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Thermodynamics of Heat Inactivation of *Listeria monocytogenes* in Soymilk of Varying Initial pH and Sugar Values

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Abstract: The thermodynamics of heat destruction of *Listeria monocytogenes* in soymilk of varying initial pH (6.0-6.8) and sugar contents (0.5-10% w/v) were studied using kinetic parameters generated via the classical thermobacteriology assumption of a log-linear relationship between *Listeria monocytogenes* survivors and heating time (0.5-30 min at 50-65°C). The activation enthalpy (ΔH^+), entropy (ΔS^+), Energy (E_a) and frequency factor (k_0) for the heat inactivation ranged, respectively, from about 275-302, 612-705 J/mol deg., 277-305 kJ/mol and 2.9×10^{43}-2.0×10^{48} min^{-1} increasing with increases in initial sugar contents and acidity of soymilk. A kinetic compensation effect was observed for both $\Delta S^+/\Delta H^+$ and ln k_0/E_a relationships with isokinetic temperature of 32.16×0.13°C and isokinetic destruction rate constant of 9.21×10^{-5} min^{-1} for the microbe in soymilk.

Key words: Kinetic compensation, *Listeria monocytogenes*, soymilk, thermodynamics, microbe

INTRODUCTION

Listeria monocytogenes is the causative agent of listeriosis. The microbe exhibits a greater resistance to thermal inactivation than many other pathogenic vegetative microorganisms in foods (Brown, 1991). Because of high fatility rate of human listeriosis and ability to grow in soymilk, the control of *L. monocytogenes* in the product is imperative. The earlier reports indicated that the heat resistance of the bacterium followed a log-linear relationship and was significantly ($p<0.05$) influenced by initial pH and sugar contents of soymilk.

Thermodynamic data from heat inactivation of *L. monocytogenes* in soymilk are essential for design and predictive purposes and for verification of kinetic compensation effect in *L. monocytogenes* destruction by heat. The kinetic compensation effect has been widely observed in various areas such as physics, chemistry, biology and food science (Rhim *et al*., 1990; Canjura *et al*., 1991). This effect consists of a correlation between the kinetic parameters and is usually observed for a family of processes which involve similar reactions or different experimental conditions. By carefully characterizing this correlation, reaction rates or other kinetic parameters could be predicated (Aguerre *et al*., 1986). This according to Rhim *et al*. (1990) would also provide insight into the mechanism of the reaction or classification. Such data are lacking for heat destruction of *L. monocytogenes* in soymilk. The objective of this study was therefore to study the thermodynamics of *Listeria monocytogenes* destruction by heat in soymilk of varying initial pH and sugar contents.

MATERIALS AND METHODS

Media: Typticase Soy Broth (TSB) (Oxoid Ltd. Basingstoke, Hants, England) plus 0.6% Yeast Extract (TSBYE) was used for preparing all experimental cultures. Trypticase Soy Agar (TSB+0.2% Agar) with 0.6% Yeast Extract (TSAYE) was used for all plate counts.

Microbial culture: Three strains of *L. monocytogenes* (all hemolysin positive at different levels) which comprised of EGD (weakly hemolytic), NCTC 7973 (strongly hemolytic) and P7 (serovar 4b) were selected for this study. The strains were kindly donated to the laboratory by Prof. W. Goebel Institute for Genetik and Microbiologic, University Wurzburg, 8700 Wurzburg, Germany. The cultures were maintained through monthly transfers on Trypticase Soy Agar (TSA) slants at 4°C.

Production, enumeration and standardization of inoculum: Each strain was grown separately by inoculating TSBYE and incubating in a static condition for 24 h at 37°C (Brown, 1991). Each culture was centrifuged (17000×g), washed twice with sterile 0.1 M Phosphate Buffered Saline (PBS) of pH 7.0. Dilutions of each harvest

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were made using the PBS to yield about 1×10^8 cells mL^{-1} of stationary phase cells. The three strains were then thoroughly mixed to produce a working inoculum stored at 4±1°C in a household refrigerator until required for challenge tests. Preliminary investigations confirmed that the three strains of *L. monocytogenes* remained viable at 4±1°C for at least 4 weeks after preparation.

Experimental design: A 3×3×4 randomized factorial design was employed to evaluate the effects of sugar concentration (0.5, 5.0 and 10.0 g/100 mL), initial pH (6.0, 6.5 and 6.8) and heating temperature (50, 55, 60 and 65°C). All variables combinations were replicated two times with duplicate samples being taken at each sampling time.

Soymilk preparation: About 10 kg soybean (*Glycine max* L.) variety Tax 1448-2E-2001 was obtained from the Experimental/Commercial Farm, University of Agriculture, Makurdi, Nigeria. Soymilk was prepared by a modified hot-grind method described by Johnson and Synder (1986). About 1 kg of sorted soybean was boiled in distilled water for 30 min, drained using a plastic basket and hot milled into a smooth slurry with aliquots of boiling distilled water in a blender (Kenwood Major, model km 230, Kenwood Ltd. Havant Hants, UK). The slurry was filtered through two layers of previously sterilized (121°C, 15 min) cheese cloth. The filtrate was made up to 6 L with hot distilled water to obtain soymilk with initial pH 6.8 and basal sugar content of 0.5 g/100 mL.

The bulk soymilk was divided into 3 lots. The sugar concentrations of Lots II and III were adjusted using sucrose to 5.0 and 10.0 g/100 mL, respectively by material balance method (Toledo, 1981). Lot I contained the basal sugar level. Each lot was subdivided into three. The pH of two of the sub lots for each group was adjusted to 6.0 and 6.5, respectively using citric acid. The remainder sub lot in each group had the basal pH 6.8. The 9 sub lots were each dispensed in 99 mL portions into 200 mL milk dilution bottles and sterilized by autoclaving at 121°C for 15 min. The sugar levels were verified by the phenol-sulphuric acid spectrophotometric method as described by Meloan and Pomeranz (1980). The soymilk sub lots were placed in storage at -10°C until utilized for *L. monocytogenes* heat resistance studies.

Heat resistance studies: The frozen soymilk from the sub lots were thawed under running tap water. About 1 mL of PBS working culture was added to 99 mL of sterile soymilk from each sub lot and mixed thoroughly by inverting several times. This achieved a net dilution of 10^2 of the starting PBS culture. The initial level of *L. monocytogenes* in each soymilk sub lot was approximately 10^6 CFU mL^{-1}.

For heat resistance studies, 1.5 mL portions of each inoculated soymilk were aseptically dispensed into sterile 2 mL borosilicate glass ampoules (75×10 mm), placed in ice and the mouths of the ampoules were sealed with a propane torch. The hermetically sealed ampoules were heated at selected temperatures (50, 55, 60 or 65°C) by submersion in a constant temperature-circulating (±0.1°C) water bath (Gallenkamp, UK) equipped with a shaker (Citenco Ltd. Manor Way, Herts Boreham Wood, UK) maintained at 50 rpm. The Come-Up Time (CUT) for each temperature was determined using copper-constantan needle thermocouples (0.1 mm diameter) carefully placed inside six unsealed ampoules containing soymilk. The top parts of the ampoules were sealed with leak-proof silicone glue. The signals from the thermocouple junctions were recorded using an Electrolaboratoriet (Ellab) Type 29-CFT Recorder (Electrolaboratoriet, Copenhagen, Denmark). The heating times at selected temperatures were determined with a stopwatch. Ampoules removed after the CUT were considered to contain the initial *L. monocytogenes* population (N_0). Experimental ampoules containing inoculated soymilk from each sub lot were heated to reach the desired temperatures and held for the desired times.

At each selected time for a selected temperature, six ampoules were removed from the heating bath, immediately cooled by submersion in ice water and prepared for *L. monocytogenes* enumeration. To reduce the number of dilutions, plates and counts necessary for accuracy in microbiological experimentation, the contents of the six ampoules were combined and appropriate serial dilutions were made resulting in duplicate samples. *L. monocytogenes* survivors were determined by the spread plate method on TSAYE plates. The entire experiment was repeated over with a coefficient of variation of 2-5%.

Kinetic data analysis: A modified steady-state procedure was employed in which data were collected only in the isothermic portion of heating. A traditional two-step method was used to calculate activation energies (Van Boekel, 1996). This method required verification of the relationship between $\log_{10}$ of *Listeria monocytogenes* remaining (N) versus time (t):

$$\text{Log}_{10}\,[N\,(t)/No] = -kt^{\beta} \quad (1)$$

to fit a kinetic model in which k is the heat destruction rate constant (min^{-1}) and β the order of the reaction. The reaction rate constants were related to temperature by the Arrhenius equation:

$$\ln k = \ln k_0 - E_a/RT \quad (2)$$

Where:
E_a = Activation energy (kJ/mole)
R = The universal gas constant (0.008314 kJ/mol °C)
T = The absolute temperature (K)
k_0 = The frequency factor (per min)

E_a and k_0 values were determined from the slopes and intercepts, respectively of the lines generated from the relationship of ln k versus 1/T by use of least square linear regression.

Enthalpy (ΔH^+, kJ/mol) and entropy (ΔS^+, kJ/mol deg.) of activation were obtained by regressing 1n (kT) on 1/T via the equation derived from Transition State Theory:

$$\ln (k/T) = (\ln k_s/h + \Delta S^+/R) - (\Delta H^+/R)\,(1/T) \quad (3)$$

Where:
k_s = Boltzmann constant (6.6256×10^{-23} J/K^{-1})
h = Planck constant (6.6256×10^{-34} Jsec)

From the slopes and intercepts of the lines, ΔH^+ and ΔS^+, respectively were obtained. From well known thermodynamic relationship:

$$\Delta G^+ = \Delta H^+ - T\Delta S^+ \quad (4)$$

where, ΔG^+ is change in free energy of the reaction. Equation 3 can be rewritten as:

$$k = (k_s/h)T \exp(-\Delta G^+/RT) \quad (5)$$

A compensation effect can be linearly expressed by a relationship between activation enthalpy (ΔH^+) and entropy (ΔS^+) which compensation each other to produce small changes in the free energy (ΔG^+) of a reaction. This behavior was expressed formally by using Absolute Rate Theory (Eq. 3) as described by Rhim *et al.* (1990). An isokinetic Temperature (T_c) was assumed to exist at which each of the destruction rate constants has the same value for *Listeria monocytogenes* in soymilk of varying initial pH and sugar concentrations. It follows that the exponent terms in Eq. 3 are equal at T_c. Therefore, the quantity $\Delta H^+ - T_c\,\Delta S^+$ is also a constant which can be denoted by C such that:

$$C = -RT_c \ln (kh/T_c k_s) \quad (6)$$

According to the Transition Reaction Rate Theory, this is the same as the free energy of activation (ΔG^+) at T_c. It therefore follows that:

$$\Delta S^+ = a\,\Delta H^+ + b \quad (7)$$

where, $a = 1/T_c$, $b = -C/T_c = -\Delta G^+/T_c$. The kinetic compensation parameters (a, b) were obtained by regressing ΔS^+ on ΔH^+ using least square linear regression.

A frequency factor/activation energy ($\ln k_0/E_a$) compensation relationship was directly deduced from the Arrhenius Model (Eq. 2). At the temperature of compensation, each heat destruction rate constant has the same value. The right side of Eq. 2 is constant which can be denoted by B. From this relationship, the compensation equation (Eq. 8) was derived as:

$$\ln k_0 = AE_a + B \quad (8)$$

where, $A = 1/RT_c$. Some researchers (Rhim *et al.*, 1989, 1990; Krug *et al.*, 1976) have noted that under certain conditions, compensation effect may occur accidentally as a computational artifact from experimental errors. To test the validity of the compensation effect for a reaction, a statistical method has been developed (Rhim *et al.*, 1989; Krug *et al.*, 1976). This consists of comparing the isokinetic Temperature (T_c) with the harmonic mean Temperature (T_H). T_H is the reciprocal of the arithmetic means of the reciprocal of the experimental temperatures:

$$T_H = \frac{n}{\sum_{i=1}^{n} 1/T_i} \quad (9)$$

where, n is number of experimental temperatures. If they are significantly different ($T_c \neq T_H$), the existence of true compensation is suggested for the heat destruction of *L. monocytogenes* in soymilk.

Statistical analysis: The tests for significant ($p \leq 0.05$) differences in the ΔH^+, ΔS^+, E_a and k_0 values among the pH, sugar levels and temperature treatments were calculated with the multiple comparison range method of Kramer and Twigg (1970). The least square linear regression analysis of the Arrhenius, transition state and kinetic compensation parameters were as described by Gupta (1979).

RESULTS AND DISCUSSION

The influence of initial pH and sugar concentration of soymilk on heat destruction rate constants (k) of *Listeria monocytogenes* are shown in Table 1. The rate constants were derived on the basis of the classical thermobacteriolgy which assumes a first order reaction kinetics (Toledo, 1981). The mean k-values were used for computing E_a, k_0, $\Delta H^\#$ and $\Delta S^\#$ values.

Arrhenius Model: Temperature dependence of the heat destruction rate constants was studied in an Arrhenius relationship (ln k vs. 1/T) via least square linear regression.

Table 1: Regression parameters* for semi-logarithmic relationship between *Listeria monocytogenes* survivors and heating time in soymilk

			Heating temperature (°C)			
Soymilk pH	Sugar level (w/v%)	Regression parameter	50	55	60	65
6.0	0.5	r^2	0.986±0.011	0.998±0.004	0.997±0.003	0.998±0.003
		k	0.0512±0.0012	0.1706±0.0021	2.3500±0.0001	5.3558±0.0022
		r^2	0.992±0.005	0.998±0.001	0.998±0.001	0.995±0.002
	5.0	k	0.0631±0.0004	0.2953±0.0011	2.6779±0.0001	7.6767±0.0004
		r^2	0.994±0.003	0.997±0.001	0.993±0.004	0.955±0.015
	10.0	k	0.0702±0.0011	0.5234±0.0003	5.0065±0.0013	8.8577±0.0004
		r^2	0.988±0.004	0.998±0.001	0.997±0.002	0.998±0.001
6.5	0.5	k	0.0511±0.0011	0.1535±0.0004	1.2121±0.0013	4.6060±0.0004
		r^2	0.987±0.003	0.995±0.001	0.998±0.001	0.997±0.001
	5.0	k	0.0574±0.0004	0.2742±0.0002	1.4394±0.0001	6.5800±0.0031
		r^2	0.978±0.002	0.968±0.002	0.976±0.001	0.954±0.004
	10.0	k	0.0694±0.0011	0.4345±0.0004	4.4288±0.0003	8.2250±0.0041
		r^2	0.983±0.004	0.998±0.001	0.907±0.011	0.955±0.006
6.8	0.5	k	0.0478±0.0015	0.1238±0.0003	1.1515±0.0023	3.7145±0.0111
		r^2	0.989±0.003	0.998±0.001	0.978±0.013	0.968±0.011
	5.0	k	0.0548±0.0044	0.2350±0.0002	1.2794±0.0007	5.7575±0.0007
		r^2	0.982±0.004	0.976±0.011	0.959±0.005	0.991±0.004
	10.0	k	0.0658±0.0022	0.3071±0.0007	3.3868±0.0125	7.1969±0.00

*Each parameter is mean±SD of four replicates. k = Heat destruction rate constant (1/min), r^2 = Correlation coefficient of regression

Table 2: Regression parameters for Arrhenius relationship between heat destruction rateconstants and absolute temperature for *Listeria monocytogenes* in soymilk of varying initial pH and sugar

		Soymilk initial sugar content (w/v%)		
Soymilk pH	Parameters	0.5	5.0	10.0
6.0	n	4	4	4
	r^2	0.962	0.986	0.957
	Intercept	109.03	109.60	111.23
	k_0 (min^{-1})	2.20×10^{47}	3.96×10^{47}	2.0×10^{48}
	Gradient	-36206.92	-36291.11	-36709.06
	E_a (kJ/mol)	301.02	301.72	305.20
6.5	n	4	4	4
	r^2	0.987	0.999	0.962
	SE	0.24	0.03	0.43
	Intercept	102.44	104.48	110.20
	k_0 (min^{-1})	3.09×10^{44}	2.38×10^{45}	7.25×10^{47}
	Gradient	-34107.70	-34679.07	-36403.61
	E_a (kJ/mol)	283.57	288.32	302.66
6.8	n	4	4	4
	r^2	0.975	0.998	0.969
	SE	0.32	0.07	0.38
	Intercept	100.06	102.86	108.90
	k_0 (min^{-1})	2.90×10^{43}	4.71×10^{44}	1.98×10^{47}
	Gradient	-33363.60	-34181.50	-36040.57
	E_a (kJ/mol)	277.38	284.18	299.64

The regression parameters are shown in Table 2. Arrhenius equation (Eq. 2) fitted well ($r^2 \geq 0.957$) for all environmental conditions of the soymilk. E_a values (kJ/mol) ranged from 277.4-305.2 and increased with an increase in initial soymilk acidity and sugar level. The E_a is a measure of sensitivity and dependence of the reaction temperature; larger magnitude of E_a is associated with higher temperature dependence. *Listeria monocytogenes* is therefore more sensitive to temperature at lower initial pH and higher initial sugar contents of soymilk. This implies that a small change in temperature produces a large change in destruction rate of *L. monocytogenes* in soymilk of increasing initial acidity and sugar contents. In reaction kinetics, the frequency factor (k_0) indicates the collision fraction between the molecules that present enough energy to lead to destruction or inactivation. The changes in E_a were accompanied by parallel increases of the collision factors. According to Speroni *et al.* (1985) whenever the E_a or k_0 values differ between two systems, it is tempting to imply dissimilarity in mechanism of heat destruction. However, the frequency factors were determined by extrapolation to values well outside the range of temperatures used experimentally with minor changes in the E_a resulting in substantial changes in k_0. Most instances of heat denaturation of proteins have been observed in the thermal death of microorganisms (Rhim *et al.*, 1990). The E_a values described by heating were within the ranges of 210-630 kJ/mol for vegetative microbial cells destruction by heat (Toukis and Labuza, 1989).

Transition State Model: The estimated activation enthalpy (ΔH^+) and entropy (ΔS^+) and statistics of fit for *L. monocytogenes* destruction in the various soymilks are shown in Table 3. The high correlation coefficients ($r^2 \geq 0.956$) indicated adequate fit and characterization of the temperature dependence of heat destruction of *L. moncytogenes* by the transition state model. ΔH^+ and ΔS^+ increased with a decrease and an increase, respectively in initial pH (6.8-6.0) and sugar contents (0.5-10% w/v) suggesting also a greater temperature sensitivity with increasing acidity and sugar concentration. Solutes such as sucrose tend to lower the water activity of aqueous systems and would enhance heat destruction of microorganisms. The ΔH^+ is a measure of the internal energy of the activated complex formed just before the transition state is reached in reactions

Table 3: Absolute reaction rate parameters for heat destruction of *Listeria moncytogenes* in soymilk

		Soymilk initial sugar content (w/v%)		
Soymilk pH	Parameters	0.5	5.0	10.0
6.0	n	4	4	4
	r^2	0.962	0.985	0.956
	SE	0.42	0.26	0.46
	Intercept	102.23	102.80	104.42
	ΔS* (J/mol deg.)	686.45	691.16	704.69
	Gradient	-35876.76	-35960.60	-36378.61
	ΔH* (kJ/mol)	298.28	298.98	302.45
6.5	n	4	4	4
	r^2	0.986	0.999	0.961
	SE	0.24	0.03	0.43
	Intercept	102.23	102.80	104.42
	ΔS* (J/mol deg.)	628.43	648.61	696.68
	Gradient	-33646.72	-34348.66	-36092.29
	ΔH* (kJ/mol)	298.28	298.98	302.45
6.8	n	4	4	4
	r^2	0.975	0.999	0.969
	SE	0.32	0.07	0.38
	Intercept	93.26	96.06	102.100
	ΔS* (J/mol deg.)	611.85	635.18	685.38
	Gradient	-33033.12	-33851.09	-35710.05
	ΔH* (kJ/mol)	274.64	281.44	296.89

occurring in solutions (Atkins, 1980). The ΔS^+ being non-zero supported the assumption that the heat destruction of *L. monocytogenes* in soymilk was spontaneous and irreversible. The ΔS^+ for all the variables combinations were positive which indicated a decrease in structural order in the molecules of the activated complexes formed during heat destruction of *L. monocytogenes* in soymilk. Higher ΔS^+ corresponds to a more probable activated complex hence the heat destruction of the microbe is faster in soymilk with higher initial acidity and sugar values.

As a result of the empirical nature of the thermal resistance and Arrhenius equations, it has been proposed that the absolute reaction rate equation should be used in the treatment of thermal rate data (Ariahu and Ogunsua, 2000). In general, ΔH^+ is a measure of the energy barrier which must be overcome by reacting molecules and is related to the strength of the bonds which are broken and made in the formation of the transition state from the reactants. ΔS^+ is related to how many molecules with the appropriate energy can actually react. The value of ΔS^+ includes steric and orientation requirement and also solvent effects and provides a better insight into the roles of the reactants in the microbial destruction than the less definite probability factor of the Collision Theory.

Kinetic compensation: In Fig. 1, the enthalpy of activation was plotted against the entropy of activation of heat inactivation of *Listeria monocytogenes* in soymilk. Though the enthalpy of activation varied from about 274-302 kJ mol^{-1}, it did not change independently from the entropy of activation. They exhibited a marked

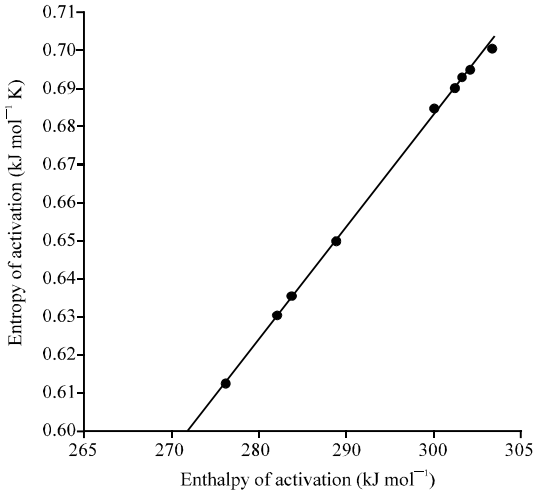

Fig. 1: Kinetic compensation effect in heat destruction of *Listeria monocytogenes* in soymilk of varying initial pH (6.0-6.8) and sugar contents (0.5-10.0% w/v) examined by $\Delta H^{\#}/\Delta S^{\#}$ relationship

compensation effect in which increase in ΔH^+ was accompanied by a linear increase of ΔS^+. The compensation parameters of a = 3.2757×10^{-3} and b = −0.2875 (Eq. 7) were determined from the straight line (r^2 = 0.998) using linear regression. From the relationship of $T_c = 1/a$, the isokinetic temperature determined from the slope of the line was 305.28 K (32.28°C). At this temperature, ΔG^+ of 87.77 kJ/mol was calculated using the relationship of $\Delta G^+ = -bT_c$.

Figure 2 shows the $\ln k_0/E_a$ compensation plot for the heat destruction of *Listeria monocytognenes* in soymilk. The Fig. 2 also shows a marked compensation effect between these two kinetic parameters. From the straight line (r^2 = 0.998), the compensation parameters of A = 0.3943 and B = −9.2925 were determined via least square linear regression analysis. The isokinetic temperature of 305.03 K (32.03°C) was calculated using the relationship of T_c = 1/AR. At this temperature, the destruction rate constant of *L. monocytogenes* in soymilk (isokinetic rate constant) of 9.21×10^{-5} min^{-1} was derived from $\ln^{-1}$ B.

The isokinetic temperature determined by the $\ln k_0/E_a$ method yielded a slightly different value from that determined by the $\Delta S^+/\Delta H^+$ compensation method. Discrepancy in T_c determined by the methods was reported Rhim *et al.* (1990) in their study of whey protein denaturation. According to the researchers, these methods for calculating isokinetic temperature are derived from different kinetic theories which are not exactly consistent. Again, computational errors involved to calculate apparent kinetic parameters (ΔH^+, ΔS^+, E_a and k_0) are propagated throughout the calculation procedure of

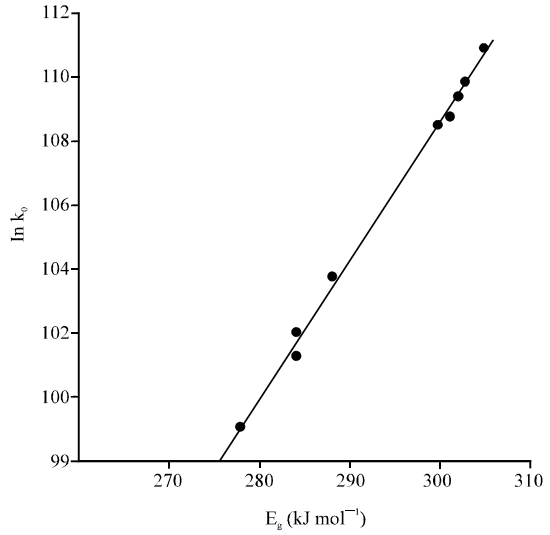

Fig. 2: Kinetic compensation effect in heat destruction of *Listeria monocytogenes* in soymilk of varying initial pH (6.0-6.8) and sugar contents (0.5-10.0% w/v) examined by in kg/E_g relationship

isokinetic temperature. The apparent kinetic parameters are derived quantities, removed from the experiment by a number of steps of computation and their values are not obtained independently but are computed from a single equation.

The mean harmonic temperature (T_H, Eq. 9) was calculated as 57.4°C consequently, $T_c \neq T_H$ which suggest existence of a true compensation effect for heat inactivation of *L. monocytogenes* in soymilk of varying initial pH and sugar contents (Canjura *et al*., 1991; Krug *et al*., 1976). In Fig. 1 and 2, data points are separated by two groups. This reflects the change in kinetic parameter values at about pH 6.5-6.0. Even though the kinetic parameters changed substantially at lower and higher pH ranges, these parameters showed overall kinetic compensation effects. According to earlier workers (Rhim *et al*., 1990), most instances of kinetic compensation behavior of heat denaturation of proteins have been observed in the thermal death of microorganisms. Although, the kinetic parameters (ΔH^+, ΔS^+, E_a and k_0) characterized a single reaction, they depend on experimental variables such as solute concentration, water activity, pH and oxygen availability, etc. However, the compensation parameters (a, b, A and B) do not depend on these variables and presumably they would better characterize the reaction.

The isokinetic temperature was below the experimental temperature (50-65°C) where the inactivation of *Listeria monocytogens* occurred. This is consistent with earlier reports (Zsako, 1976) that the isokinetic temperature is never in the temperature range where denaturation occurs. The isokinetic temperature for acid catalyzed hydrolysis of disaccharides was also beyond the experimental temperature ranges (Rhim *et al*., 1989).

CONCLUSION

The sensitivity to heat destruction of *Listeria monocytogenes* in soymilk increases with initial acidity and sugar levels. Existence of kinetic compensation effect for the inactivation of the microbe suggested that *Listeria monocytogens* is destroyed by similar mechanisms within initial pH 6.0-6.8 and sugar contents of 0.5-10% w/v of soymilk.

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