

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.703.205>

Chemical Constituents and Autoxidative Stability of Milk Lipids of Kankrej Cow

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ABSTRACT

Keywords

Kankrej milk fat,
Unsaponifiable matter,
Cholesterol, β -Carotene,
Vitamin A, Moisture and
free fatty acids

Article Info

Accepted:

16 February 2018

Available Online:

10 March 2018

Various chemical constituents of Kankrej milk fat namely moisture, free fatty acids, carbonyl value and unsaponifiable matter were analyzed in the present study for a period of 12 months. The average unsaponifiable content of Kankrej milk fat sample was 423 mg/100 g, while cholesterol content was found to be 320 mg/100g. β -Carotene content of Kankrej milk fat was observed to be lower (2.9 μ g/g) while vitamin A level of Kankrej milk fat was observed to be higher (34.5 IU/g) than those reported for other indigenous breeds. An average moisture content of Kankrej milk fat sample was 0.19 per cent, while free fatty acids content was found to be 0.20 per cent. Total carbonyl value of Kankrej milk fat was 4.9 μ M/g of milk fat. Autoxidative study at accelerated storage conditions ($80\pm 1^\circ\text{C}$) revealed that Kankrej milk fat showed better oxidative stability may be due to lower content of unsaturated fatty acids. The average value for unsaponifiable and cholesterol content in Kankrej milk fat was highest during November to February while levels of β -carotene and vitamin A were higher during July to October reflecting seasonal variations.

Introduction

Kankrej breed of cattle is one of the 26 recognized cattle breed (*Bos indicus*) of India. They are one of the heaviest dual purpose breed in India. The breed originated in north Gujarat in the erstwhile Bombay province of India. They takes their name from geographical area i.e. Kankrej taluka of Banaskantha district in Gujarat. They are found in the area southeast of Rann of Kutch comprising Mehsana, Ahmedabad, Kheda, Anand, Sabarakantha and Banaskantha districts of Gujarat and Barmer and Jodhpur districts of Rajasthan. Milk fat obtained from

Kankrej cow shows seasonal variation in chemical constituents in which unsaponifiable matter includes cholesterol, carotene and vitamins.

The unsaponifiable matter which can be obtained from oils and fats after saponification with alkali and subsequent extraction by a suitable organic solvent constitutes less than 2 per cent by weight of fat. It is a repository of so many valuable constituents, like sterols (cholesterol and phytosterols), fat-soluble vitamin (A, D, E and K), hydrocarbons such as squalene, pigments, etc. Milk fat contains unsaponifiable matter in the range of 0.30 to

0.45 per cent by weight (Jeness and Patton, 1969) chiefly consisting of cholesterol (0.25 to 0.40 per cent by weight of fat). Cholesterol is the major constituent of the unsaponifiable matter of milk fat. It exists both in the esterified and in free forms.

Carotene is a chief colouring material in milk fat, although other related carotenoids (Xanthophyll) are present in small amount. Carotene is derived from green plant sources in the feed of the lactating cow, which is the only mammal to transfer such colour to milk fat. It is found free from chemical combination in milk fat. It is stable to oxidative agents but in fat it may be oxidized and bleached under unfavourable storage conditions. Carotene present in milk fat is of considerable importance as a precursor of vitamin A. further, it plays an important role in the autoxidative stability of milk fat (Kempanna and Unnikrishnn, 1986; Surendra Nath and Rama Murthy, 1983).

Ghee owes its pleasing flavour to several free fatty acids (FFA). These compounds are produced from fatty acid glycerides by the mechanism involving lipolysis (through lactic streptococci)/hydrolysis during the fermentation of milk or cream and/or processing treatments while preparing ghee (Wadhwa and Jain, 1990).

Carbonyls play an important role in contributing to the flavour of ghee. This class of compounds includes, broadly, monocarbonyls and dicarbonyls. Monocarbonyls further constitute alkan-2-ones (90 per cent), alkanals (6 per cent), alk-2-enals (2 per cent) and alk-2, 4-dienals (2 per cent). Alkan-2-ones are produced by the hydrolysis of ketonogenic glycerides (β -keto glycerides) followed by decarboxylation of β -keto carboxylic acids during various processing treatments involved in the preparation of ghee (Wadhwa and Jain, 1989).

Materials and Methods

Feeding and management of animals

Milk samples were collected from group of pure breed of Kankrej cow maintained at the LRS (Livestock research station), Anand Agricultural University, Anand. The herd consisted of 18 cows at different stages of lactation. The cows were fed *ad libitum* amount of mixed green fodder consisting of maize, sorghum and Napier grass (Coimbatore-2) during summer and monsoon under farm conditions.

During summer the proportion of Napier grass fed to the animal was relatively high compared to other seasons. During winter along with sorghum sunflower, oats and cowpea was also fed. The proportion of legume, cowpea was more than 70 per cent of total green fodder during winter. The cows were also given concentrate (Amul dhan) at the rate of 0.5 kg / litre of milk / day. During all season sorghum hay was fed. Each animal was fed 17-18 kg of dry matter/ day.

Collection of milk sample

The pooled Kankrej cow milk sample was collected at 15 days interval from Livestock Research Station (LRS), AAU, Anand for a period of one year, from March 2016 to February 2017. Total 24 replications for milk of Kankrej cow were carried out. All cows were maintained under identical conditions of feeding and management. The feed given to animals throughout the year consisted of normal concentrate and roughages.

Preparation of ghee sample

The milk sample were warmed to 40°C and then separated into cream using cream separator. Ghee sample was prepared in laboratory by direct cream method (Heat

clarification at 120°C/ no hold). Ghee was then filtered through 6-8 fold muslin cloths followed by Whatman No. 4 filter paper, filled in glass bottles, cool to room temperature and kept in a refrigerator at a temperature of 7±1°C till further analysis.

Analysis of ghee

Ghee samples were analysed for Chemical constituents such as unsaponifiable matter in which cholesterol; β -Carotene and vitamin A content, moisture content, free fatty acids, carbonyl value and also peroxide value by using the method specified in BIS (1981).

Statistical analysis of data

The data were analysed using Completely Randomized Design (CRD), while data on storage studies were analysed by two factorial CRD as per the methods described by Steel and Torrie (1980).

Results and Discussion

Various chemical constituents of Kankrej milk fat namely unsaponifiable matter, moisture, free fatty acids and carbonyl value and as well as autoxidative stability were analyzed in the present study for a period of 12 months. The unsaponifiable matters namely cholesterol content, vitamin A and β -carotene were determined for Kankrej milk fat. The results and findings are discussed below.

Unsaponifiable matter

The unsaponifiable matter consists mainly of cholesterol, carotene, vitamin A, D, E and K certain sterols and hydrocarbons such as squalene. Milk fat contains unsaponifiable matter in the range of 0.30 to 0.45 per cent by weight (Jenness and Patton, 1969) chiefly consisting of cholesterol (0.25 to 0.40 per cent by weight of fat).

The unsaponifiable content of Kankrej milk fat sample ranged from 415 to 435 mg/100 g with an average 423 mg/100 g (Table 1).

Deshpande *et al.*, (2008) found unsaponifiable matter content of 780 mg/100 g in cow milk fat while Dhurvey *et al.*, (2012) reported unsaponifiable matter of 923 mg/100 g of cow ghee.

Unsaponifiable matter in Kankrej milk fat was comparable with the values reported reported by other authors.

It also showed seasonal variations the average value for unsaponifiable matter content was highest during November to February (432.7 mg/100 g) and lowest during the period from March to June (415 mg/100g), while it has 421.7 mg/100 g during the period from July to October for Kankrej milk fat (Table 2).

Sharma (1989) in his study reported that cow ghee contained higher level of unsaponifiable matter in winter which was 444 to 574 mg/100 g; average, 507mg/100 g and lower level in summer 422 to 548 mg/100 g; average, 475 mg/100 g.

The observed result of unsaponifiable matter in the present study is similar to the values reported in the literature.

Cholesterol

The average cholesterol content of Kankrej milk fat samples was found to be 320 mg/100g with a range of 318 to 334 mg/100 g. It was also observed that bulk of the unsaponifiable matter was constituted with cholesterol (Table 1); on an average, 75.80 per cent of unsaponifiable matter was cholesterol. The cholesterol content of Kankrej milk fat was not high and was comparable with the values reported by other researchers (Kumar *et al.*, 2010; German and Dillard, 2013).

It can be seen from Table 2 that the average value for cholesterol content was highest during the period from November to February (327.2 mg/100 g) and lowest during the period from July to October which is 316 mg/100 g, while an average cholesterol content of 319 mg/100g was observed during March to June for Kankrej milk fat.

Bindal and Jain (1973) in their study reported cholesterol content of 342.6 mg/100 g in spring (March to May), 315.3 mg/100 g in summer (June to August.), 330.6 mg/100 g in autumn and 340.8 mg/100 g of cow milk fat in winter (December to February). They correlated higher content of cholesterol during winter and spring season to high proportion of leguminous feed given to animals during these seasons.

In case of Kankrej cows they were fed more proportion of cow pea and oats during November to February and inclusion of higher proportion of leguminous fodder have resulted in higher observed cholesterol levels during this period and as observed by Bindal and Jain (1973).

β -carotene and vitamin A

The average β -carotene and vitamin A content of Kankrej milk fat sample was found to be 2.9 $\mu\text{g/g}$ with a range from 2.4 to 3.7 $\mu\text{g/g}$ and 34.5 IU/g with a range from 24.5 to 45.0 IU/g, respectively (Table 3).

From the data given by Kumar *et al.*, (2010); Rafalowski *et al.*, (2014) we can say that β -Carotene content in Kankrej cow milk fat is lower (3.0 $\mu\text{g/g}$) than that reported in literature. The probable reason can be attributed to higher bioconversion of β -carotene to vitamin A in Kankrej cow. Assuming a bioconversion of β -carotene to retinol of 1:0.3 (FAO/WHO, 1988) or 1:0.5 (Institute of Medicine (IOM), 2001), it can be

calculated that β -carotene contributes 20% to the vitamin A activity in milk according to the FAO/WHO conversion factors or 33% according to the IOM conversion factors.

Breed appears to be one of the major factors affecting the carotene level of milk fat; other important factor being feed. In our case β -carotene content during July to October was higher compared to rest of the period of study. This may be due to availability of more carotene in the green fodder during rainy season. The level of vitamin A observed for Kankrej milk fat in the present study is on higher (34.5 IU/g) side of the values reported for milk fat of other breeds of cattle. The probable reason can be attributed to higher content of β -carotene in the milk fat during these periods resulting in higher bioconversion to vitamin A.

It can be seen from Table 4 that the average value for β -Carotene and vitamin A content in Kankrej milk fat was highest during the period from July to October (3.4 $\mu\text{g/g}$ and 44.2 IU/g respectively) and lowest during November to February (2.5 $\mu\text{g/g}$ and 24.6 IU/g) i.e. winter season. During the period from March to June, the mean value of β -Carotene (2.9 $\mu\text{g/g}$) and vitamin A (24.60 IU/g) content in Kankrej milk fat was in range between the above two periods.

In the present study highest β -carotene and vitamin A content was observed during the month of July to October i.e. rainy season. Although almost same type and quantity of green fodder was fed to the animals during the period March to June (summer season) and July to October (rainy season), the carotene and vitamin A content was higher during July to October. This can be very well justified by the fact that carotene content is not only influenced by the quantity of green fodder but also by the carotene present in green fodder fed to the animals.

Table.1 Unsaponifiable matter and cholesterol contents in Kankrej milk fat

Period	Unsaponifiable matter (mg/100 g)	Cholesterol (mg/100 g)	Proportion of cholesterol in unsaponifiable matter (%)
March 2016	415	319	76.86
April 2016	415	319	76.86
May 2016	415	318	76.62
June 2016	415	320	77.10
July 2016	425	314	73.88
August 2016	422	317	75.11
September 2016	420	318	75.71
October 2016	420	315	75.00
November 2016	435	320	73.56
December 2016	435	322	74.02
January 2017	430	333	77.44
February 2017	431	334	77.49
Average	423	320	75.80
SEm±	0.8	1.0	-
CD (0.05)	2.5	3.0	-
CV %	0.2	0.4	-

Table.2 Seasonal variations in unsaponifiable matter and cholesterol

Period	Unsaponifiable matter* (mg/100g)	Cholesterol* (mg/100g)	Proportion of cholesterol in unsaponifiable matter* (%)
March 2016 to June 2016 (Summer season)	415.0 ^b	319.0 ^b	76.6
July 2016 to October 2016 (Rainy season)	421.7 ^a	316.0 ^a	74.9
November 2016 to February 2017 (Winter season)	432.7 ^c	327.2 ^c	75.6
SEm±	0.7	1.4	-
CD (0.05)	2.1	4.3	-
CV %	0.4	1.3	-

* The values are average of samples collected during four months for each period
 Values within columns (treatments) with same lowercase superscript did not differ significantly (P<0.05) from each other

Table.3 β - Carotene and vitamin A content in Kankrej milk fat

Period	β – Carotene ($\mu\text{g/g}$)	Vitamin A (IU/g)
March 2016	3.0	33.5
April 2016	3.0	33.5
May 2016	3.0	37.5
June 2016	3.0	35.0
July 2016	3.1	45.0
August 2016	3.6	43.0
September 2016	3.5	45.5
October 2016	3.7	43.0
November 2016	2.5	24.5
December 2016	2.6	24.5
January 2017	2.5	25.0
February 2017	2.4	24.5
Average	2.9	34.5
SEm \pm	0.1	1.4
CD (0.05)	0.2	4.3
CV %	3.8	5.7

Table.4 Seasonal variation in β -carotene and vitamin A

Period	β – Carotene* ($\mu\text{g/g}$)	Vitamin A* (IU/g)
March 2016 to June 2016 (Summer season)	2.90 ^b	34.80 ^b
July 2016 to October 2016 (Rainy season)	3.40 ^c	44.20 ^c
November 2016 to February 2017 (Winter season)	2.50 ^a	24.60 ^a
SEm \pm	0.05	0.68
CD (0.05)	0.17	2.00
CV %	5.53	5.58

* The values are average of samples collected during four months for each period

Values within columns (treatments) with same lowercase superscript did not differ significantly (P<0.05) from each other

Table.5 Moisture, free fatty acids content and Total carbonyl value in Kankrej milk fat

Period	Moisture content (%)	Free fatty acids* (% oleic acid)	Total carbonyl value (µm/g)
March 2016	0.19	0.20	4.70
April 2016	0.19	0.20	4.85
May 2016	0.19	0.20	4.75
June 2016	0.20	0.20	4.85
July 2016	0.20	0.20	5.25
August 2016	0.20	0.20	5.55
September 2016	0.20	0.20	5.25
October 2016	0.20	0.20	5.25
November 2016	0.20	0.20	4.60
December 2016	0.20	0.20	4.63
January 2017	0.20	0.20	4.73
February 2017	0.20	0.20	4.94
Average	0.19	0.20	4.94
SEm±	0.01	0.00	0.16
CD (0.05)	NS	NS	0.49
CV %	3.58	0.07	4.58
NS: non-significant			

Table.6 Rate of autoxidation of Kankrej milk fat stored at 80 ± 1°C

Treatments (Milk fat samples)	Storage period (Days)										Average of treatments
	1	2	3	4	5	6	7	8	9	10	
	Peroxide value (millimoles of O ₂ /kg of fat)										
March 2016	0.1	0.29	0.42	0.62	0.85	1.25	1.52	1.84	2.25	2.60	1.17
April 2016	0.1	0.26	0.43	0.62	0.86	1.25	1.53	1.81	2.24	2.60	1.17
May 2016	0.1	0.26	0.42	0.63	0.86	1.20	1.55	1.83	2.24	2.60	1.17
June 2016	0.1	0.27	0.42	0.63	0.86	1.25	1.53	1.85	2.23	2.59	1.17
July 2016	0.1	0.29	0.44	0.64	0.85	1.25	1.55	1.85	2.24	2.65	1.18
August 2016	0.1	0.29	0.47	0.66	0.86	1.24	1.54	1.87	2.27	2.64	1.19
September 2016	0.1	0.29	0.45	0.68	0.87	1.26	1.57	1.85	2.27	2.63	1.20
October 2016	0.1	0.3	0.45	0.67	0.87	1.28	1.58	1.86	2.28	2.67	1.20
November 2016	0.1	0.28	0.44	0.66	0.85	1.25	1.55	1.84	2.27	2.64	1.19
December 2016	0.1	0.28	0.47	0.62	0.84	1.25	1.54	1.88	2.26	2.62	1.18
January 2017	0.1	0.27	0.48	0.63	0.84	1.26	1.55	1.87	2.25	2.62	1.18
February 2017	0.1	0.27	0.45	0.62	0.86	1.25	1.54	1.84	2.27	2.63	1.18
Average of PV	0.1	0.28	0.44	0.64	0.85	1.25	1.54	1.85	2.25	2.62	1.18
CD(0.05) T=NS; P=NS; TxP=NS											
NS: non-significant											

As reported by Hulshof *et al.*, (2006) carotene content in the plant is subjected to seasonal variations; decreases during summer and is highest during rainy season. The reason for higher content can be correlated to availability of high carotene content in the green fodder fed to the animals during the period July to October i.e. rainy season. Subsequently, a higher content of β -carotene paved way for greater bioconversion into vitamin A resulting in its increased average content during July to October.

Moisture Content

The moisture content of the Kankrej milk fat sample was in the range of 0.19 to 0.20 per cent with an average 0.19 per cent (Table 5). Thus, the Kankrej milk fat samples meet the standards for moisture given by FSSA (2011) (maximum permitted value is 0.5 per cent) and AG Mark (1938) (maximum permitted value is 0.3 per cent). The moisture content was not affected by seasons.

Changade *et al.*, (2006) reported that moisture content of 0.24 per cent in ghee. Meghwal *et al.*, (2012) reported moisture content of 0.122 per cent in cow ghee.

The presence of moisture accelerates the hydrolysis of fats, and thereby released free fatty acids, which were prone to quicker autoxidation than intact glycerides (Sen, 2011).

Thus, the lower moisture content of Kankrej milk fat can give good keeping quality to the milk fat by preventing occurrence of rancidity in the ghee.

Free Fatty Acids (FFA)

The free fatty acids content of Kankrej milk fat samples was found to be 0.20 per cent oleic acid (Table 5).

Small portion of fatty acids are always present in fresh milk fat; larger percentage found in the fat isolated from milk or cream that has been subjected to bacterial action or in fat that has been stored for some time. The presence of free fatty acids in unlipolyzed fat may be the result of an incomplete esterification of glycerides in the mammary gland (Kurtz, 1980).

The level of FFA in Kankrej milk fat was lower than the maximum limit suggested by FSSA (2011) (maximum permitted value is 3 per cent as oleic acid) and AG Mark (1938) (maximum permitted value is 3 per cent oleic acid for standard grade ghee) reflecting its quality and freshness.

Total Carbonyl Compounds

The total carbonyl value for Kankrej milk fat samples ranged from 4.6 to 5.5 $\mu\text{m}/\text{g}$ with an average value of 4.9 $\mu\text{m}/\text{g}$ (Table 5).

Carbonyl compounds are considered to play an important role in the development of flavor profile of dairy products. Rama Murthy and Jain (1973) observed that total carbonyls in cow milk fat ranged from 4.2 to 4.5 $\mu\text{m}/\text{g}$.

Rate of Autoxidation of Kankrej milk fat

In general, most of fats/oils have reasonably long induction periods under normal condition of their storage. However, induction period, which is a measure of keeping quality of a fat, is influenced by many factors. Therefore, measurement of induction period by accelerated shelf life test is commercially very important to predict the shelf life of oils and fats. In all these tests, the rate of autoxidation is known to increase with the increase in the temperature of reaction and it is assumed that a definite relationship exists between the rate of increase in temperature of storage and rate of increase in autoxidation.

Therefore, increase of temperature is usually employed in accelerated shelf life tests. Peroxide value is monitored to assess the rate of autoxidation.

In the present study, the rate of autoxidation of Kankrej milk fat samples was assessed by storing the sample at $80 \pm 1^\circ\text{C}$ and then analysing them regularly until the peroxide value exceeded 2.6 millimoles of O_2/kg of fat (BIS 1981). According to BIS the ghee sample having a peroxide value of 2.6 to 3.5 millimoles of O_2/kg , is interpreted as of poor quality. The result of analysis is presented in Table 6.

During accelerated storage ($80 \pm 1^\circ\text{C}$) study, the peroxide value (millimoles of O_2/kg of fat) of the sample increased sharply from the 2nd day and continued to increase throughout the storage period. On the 10th day of accelerated storage of ghee at $80 \pm 1^\circ\text{C}$, the peroxide value was 2.60 and above, which as per BIS (1981) is interpreted as 'poor quality'. So the accelerated storage study was discontinued on reaching this peroxide value. The statistical analysis showed that changes in the peroxide value were not significant for the period of storage (days) as well for experimental samples (milk fat samples) while interaction between period of storage and treatments was non-significant.

Gandhi *et al.*, (2013) in their study reported peroxide value for ghee sample was 10 millimoles of O_2/g of fat on the 10th day of storage at $80 \pm 1^\circ\text{C}$. Parmar *et al.*, (2013) observed that shelf life of Arjuna ghee sample was 8 days at $80 \pm 1^\circ\text{C}$ as compared 2 days in the control. Mehta *et al.*, (2015) reported that peroxide value for ghee sample was 6.38 meq of O_2/kg of fat on the 10th day of storage at $80 \pm 1^\circ\text{C}$. Vaghela (2017) reported that peroxide value for ghee sample was in the range of 3.5 to 4.2 with an average 3.8 millimoles of O_2/kg of fat on the 10th day of storage at $80 \pm 1^\circ\text{C}$.

The rate of autoxidation of Kankrej milk fat observed in the present study was lower than the reported earlier for milk fat stored at $80 \pm 1^\circ\text{C}$. Kankrej milk fat may have a high concentration of saturated fatty acids and low concentration of unsaturated fatty acids.

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How to cite this article:

Minal Bharwade, Smitha Balakrishnan and Nisha Chaudhary. 2018. Chemical Constituents and Autoxidative Stability of Milk Lipids of Kankrej Cow. *Int.J.Curr.Microbiol.App.Sci.* 7(03): 1732-1742. doi: <https://doi.org/10.20546/ijcmas.2018.703.205>