Kinetic Reaction Estimation of Heat Treated Milk

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ABSTRACT

The quantitative data on hysteresis size in heat treated milk are scarce mainly because of the lack of suitable methods to quantify the degree of changes in it. The kinetic reactions of heat treated milk were studied at processing of milk on the range of 60-80°C for holding time 5-30 min. The hysteresis loops were determined from acid-base potentiometric titration. The values of the hysteresis size were increased with increasing heat treatment or holding times. Kinetic parameters *i.e.*; the velocity constants (k), the decimal reduction times value (Dϑ), the activation energy (Ea), the thermal coefficient values (Z-value) and temperature coefficient value (Q_{10}) were calculated. The k and Z values were increase with increasing of heat treatment. While, Dϑ and Q_{10}-values illustrated decrease trend with increasing of heat temperature up to 80°C. The mean values of K, Dϑ, Z and Q_{10}-values values were 0.239 min^{-1}, 12.50 min, 34.71°C and 1.94 during complete temperature range, respectively. The activation energy required to complete the loss reaction of hysteresis size in standardized buffalo's milk samples after heat treatment under the studied temperature range was 64.834 KJ/mol.K. In all heat treatment milk samples, the reaction could be described as first order reaction kinetics during studied temperature range.

Key words: Physico-chemical parameters, Hysteresis, Potentiometric Acid-base titration, Heat treatments, Kinetic reaction.

INTRODUCTION

Chemical, microbiological and physical tests are being used widely in the study of milk quality. The temperature dependency of the death rate constant can best be expressed by the Arrhenius equation **(Burton, 1982)**. The reaction rate constant values (k), activation energy (Ea) and other thermodynamic parameters are very useful to evaluate the performance of heat treated milk processing plant or to select a holding time at a given processing temperature. The quantitative measurement of hysteresis size can be predicted at a given time/temperature combination with the help of this data. The principle depends on the performance of pH-dependent phase transitions, which are recorded by a single titration cycle and evaluated quantitatively by means of hysteresis loops (**Kirchmeier, 1977** and **Mansour *et al*, 2008**). The quantitative data on heat treated milk are, however, scarce mainly because of the lack of suitable methods to quantify the degree of changes in it. This paper presents the potentiometric hysteresis during processing heat treatment of standardized buffalo's milk. Hence, this study was undertaken to estimate kinetic reaction of heat treated milk held at 60, 65, 70, 75 and 80°C for 5, 10, 15, 20, 25 and 30 min, respectively.

MATERIALS AND METHODS

Milk source:

Bulk samples of buffalo's milk used in the present study were obtained from the fresh morning bulk milk of herds of the Animal Production Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch).

Heat treated milk samples:

The whole milk samples were defatted by using electrical cream separator at 4°C, and then adjusted to 3% fat. Milk samples were heated in the scale range of 60-80°C for 5-30 min.

Methods:

Titration procedure:

The acid-base titration of milk and its products was well intensive studied through various applications (**Cox *et al.*, 1956; Kirchmeier, 1979; El-Shobery, 1983 and Mansour *et al.*, 2010**). Titration was obtained using an electronic computerized titration system (Metrohm AG, Herisau / Switzerland) consisted of Titroprocessor 686, Dosimat 665, magnetic stirrer 649, and combined glass electrode.

Kinetic reaction estimation of heat treated milk:

The kinetic reaction estimation of heat treated milk was calculated as described by **Anap *et al.* (1987); Bahbo (1999) and Mansour *et al.* (2010)**.

RESULTS AND DISCUSSIOS

Potentiometric acid-base titration:

The protein molecule, such as the peptide group, also possess acidic or basic properties, one out of every three or four amino acid residues contains a titratable acidic or basic group. The maximum amounts of acid and base with which proteins can combine, as indicative of the number of dissociable cationic and anionic groups which they possess. Actually the terms [acid-binding "and" base-binding] as used really refer to the binding and release of protons. In the casein, acid titration passes not only through the protonation reaction, but also secondary reactions. The approximation to the isoelectric point leads to aggregation and finally to clotting of casein molecules. This aggregation process is often obtained irreversible partial-steps, so that throughout acid-base titration can be appeared **(Anema, 2007)**. The simplest information gained from titration curves is kind and count of the number of groups titrated. The most interesting applications of groups containing is detecting the occurrence of conformational change of protein molecules. The acid-base hysteresis of milk and its products was well recognized through specific aggregation-disaggregation processes **(Kirchmeier, 1979; El-Shobery, 1983 and Mansour, 2013)**.

Table 1: Hysteresis size of milk samples heated at different temperatures for different times.

Heating period (min)	$\oint \alpha$ dpH value*				
	60°C	65°C	70°C	75°C	80°C
5 min	0.100	0.102	0.105	0.107	0.114
10 min	0.103	0.111	0.114	0.120	0.146
15 min	0.108	0.116	0.125	0.134	0.181
20 min	0.112	0.128	0.136	0.154	0.218
25 min	0.117	0.139	0.154	0.171	0.271
30 min	0.124	0.149	0.161	0.194	0.337

* Control sample: 0.094

The potentiometric hysteresis behavior was obtained using standardize buffalo's milk during the studied conditions of heat treatments. The results of acid-base potentiometric titration of heated milk samples were plotted in form of integral curve, in terms of α as a function of pH indicated different width in the shape of the hysteresis loop. The quantitative measurements of hysteresis size in relation to the increasing of heat treatments are shown in Table 1. It could be noticed that, the heating caused more of these values. The values of hysteresis size were increase with increasing heat treatment or holding times. The obtained data were in accordance with that reported by **Mansour *et al.* (2010).**

A comparison of the hysteresis loop as $\oint \alpha$ dpH of raw milk and corresponding heat treated milk samples indicated that, the control sample had higher values than that of heat treated milk. The obtained data showed that, the highest value of hysteresis loop was found at the sample heated at 80°C for 30 min, while the lowest value was found at the samples heated to 60°C for 5 min. These results were attributed to the occurred association of denatured whey proteins with casein micelles at high heat treatment **(Anema & McKenna, 1996 and mansour *et al.*, 2010).**

Kinetic estimations:

The present study aim to follow the change of the hysteresis size in heat treated milk subjected to controlled time/temperature combinations (60-80°C at 5-30 min) of treatments establish the reaction kinetics of the process.

The concentration of quantitative measurement of hysteresis size at each measurement time was subjected to kinetic analysis. These calculations were obtained for standardized buffalo's milk samples at temperatures range of 60, 65, 70, 75 and 80°C for 5, 10, 15, 20, 25 and 30 min.

Firstly, to determine the order of the reaction responsible for the hysteresis size of standardized buffalo's milk, the standard graphical method was used. The data in Table 2 and Fig 1 concludes that, the reaction order of heated milk at the studied range is that which yields a straight line plot. From plotted data of the different heated milk samples, it was found that the reaction could adequately be described by first order kinetics during the temperature range of 60-80°C. This finding is in full agreement with that ascribed by **Mansour *et al.* (2010).**

The velocity constants (k), it is defined as the rate of hysteresis size influenced by temperature / time. It was calculated from the slopes of the obtained straight lines. As expected, it can be declared that the velocity constants (k) increase with increasing of heat treatment up to 80°C. The present study indicated also that, the rate constants (k) of hysteresis size were in the highest values of heat treated milk at 80°C and the lowest values at 60°C. This finding is in full agreement accordance with that reported by **Mansour *et al.* (2010).**

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Table 2: Kinetic data of hysteresis size of milk samples heated at different temperatures for different times.

Temp. °C	Time(min)	∫αdpH	Change (%)	C	Log C	1/C	$1/C^2$
-	-	0.094	-	1.000	0.000	1.000	1.000
60	5	0.100	6.38	1.064	0.027	0.940	0.884
	10	0.103	9.57	1.096	0.040	0.912	0.832
	15	0.108	14.89	1.149	0.060	0.870	0.757
	20	0.112	19.15	1.191	0.076	0.840	0.706
	25	0.117	24.47	1.245	0.095	0.803	0.645
	30	0.124	31.91	1.319	0.120	0.758	0.575
65	5	0.102	8.51	1.085	0.035	0.922	0.850
	10	0.111	18.09	1.181	0.072	0.847	0.717
	15	0.116	23.40	1.234	0.091	0.810	0.656
	20	0.128	36.17	1.362	0.134	0.734	0.539
	25	0.139	47.87	1.479	0.170	0.676	0.457
	30	0.149	58.51	1.585	0.200	0.631	0.398
70	5	0.105	11.70	1.117	0.048	0.895	0.801
	10	0.114	21.28	1.213	0.084	0.824	0.679
	15	0.125	32.98	1.330	0.124	0.752	0.566
	20	0.136	44.68	1.447	0.160	0.691	0.477
	25	0.154	63.83	1.638	0.214	0.611	0.373
	30	0.161	71.28	1.713	0.234	0.584	0.341
75	5	0.107	13.83	1.138	0.056	0.879	0.773
	10	0.120	27.66	1.277	0.106	0.783	0.613
	15	0.134	42.55	1.426	0.154	0.701	0.491
	20	0.154	63.83	1.638	0.214	0.611	0.373
	25	0.171	81.91	1.819	0.260	0.550	0.303
	30	0.194	106.38	2.064	0.315	0.484	0.234
80	5	0.114	21.28	1.213	0.084	0.824	0.679
	10	0.146	55.06	1.553	0.191	0.644	0.415
	15	0.181	92.55	1.926	0.285	0.519	0.269
	20	0.218	131.91	2.319	0.365	0.431	0.186
	25	0.271	188.30	2.883	0.460	0.347	0.120
	30	0.337	258.51	3.585	0.554	0.279	0.078

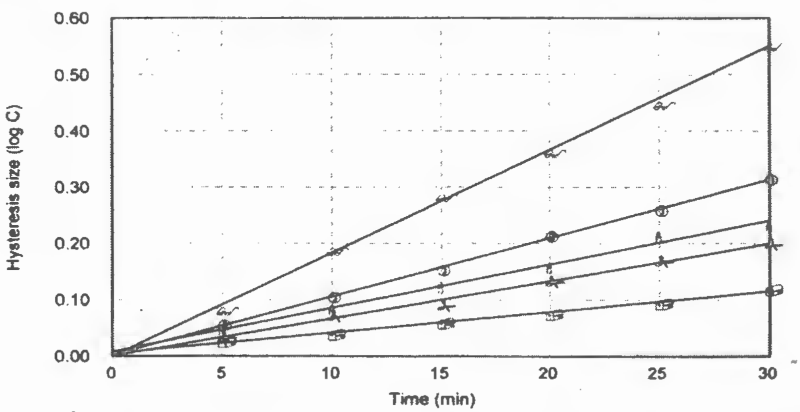

Fig 1: Plot according to 1st order reaction of hysteresis values of milk samples heated at pasteurization scale.

Moreover, the mean value of velocity constant of standardized buffalo's milk samples at studied treatments was 0.239 min^{-1}.

Table 3: Values of velocity constant (K) and decimal reduction time (Dv) of milk samples heated at different temperatures for different times.

Temp (°C)	K (min^{-1})	D_v (min)
60	0.098	23.50
65	0.163	14.13
70	0.207	11.13
75	0.261	8.82
80	0.467	4.93
Mean	0.239	12.50

The decimal reduction times (D-value: times required for reducing the number of organisms or concentration to 1/10 of the original value at a specific temperature ϑ) were calculated for standardized buffalo's milk samples during heat treatments at 60, 65, 70, 75 and 80°C, using the plot of concentration / weight unit versus holding time (Fig 2) used in the heat treatment at the constant temperature levels (semi log plot). The slope of each temperature line (k / 2.303) was measured. The reciprocal of these values could be expressed the D values of each milk sample.

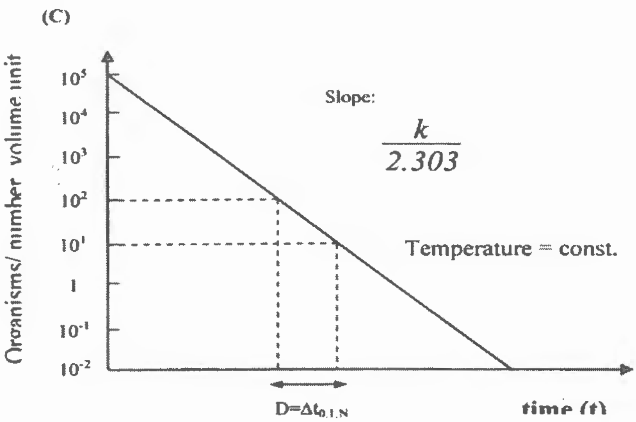

Fig 2: Semi logarithm plot of organism number or concentration/volume versus heating time at constant temperature level.

The mean values of decimal reduction time (Dϑ) of all studied milk samples are presented in Table 3. From these data, it was found that the values of decimal reduction time (Dϑ) for the hysteresis size concentration in standardized buffalo's milk samples were decrease regularly with increasing heat treatment up to 80°C. These finding agreed with the results obtained by **Mansour *et al.* (2010)**. These could be in a logic harmony with the increase rate constant (k) in the all studied milk samples at the different temperatures. Moreover, the mean value of Dϑ of standardized buffalo's milk samples at studied treatments was 12.50 min.

The temperature dependency of the death rate constant (k) is expressed by Arrhenius equation. Basically, the log of the rate constant is proportional to the inverse of the absolute temperature (Fig 3).

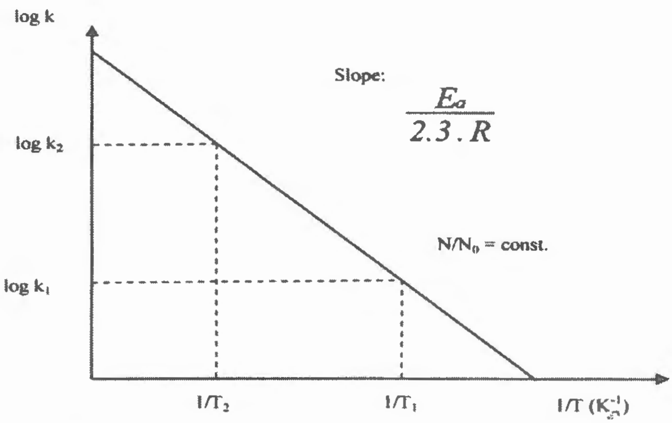

Fig 3: Logarithm of rate constant as a function of the reciprocal of absolute temperatures at constant C/C_0 level (Arrhenius plot).

The activation energy (Ea, extra energy needed by the reaction component to have a high probability of completing the reaction successfully) was calculated by equating the slope of these plots using the following relationship:

$$\log k_2 - \log k_1 = - E_a / 2.3\ R\ (1/T_2 - 1/T_1)$$

Where: k = velocity constant, (min^{-1})

R = universal gas constant (8.314 KJ/mol)

T = absolute temperature.

It is evident that, the relationship (log k versus 1/T) is strictly linear in a given temperature range which makes it possible to determine the activation energy (Ea). The energy of activation for the hysteresis size concentration in standardized buffalo's milk samples calculated from the slope of the straight lines (Fig 3) and presented in Table 4.

Table 4: Activation energy (Ea) value of the studied treatments.

Ea (KJ/mol.K)
64.834

The obtained data presented in Table 4, demonstrated that the activation energy required to complete the loss reaction of hysteresis size in standardized buffalo's milk samples after heat treatment under the temperature range between 60 and 80°C was 64.834 KJ/mol.K.

The thermal destruction coefficient (Z-value) [the increase in temperature required for obtaining the same lethal action or the same effect (*i.e.* C/C_0 = const.) in one tenth of the time]. Using the semi logarithmic plot of times versus temperature of heating, when the ratio of times is 10 the logarithm to the base 10 becomes equal to 1. This corresponded to a certain temperature difference ($Z = \Delta\vartheta$) on the abscissa, the value of which depends on the slope of the straight line (Fig 4). In other words Z-value could be expressed as the following relation:

$$Z = \frac{2.3 \, . \, R \, T_1 T_2}{E_a}$$

The calculated Z-values for the reaction of hysteresis size in standardized buffalo's milk induced throughout heat treatment of buffalo's milk were listed in Table 5 and showed in Fig 4.

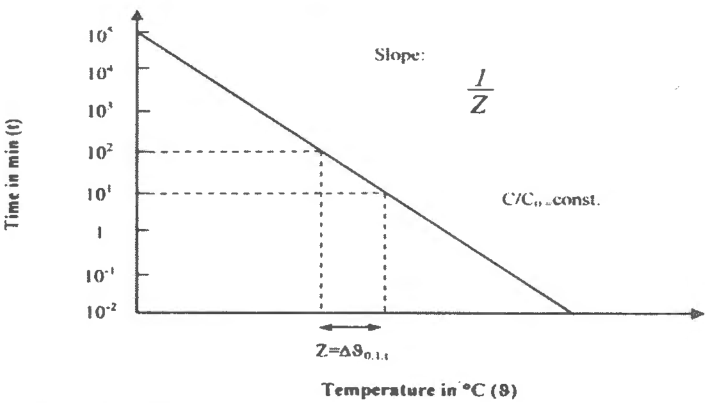

Fig 4: Semi logarithm plot of heating time versus heating.

From the presented data, the thermal coefficient values (Z-values) are ranged between 33.20 and 36.23°C (with mean value of 34.71°C) in the temperature range of heat treatment (60-80°C) for all studied milk samples. Generally, Z-values illustrated an increase trend with the increase of heating temperature in all treatments.

The temperature coefficient (Q_{10} value) that denote how much faster a reaction takes place when the temperature is raised by 10°C, can be expressed as the factor by which D value decreases for 10°C rise in temperature. The temperature coefficient (Q_{10} values) is much more flexible as compared to Z-value. In the same trend, Q_{10} value can also be expressed by means of the Z-value as the following relationship:

$$Z = \frac{10}{\log Q_{10}} \qquad \text{Then} \qquad Q_{10} = 10^{10/Z}$$

Table 5: Thermal destruction coefficient (Z) and temperature coefficient (Q_{10}) values of the different treatments.

Temperature range (°C)	Z-value (°C)	Q_{10}-value
60 – 65	33.20	2.00
65 – 70	34.19	1.96
70 – 75	35.21	1.92
75 – 80	36.23	1.89
Mean	34.71	1.94

The calculated data of Q_{10}-values of the hysteresis size in standardized buffalo's milk samples after heat treatment under the temperature range between 60 and 80°C are tabulated in Table 5. Generally, the results of Q_{10}-values for hysteresis size in standardized buffalo's milk samples after studied heat treatment varied from about 1.89 to 2.00 (with mean value of 1.94). These could be correlated with the variations between the loss rates in the different milk samples during the heat treatment range (60-80°C). Additionally, Q_{10}-values for the hysteresis size in standardized buffalo's milk samples after studied heat treatment are decrease with increasing the temperature of milk samples.

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تقدير حركية التفاعل في اللبن المعامل حراريا

علي إبراهيم منصور

قسم الألبان - كلية الزراعة - جامعة الأزهر (أسيوط)

- تعتبر المعاملات المختلفة التي تجرى على اللبن مثل المعاملات الحرارية من العمليات الأساسية ، وذلك بغرض إبادة الميكروبات المرضية وكذا البكتريا غير المرغوب فيها . وبمعاملة اللبن حرارياً تحدث فيه عدة تغيرات سواء في النظام البروتيني أو التوازن الملحى وكذا انخفاض ثبات النظام

الغروي ، ومن أهم هذه التغيرات انخفاض رقم الأس الأيدروجيني pH ، ترسيب فوسفات الكالسيوم ، دنترة بروتينات الشرش وارتباطها مع الكازين ، حدوث تغير اللون إلى البني من خلال تفاعل ميلارد ، فقد بعض الأحماض الأمينية والفيتامينات ... إلخ ، ولما كان لتتبع هذه التغيرات بصورة وصفية لا تعطى المعلومات الكمية التي من خلالها يمكن التحكم في درجة حدوث التفاعل ، فقد تم التتبع بصورة كمية عن طريق تطبيق قوانين حركية التفاعلات.

وتهدف هذه الدراسة إلى معرفة تأثير المعاملات الحرارية بدرجات حرارة 60-80°م لفترات زمنية 5-30 دقيقة على اللبن الجاموسي المعدل (3% دهـن) وذلك باستخدام الظاهرة الهستيريزية الناتجة عن أسلوب التنقيط الحامضي- القلوي للتقييم النوعي والكمي للتغيرات الكيموطبيعية نتيجة للمعاملات الحرارية تحت الدراسة ، وبصفة عامة أظهرت النتائج أن حجم الظاهرة الهستيريزية يزداد بزيادة درجات الحرارة ووقت المعاملة ، وقد اهتم البحث أيضا بعمل صورة كاملة لكل ثوابت التفاعلات بحساب قيم ثوابت معادلة أرهنيوس مثل ثابت سرعـة التفاعل (k) ، قيم وقت التخفيض العشري (D9) ، قيم طاقة التنشيط (Ea) ، قيم المعامل الحراري (Z) وكذا معامل إسراع درجة الحرارة (Q_{10}) ، وقد لوحظ أثناء دراسة الحركية لهذه التغيرات أن التفاعل الذي يحدث يتبع الرتبة الأولى .

وقد أوضحت النتائج الحسابية زيادة كلاً من ثابت سرعة التفاعل ، المعامل الحراري وانخفاض كلاً من وقت التخفيض العشري ، معامل الإسراع بزيادة درجة الحرارة ، ومن جهة أخرى كان متوسط قيم كلاً من ثابت سرعة التفاعل ، وقت الخفض العشرى ، المعامل الحرارى ، معامل الإسراع 0.239 دقيقة$^{-1}$ ، 12.50 دقيقة ، 34.71°م على التوالي ، بينما كانت قيمة طاقة تنشيط اللازمة لاتمام التفاعل 64.834 كيلوجول / مول . كلفن .