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NUTRITIONAL CHANGES IN MILK DURING HEAT TREATMENT (CHANGES IN CARBOHYDRATES AND LYSINE CAUSED BY HEATING MILK)

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Degradation of lactose and lysin in milk during heating at various temparatures (90-150°) for different intervals of time was investigated. Reduction in lactose and available lysine contents alongwith the formation of lactulose, galactose and hydroxymethyl furfural (HMF) was observed due to heat treatment. The concentration of lactose and lysine was reduced from 4.81-3.52% and 21.0-18.63 m. mole respectively when milk was heated at 150° for 10 mins. Maximum concentration of lactulose, glactose and HMF in 150°-10 mins. heated milk were 791.5, 178.1 mg% and 166.21 μ M. The energy of activation of the reduction in lactose and available lysine contents was 102.32 and 50.87 KJ/mole respectively. However, the energy of activtion was 101.57 KJ/mole for the formation of lactulose, 60.68 KJ/mole for galactose and 122.21 KJ/mole for HMF in heated milk.

Key words: Milk, Heat treatment, Lactose, Lysine.

Introduction

Raw milk is always heated to preserve for a longer time. Now-a-days, various processes differing from each other in temperature and holding time are used to prolong the storage life of the milk. A number of physical and chemical changes occur during these processes. The milk becomes browner with increased severity of heating due to Maillard reaction between lysine and lactose. As a result of this reaction, hydroxymethyl furfural, furfural, furfurly alcohol and other orgnic acids are formed [1]. Lactulose and galactose are also formed due to degredation of lactose in milk after heating [2-5]. Reports in the literature, on the extent of such chemical changes during heat treatment of milk are incomplete. Therefore, the aim of the present work was to investigate extensively the effect of heating milk on lactose and lysine. Reduction in lactose and available lysine concentration alongwith the formation of lactulose, galactose and hydroxymethyl furfural was studied during this research work. In addition, attempts were also made to establish the reaction kinetics of some of the processes involved during heat treatment of milk.

Materials and Methods

Skim milk was heated in sealed stainless steel tubes (3.5 ml capacity) in a thermostatically controlled oil bath in a temperature range of 90 to 150° with holding times ranging from 2 to 120 mins. The tubes were continueously rotated during heat treatment of milk. After heating for a specified period, tubes were placed an ice bath immediately in to stop the reaction before further analysis.

Estimation of sugars. Lactose, lactulose and galactose were analyzed by high performance liquid chromatography (HPLC) technique.

Raffinose (0.75%) solution was used as internal standard. Bigg's reagent containing Zn acetate, (12.5%) phosphotungstic acid (6.25%) and v/v glacial acetic acid (10%) was used as precipitating agent. 3 Gram skim milk was diluted to 35 ml with water. Diluted skim milk was combined with the internal standard and the Bigg's reagent in a 7:2:1 volumetric ratio. The resulting precipatate was removed by contrifugation and the clear supernatant (20 μl) was injected onto the HPLC column (Amine HP x 87 PA Column). The eluant used was millipore water with flow rate of 0.6 ml/ minute. The concentration of sugars (Lactose, Lactulose galactose) in milk samples were estimated by applying the following relation.

$$\text{Unknown mg of sugars} = \frac{C \times Pa \times D.F.}{P'a \times W}$$

where C = Conc. of standard in mg/1; Pa = Peak area of unknown; Pa' = Peak area of standard; DF = Dilution factor; W = Weight of sample in grams.

Estimation of HMF. Hydroxymethyl furfural in heated milk was estimated by HPLC method. Five ml of milk was mixed with 2.5 ml acetic acid (6N) in a tube and was heated in a boiling water bath for 20 min. After cooling, 2.5 ml of trichloro acetic acid solution (40% w/v) was added to the tube and mixed well. The resulting precipitate was removed by centrifugation and clear supernatant was injected onto the HPLC column.

The cencentration of HMF was calculated by measurig the peak area as already described by Van Boekel and Rehman [6].

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Estimation of available lysine. The Udy-dye method was used for the estimation of available lysine in heated milk [7]. The principle of the method is a quantitative binding of the azo dye acid orange-12 by the basic amino groups of proteins. Lysine concentration were determined by a difference method in which colorimetric measurements at 482 nm were done before and after blocking the lysine with propionic anhydride. Measurements were made in duplicate.

Results and Discussion

Effect of heating on lactose. It is apparent from Table 1 and 2 that the concentration of lactose decreased with the rise in temperature and time period of heating. It is also evident from these results that the decrease in lactose contents was not too much significant at lower temperatures. The concentration of lactose in unheated milk was 4.81% which decreased to 4.13% on heating at 90° for 120 mins. The concentration of lactose was 4.38% at 100° after 30 mins. which was further decreased to 4.0% after 120 minutes. Similarly, the concentration of lactose in heated milk at 110° after 120 minutes was 3.88%. A significant reduction in lactose contents can be seen at higher temperatures (120-150°) even after a shorter period of heating. The concentration of lactose was 4.49% at 120°,

TABLE 1. REDUCTION IN LACTOSE AND AVAILABLE LYSINE CONCENTRATION DURING HEAT TREATMENT OF SKIM MILK.

Heating time (mins.)	Lactose (%)			Available Lysine (m.M)		
	Temperature(°C)					
	90	100	110	90	100	110
Control	4.81			21.00		
30	4.59	4.38	4.27	20.58	20.36	21.15
45	4.54	4.29	4.23	20.15	20.15	19.71
60	4.51	4.25	4.13	19.71	19.50	19.28
75	4.49	4.17	4.06	19.50	19.28	18.85
90	4.40	4.08	4.01	19.88	19.06	18.41
105	4.28	4.01	3.97	19.06	18.63	17.98
120	4.13	4.00	3.88	18.85	18.41	17.55

4.36% at 130° and 3.42% at 140° when milk was heated for 20 minutes only. However, it was 3.52% in milk heated at 150° for 10 minutes. Martinez Castro and Olano [8] suggested that the reduction in lactose contents was due to formation of other carbohydrates, as a results of heat treatment of milk.

When the results of lactose in the form of logarithm concentration were plotted against time, the graph obtained was a straight line which means that the degradation of lactose can be described as a reaction of first order. The rate constants (K) were obtained from the slopes of the straight line of the first order. On plotting lnk values against 1/T, it was found that all values for the rate constant (K) in the temperature range of 90-150° were lying on a straight line (Fig. 1). It follows therefore, that the Arrhenius equation is valid in this large temperature range. The rate constant (K_o) was obtained from the K values found at the different temperatures by applying the Arrhenius equation

$$K = K_o \exp \frac{(-Ea)}{RT}$$

where Ea = Energy of activation (KJ/mole); R = Universal gas constant (8.314 J/mole, K); T = Absolute temperature.

The energy of activation calculated from the slope of the straight line of the Arrhenius plot (Fig. 1) was 102.32 KJ/mole and the rate constant (K_o) was 1.67×10^9/s.

Effect of heating of available lysine contents. A significant reduction in available lysine was observed with the increase in temperature and time period of heating. Available lysine contents in raw milk were 21.0 m. mole/l (Table 1). On heating, lysine contents were reduced to 18.85 m. mole/l at 90° after 120 mins. Similarly the gradual decrease in lysine contents was also observed at 100° and 110°. However, losses in lysine contents were quite significant at higher temperatures (120-150°) even after 20 mins heating. The concentration of available lysine was 19.06 at 120° and 18.41 at 130° while it was 17.65 m. mole/l at 140°, when milk was heated for 20 mins. (Table 2).

TABLE 2. REDUCTION IN LACTOSE AND AVAILABLE LYSINE CONCENTRATION DURING HEAT TREATMENT OF SKIM MILK.

Heating time (mins.)	Lactose (%)				Available Lysine (m. M)			
	Temperature (°C)							
	120	130	140	150	120	130	140	150
2	4.77	4.71	4.15	4.20	20.58	20.58	20.36	20.15
4	4.70	4.65	4.00	4.12	20.36	20.15	19.93	19.71
6	–	–	–	3.88	–	–	–	18.50
8	4.62	4.60	3.90	3.60	20.15	19.71	19.50	19.06
10	–	–	–	3.52	–	–	–	18.63
12	4.58	4.56	3.95	–	19.71	19.28	18.85	–
16	4.55	4.44	3.83	–	19.50	18.85	18.20	–
20	4.49	4.36	3.42	–	19.06	18.41	17.65	–

These results also revealed that the concentration of available lysine in heated milk at 150° was 20.15 and 18.63 m. mole/1 after 2 and 10 mins respectively. Reduction in available lysine contents might be due to formation of complex compound between lysine and lactose as a result of Maillard reaction.

When the reciprocal of the concentration of lysine was plotted against time, graph obtained was a straight line which indicates that the loss in available lysine was a second order reaction. The energy of activation and K_o values calculated from Arrhenius plot (Fig. 2) for available lysine were 50.87 KJ/mole and 1739 x 10^{-2}/S respectively.

Formation of lactulose during heating. Results mentioned in Table. 3 and 4 show that lactulose was formed when milk was heated at different temperatures (90-150°) for different intervals of time. The concentration of lactulose was 65 mg% in milk heated at 90° for 45 mins. Which increased to 109 mg% after 120 mins. (Table 3). The concentration of lactulose was 784.72 mg% at 110° after 120 mins, while it was 70.7 and 177.3 at 120° for 2 and 20 mins heating respectively. Maximum oncentration of lactulose was 791.5 mg% on heating milk at 150° for 10 minutes (Table 4). These results are supported by the findings of Andrews [9] who reported the formation of lactulose due to degradation of lactose during heat treatment of milk. The formation of lactulose was found to be a first order reaction. The Arrhenius plot of the logrithum rate constant (K) as a function of the reciprocal of the temp. (K) enable calculation of the activation energy and K_o values for lactulose formation were 101.57 KJ/mole and 9.81 x 10^{12}S respectively (Fig.3).

Formation of galactose during heating. Galactose is also formed during heating of milk (Table 3 and 4) The galactose concentration was 30.9/mg% in milk on heating at 90° for 60 mins which was slightly increased to 37.0 mg% after 120 mins. Increase in galcatose formation was more significant at higher temperatures i.e. 239.0 mg% at 110° for 120 mins. The concentration of galactose increased from 46.7 to 178.1 mg% on heating at 150° for 10 mins. Calvo and Olano [10] reported that galactose was formed in heated milk by the alkaline epimerization of lactose catalysed by the free amino groups of casein. The formation of galactose during heat treatment of milk was also a first order reaction. The energy of activation and K_o values were found to be 60.68 KJ/mole and 9.7x 10^6/S respectively (Fig.4).

Formation of hydroxymethyl furfural in heated milk. Hydroxymethyl furfural is an-other important compound which is formed during heat treatment of milk (Table-5 - 6). The

TABLE 3. FORMATION OF LACTULOSE AND GALACTOSE DURING HEAT TREATMENT OF SKIM MILK.

Heating time (mins)	Lactulose mg %			Galactose mg %		
	Temperature (°C)					
	90	100	110	90	100	110
30	–	–	273.44	–	29.24	74.18
45	65.0	119.31	443.19	–	36.01	105.63
60	68.65	245.50	650.82	30.91	56.84	163.97
75	64.39	247.07	757.70	32.52	66.56	186.83
90	75.09	285.84	617.26	35.37	72.96	–
105	90.30	257.89	744.57	36.62	88.67	212.9
120	109.0	372.17	784.72	36.96	97.74	239.01

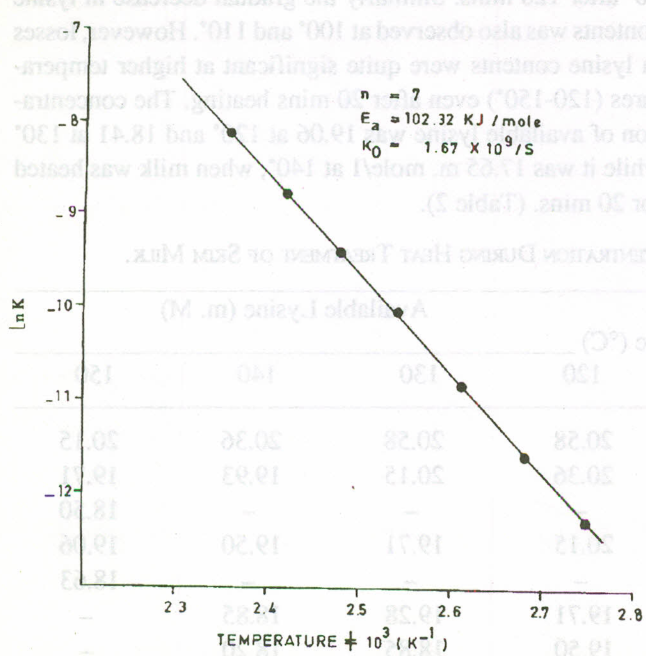

Fig. 1. Arrhenius plot for the reduction in lactose in heated milk.

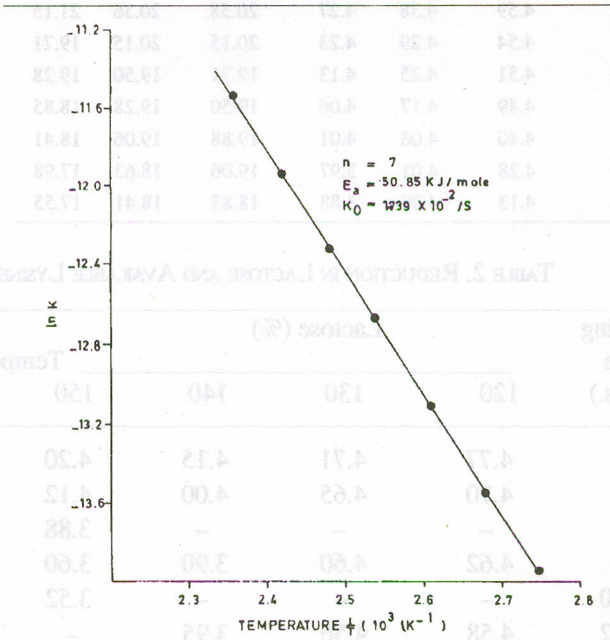

Fig. 2. Arrhenius plot for the reduction in available lysine in heated milk.

treatment of raw milk with acetic acid (during estimation) produces a blank HMF value (3.96 μ mole/1). Therefore, to obtain an accurate HMF value after heat treatment, this blank value was substrated from that measured in the heated samples. It is evident from the results that HMF values increased with an increase in the severity of heat treatment and the time period of heating. The concentration of HMF was 5.52 μ mole/litre at 90° after 30 mins which increased to 22.71 μ mole/1 at 90° after 105 mins. The concentration of HMF in heated milk was 9.33 and 31.09 μ mole/litre at 110° after 30 and 105 mins respectively. A significant increase in the concentration of HMF at 110° (from 31.09 to 50.61 μ mole/litre) was observed when time of heating was increased from 105-120 mins. A further increase in the formation of HMF was noted when temperature was raised from 110 to 150°. The concentration of HMF was 53.5 μ mole/litre in milk heated at 120° for 20 mins. However, it was 76.19 and 166.21 μ mole/litre after heating at 150° for 2 and 10 mins respectively. It is clear from these results that the formation of HMF was very small as compared to the initial concentration of the substance taking part in the reaction (e. g. lactose and lysine). Kessler and Fin [11] also found HMF values of 130 μ mole/litre when milk was heated at 120° for 40 mins.

The formation of HMF in heated milk was found to be a zero order reaction as a straight line was obtained by plotting the concentration of the HMF against time. When lnK values were plotted against reciprocal of absolute temperature, a straight line was obtained which indicated that the Arrhenius equation is valid in this wide temperature/time range (90-150° for 2-120 mins). The energy of activation calculated from the slope of this straight line was found to be 122.21 KJ/mole and rate constant (K_0) was 1.05×10^4/S. Fink and Kesseler [5] also

TABLE 4. FORMATION OF LACTULOSE AND GALACTOSE DURING HEAT TREATMENT OF SKIM MILK.

Heating time	Lactulose (mg %)				Galactose (mg %)			
	Temperature (°C)							
(mins.)	120	130	140	150	120	130	140	150
Control	–	–	–	–	–	–	–	–
2	70.70	95.57	103.40	173.25	16.92	18.32	21.83	46.67
4	75.23	99.09	201.22	257.18	17.69	19.90	38.83	83.51
6	–	–	–	537.09	–	–	–	90.46
8	89.90	203.37	373.17	651.94	18.88	27.73	44.78	136.10
10	–	–	–	791.45	–	–	–	178.05
12	159.27	267.27	553.63	–	19.90	47.09	80.29	–
16	168.05	359.56	594.47	–	21.39	79.09	101.23	–
20	177.34	382.60	622.99	–	25.35	85.15	140.38	–

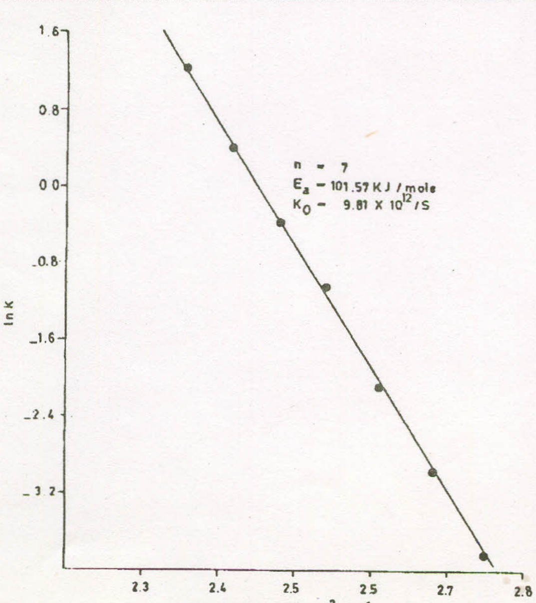

Fig. 3. Arrhenius plot for the formation of lactulose in heated milk.

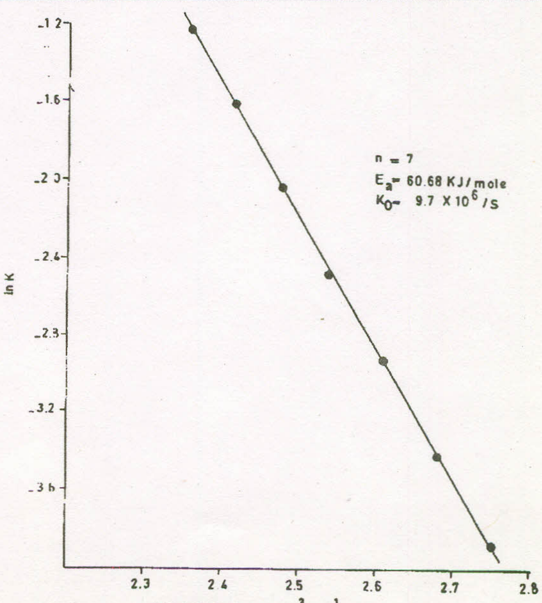

Fig. 4. Arrhenius plot for the formation of galactose in heated milk.

found the formation of HMF as a zero order reaction with an activation energy of 139 KJ/mole during heat treatment of milk.

TABLE 5. FORMATION OF HMF DURING HEAT TREATMENT OF SKIM MILK.

Heating time (mins.)	HMF (m M) Temperature °C		
	90	100	110
Control	3.96	–	–
30	5.25	6.66	9.33
45	7.98	8.91	12.17
60	9.27	10.11	18.12
75	15.50	17.13	21.23
90	18.88	20.67	27.77
105	22.71	23.01	31.09
120	35.01	39.99	58.61

TABLE 6. FORMATION OF MMF DURING HEAT TREATMENT OF SKIM MILK.

Heating time (mins.)	HMF (µM) Temperature (°C)			
	120	130	140	150
2	12.20	23.80	33.	76.19
4	15.87	31.77	51.98	95.23
6	23.80	–	59.52	119.04
8	27.77	48.01	86.90	124.04
10	–	–	–	166.21
12	43.65	62.69	98.10	–
16	51.59	73.80	122.61	–
20	53.57	73.90	123.80	–

It can be concluded from these results that the reduction in lactose and available lysine was observed alongwith the formation of lactulose, galactose and hydroxymethyl furfural during heat treatment of milk. It is also evident from these findings that the extent of changes in carbohydrates and lysine in heated milk was dependent upon temperature and time period of heating. These changes showed a linear relationship with the rise in temperature and time period of heating. These changes were significant at higher temperature (120-150°) after a shorter period of heating. However, these changes were also found to be quiet significant at lower temperature (90-110°) but after longer period of heating.

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