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Effect of milk adulteration on chemical composition of Khoa

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Abstract
Milk is a perishable in nature and cannot be stored for a very long period. The lack of cooling facilities to keep the liquid milk fresh led to the diversion of milk for preparation of khoa as an indigenous milk product with comparatively longer shelf life. Despite its high economic and nutritional importance, it has not received adequate attention for monitoring its quality. As there is no strict quality parameters prescribed to khoa, adulteration is a common practice. Study was conducted to evaluate effect of adulteration of milk on gross composition of khoa prepared from such adulterated milk. From amongst the different parameters of gross composition (moisture, fat, protein, carbohydrate and ash content) of khoa, the true protein content of khoa was found the most promising parameter to develop a strategy for detection of non protein nitrogenous compound like urea used as an adulterant in khoa.

Keywords: Adulteration, Khoa, Urea

Introduction
India is the world's largest dairy producer, 165.4 million tonnes in the year 2016-17 (Annual report, 2016-17). About 50% of milk is converted in to traditional dairy products. Amongst the various indigenous milk products, Khoa occupies the top most position (Choudhary *et al*., 2015) [10]. Khoa production is the easiest way of preserving rurally produced milk by its conversion into khoa, a semi-solid heat desiccated product. Khoa is an important intermediate product utilized for the production of variety of sweets (Choudhary *et al*., 2017) [8]. Food adulteration is fast spreading disease and khoa is not an exception. The demand of market khoa is enhanced remarkably during festive seasons and adulteration of khoa becomes more rampant (Wanjari *et al*., 2016) [26]. Manufacturing and marketing of khoa is mainly handled by an unorganised sector and its high price often makes it susceptible to adulteration for gaining monetary profit. In recent years, the dimension of milk adulteration is changing from individual adulterant to a blend of adulterants in form of artificial fluid - so called synthetic milk. Till the date, there is limited research work carried out on detection of adulteration in khoa. Therefore, study was undertaken to evaluate effect of adulteration of milk with the artificial fluid on composition of 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) [11]. The khoa was also prepared from pure milk to act as control.

Analysis of khoa
The samples of the khoa were prepared from the adulterated milk as well as from pure milk and analysed for moisture, fat, protein, carbohydrate and ash content.

Moisture content
The moisture content of khoa sample was determined by Gravimetric method (BIS, 1981) [4].

Fat content
Fat content of khoa was estimated by the Gerber method described for cheese (BIS, 1979) [5].

Protein content
Crude and true protein contents of khoa were calculated from its total nitrogen content and protein nitrogen content respectively by multiplying the value of %N by the factor of 6.38. The nitrogen content was determined by semi-micro Kjeldahl method (BIS, 1981) [4] using Kjel-plus digestion system (Model-KES 20L VA DLS, M/s. Pelican Instruments, Chennai) and Kjel-plus semi-automatic distillation system (Model-Distil M, M/s. Pelican Instruments, Chennai). For crude protein nitrogen the sample of khoa was taken, whereas, for protein nitrogen content khoa slurry was prepared and further procedure were carried out using A.O.A.C method (A.O.A.C, 1990) [3].

Ash content
The ash content of khoa sample was determined by Gravimetric method (BIS, 1969) [6].

Carbohydrate content
The carbohydrate content of khoa sample was determined by difference method.
Carbohydrate (%) = 100 – (% moisture + % fat + % protein + % ash)

Statistical analysis
The data obtained during the present investigation were analyzed by Completely Randomized Design (CRD) (Steel and Torrie, 1980) [24] with equal number of observation.

Results and Discussion
The data obtained on gross composition (moisture, fat, protein, carbohydrate and ash content) of khoa are presented in Table 1.

Table 1: Gross Composition of Khoa

Rate of adulteration (%)	Moisture (%)	Fat (%)	Protein (%)		Carbohydrate (%)	Ash (%)
			Crude	True		
0	27.18	27.08	12.46	9.44	28.79	4.49
20	26.86	27.35	12.23	8.61	28.49	5.07
30	25.62	26.85	12.06	7.66	28.60	5.16
40	23.93	26.66	12.15	7.08	29.34	5.92
Annova Table						
S.Em.	0.18	0.21	0.19	0.14	0.20	0.14
CD	0.55	0.63	0.59	0.44	0.61	0.45
Test	*	NS	NS	*	*	*
CV %	1.39	1.53	3.15	3.52	1.34	5.71

Effect on moisture content of khoa
The examination of data showed that the average value of moisture content of control sample of khoa was 27.18 per cent. Use of urea as a source of nitrogen had significant effect on moisture content of the resultant khoa. When the artificial fluid containing urea was mixed at the rate of 30 per cent or above, the moisture content of the khoa decreased significantly compare to the khoa prepared from pure milk.
The values of moisture content in khoa are reported to vary 18.5 (Sai Prakash, 1981; Gothwal and Bhavdasan, 1992) [22, 12] and 35.5 (Bhadania, 2005; Sapre and Deodhar, 1991; Miyani, 1988; Kumar and Srinivasan, 1982) [7, 23, 19, 15]. However, majority of the data are within the range of 17-36 (Lad, 2016) [16]. Therefore, moisture content of khoa prepared from pure milk obtained in present was very well within the normal range reported in the literature. However, moisture content in adulterated samples of khoa is not reported in the literature. Therefore, moisture content of the khoa prepared from the adulterated khoa could not be compared with those reported in the literature.
McGann & Fox (1974) [18] reconstituted micelles obtained from urea-treated milk. The authors observed that reconstituted micelles were considerably smaller than native micelles. Therefore, lower moisture content in khoa containing urea might be attributed to possible such alteration in casein micelles by the presence of urea. Probably similar phenomenon might also be playing role in case of melamine due to presence of its free amine groups.

Effect on fat content of khoa
Since all natural oils and fats are chemically mainly contains triglycerides (esters of fatty acids with glycerol). Therefore, bulk of the oils and fats are chemically are similar to a larger extent in their chemical makeup. Moreover, quantity wise in preparation of the artificial fluid, vegetable oil was taken in amount equivalent to fat content of the pure milk. The examination of data showed that the average value of fat content of control sample of khoa was 27.08 per cent. The fat content of khoa prepared from adulterated milk was almost similar to that of the khoa prepared from pure milk. The results indicated that neither any NPN compounds nor any carbohydrate had effect on fat content of the khoa.
The values of fat content in khoa are reported to vary 20.5 (Miyani, 1988; Kumar and Srinivasan, 1982; Sapre and Deodhar, 1991) [19, 15, 23] and 39.4 (Narain and Singh, 1981; Hemavathy and Prabhakar, 1983) [20, 13]. However, majority of the data are within the range of 21 and 39.5 (Lad, 2016) [16]. Therefore, fat content of khoa prepared from pure milk obtained in present was very well within the normal range reported in the literature. However, fat content in adulterated samples of khoa is not reported in the literature. Therefore, fat content of the khoa prepared from the adulterated khoa could not be compared with those reported in the literature.

Effect on crude and true protein content of khoa
The examination of data showed that the average value of crude protein content of khoa prepared from pure milk was 12.46 per cent. The crude protein content of khoa prepared from adulterated milk containing urea was almost similar to

that of the khoa prepared from pure milk. On the other hand, examination of data showed that the average value of true protein content of khoa prepared from pure milk was 9.44 per cent. The true protein content of khoa prepared from adulterated milk containing urea progressively decreased with increasing rate of adulteration of milk with artificial fluid. Even at the lowest rate (20 %) of adulteration the true protein content of the resultant khoa decreased significantly both in case of urea. Such decreasing true protein content was attributed to dilution of the true protein due to mixing of the artificial fluid.

The values of crude protein content in khoa are reported to vary 11.3 (Patel, 1982; Bhadania, 2005; Gothwal and Bhavdasan, 1992) [21, 7, 12] and 23.39 (Narain and Singh 1981; Abhaykumar *et al*., 1975) [20, 1]. However, majority of the data are within the range of 11 and 25 (Lad, 2016) [16]. Therefore, crude protein content of khoa prepared from pure milk obtained in present was very well within the normal range reported in the literature. However, crude protein content in adulterated samples of khoa is not reported in the literature. Therefore, crude protein content of the khoa prepared from the adulterated khoa could not be compared with those reported in the literature.

The almost similar crude protein content of khoa prepared from pure milk and adulterated milk, might be due to the fact that amount of urea was taken in a calculated amount to supply nitrogen almost similar to the nitrogen content of the pure milk. The results indicated that when protein content in khoa determined from total nitrogen content it did not distinguish between non protein nitrogen (NPN) and protein nitrogen. Similar views where expressed by Xue *et al*. (2011) [27]. These authors opined that Kjeldahl method directly does not measure the protein content. In fact, it quantitatively determines nitrogen content, which intern indicates the protein content. Generally, in analysis of protein is not fractionated from the food, prior to determination of nitrogen by Kjeldahl method, owing to very complex nature of food. As a result it determines protein nitrogen as well as non-protein nitrogen to gather as total nitrogen. Therefore, it determines crude protein instead of true protein content. Lad & Aparnathi (2017) [17] stated that such limitation of the method encourages the fraudulent addition of NPN compounds in food to elevate its apparent protein content. Therefore, protein-specific analytical procedures are adopted. Approaches to mitigate future fraudulent or harmful adulteration of food protein ingredients, to protect public health and to facilitate trade will require the development and adoption of reliable, specific quantification of protein.

Thus, it was clearly evident from forgoing results obtained in the study and supportive evidences in the literature that true protein content determination in khoa can serve as a promising tool to detect adulteration by mixing commonly used NPN compound like urea.

Effect on carbohydrate content of khoa

The examination of data showed that the average value of total carbohydrate content of khoa prepared from pure milk was 28.79 per cent. The total carbohydrate content in khoa was calculated by difference. Thus total carbohydrate content was derived by subtracting moisture, fat, crude protein and ash content from the value of respective TS content of the samples. Therefore, any change in moisture, fat, crude protein and ash content of the khoa due to adulteration resulted in to corresponding change in carbohydrate content of the sample of khoa. From examination of data given in Table 1 for moisture, crude protein and ash content of khoa respectively; it was evident that all these three constituents decreased in adulterated sample of khoa. Therefore, increase in total carbohydrate content of the khoa upon adulteration was attributed to decrease in moisture, crude protein and ash content of khoa.

The values of carbohydrate content in khoa are reported to vary 18.8 (Patel, 1982; Sapre and Deodhar, 1991) [21, 23] and 36.2 (Narain and Singh, 1981; Srinivasan and Anantakrishnan, 1964) [20]. However, majority of the data are within the range of 18.3 and 36 (Lad, 2016) [16]. Therefore, carbohydrate content of khoa prepared from pure milk obtained in present was very well within the normal range reported in the literature. However, carbohydrate content in adulterated samples of khoa is not reported in the literature. Therefore, carbohydrate content of the khoa prepared from the adulterated khoa could not be compared with those reported in the literature. Upon adulteration of milk with the artificial fluid, the total carbohydrate content of the resultant khoa increased progressively. Its effect on total carbohydrate content was significantly significant.

Effect on ash content of khoa

The examination of data showed that the average value of ash content of khoa prepared from pure milk was 4.49 per cent. The ash content of khoa prepared from adulterated milk containing urea progressively increased with increasing rate of adulteration of milk with artificial fluid. Even at the lowest rate (20 %) of adulteration the ash content of the resultant khoa increased significantly both in case of urea.

The values of ash content in khoa are reported to vary 2.92 (Patel, 1982; Abhaykumar *et al*., 1975; Sai Prakash, 1981) [21, 1, 22] and 4.84 (Narain and Singh, 1981; Hemavathy and Prabhakar, 1983; Miyani, 1988) [20, 13, 19]. However, majority of the data are within the range of 2.9 and 4.85 (Lad, 2016) [16]. Therefore, ash content of khoa prepared from pure milk obtained in present was very well within the normal range reported in the literature. However, ash content in adulterated samples of khoa is not reported in the literature. Therefore, ash content of the khoa prepared from the adulterated khoa could not be compared with those reported in the literature.

From the list of different ingredients (urea/melamine, sucrose/maltodextrin/starch, ammonium sulphate/sodium chloride/potassium nitrate, vegetable oil, detergent and formaldehyde/hydrogen peroxide/sodium hydroxide) used in preparation of the artificial fluid, theoretically only inorganic compounds (ammonium sulphate, sodium chloride, potassium nitrate or sodium hydroxide) could contribute to ash content. However, these inorganic compounds were taken in a calculated amount in such a way their weight was almost equivalent to the inorganic compounds of the milk replaced by them.

As per the logic discussed above values of ash content of the resultant khoa from adulterated milk were expected to remain same as that of the khoa from pure milk samples. The probable reasons for increase of ash content in khoa from adulterated milk might be resulted from formation of oxides of some elements during ashing process. The two most probable elements are nitrogen (N) and sulphur (S). The source of nitrogen could be two amine groups in case of urea and three amine groups as well as three nitrogen atoms of the ring structure in case of melamine. Similarly, the source of sulphur atom could be sulphur group of the detergent.

This logic supported by the opinion expressed by Jenness and pattern (1959) [14]. These authors opined that the ash does not

truly represent the salt system present in the original milk. Oxidation during incineration results in the formation of oxides of the metal.

Ahirwar *et al*. (2015) [2] studied the quality changes in milk adulterated with detergent, urea, ammonium sulphate and neutralizers. Data of the study indicated that ash content of the milk increased progressively with increased in urea content (0.5 to 1, 3, 5 and 7 %). Similarly, ash content of the milk increased progressively with increased in detergent content (0.1 to 0.3, 0.5, 0.7 and 0.9 %). Therefore, in present study increase of ash content of khoa prepared from adulterated milk was very well collaborated with the results reported by these authors.

Conclusion

From amongst the different parameters of gross composition (moisture, fat, protein, carbohydrate and ash content) of khoa, the true protein content of khoa was found the most promising parameter to develop a strategy for detection of non protein nitrogenous compounds used as an adulterant in khoa.

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