Heat Induced Changes on Levels of Bound Sialic Acid and Hexose of Whole Acid and K-caseins Obtained from Milk of Kankrej and Jersey Cows*

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ABSTRACT

When different heat treatments were applied to milk in order to assess the level of sialic acid and hexose content in whole acid and k-caseins, it was found that sialic acid decreased due to heat treatments. Further, reduction in sialic acid in sterilized milk whole acid and k-caseins was more pronounced compared to other heat treatments. Decrease was more prominently seen in sterilized milk k-caseins 62.2 and 46.5 per cent compared to whole acid caseins 19.6 and 21.6 per cent of Kankrej and Jersey breeds of cow, respectively.

Results obtained for hexose content indicated that pasteurisation and boiling treatments had a tendency to lower the hexose content while sterilization increased the same. The increase in case of later was suspected to be due to the formation of a lactose-protein complex.

INTRODUCTION

It has now been established that the carbohydrates attached to proteins can contribute to, or modify properties and functions of the proteins (Gottschalk, 1969). Out of the major protein bound carbohydrates, sialic acid has been studied from different angles to a greater extent compared to hexoses or hexosamines (Hindle and Wheelock, 1970, Wheelock and Hindle 1971 and Sabarwal and Ganguli 1973). This is partly attributed to the fact that determination of sialic acid can be used as a measure of the k-casein content (Sullivan, Fitzpatric and Stanton, 1959) and that the sialic acid is supposed to be the essential food element, particularly important for the development of gangliosides during early post natal life (Ziegler, 1960). K-casein is known to contain substantial amount of carbohydrates, particularly sialic acid (Marier, Tassier and Rose, 1963), which is assumed to be related to its stabilizing power towards α_s casein (Hill and Wake, 1969). A critical review of the literature indicates that interest regarding hexose has been mainly oriented around its compositional aspects (Albonico, Saracchi and Zamini, 1964 and Sabarwal and Ganguli 1968). In the present investigation an attempt has been made to evaluate the effect of heating the milk of Kankrej and Jersey breeds on the level of sialic acid and hexose in the casein moieties.

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METHODS

Milk samples:

Six composite whole milk samples (six liters each) were collected from Kankrej and Jersey breeds of cow maintained at the Anand campus, Gujarat Agricultural University, Anand, (Gujarat). Milk samples were skimmed with the help of hand driven Alfa-laval cream separator.

Preparation of whole acid and K-casein samples from raw and heated skim milk.

Skim milk was divided equally into four lots according to different treatments under consideration i.e., T_0, T_1, T_2 and T_3 as under:

T_0: Control (raw skim milk samples).

T_1: Pasteurized skim milk samples, heated to 71°C for a moment and cooled to room temperature.

T_2: Boiled skim milk samples, heated to its boiling point and cooled to room temperature.

T_3: Sterilized skim milk samples, sterilized at 115°C under 8.6 kg/cm², pressure for 20 minutes and cooled immediately to room temperature.

These milk lots were taken separately for the preparation of whole acid and k-casein samples. Whole acid casein was prepared by following the acid precipitation procedure of Gupta and Ganguli (1965) while k-casein was isolated from respective caseins by following the method of Zittle and Guster (1963).

Sialic acid estimation:

Sialic acid was estimated using 2 thiobarbituric acid assay of Warren (1959) with certain modification according to Gupta and Ganguli (1965).

Hexose estimation:

Method followed for the determination of protein bound hexose was that of Winzler (1955) using orcinol sulphuric acid reagent.

RESULTS AND DISCUSSION

The average sialic acid content in acid and k-casein isolated from six untreated and treated Kankrej and Jersey milk samples are given in Table 1.

TABLE—1

Sialic acid content in whole acid and k-caseins of Kankrej and Jersey milk.

Breed of cow	*Casein components*	*Treatments* T_0 (*Raw*)	T_1 (*Pasteurized*)	T_2 (*Boiled*)	T_3 (*Sterilized*)	*F Value*	*C.D.*
		(per cent)*a					
Kankrej	whole acid casein	0.281	0.268	0.258	0.226	20.11†	0.0075
		—	(4.6)	(8.2)	(19.6)		
	K-casein	0.946	0.900	0.852	0.358	0.25†	0.127
		—	(4.8)	(9.9)	(62.2)		
Jersey	whole acid casein	0.318	0.292	0.281	0.246	3.809*	0.020
		—	(7.3)	(10.8)	(21.6)		
	K-casein	0.748	0.701	0.495	0.348	333.09†	0.014
		—	(6.3)	(33.8)	(46.5)		

*a—Average of six samples.

( ) — represent per cent reduction in sialic acid respectively from raw milk samples.

* Significant at 5 per cent level.

† Significant at 1 per cent level.

Data in Table 1 indicate that sialic acid in whole acid casein as well as k-casein of both the breeds was lowered by heat treatments and this reduction in sialic acid was directly proportional to the heat treatment given to milk particularly in whole acid casein samples. K-casein isolated from sterilized milk of Kankrej and Jersey breeds showed remarkable reduction in sialic acid i.e., 62.2 and 46.5 per cent, respectively. Sialic acid level in k-casein isolated from Jersey boiled milk declined severely at boiling temperature compared to Kankrej boiled milk k-casein samples. Sialic acid was found to be about 2.5 to 3.5 times in k-casein compared to whole acid casein (Table 1).

Data on average hexose content in whole acid and k-caseins isolated from raw, pasteurized, boiled and sterilized milk of Kankrej and Jersey cows are presented in Table 2, which indicate that on pasteurization hexose content declines, while sterilization causes an increase in its level in both, whole acid and k-casein components of the Kankrej milk. This implies a decrease of about 7.9 per cent in pasteurized and an increase of about 2.3 times in sterilized milk compared to raw milk whole acid casein (Table 2). Whereas data related to hexose content in k-casein showed a reduction of about 3.3 and 3.6 per cent in pasteurized and boiled milk samples and increase of about 1.9 times in sterilized milk k-casein samples compared to raw milk samples.

The reduction in hexose content in Jersey boiled milk whole acid casein was much higher compared to Kankrej boiled milk whole acid casein. Jersey milk whole acid casein showed a reduction in hexose by about 3.2 and 14.3 per cent in case of pasteurized and boiled samples whereas 1.4 times higher values were obtained in sterilized milk whole acid casein samples. It is apparent from the results obtained for hexose content in k-casein (Table 2) that pasteurization and boiling treatments tended to lower the hexose content by about 3.3 and 11 per cent, respectively whereas sterilization increased the same by 1.8 times.

TABLE—2

Hexose content in whole acid and k-caseins of Kankrej and Jersey milk.

Breed of cow	*Casein components*	*Treatments*				*F. value*	*C.D.*
		(T_o *(Raw)*	T_1 *(Pasteurised)*	T_2 *(Boiled)*	T_3 *(Sterilized)*		
		(per cent)*a					
Kankrej	whole acid casein	0.317	0.292	0.317	0.741	29.14†	0.098
	K-casein	0.330	0.319	0.318	0.627	141.08†	0.018
Jersey	whole acid casein	0.477	0.462	0.409	0.685	4.25*	0.083
	K-casein	0.336	0.325	0.299	0.596	11.38†	0.058

*a—Average of six samples.

*—Significant at 5 per cent level.

†—Significant at 1 per cent level.

Increase in hexose content on sterilization was statistically significant in all the cases.

Lower sialic acid content in whole acid casein and k-casein isolated from heated milks might presumably be due to the release of heat casein glycopeptides during the heating process (Koning, Jenness and Wijnand, 1963 and Hindle and Wheelock, 1970).

Reduction in sialic acid content due to heat has also been indicated by many workers (Nakanishi and Itoh, 1970) and Sabarwal and Ganguli (1973). According to Wheelock, Sinkinson and Hindle (1972) if a complex is formed between β-lactoglobulin and k-casein in whole milk as a result of heating, it is formed preferentially with the k-casein, which lacks in carbohydrates, this might also be one of the reasons in lowering the sialic acid after heating the milk.

Further it is interesting to note that k-casein which contained a higher sialic acid originally, showed a significant reduction in sialic acid at elevated temperature compared to whole acid casein. This might lead to conclude that at elevated temperatures, it is the k-casein which is mainly affected and this may be the cause which hampers its genuine stability character towards the α_s-casein fraction.

Results for hexose content in untreated samples obtained under present investigation tally with the reported values by Sabarwal and Ganguli (1968). The increase in hexose level of sterilization treatment could be suspected to the formation of lactose-protein complex and thereby interfering in the estimation of hexose. Data regarding hexose content in whole acid and k-casein isolated from heated milks are lacking for comparison. Further approach in this line would be helpful to uncoil the nature of chemical reactions that take place when milk is heated.

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