28^{B}	86.00 ± 0.71^{A}

Data are presented as mean ± Standard Error Mean (n = 15)

ABMK acidic buffalo milk *khoa*, *FBMK* fresh buffalo milk *khoa*, *NBMK* neutralized buffalo milk *khoa*, *ACMK* acidic cow milk *Khoa*, *FCMK* fresh cow milk *khoa*, *NCMK* neutralized cow milk *khoa*

$^{A–C}$ Means within column with different upper case superscript are significantly different ($p < 0.05$) from each other were highest in fresh samples. However, total sensory scores of buffalo milk *khoa* samples were comparatively higher than cow milk *khoa* samples.

Texture parameters

Texture is an important attribute in *khoa* and *khoa* based sweets as it decides the ultimate acceptability of a product by the consumers. The changes in the textural attributes (hardness, springiness, cohesiveness, chewiness, gumminess) of cow and buffalo milk *khoa* as affected by the development of acidity and neutralization of milk were also determined. Changes in these textural attributes directly affected the quality of *khoa* e.g. higher hardness in *khoa* will result in brittle texture of *khoa* and *khoa* based sweet. Similarly, higher gumminess and springiness will result in elastic and gummy texture of *khoa* which is not desirable in *khoa* based sweets. In present study, texture changes were also visible in chopped diced samples of *khoa* (Fig. 1) as the *khoa* prepared from acidic milk wasgranular as compared to fresh cow and buffalo milk *khoa* while, in case of neutralized sample khoa prepared was smooth and plain surfaced when compared with fresh milk *khoa* samples.

Hardness

Significant difference ($p < 0.05$) was observed in the hardness of *khoa* prepared using fresh, acidic and neutralized milk from both buffalo and cow. Hardness of *khoa* prepared from acidic milk was significantly higher ($p < 0.05$) than *khoa* from fresh and neutralized milk. This might be due to the higher amount of total solids mainly contributed by low moisture content in acidic samples, as a decrease in pH resulted in lower moisture retention (Choudhary et al. 2016). Sachdeva and Singh (1988) reported that with a fall in pH, moisture retention and yield of buffalo milk *paneer* decreased. Adhikari et al. (1994) also reported negative correlation between moisture and instron hardness of *khoa*. Our results were also supported by Rajorhia et al. (1990) who reported higher hardness values in *khoa* prepared from acidic buffalo milk. Patel et al. (1990) reported that moisture content of *peda* (a *khoa* based sweet) had a direct relationship with hardness. Our findings were in accordance with the reports of Gupta et al. (1990) and Suresh and Jha (1994) who reported that the increased hardness of *khoa* correlated highly with the total solids and by increasing the total solids, hardness also increased.

Cohesiveness

Cohesiveness expresses the strength of internal structure of food and can be calculated as area of work during the second deformation divided by the area of work during the first deformation. Cohesiveness values were significantly higher ($p < 0.05$) in *khoa* prepared from acidic milk and lowest in *khoa* prepared from neutralized milk as compared to *khoa* from fresh milk which might be due to difference in moisture and total solids of respective *khoa* samples (Choudhary et al. 2016). Adhikari et al. (1994) and Garg et al. (1989) also reported negative correlation between cohesiveness and moisture content in *khoa*. Gupta et al. (1990) reported that cohesiveness of *khoa* tended to decline with increasing total solids. Puranik et al. (1998) reported cohesiveness value of 0.260 in cow milk *khoa*. Rajorhia et al. (1990) also reported that cohesiveness was highest in *khoa* prepared from acidic milk and lowest from neutralized milk.

Gumminess

The gumminess expresses the force needed to disintegrate a semi solid food to a state ready for swallowing. It is the product of hardness and cohesiveness. Gumminess of acidic samples was significantly higher ($p < 0.05$) than *khoa* prepared using fresh and neutralized cow and buffalo milk. Since gumminess is a secondary parameter derived from hardness and cohesiveness, hence a slight change in these two textural parameters also affected it. Gupta et al. (1990) also reported an increase in total solids, which resulted in increase in instron gumminess in *khoa*. Adhikari et al. (1994) also reported negative correlation between moisture and instron gumminess of *khoa*. Similar results were also reported by Rajorhia et al. (1990) that the gumminess of *khoa* from acidic buffalo milk was higher than *khoa* from fresh and neutralized buffalo milk.

Springiness

Springiness expresses the rate at which the deformed food material returns to its original condition after removal of force. Significant difference ($p < 0.05$) was observed in springiness values of *khoa* prepared from fresh, acidic and neutralized buffalo and cow milk samples. Springiness values of *khoa* from acidic milk was significantly higher ($p < 0.05$) than *khoa* prepared using fresh and neutralized milk. It may be due to the better defined granular texture in acidic samples as compared *khoa* from fresh and neutralized milk samples owing to their moisture difference (Choudhary et al. 2016). Jha et al. (2014) reported that lower springiness value in *lalpeda* was due to the porous texture of *lalpeda*. Results were in accordance with Rajorhia et al. (1990) who reported higher springiness value in *khoa* from acidic buffalo milk followed by *khoa* from fresh and neutralized milk.

Chewiness

Chewiness expresses the work needed to masticate a solid food to a state ready for swallowing. Chewiness (N mm)

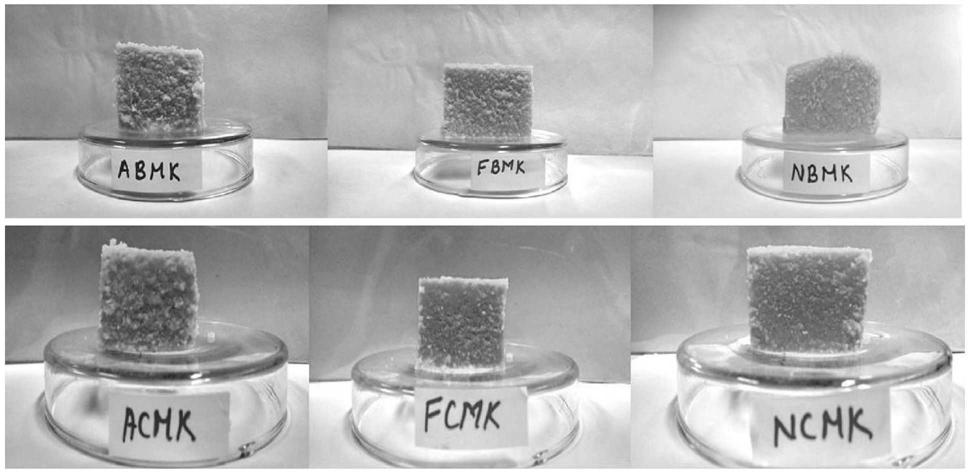

Fig. 1 Chopped diced sample of *khoa* ABMK- acidic buffalo milk *khoa*, FBMK- fresh buffalo milk *khoa*, NBMKneutralized buffalo milk *khoa* ACMKacidic cow milk *khoa*, FCMK- fresh cow milk *khoa*, NCMK- neutralized cow milk *khoa*

values were higher in cow milk *khoa* as compared to buffalo milk *khoa* which might be due to higher protein and ash content as compared to buffalo milk *khoa.* Wasnik et al. (2015) reported positive correlation between chewiness and protein content of *santraburfi*. Acidic samples had significantly higher ($p < 0.05$) values for chewiness as compared to *khoa* samples from fresh and neutralized milk which might be due to higher total solids and granular texture of acidic *khoa* samples as compared to fresh and neutralized samples (Choudhary et al. 2016). Since chewiness is a secondary parameter derived from hardness, cohesiveness and springiness, hence a slight change in these textural parameters also affected it. Gupta et al. (1990) also reported that the increase in total solids resulted in increase of instron chewiness in *khoa*. Adhikari et al. (1994) also reported negative correlation between moisture and instron chewiness of *khoa*. Rajorhia et al. (1990) observed similar findings and reported that chewiness was highest in acidic buffalo milk *khoa* followed by *khoa* samples prepared from fresh and neutralized buffalo milk. Puranik et al. (1998) reported chewiness values up to 1.60 in cow milk *khoa.*

Effect of acidity and neutralization on microstructure of cow and buffalo milk *khoa*

Scanning electron microscopy is an important tool in the study of surface topography as it produces a 3-D impression of the rough surface. This technique is especially useful in the study of various microstructural changes taking place during the various stages of processing of a product. Casein, organized in micellar form in fluid milk, in conjugation with other milk components is capable of forming structures widely ranging in density depending on the manufacturing conditions. During the preparation of sample for SEM, they were chemically dehydrated by passing through a graded series of alcohol-water mixture which resulted in removal of fat. The void spaces as evident from the photographs might have developed as a result of the fat extraction during sample preparation for electron microscopy. Microstructure of *khoa* revealed an agglomerated, dense, threaded protein bodies forming a ragged surface. The gritty, loose matrix of coagulated milk protein vanished completely during patting stage.

Uniform rounded shaped protein complexes (Fig. 2c) were observed in buffalo milk *khoa*. Surface of buffalo milk *khoa* was slightly granular and smooth as compared to cow milk *khoa* (Fig. 3c) which had rough and dry native surface presumably due to lower initial fat content. In addition to this, smaller rounded protein aggregates and an irregular surface pattern were observed in cow milk *khoa*. The microstructure of buffalo milk *khoa* was characterized with loose matrix, uneven surfaces and thick protein complexes joined together. Acidification of milk up to 0.18% lactic acid brought about shrinkage of native microstructure. Native protein aggregates in *khoa* samples prepared from acidic buffalo milk consisted of compact agglomerated irregular protein complex (Fig. 2a, b) as compared to control buffalo milk *khoa*. Similarly in case of *khoa* prepared from acidic cow milk (Fig. 3a, b) very dense, compact and relatively uniform pattern of agglomerated protein mass was observed. Shrinkage of microstructure can be explained by the fact that acidic milk has tendency to form granular *khoa* and has comparatively higher hardness, chewiness and springiness as compared to fresh milk *khoa*. The microstructure appeared to be hard and compact as compared to fresh *khoa* samples which might be due to higher TS in acidic *khoa* samples as compared to fresh *khoa* samples. The coarse structure of *khoa* increased with increase in the acidity of milk, also the

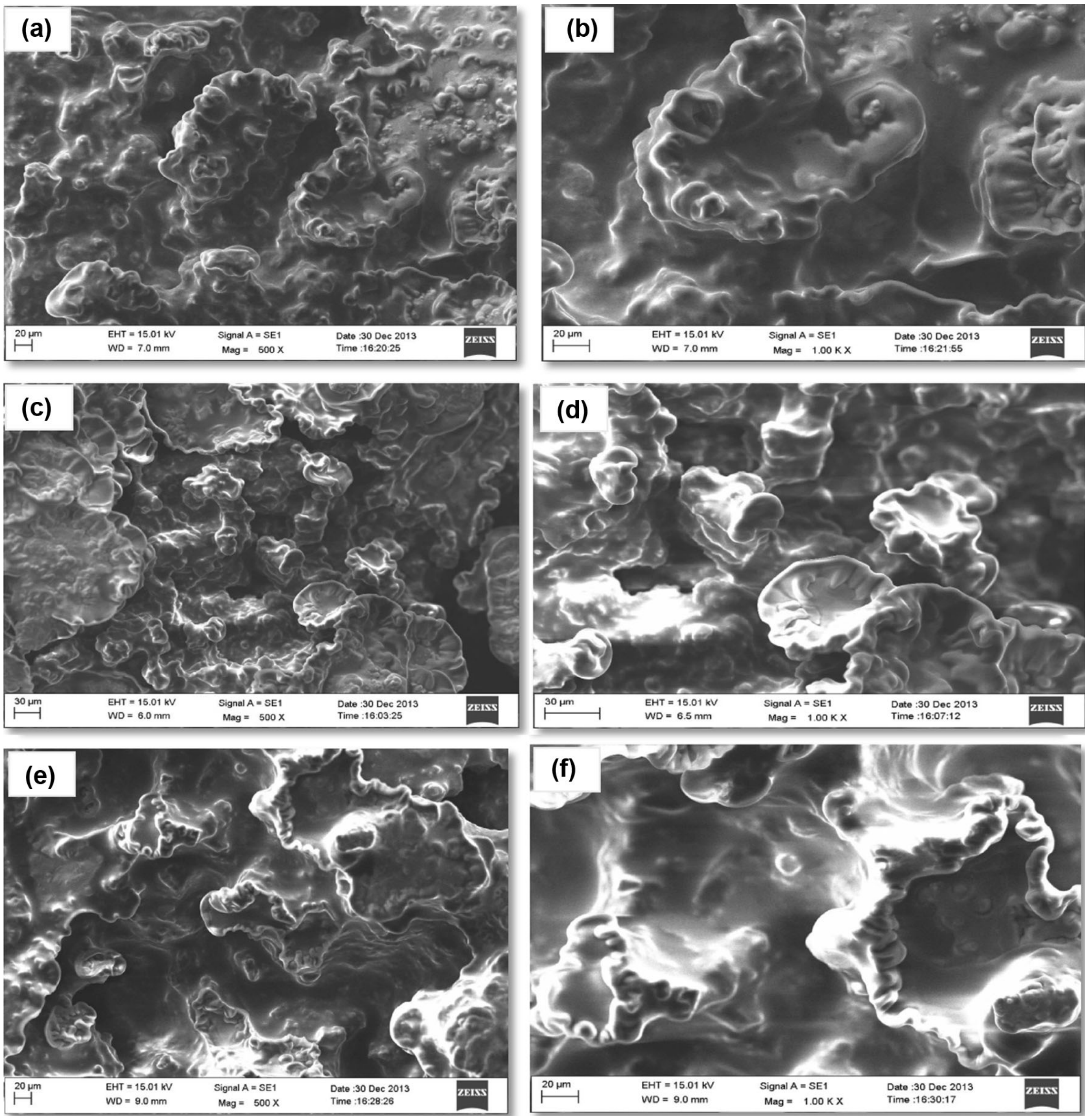

Fig. 2 *Khoa* prepared from fresh, acidic and neutralized buffalo milk. **a** Acidic buffalo milk *khoa* at 500X. **b** Acidic buffalo milk *khoa* at 1.00KX. **c** Fresh buffalo milk *khoa* at 500X. **d** Fresh buffalo milk *khoa* at 1.00KX. **e** Neutralized buffalo milk *khoa* at 500X. **f** Neutralized buffalo milk *khoa* at 1.00KX

in size and hardness of grain leads to poor body and texture of *khoa* (Rajorhia et al. 1990). *Khoa* from neutralized milk had comparatively higher moisture, smooth surface and pasty consistency which resulted in slightly elongated loose matrix. *Khoa* prepared from neutralized buffalo milk (Fig. 2e, f) indicated elastic and loose round shaped protein agglomerates and granules which were comparatively smooth surfaced as compared to *khoa* prepared using neutralized cow milk.

Khoa prepared from neutralized cow milk (Fig. 3e, f) possessed uniform, plain and smooth surface as compared to control cow milk *khoa* (Fig. 3c, d). Besides, large rounded granules with inner microvilli were observed which were frequently found spread over the whole surface. The microstructure appeared to be open or loosely held which can be ascribed to the greater moisture retention and lower hardness in neutralized samples (Pal 2006) resulting in smooth texture as compared to fresh *khoa* samples. Microstructural changes correlated with textural attributes as loose networks in neutralized samples can be explained by the low hardness, cohesiveness and accordingly lower gumminess and chewiness in *khoa* samples from neutralized milk and shrinkage of microstructure in acidic samples might be due to greater hardness, cohesiveness and accordingly lower gumminess and chewiness values in *khoa* samples from acidic milk (Tables 3a, b).

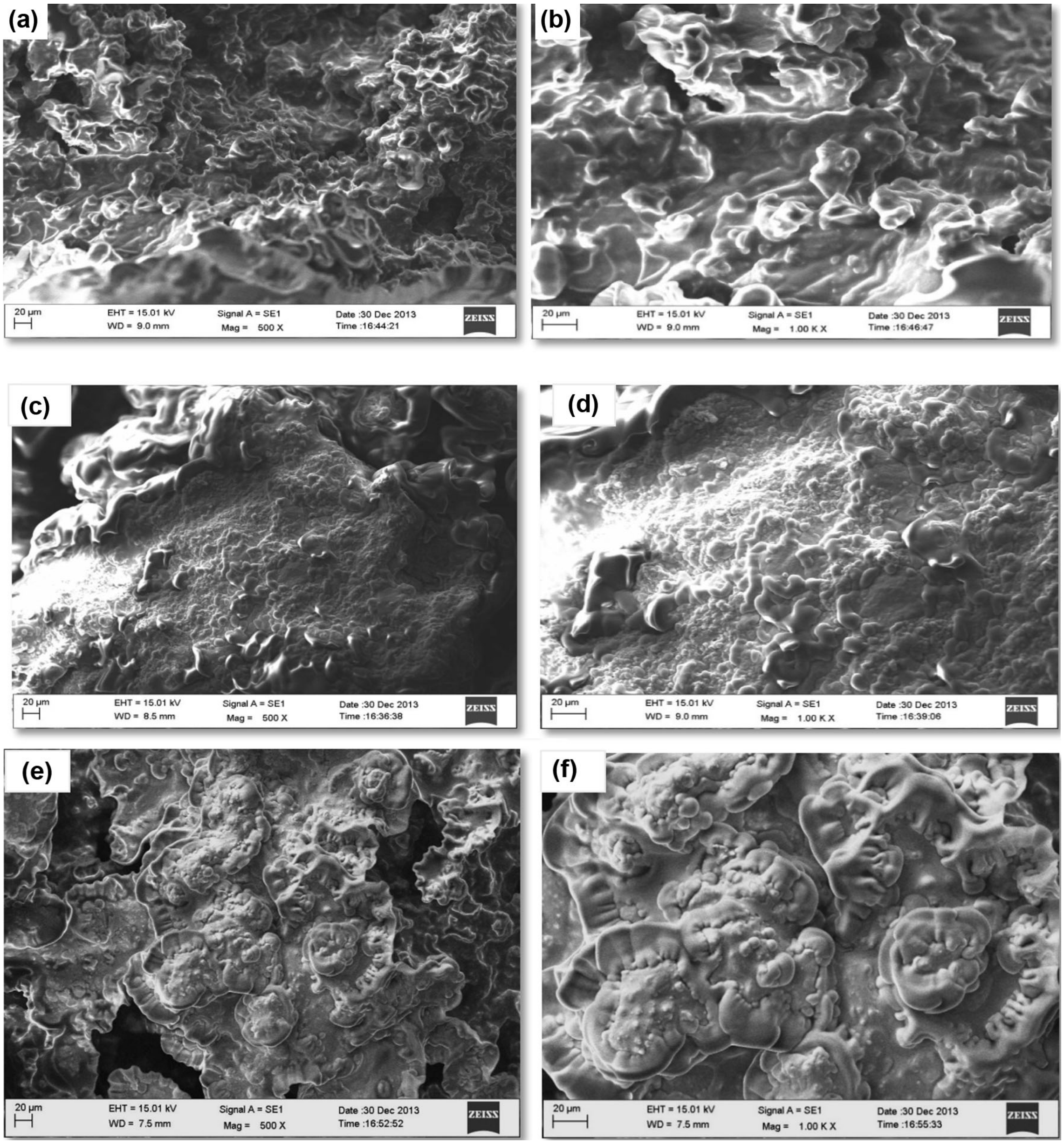

Fig. 3 *Khoa* prepared from fresh, acidic and neutralized cow milk. **a** Acidic cow milk *khoa* at 500X. **b** Acidic cow milk *khoa* at 1.00KX. **c** Fresh cow milk *khoa* at 500X. **d** Fresh cow milk *khoa* at 1.00KX. **e** Neutralized cow milk *khoa* at 500X. **f** Neutralized cow milk *khoa* at 1.00KX

Adhikari et al. (1994) also reported that moisture *was* negatively correlated with hardness, cohesiveness, gumminess and chewiness and the observed values for these parameters were the lowest in *khoa* samples from neutralized milk. Rajorhia et al. (1990) also reported lower cohesiveness, gumminess and chewiness values in *khoa* samples prepared using neutralized buffalo milk as compared to *khoa* samples from fresh and acidic milk. Tunick et al. (1993) also reported that higher moisture level resulted in lower hardness in low fat mozzarella cheese.

Such changes were also visible in chopped diced samples of *khoa* as the *khoa* prepared from acidic milk (Fig. 1) was granular as compared to fresh cow and buffalo milk *khoa*. *Khoa* from neutralized milk possessed a smooth and plain surface as compared to fresh *khoa* samples. Arora et al. (2007) also reported that the loose network in microstructure in *burfi* could be due to lower hardness, cohesiveness and accordingly, gumminess and chewiness. Adhikari (1993) reported that constant boiling of milk during *khoa* manufacture led to the formation of casein-whey protein complexes, which coalesced gradually with the progress of boiling, forming a fuzzy-agglomerated mass and finally precipitating as heat-induced milk gels, joined together by thick protein bridges. Further heat

Table 3 Effect of acidity and neutralization on textural properties of buffalo milk *khoa* and cow milk *khoa*

Parameters Sample↓		Textural attributes				
		Hardness (N)	Cohesiveness	Gumminess	Chewiness (N mm)	Springiness
Buffalo Milk	FBMK	9.60 ± 0.67^{B}	0.352 ± 0.001^{B}	2.62 ± 0.01^{B}	0.96 ± 0.003^{B}	0.351 ± 0.003^{B}
	ABMK	13.53 ± 0.76^{C}	0.412 ± 0.002^{C}	3.36 ± 0.31^{C}	1.25 ± 0.091^{C}	0.369 ± 0.002^{C}
	NBMK	7.96 ± 0.34^{A}	0.299 ± 0.005^{A}	1.75 ± 0.14^{A}	0.62 ± 0.005^{A}	0.310 ± 0.002^{A}
Cow milk	FCMK	18.34 ± 0.61^{B}	0.178 ± 0.001^{B}	3.25 ± 0.021^{B}	1.41 ± 0.033^{B}	0.290 ± 0.004^{B}
	ACMK	23.06 ± 0.26^{C}	0.195 ± 0.002^{C}	3.88 ± 0.292^{C}	1.94 ± 0.045^{C}	0.320 ± 0.005^{C}
	NCMK	15.75 ± 0.70^{A}	0.158 ± 0.003^{A}	2.71 ± 0.084^{A}	0.91 ± 0.035^{A}	0.261 ± 0.006^{A}

Data are presented as mean ± Standard Error Mean (n = 3)

ABMK acidic buffalo milk *khoa*, *FBMK* fresh buffalo milk *khoa*, *NBMK* neutralized buffalo milk *khoa*, *ACMK* acidic cow milk *khoa*, *FCMK* fresh cow milk *khoa*, *NCMK* neutralized cow milk *khoa*

$^{A–C}$ Means within column with different upper case superscript are significantly different ($p < 0.05$) from each other

desiccation of this gel resulted in the compaction of the protein agglomerates with reduction in void spaces and fat globules interspersed in between (i.e. *khoa*). Adhikari (1992) reported that structure of *khoa* from cow milk showed agglomerated protein complexes interlinked together with thick bridging material with minimal void spaces. However, buffalo milk *khoa* showed comparatively loose matrix with thick protein bodies joined together forming more uneven surfaces and more voids in between.

Summary and conclusion

The results of the present investigation have established that the type and quality of milk used for preparation of *khoa* produced noticeable changes in sensory, textural and microstructural changes in the product. Development of acidity in milk resulted in bigger granules, shrinkage of native microstructure due to lower acidity of milk with slight acidic flavor of resulting *khoa*. Neutralization of milk resulted in *khoa* with plain and smooth texture, elongation of native structure and comparatively higher moisture retention and salty taste and yellowish tint. Textural attributes such as hardness, cohesiveness, springiness, chewiness and gumminess were significantly affected by development of acidity and neutralization of milk, indicating lower values for hardness, cohesiveness, springiness, chewiness and gumminess in *khoa* manufactured using neutralized milk and higher values in *khoa* manufactured using acidic milk.

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