STUDIES ON THE EFFECT OF A NEUTRALIZER ON THE PHYSICO-CHEMICAL PROPERTIES OF KHOA

DISSERTATION
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
Master of Science IN
DAIRYING (DAIRY CHEMISTRY)
TO THE KURUKSHETRA UNIVERSITY
KURUKSHETRA

By
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B.Sc.
DIVISION OF DAIRY CHEMISTRY
NATIONAL DAIRY RESEARCH INSTITUTE (I. C. A. R.)
KARNAL (Haryana) INDIA
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Registration No. 79-dk-72
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February 10th, 1982

I certify that the work recorded in this dissertation entitled "Studies on the Effect of a Neutralizer on the Physico-chemical Properties of Khoa" was carried out by Shri Satish Chand Rajput, in partial fulfilment of his M.Sc. Dairy Ing(Dairy Chemistry) course of the Kurukshetra University under my guidance and supervision.

(K.C. TANDON)
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10th February, 1982
Karnal.

SCRAPPUT
(SATISH CHAND RAJPUT)
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1-2</td>
</tr>
<tr>
<td>II. REVIEW OF LITERATURE</td>
<td>3-12</td>
</tr>
<tr>
<td>III. MATERIALS AND METHODS</td>
<td>13-19</td>
</tr>
<tr>
<td>IV. SCOPE AND PLAN OF WORK</td>
<td>20-21</td>
</tr>
<tr>
<td>V. RESULT, AND DISCUSSION</td>
<td>22-35</td>
</tr>
<tr>
<td>VI. SUMMARY</td>
<td>36-37</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>1-111</td>
</tr>
</tbody>
</table>
INTRODUCTION

'Khoa' or Mawa, an indigenous preparation, refers to the partially dehydrated whole milk product prepared by continuous heating of milk with constant stirring-cum-scrapping till it reaches a semi-solid mass. According to the Food Adulteration Act (1977), khoa is the product obtained from cow, buffalo, goat or sheep milk or a combination thereof by rapid drying. The milk fat of khoa should not be less than 20% of the finished product as enunciated by Indian Standard Institution (ISI).

Mostly buffalo milk is used for khoa production because it not only gives more yield but also produces better body and texture in the finished product, which ultimately gives better quality khoa sweets.

Generally, there is a time gap between the place of milk production and the place where khoa is to be prepared and because of the temperate climate of our country, the acidity in milk develops so much, so as to make it unfit even for boiling. Such milk which has developed acidity can not, therefore, be utilized for drinking as well as for making any product. So, people dealing in milk usually add some alkaline substances for neutralizing the developed acidity so as to make it fit for boiling and for making khoa like products. The physico-chemical aspects of khoa made from such
neutralized milk has not been studied at all. The present study has, therefore, been undertaken to elucidate the changes in khoa made from buffalo and cow milk. The data may be useful for the identification of neutralized milk being used in the preparation of khoa.
REVIEW OF LITERATURE

In spite of its high economic importance as an indigenous milk product, khoa has not received the attention it richly deserves from the research workers. Only recently, a few studies have been made by Indian workers on the method of preparation and quality of the product.

EFFECT OF AGING AND NEUTRALIZATION OF MILK

In the acid-, rennet- or heat coagulation of milk, the casein is precipitated as a gel, albumin and globulin are precipitated by acid or rennet coagulation. With increase in acidity of milk (by addition of acid or by development of lactic acid during bacterial fermentation of milk sugar), two changes take place, viz. (1) a progressive removal of the colloidal tricalcium phosphate from the surface of the casein and its conversion into soluble mono-calcium phosphate and soluble-calcium salt (of the acid), and (2) a progressive removal of calcium from the calcium hydroxy caseinate to form soluble calcium salt (of the acid) and liberating free casein. The soluble calcium salts pass into true solution, when the acidity reaches to pH 4.6 or 4.7 and the colloidal particles become iso-electric, i.e., the electric charge becomes effectively zero. Under these conditions the dispersion is no longer stable, the casein is precipitated and coagulation (or curdling) takes place.

The addition of neutralizer to the milk, which has developed acidity, helps in bringing back the initial acidity or pH of milk by
neutralization. Neutralizers are usually added in solution form for easy mixing.

**HEAT-INDUCED CHANGES IN MILK**

Casein is not a heat coagulated protein, is very much stable and is not denatured by boiling the milk for short duration. Its dispersion in normal fluid milk is so stable that it may resist coagulation even if heated for several hours at boiling temperatures (Dobb et al., 1974).

Morr (1965) observed that heating skim-milk at 80°C for 10 min. reduced the magnitude of differences in electrophoretic composition of supernatant fraction indicating the interaction of β-casein with β-lactoglobulin among smaller skin milk micelles. Mill et al. (1963) observed that 5 to 80% of the whey proteins were denatured when skim-milk preheated to 110°F was heated to 160-330°F by steam injection for 5 to 53 seconds. Harland et al. (1952) reported that for every 13.5°F increase in temperature, the time for the same amount of denaturation decreased 10-fold. Harland et al. (1955) again reported that the amount of undenatured protein in dried skim milk of unknown history, was not a reliable indication of the amount of heat treatment received and at any degree of whey protein denaturation, there was a curvilinear relationship between time and temperature of heating.

On electrophoretic studies, Damitz (1966) observed that blood-serum-albumin denatured completely, when heated at 75°C for more than 10 min. or at 80°C for more than 30 seconds, whereas
\(\beta\)-lactoglobulin denatured completely at 80°C for more than 10 minutes or 90°C for more than 30 seconds. Ninety percent of immunoglobulins were denatured at 75°C for more than 10 minutes and the most heat resistant was \(\alpha\)-lactalbumin, 22% of which remained undenatured at 90°C for 1 minute. Kieza and Rotkiewicz (1974) could not detect free sulphhydryl compounds in raw milk but it was highest when milk was heated to 90°C for 1 minute.

**CHEMICAL COMPOSITION OF Khoa.**

The chemical quality of khoa varied considerably from place to place depending upon the type and quality of milk used. De and Ray (1952) reported that the best quality khoa was obtained by boiling milk, with stirring, until it reaches a pasty consistency, then lowering the temperature to 130°C-190°F until a pat was formed. The authors also indicated the yields and the average composition of khoa of cows' and buffaloes' milk respectively, as follows: yield-13.3 and 21.6%; moisture-25.6 and 19.2%; butterfat-34.3 and 45.9%; protein-25.8 and 22.1%; lactose-34.4 and 27.3%; ash-5.2 and 4.5%; Iron-139 and 125 ppm. Dadur and Lakhani (1971) observed the average composition of khoa obtained from local shops in Poona as follows: moisture-25.8%, fat-27.24%; ash-3.36%; ash-soluble in HCl-0.11% and protein-19.58%. Rajurhia (1971) observed that the yield of khoa from cows' milk (17.5 to 21.0%) was significantly lower than those from buffaloes' milk (20.5 to 23.35%). Cows' milk (4 to 5.8% fat and 8.3 to 10.28% SNF) yielded khoa containing 23.3 to 29.8% fat and 26.4 to 33.5% moisture, whereas buffaloes' milk (4 to 7.8% fat, and 8.8 to 10% SNF) resulted in khoa of 20.1 to 23.35% fat and 28.3 to
34.4% moisture content. Zariwalla et al. (1974) reported an average composition (± 5.0%) of khoa samples by analysing 511 samples in Bombay city, as follows: moisture—28.13 ± 2.39%; fat—27.14 ± 3.12%; and lactose—17.67 ± 1.30%. Similar variations were also observed in other constituents of khoa samples.

**PHYSICO-CHEMICAL CHANGES DURING KHOA MAKING**

Khoa being essentially a heat desiccated product, number of changes take place during its manufacture. Rajoria and Srinivasan (1974) listed the following changes during khoa making. Progressive increase in total reducing capacity, linear decrease in pH from 6.00 to 6.35 with the corresponding rise in the acidity from 0.135 to 0.50%; lactic acid, increase in soluble nitrogen (at pH 4.3) and formol titre; and loss of lactose accompanied by colour development (browning) and intensive cooked flavour in the final product.

(a) **Heat coagulation**

Major changes in khoa making are the heat denaturation and heat coagulation of milk proteins, as evidenced by an abrupt change in colour and consistency of the product towards the end of the process. Cole and Tarassuk (1946) observed that the coagulation was linear with respect to temperature and logarithmic with respect to heating. De and Ray (1952) found that the heat coagulation of cow milk starts at a ratio of concentration of 2.83 and ends at 3.57, whereas in case of buffalo milk it was 2.48 and 3.05, respectively. Grimbleby (1954) reported that when milk was heated at 60°C to 80°C for 4 hours, its titratable acidity increased.
rapidly at first and then remained steady. At 90° or 100°C, the acidity continued to increase the whole time and the rate of increase rose with temperature. The rate of lactose disappearance increased with temperature. It was, therefore, evident that much more lactose combines with protein, increasing the acidity and the protein-lactose bond became stronger leading to browning above 90°C.

Gould (1945) observed that when samples of skim milk were heated in cans for 1 to 2 hours at 116°C, volatile acids increased appreciably. Redistillation of the milk distillate showed that formic acid constituted 80 to 85% of the total volatile acids. Evenhuis (1953) exhibited that the particle size of casein increased on heating and decreased on cooling the milk and it was probably due to the dissociation and association of the calcium-citrate complex.

Pyne and McHenry (1960) observed that calcium ions and colloidal phosphate were the substances most favouring coagulation, which was very slow in milk with a low content of one or the other. They showed that half of the acidity developed on heating was due to lactose decomposition. Little et al. (1958) found that no calcium was bound with casein at pH 5.3, but binding is increased with increasing pH. Calcium was bound only above pH 6, in solutions containing phosphate but more calcium was bound in the absence of phosphate. The authors showed that neither time nor temperature had any effect on the amount of bound calcium.

Puri and Prakash (1963) suggested that the time required for coagulation of buffalo milk at temperatures 110°, 115°, 120°, 130°
and 140°C was significantly higher than cow and goat milk. Cow milk and goat milk did not differ much from one another.

(b) *Milk proteins*

The exposure of milk to heat processing has been shown to affect its proteins. The extent to which proteins get denatured during heat processing of milk and manufacture of concentrated as well as dried milk products have been the subject of innumerable research workers.

(i) *Caseins:*

It is not a heat coagulated protein, in the strictest sense. Its dispersion in normal milk is so stable that it may resist coagulation for as long as fourteen hours of boiling temperature (Wubb et al., 1974). However, since it has a native structure mostly of quaternary type, temperature and pH can drastically affect casein association and result in micelle alteration. The heat coagulation of casein in milks of normal stability occurs largely as a result of compositional changes in milk caused by sustained exposure to high temperature.

Miller and Sommer (1940) reported that the pH of milk decreased with the increase in temperature, partially as a result of changes in the buffer capacity of milk salts and the expulsion of carbon-dioxide on heating.

(ii) *Whey proteins*

Serum proteins, which constitute approximately 0.6% of milk or nearly 20% of the total milk proteins, are much more heat labile than
casein. Kieferle and Gloetzal (1930) showed that serum proteins were completely denatured by heat treatments at 77.5°C, 80°C and 90°C, when heated for 60, 30 and 5 minutes, respectively. Howland (1937) also reported similar extents of denaturation of albumin and globuline at temperatures above 75°C. Larson and Jenness (1950) exhibited that heat treatments decreases the sulphydryl reducing capacity of milk serum proteins. It was thought that the decrease might be due to oxidation since it was largely prevented by excluding air from the sample during heating.

(c) Milk Fat

Although milk fat is not known to be affected by the processing treatments commonly employed, they do have an effect on the fat globule membrane. Lowenstein and Gould (1954) reported that fat globules in milk heated to 82°C for 15 minutes lost some membrane material. Radema (1956) observed that the stirring of thin cream in cold did not alter the partition of the phosphatides, whereas heat treatments, with and without stirring caused some transfer of phosphatides from the fat globules to the serum.

Greenbank and Pallansch (1959) also reported the migration of phosphatides during pasteurization, homogenization and concentration of whole milk. Homogenization of whole milk caused migration of phosphatides from the fat globule membrane to the skim-milk, with maximum effect in the range from 1,000 to 3,000 psi. The authors further reported (1961) that any form of agitation apparently caused a migration. The greatest change in the distribution of the
phosphatides occurred during condensing, where the turbulence encountered is great and prolonged.

The relative ease with which phospholipids could be removed from the fat-globule surface showed that a large fraction of phosphatides initially associated with the fat in fresh milk was held by weak forces. On heat denaturation of membrane protein, breaking of various linkages may occur, e.g., salt linkages, hydrogen bonds between neighboring polypeptide chains etc. The sulfur bridge of cystine residues may be broken causing liberation of sulfides, which in turn was responsible for cooked flavour (King, 1955).

Davies (1948) indicated that homogenizing action of stirring of hot milk on the fat globules was quite appreciable and therefore at the coagulation stage, a major portion of the fat globules was entrapped in the coagulum. Choi et al. (1951) reported that when dried whole milk was treated with 95% ethanol, the amount of hydrated lactose did not increase, and it was suggested that the increase in extractable fat produced by this treatment was due to protein coagulation rather than to lactose crystallization. Kharitonov (1976) exhibited that the dispersibility of dried whole milk reduced due to increased free fat content. No studies on free fat content of khoa have so far been attempted.

Agora and Sindel (1976) studied the lipid constituents in detail during khoa making. They observed that total phospholipids were higher in cow milk (0.44%) than buffalo milk (0.28%) but at all stages of khoa making, the values were almost comparable in both cow's and buffaloe's milk. No variations were observed in the level and
distribution of cholesterol at various stages of whey making.

(d) Lactose

Whittier and Benton (1926) concluded from their experiments that heating of skim milk at temperatures near the boiling point causes first a drop and then a rise in the titratable acidity. The authors investigated that the reason of initial drop in titratable acidity had been due to loss of carbon-dioxide from the milk; the increase in titratable acidity was due to the formation of acids from certain constituents of the milk. During coagulation, the rate of change of hydrogen-ion concentration was considerably lessened due to buffer readjustment, not yet explained in detail. Again in 1927, they observed that acid was formed during heating of milk at a rate having direct function of the time and temperature of heating and of lactose concentration. The rate of acid formation appeared to be unchanged by the coagulation of casein. The loss of lactose in heated milk was more than sufficient to account for the acid production. More et al. (1957) postulated that the thermal decomposition of lactose resulted into the formation of compounds such as formic acid, acetic acid, butyric acid, pyruvic acid and lactic acid. Methods of heat treatment may influence the acid productions in milk heated to 121°C, i.e. in sealed containers it was higher than in unsealed containers heated to same temperature.

Patton and Josephson (1949) exhibited the presence of furfuryl alcohol from heated skim milk, although its precise mechanism could not be explained. It was suggested that it might be connected with caramelization and browning phenomenon. Patton (1954), on prolonged
heating of skim-milk at high temperatures observed the presence of maltol (hydroxy-methyl-pyran). Its formation was correlated with the browning of milk, where lactose molecule was necessary for the reaction. Adachi (1959) observed that lactulose was produced from lactose in heated milk in amounts proportional to the rise in temperature and with casein-lactose mixtures in amounts which was directly related to both with rise in temperature and pH from 6 to 7. Kumar and Haneen (1972) reported that the precise nature of colour changes during cheese making was little understood, but in milk it was known that colour was produced as a result of several complex reactions.
CHAPTER III
MATERIALS AND METHODS

Materials

Preparation of khoa sample

Milk

Fresh raw milk from the herds of various breeds of cows and buffaloes were obtained from the 'Cattle Yard' of the Institute. After determining the natural acidity, the sample was divided into four parts. First part of the sample was used to prepare controlled khoa sample. Other parts were kept at room temperature for acid development up to 0.2%, 0.25% and 0.3% lactic acid respectively. After acid development, they were treated with requisite amount of a neutralizer (sodium-carbonate) to lower down the titratable acidity up to 0.15% lactic acid level in all the three cases. The neutralizer was added in the form of a solution in water, stirring the milk all the time. The amount to be added in each part was determined by preliminary titration. All neutralizer treated samples of milks were again tested for titratable acidity and then converted into khoa.

Milk (500 gms) was poured into a small, shallow, open, round bottom stainless steel karahi and brought to boil by placing over a brisk (non-smoky) fire. It was stirred continuously with circular motion by the flattened end of an stainless steel-stirrer called 'khunti'. During this operation, all parts of the pan with which
the milk came into contact were lightly scraped. As a result, a
constant evaporation of moisture took place and the milk thickened
progressively. At a certain stage, the thickened mass showed an
abrupt change of colour indicating occurrence of heat-coagulation of
milk proteins. The vigorous stirring and desiccation was continued
till a viscous product reached a pasty consistency and began to dry up.
Very close attention was given at the later stages. The final product
was considered to be ready when it showed signs of leaving the sides of
the pan and sticking together so as to make pat formation possible.
The khoa-pat was made after removing the pan completely off the fire
and by working the contents up and down.

Methods

1. Acidity of khoa

The acidity of khoa was determined by indirect method as given
below:

Two gms of khoa was weighed in a conical flask. The product was
made into paste by adding 3 ml of hot distilled water and diluting by
another 17 ml of hot distilled water. The contents were mixed with
a small glass rod and cooled to the room temperature. Five drops of
0.5% phenolphthalein solution was added and the contents titrated
against N/10 NaOH solution with continuous stirring till a faint pink
colour persisted. The acidity of khoa was expressed as lactic acid
equivalents per 100 g of khoa.
\[ L.A. = \frac{0.009 V}{w} \times 100 \]

where \( V \) = Volume of \( \text{H}_2\text{SO}_4 \) required for titration

\( w \) = weight of sample of khoa (2 g)

2. Moisture content of khoa

The % moisture in khoa was determined by adopting the method described for cheese in ISI (1964).

3. Fat of khoa

Fat percentage was determined by the Gerber method. Weighed accurately about 3 g of fairly divided khoa sample into a cheese butyrometer. Added 10 ml of Gerber sulphuric acid (of density 1.307 to 1.312 at 27°C) to which added 10 ml of distilled water and 1 ml of iso-amyl alcohol, respectively. The butyrometer was closed with the stopper and shaken well till all the contents were well mixed. Placed the butyrometer in a water bath at 65 ± 2°C for tempering, shaken periodically until the solution of khoa was complete. Centrifuged at 1,200 rpm for 5 minutes and read the percentage of fat by adjusting the fat column within the scale of the butyrometer.

4. Protein content of khoa

Reagents: (a) Concentrated sulphuric acid

(b) Copper sulphate

(c) Anhydrous potassium sulphate or sodium sulphate

(d) Sodium hydroxide - 50 percent by weight

(e) Standard sulphuric acid - 0.1N

(f) Boric acid (Analar) containing Tashiro's Indicator

20 g of boric acid was dissolved in water, 10 ml of Tashiro's
indicator added and made up to 1 litre. The pH of the solution should be 4.8, when a sample of it diluted in water was grey in colour. If it was too acidic (purple) or too alkaline (green), drops of \( \frac{1}{10} \) \( \text{N} \) \( \text{NaOH} \) or \( \frac{1}{10} \) \( \text{HCl} \) were added until the grey colour was produced.

**Ishiro's indicator:** 30 mg of methyl red and 20 mg of methylene blue dissolved in 100 ml alcohol.

**Procedure**

0.2 gm of the sample was weighed accurately and transferred into a 100 ml Kjeldahl flask. Five ml of concentrated sulphuric acid and 1 gm of the digestion mixture (potassium sulphate and copper sulphate 20:1 parts respectively). Gently rotated the flask so that the whole of the contents were well mixed. The sample was digested over a burner till the contents of the flask was clear, green and free from any yellowish tinge. The digested material was dissolved in 10-15 ml of distilled water and transferred to the semi micro-Kjeldahl distillation apparatus, and distilled after adding 15 ml of sodium hydroxide (50%). The ammonia liberated as adsorbed in saturated solution of boric acid containing mixed indicator. About 25-30 ml of the distillate was collected in a 100 ml conical flask. The distillate was then titrated against \( \frac{1}{10} \) \( \text{N} \) \( \text{H}_2\text{SO}_4 \).

A blank was also carried out simultaneously. The percent of total nitrogen was calculated as follows and results were expressed as percent total protein by multiplying the nitrogen percent by 6.28.
Calculations

\[ \% \text{ Nitrogen} = \frac{1.4 \times N (V - v)}{w} \]

where \( N \) was the normality of the titre acid, \( V \) and \( v \) were the titre values of the sample and the blank respectively, and \( w \) was the dry weight of the sample.

\[ \% \text{ protein} = \% \text{ Nitrogen} \times 6.28 \]

5. Determination of lactose in khoa

The lactose present in khoa samples was analysed by Lange-Zynon method as given below:

Reagents

Fehling's solution

This was prepared immediately before use by mixing equal volumes of Fehling solutions A and B prepared as described below:

(i) Fehling solution A: 34.639 gm of Anal. copper sulphate (CuSO\(_4\) \( \cdot \) 5H\(_2\)O) was dissolved in distilled water and diluted to 500 ml in a volumetric flask. The solution was filtered through asbestos.

(ii) Fehling solution B: 173 gas of Rochelle salt (sodium potassium tartarate) and 50 gm of sodium hydroxide (analytical reagent grade) were dissolved in water, diluted to 500 ml in a volumetric flask and allowed to stand for two days. The solution was filtered through asbestos.

(iii) Acetic acid solution: 10% v/v

(iv) Methylene blue indicator: 1 gm of methylene blue was dissolved in water and diluted to 100 ml.
(v) Standard lactose solution: 0.5% solution was prepared by dissolving 5 gms of pure lactose in distilled water and the volume was made up to 1 litre.

**Standardization of Fehling's solution**

5 ml each of Fehling solutions A and B were pipetted in a 250 ml conical flask. The contents of the conical flask were heated on a burner to boiling within 2 minutes. 0.5% standard lactose solution was then added from the burette to effect reduction of all the copper, so that not more than 0.5 to 1.0 ml was required later to complete the titration. 2-3 drops of methylene blue indicator was then added and the titration was completed with last drop of lactose solution to the point of decolourisation of indicator within a total boiling time of 3 to 4 minutes.

**Preparation of khoa filtrate**

5 gms of the khoa sample accurately weighed was diluted with 200 ml of warm distilled water, 3.75 ml of 10% acetic acid was added in it and boiled. After cooling, the volume was made up to 250 ml. It was then filtered through dry Whatman No.42 filter paper. The clear filtrate was titrated against standardised Fehling solution to find out the lactose content in the khoa sample as given below.

**Procedure for the lactose estimation of khoa**

Approximate quantity of the khoa filtrate required to reduce all the copper present in 10 ml of Fehling solution was determined in the manner described for the standardization of Fehling solution. 10 ml of
Fehling solution was taken in a conical flask, the required amount of \textit{khao} filtrate was added and heated the contents of the flask to boiling over a burner. The contents were boiled for 15 seconds and further quantity of \textit{khao} filtrate was added rapidly until faint blue colour remained. Then 2-3 drops of methylene blue indicator was added and the titration was completed by adding \textit{khao} filtrate drop-wise. Total quantity of \textit{khao} filtrate used for 10 ml of Fehling solution was recorded.

\textbf{Calculations}

Let \( A = \) Volume of 0.5\% standard lactose consumed for 10 ml of Fehling solution

\[ A = \frac{B}{250} \times 0.005 \times 250 \]

\( B = \) Volume of \textit{khao} filtrate consumed for 10 ml of Fehling solution

10 ml of Fehling solution = \( A \) ml of 0.5\% lactose solution

= \( B \) ml of \textit{khao} filtrate

8 ml of \textit{khao} filtrate = \( A \) ml of 0.5\% lactose solution

= \( 8 \times 0.005 \) gm of lactose

250 ml of \textit{khao} filtrate = \( \frac{A \times 0.005 \times 250}{B} \) gm of lactose

or 5 gms of \textit{khao}

= \( \frac{A \times 0.005 \times 250}{9} \) gm of lactose

\% Lactose in \textit{khao} = \( \frac{A \times 0.005 \times 250}{8} \times \frac{100}{5} = \frac{25A}{8} \)
CHAPTER IV

SCOPE AND PLAN OF WORK
SCOPE AND PLAN OF WORK

Khoa is an indigenous whole milk product which constitutes an important base and filler for the preparation of milk sweets. It is prepared by the continuous heating of milk in an open pan with constant stirring-gum scraping till it reaches a semi-solid consistency. This partially dehydrated solid product is called khoa. Fresh buffalo milk yields the best khoa, whereas milk with developed acidities progressively tend to produce sour taste and undesirable flavour and coarse texture in the product, which thereby produces lower quality sweet preparations.

This investigation was undertaken to find out the physico-chemical changes in khoa samples made from neutralized milks. The necessity for such a study was felt because of the temperate climate of our country, which leads to developed acidity and reduces the keeping quality of fresh milk. No work has been conducted so far on these aspects of khoa preparation using neutralized milk samples.

The plan of work for the present study using cow and buffalo milks have been shown below:

(1) Fresh milk will be kept at room temperature for acid development. Titratable acidity (% L.A.) will be allowed to develop upto 0.2, 0.25 and 0.3% levels in different containers.

(2) The different acidified milk samples will be neutralized with sodium carbonate solution (M approx.) to 0.15% acidity (% L.A.) level and khoa prepared.
(3) Titratable acidities of fresh and neutralized cow and buffalo khoa samples will be conducted.

(4) Lactose content of fresh and neutralized cow and buffalo khoa samples will be seen.

(5) Fresh and neutralized cow and buffalo milk khoa samples will be analysed for their total proteins.

(6) Fat content of fresh and neutralized cow and buffalo khoa samples will be seen.

(7) Physical properties such as general appearance, body and texture, and their flavour score will be studied.
CHAPTER XV

RESULTS AND DISCUSSION
RESULTS AND DISCUSSION

Effect of neutralization on titratable acidity of khoa.

Data pertaining to the effect of neutralization on titratable acidity of khoa made from cow and buffalo milks have been presented in Table 1. It is evident from the table that acidity of the fresh samples of khoa made from cow and buffalo milks were observed to be 0.45 and 0.30% (average) respectively. Neutralizer was used to reduce the developed acidity from 0.26, 0.25% and 0.3% lactic acid level to 0.15% lactic acid in each case.

Khoa thus prepared from these neutralized milk samples exhibited an increasing trend. The acidity of khoa made from these neutralized cow milk samples increased by 6.6%, 11.1% and 22.2% when compared against the control khoa made from fresh milk. Similarly in khoa made from neutralized buffalo milk the acidity increased by 10%, 16.6% and 26.2% as against fresh khoa from buffalo milk. The change in acidity was observed to be more in case of buffalo milk khoa as compared to the acidity observed in case of khoa made from cow neutralized milk samples. It was also observed that cow milk took more time for the development of the required acidity as compared to the time taken by buffalo milk. Increases in acidity of khoa samples from neutralized cow and buffalo milks are being attributed to the following two reasons (i.e.), firstly the conversion of
### Table 1

Effect of neutralization on titratable acidity of cow and buffalo milk khoa (% Titratable acidity).

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Acidity (% L.A.) Before Neutralization</th>
<th>Acidity (% L.A.) After Neutralization</th>
<th>Cow milk khoa On dry matter basis</th>
<th>Buffalo milk khoa On dry matter basis</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cow</td>
</tr>
<tr>
<td>0.14</td>
<td>-</td>
<td>0.32-0.61 (0.45)</td>
<td>0.27-0.37 (0.30)</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>0.2</td>
<td>0.15</td>
<td>0.37-0.64 (0.48)</td>
<td>0.24-0.38 (0.33)</td>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td>0.41-0.63 (0.50)</td>
<td>0.27-0.42 (0.35)</td>
<td></td>
<td>11.1</td>
</tr>
<tr>
<td>0.3</td>
<td>0.15</td>
<td>0.36-0.74 (0.55)</td>
<td>0.24-0.44 (0.38)</td>
<td></td>
<td>22.2</td>
</tr>
</tbody>
</table>

Average of six khoa samples each from cow and buffalo milk are given in brackets.
soluble calcium and phosphate on severe heat treatment to colloidal calcium and phosphates releasing hydrogen ions as follows:

$$3 \text{Ca}^{++} + 2\text{HPO}_4^{2-} \rightarrow \text{Ca}_3(\text{PO}_4)_2 + 2\text{H}^+$$

(Soluble) \hspace{2cm} (Colloidal)

Thus the releasing $\text{H}^+$ ions are responsible for the decrease in pH and increase in titratable acidity. Secondly high temperature heat treatment given to milk in khoa preparation also results in the production of lower chain acids by the degradation of lactose present in milk. Rajorhia and Srinivasan (1974) also prepared and analysed khoa samples from cow and buffalo milks and observed a linear pH reduction from 6.60 to 6.35 with corresponding average increases in titratable acidity from 0.135 to 0.35 lactic acid. They also indicated the range of acidity from 0.26 to 0.61% L. for normal milk. This might be due to the preparation of khoa from more acidified milk. Similar increases in titratable acidity were also noticed in the heat treatment of milk by Chitter & Benton (1926), Gould (1945), Grimbleby (1954) and Pyne & McHenry (1960).

Effect of neutralization on lactose content of khoa samples

Results regarding the effect of neutralization on lactose contents of cow and buffalo milks khoa have been presented in Table 2. The khoa made from fresh cow milk was observed to have the average lactose content of 39.86% as
Table-2

Effect of neutralization on lactose content of cow and buffalo milk khoa (gm/100 gm).

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Acidity (° L.A.) Before Neutralization</th>
<th>Acidity (° L.A.) After Neutralization</th>
<th>Cow milk khoa On dry matter basis</th>
<th>Buffalo milk khoa On dry matter basis</th>
<th>% Decrease Cow</th>
<th>% Decrease Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.14</td>
<td></td>
<td>-</td>
<td>39.34-41.38 (39.86)</td>
<td>28.80-30.36 (29.62)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.2</td>
<td></td>
<td>0.15</td>
<td>36.90-38.23 (37.16)</td>
<td>28.79-29.39 (29.09)</td>
<td>6.7</td>
<td>1.7</td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td>0.15</td>
<td>36.29-41.23 (36.80)</td>
<td>28.24-28.68 (28.41)</td>
<td>7.6</td>
<td>4.06</td>
</tr>
<tr>
<td>0.3</td>
<td></td>
<td>0.15</td>
<td>34.26-38.49 (35.92)</td>
<td>26.57-29.35 (28.01)</td>
<td>9.7</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Average of six khoa samples each from cow and buffalo milk are given in brackets.
compared to the lactose content of 29.62% in case of buffalo milk. Khoa samples made from all the three neutralized cow samples exhibited higher lactose contents as compared to khoa made from the corresponding neutralized buffalo milk samples. A continuous reduction in the lactose content of khoa samples made from the graded acidified developed cow milks were observed to be higher (6.74, 7.61 and 7.76) when compared with the decreasing lactose contents in the khoa samples made from corresponding buffalo milk samples (1.76, 4.06% and 5.4%). This showed greater break-down of lactose in khoa samples from cow milk as compared to its breakdown in khoa samples made from buffalo milk.

This may be due to the higher initial content of lactose present in cow milk. Reductions in lactose content of khoa may also be attributed to the thermal decomposition of lactose on severe heating with the formation of acids and alcohols and also due to its participation in Maillard reaction with proteins leading to caramelization during heat treatment. The results were in conformity with the results obtained by De and Ray (1952) who also recorded more lactose in khoa made from fresh cow milk (34.34) when compared against a value of 27.44% lactose present in khoa made from buffalo milk. Lariwala et al. (1974) also worked out the lactose contents of 551 samples of khoa obtained from retailers at Bombay between 1966-71, which was 17.67 ± 1.80%. The lower values obtained by the authors may be due to the inferior quality of milk containing less percentage of lactose or the khoa might have been less dehydrated. Rajorhia and Srinivasan (1974)
also recorded the loss of lactose in the preparation of khoa from cow and buffalo milks. Similarly reductions in the concentration of lactose in milk as a result of heat treat-ment have also been recorded by Patton & Josephson (1949), Patton (1950) and Morr et al. (1957).

**Effect of neutralization on the protein content of khoa samples.**

The effect of neutralization on the protein content of khoa samples has been shown in Table 3. The protein contents of fresh cow and buffalo milk khoa samples on an average were observed to be 34.95 and 24.66% respectively. The corresponding figures for cow and buffalo milk khoa samples from three neutralized milks were found to be 34.81, 34.73, 33.64 and 24.63, 24.53 and 24.16% respectively. The marginal reductions in the case of cow and buffalo khoa samples from neutralized milk samples were observed to be 0.38, 0.62, 3.7% and 0.12, 0.52, 2.0% respectively when compared against the respective control samples.

It was, therefore, clear that the degradation of protein contents were more in samples neutralized from the 0.3% developed L.A. milk samples, cow milk khoa being more vulnerable than buffalo milk product. The reduction of protein in khoa samples may be attributed to the dephosphorylation of casein, denaturation of serum proteins, interaction between proteins and lactose etc. De and Ray (1952) reported the % proteins in cow and buffalo milk khoa samples as 25.8 and 22.1% respectively. Dratur and
Table 3

Effect of neutralization on protein content of cow and buffalo milk khoa samples (gm/100 gm).

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Acidity (% L.A.) Before Neutralization</th>
<th>Acidity (% L.A.) After Neutralization</th>
<th>Cow milk khoa On dry matter basis</th>
<th>Buffalo milk khoa On dry matter basis</th>
<th>% Decrease Cow</th>
<th>% Decrease Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>33.95-36.37</td>
<td>23.95-25.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(34.95)</td>
<td>(24.66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.14</td>
<td></td>
<td>0.15</td>
<td>33.85-35.85</td>
<td>23.70-25.58</td>
<td>0.38</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(34.81)</td>
<td>(24.63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.15</td>
<td></td>
<td>33.68-36.52</td>
<td>24.54-25.01</td>
<td>0.62</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(34.73)</td>
<td>(24.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td></td>
<td>33.20-34.17</td>
<td>22.26-27.08</td>
<td>3.7</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(33.64)</td>
<td>(24.16)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average of six khoa samples each from cow and buffalo milks are given in brackets.
Lakhani (1971), after analysing 30 samples of khoa obtained from local shops at Poona could only get an average value of 19.58% protein which might reflect the composition of milk samples used for khoa preparation. Similarly reductions in the protein of milk on heating were observed by Kieferle and Gilostzd (1930).

**Effect of neutralization on the fat content of khoa samples.**

Data regarding the fat contents of khoa samples prepared from fresh and neutralized cow and buffalo milks have been presented in Table 4. It is evident from the table that the fat content of fresh khoa samples from cow and buffalo milks were 33.77 and 40.97% respectively. Fat content of khoa samples from cow and buffalo neutralized milk samples showed a decreasing trend. The khoa made from cow and buffalo milks, neutralized after attaining 0.24 developed acidity as % L.H., showed fat percentages (on dry matter basis) as 30.38 and 40.26 showing thereby a reduction of 8.5 and 1.76 respectively when compared against their control samples. Similarly, khoa samples made from cow and buffalo milks, after neutralizing the developed acidities of 0.25 and 0.34 L.H., exhibited a reductions of 11.9 and 15.06 in case of cow milk khoa and 27 and 3.2% in case of buffalo milk khoa samples. This indicated greater breakdown of fat molecules in cow milk khoa as compared to the buffalo milk khoa samples. The variations in the fat content of cow and buffalo (fresh and
### Table-4

Effect of neutralization on fat content of cow and buffalo milk khoa samples (gm/100 gm).

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Acidity (% L.A.) Before Neutralization</th>
<th>Acidity (% L.A.) After Neutralization</th>
<th>Cow milk khoa On dry matter basis</th>
<th>Buffalo milk khoa On dry matter basis</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Buffalo</td>
</tr>
<tr>
<td>0.14</td>
<td>-</td>
<td>21.21-48.53</td>
<td>38.30-42.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33.77)</td>
<td>(40.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.15</td>
<td>28.15-31.62</td>
<td>36.79-43.41</td>
<td>8.5</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(30.88)</td>
<td>(40.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td>28.78-30.83</td>
<td>34.25-45.27</td>
<td>11.9</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(29.75)</td>
<td>(39.83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>0.15</td>
<td>25.53-29.66</td>
<td>35.78-44.74</td>
<td>15.0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(28.69)</td>
<td>(39.66)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average of six khoa samples each from cow and buffalo milks are given in brackets.
neutralized) milk khoa samples were due to the differences in the fat content of the milks from two species. De and Roy (1952) reported the butterfat contents of normal cow and buffalo milk khoa samples as 34.8 and 45.9% respectively. In analysing 39 samples of khoa obtained from the local market of Poona, Desur and Lakhani (1971) observed the average value of fat as 27.24%. Rajorhia (1971) also concluded from his studies that the fat percentage of khoa made from cow milks (4 - 5.3% fat and 8.3 - 10.28% SNF) ranged from 22.3 to 29.3% in the samples which contained moisture from 26.4 - 33.5%. Similarly, the fat of khoa made from buffalo milks (4 - 7.8% fat and 8.9 - 10.6 SNF) indicated a range of 20.1 - 23.35%, with a moisture range of 28.4 - 34.4% in various samples. Zariwala et al. (1974) reported an average value of 27.14 ± 3.12% fat from 531 samples of khoa obtained from retailers in Bombay City having a moisture average of 23.12 ± 2.39%. The higher value for fat contents observed in our studies were due to the calculations on the dry matter basis of the khoa samples.

**Effect of neutralization on the physical properties of cow milk khoa samples.**

The object was to record the effect of neutralization on the physical properties namely, general appearance, body-texture and flavour etc. of khoa samples made from neutralization of the various degrees of developed acidities in cow milk as a result of time gap especially in hot seasons. Various physical parameters of different khoa samples made from cow milks have been presented in Table S.
Table 5

Effect of neutralization on physical quality of cow milk khoa samples.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Acidity (% L.A.) Before Neutralization</th>
<th>Acidity (% L.A.) After Neutralization</th>
<th>General Appearance</th>
<th>Body and Texture</th>
<th>Flavour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.14</td>
<td>0.14</td>
<td>-</td>
<td>Pale yellow colour, moist surface</td>
<td>Slightly hard sticky body &amp; sandy texture</td>
<td>Desirable smell, sweetish taste</td>
</tr>
<tr>
<td>0.2</td>
<td>0.15</td>
<td>0.15</td>
<td>Yellow colour, moist surface</td>
<td>Soft body, coarse texture</td>
<td>Slightly sour smell &amp; saltish taste</td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td>0.15</td>
<td>More yellow colour, moist surface</td>
<td>Soft body, coarse texture</td>
<td>Sour smell &amp; saltish taste</td>
</tr>
<tr>
<td>0.3</td>
<td>0.15</td>
<td>0.15</td>
<td>Intense yellow colour, moist surface</td>
<td>Very soft body coarse texture</td>
<td>Very sour smell &amp; too much saltish taste</td>
</tr>
</tbody>
</table>
(i) **General appearance**: Khoa prepared from fresh cow milk was found to lack the characteristic appearance. It had sticky appearance and high percentage of moisture and colour of the product was pale yellow. Its yield was also very less because of the lower fat and SNF contents of the milk. Rajorhia (1971) also observed lower yield of khoa from cow milk which was significant when compared against the yield of khoa from buffalo milk. The colour of khoa obtained from the neutralized milk was more yellow and the intensity increased as the developed acidity was higher. This might be due to the fact that the carbonate tended to increase the caramelization reaction rate in the production of khoa made from neutralized milk.

(ii) **Body and Texture**: Khoa made from fresh cow milk was slightly hard and had sticky body and sandiness. As regards khoa made from neutralized milks, the body of the product was soft and the softness increased with the degree of developed acidity and neutralization. The texture of khoa from the neutralized milk was observed to be of coarse nature.

(iii) **Flavour**: The flavour component of khoa from fresh cow milk was normal, desirable and sweet. Khoa made from neutralized milk (0.2% L.A.) was having slight sour small and saltish taste, whereas khoa obtained from more neutralized milk (0.25 L.A.) was marginally more sour and saltish in taste. However khoa from the neutralized milk having 0.3% developed acidity (as L.A.) was very sour and saltish in smell and taste respectively. Heating at higher temperatures in the preparation of khoa produced cooked flavour in
the products due to the liberation of sulphydryl groups from the serum proteins.

**Effect of neutralization on the physical properties of buffalo milk khoa.**

The object again was to study the effect of neutralization on the physical parameters, such as general appearance, body-texture and flavour etc. of buffalo neutralized milks having different developed acidities (Table 6). Khoa produced from fresh buffalo milk had white colour, soft body, and appreciable flavour, smooth texture. Its yield also was significantly more as against that from cow milk because of high fat and SNF percentages of the milk. Similarly, Rajorhia (1971) also observed higher yield of khoa from buffalo milk. Khoa from neutralized milk with 0.2% developed acidity as 6 L.A., was also observed to be white in colour, having nearly the similar soft body and texture but it had slight sour smell and saltish taste. Khoa prepared from buffalo neutralized milk having 0.25% L.A., was slightly yellow in colour, had loose body and sticky-naseness and comparatively more sour and saltish taste. In case of neutralized buffalo milk having 0.3% L.A., khoa made was more yellowish in colour, had very loose body, very sticky nature and possessed more sour and saltish taste. High heat treatment here again, if given, in the preparation of khoa from fresh or neutralized milks gave cooked flavour due to the liberation of sulphydryl groups from the whey proteins.
Table-6

Effect of neutralization on physical quality of buffalo milk khoa samples.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Acidity (% L.A.) Before Neutralization</th>
<th>Acidity (% L.A.) After Neutralization</th>
<th>General Appearance</th>
<th>Body and Texture</th>
<th>Flavour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.14</td>
<td>-</td>
<td>white colour</td>
<td>Soft body, smooth texture</td>
<td>Sweet flavour &amp; taste</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.15</td>
<td>white colour</td>
<td>Soft body, smooth texture</td>
<td>Slight sour smell &amp; saltish taste</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td>Slightly yellow colour</td>
<td>Loose body, less sticky texture</td>
<td>Sour smell &amp; saltish taste</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>0.15</td>
<td>More yellow colour</td>
<td>Very loose body &amp; sticky texture</td>
<td>Very sour smell &amp; too much saltish taste</td>
<td></td>
</tr>
</tbody>
</table>
SUMMARY

(1) Titratable acidity (° L.A.) of khoa prepared from different acidified cow and buffalo milk samples showed an increasing trend. The acidity developed in khoa made from buffalo neutralized milk was comparatively more when compared against the acidity from khoa made from corresponding cow neutralized milk.

(2) Lactose was observed to be higher in khoa prepared from cow milk as compared from buffalo milk. It also exhibited a decreasing trend, depending upon the neutralizer used. The reductions were comparatively higher in case of khoa from cow neutralized milk as against the reductions in case from buffalo neutralized milk.

(3) Total protein was reduced marginally in both the types of khoa, but the reduction in cow neutralized milk khoa was more.

(4) Fat content of khoa from cow milk was higher as against that from buffalo milk. More reduction of fat was observed in khoa made from cow milk, when compared against fat contents of khoa from buffalo neutralized milk samples.

(5) Khoa made from fresh buffalo milk was white in colour, having soft body, smooth texture and possessed a pleasant flavour, whereas khoa made from fresh cow milk was slightly pale in colour, with comparatively hard and sticky body, and a sweet and desirable
odour. Khoya made from neutralized cow and buffalo milks was observed to have a yellowish colour, loose body, more sticky texture and was observed to have saltish taste and sour smell.
BIBLIOGRAPHY


