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Research Article

Thermodynamics of Heat Inactivation of *Aeromonas hydrophila* in Soymilk of Varying Initial pH and Sugar Levels

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Abstract

The thermodynamics of thermal inactivation of *Aeromonas hydrophila* in soymilk of varying pH (6.0-7.0) and sugar concentration (0-10%) were studied at a temperature of 50-65℃ using kinetic parameters generated through the Classical thermobacteriology assumption of a log-linear relationship between *A. hydrophila* survivors and heating time. The activation enthalpy ($\Delta H^{\#}$), activation entropy ($\Delta S^{\#}$), activation energy (E_a) and frequency factor (K_o) for thermal inactivation of *A. hydrophila* in the soymilk samples were also obtained. Thermal inactivation of the organism followed first order reaction kinetics. The heat destruction rate constant (k) decreased with increase in heating temperature. The activation energy ranged from 210.98 to 215.28 kJ/mol increasing with decrease in pH and increase in sugar concentration of soymilk. The isokinetic temperature (T_C) obtained varied from 55.95 to 56.62 ℃ with inactivation of *A. hydrophila* exhibiting true compensation effect, with a Gibbs free energy of 82.86 kJ/mol. A combination of temperature, pH and sucrose significantly influenced inactivation of *A. hydrophila* in soymilk, following a similar mechanism being driven by entropy. Optimum safety from *A. hydrophila* can be achieved through application of multifactorial hurdles in soymilk processing. The thermodynamic data obtained will be useful to optimize thermal processing conditions for soymilk targeting *A. hydrophila.*

Keywords

Soymilk, Hurdles, *Aeromonas hydrophila*, Thermodynamics, Arrhenius, Activation Enthalpy, Safety, Inactivation

1. Introduction

Food processing targets mainly pathogenic microorganisms due to the huge burden posed by microbial foodborne diseases [1]. As reported by van Liverloo *et al*. [2] and Hii *et al*. [3], thermal processing of foods has over time played a significant role in maintaining nutritional quality, limiting spoilage and safeguarding microbial safety of foods, which may have resulted in serious health concerns.

Thermal processing is among the most used methods applied to ensure shelflife extension and microbial safety of soymilk amidst the recent techniques used in food. Soymilk, the rich creamy liquid extract of soybean is a popular nutritive alternative to cow milk. It is consumed widely especially in developing countries, as a cheaper source of protein and calories, and as a substitute to cow milk in solving malnutrition due to its nearly balanced content of essential amino acids. It has similar physical and chemical attributes to cow milk and is suitable for all ages from infants to the elderly, therefore it is consumed even by populations who cannot digest cow milk

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due to lactose intolerance, allergy to milk proteins or vegetarian way of diet [4-6]. Being a high-moisture, highly nutritious, low acidity fluid with a pH ranging between 6.0 and 7.0, it is prone to microbial growth and activity.

Aeromonas hydrophila, an emerging food borne bacteria of great concern to human health [7-9] has been reported to grow in soymilk [10]. It has been shown to form biofilms on various biotic and abiotic surfaces, allowing for the persistence of this pathogen in water distribution systems as well as human body and the environment [11]. Its contamination of food can cause various long-term food borne illness [9], lead to economic losses to food industries and pose a critical threat to human health [12]. Although the organism has been described as a pathogen of fish [13], it can be transmitted to human through ingestion of contaminated foods [14]. Being a ubiquitous waterborne bacterium and given its pathogenic qualities [15, 16] and its ability to grow in soymilk, the control of *A. hydrophila* in food products is imperative.

In addition to heat, other hurdles have also been combined and applied mildly in food to ensure safety, including addition of solutes and reduction in pH. Generally, it has been observed that the thermal resistance of microorganisms has been highly variable depending on the heating medium and strain [17, 18]. Earlier studies by researchers have revealed low thermal resistance of *A. hydrophila* [17-19] in various foods. Recently, Tersoo-Abiem *et al.* [10] showed an increase in the thermal resistance of the organism in soymilk, influenced by initial pH, heating temperature and sugar contents of soymilk. Obviously, studies of the thermal death kinetic parameters of microorganisms in specific foods is important in food processing to prevent excessive heating which may denature or degrade proteins or other nutrients in foods but at the same time ensure its safety.

Generally, the mechanisms for quality changes in food during heating can be evaluated using kinetic and thermodynamic properties. The application of thermodynamics in microbial systems provides information needed in the calculation of the system reactions and processes [18]. The thermodynamic parameters of heat inactivation of pathogenic organisms in food enable prediction of the effectiveness of certain heat treatments in achieving safety and shelflife extension. According to Igyor *et al.* [21], thermodynamic data from microbial heat inactivation studies is essential for the design of a heat process, predictive purposes and for verification of kinetic compensation effect in microbial destruction by heat. This effect consists of a correlation between the kinetic parameters and is usually observed for a family of processes which involve similar reactions of different experimental conditions. Reaction rates and other kinetic parameters can be predicted by characterizing this correlation. Furthermore, thermodynamic parameters provide insight into the mechanism of the reactions. For a microbial inactivation to occur, the interacting molecules require a minimum amount of energy referred to as the activation energy (E_a). Other thermodynamic parameters including the activation enthalpy ($\Delta H^{\#}$), activation entropy ($\Delta S^{\#}$) and Gibbs free energy of activation are also useful in improving the performance of the preservation processes. Thermodynamic parameters have been documented in literature for several bacteria pathogens in various foods including soymilk, but such data are lacking for heat destruction of *A. hydrophila* in soymilk.

The objective of this study was therefore to study the thermodynamics of *A. hydrophila* destruction by heat in soymilk of varying initial pH and sugar contents.

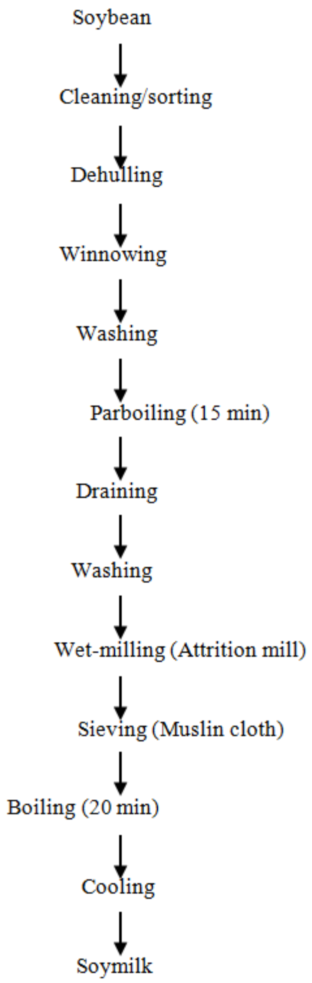
2. Materials and Methods

2.1. *A. hydrophila* Inoculum Preparation

The inoculum was prepared according to the procedure described by Pin *et al.* [22]. *Aeromonas hydrophila* M39a isolated from a drinking water source [16] was cultivated on starch ampicillin agar and incubated at 30°C for 48 h to obtain stationary phase organisms. A dilution of the harvested colonies picked using a sterile wire loop was made using phosphate buffer saline in a corked tube to achieve a working inoculum with a concentration equivalent to 0.5 MacFarland standard (10^8 CFU/ml). This was stored in a refrigerator until required for inoculation.

2.2. Soymilk Preparation

Soymilk was prepared as described by Tersoo-Abiem *et al.* (2022) as shown on Figure 1. One kilogram of cleaned soybean was dehulled, winnowed and washed using clean water. The washed beans were blanched in hot water at 90 ℃ for 15 minutes, washed and milled using an attrition mill. Hot distilled water (5 Litres) was added to make a slurry. This was sieved using muslin cloth and boiled for 20 min. It was then packaged, allowed to cool, and refrigerated. The bulk soymilk was divided into portions to make 0, 5.0 and 10.0 w/v sugar concentration. Each of the samples was further divided to make pH 6.0, 6.5 and 7.0 using citric acid. These were dispensed in McCartney bottles properly corked, labelled and sterilized by autoclaving at 121 ℃ for 15 min, then cooled and stored in a freezer until utilized for the study.



Source: Tersoo-Abiem *et al*. [10]

Figure 1. *Flowchart for the production of soymilk.*

2.3. Experimental Design

A 3 x 3 x 7 randomized factorial design was employed to evaluate the effect of sucrose concentration (0, 5.0 and 10.0%W/V), initial pH (6.0, 6.5 and 7.0) and heating temperature (50, 52.5, 55, 57.5, 60, 62.5 and 65 ℃) on the survival of *Aeromonas hydrophila* in soymilk. All variable combinations were replicated twice with duplicate samples being taken at each sampling time.

2.4. Heat Resistance Studies

The sterile soymilk in screw-capped McCartney bottles (10 ml) were inoculated with 10 ul *A. hydrophila* working culture to achieve a net dilution of approximately 10^5 CFU/ml which was used for heat resistance studies as described by Harrigan and McCance [23]. The screw capped tubes were heated at the selected temperatures (50, 52.5, 55, 57.5, 60, 62.5 and 65 ℃) by placing them in a constant temperature water bath for 1 h. The inoculum was added when the heating medium (soymilk) had attained the desired temperature. One millilitre of the inoculated soymilk was immediately taken, diluted serially and plated to determine the initial *A. hydrophila* population. The come-up time (CUT) for each temperature was determined using thermometer carefully placed inside a test tube containing uninoculated soymilk. For each selected temperature, 1 ml of inoculated soymilk samples were removed from the heating bath at regular intervals, immediately cooled by submerging into ice water and serial dilutions carried out for *A. hydrophila* enumeration. *A. hydrophila* survivors were determined by the pour plate method using starch agar and incubated for 24-48 h at 30 ℃. All experiments were carried out in triplicate to determine the mean colony forming units per ml.

3. Thermodynamic Data Analysis

A modified steady-state procedure was employed in which data was collected only in the isothermic portion of heating. D-values and thermal destruction rate constants (k) of *A. hydrophila* were determined using the partial sterilization technique described by Toledo 2007 [24]. A traditional two-step method was used to calculate activation energies [21]. This method requires verification of the relationship between $\log_{10}$ of *A. hydrophila* remaining (N) versus time (t) to fit a kinetic model in which k is the heat destruction rate constant (min^{-1}) and β the order of the reaction:

$$\text{Log}_{10}\ [N\ (t)/N_0] = -kt^{\beta} \tag{1}$$

The reaction rate constants were related to temperature by the Arrhenius equation:

$$\text{In } k = \text{In } k_o - E_a/RT \tag{2}$$

where E_a = activation energy (kJ/mol), R= Universal gas constant, T = absolute temperature (K) and K_o = frequency factor.

E_a and K_o values were determined by use of linear regression.

Enthalpy ($\Delta H^{\#}$, kJ/mol) and entropy ($\Delta S^{\#}$, kJ/mol deg) of activation were obtained using the transition state theory as follows:

$$\text{In } (k/T) = (\text{In } k_s/h + \Delta S^{\#}/R) - (\Delta H^{\#}/R)\ (1/T) \tag{3}$$

where k_s is Boltzman constant (1.38 x 10^{-23} JK^{-1}), and h is Planck constant (6.6256 x 10^{-34} Js). From the slope and intercepts of the lines, $\Delta H^{\#}$ and $\Delta S^{\#}$ respectively, were obtained [21, 25].

From the thermodynamic relationship:

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \tag{4}$$

Where $\Delta G^{\#}$ is change in free energy of the reaction

Equation (3) can be rewritten as:

$$k = (k_s/h)\ T\ \exp\ (-\ \Delta G^{\#}/RT) \tag{5}$$

3.1. Kinetic Compensation Effect

A compensation effect between activation enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) was determined using absolute rate theory as described by Igyor *et al.*, [21]. An isokinetic temperature (T_c) was assumed to exist, at which each of the destruction rate constants had the same value. This follows that the exponent terms 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:

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

According to the transition rate theory, this is the same as the free energy of activation ($\Delta G^{\#}$) at T_c. It therefore follows that

$$\Delta S^{\#} = a\ \Delta H^{\#} + b \quad (7)$$

where $a = 1/T_c$,

$$b = -C/T_c = -\Delta G^{\#}/T$$

The kinetic compensation parameters (a and b) were obtained by regressing $\Delta S^{\#}$ on $\Delta H^{\#}$ using linear regression.

Frequency factor/activation energy (In k_o/E_a) compensation relationship was directly deduced from the Arrhenius model using the relationship:

$$In\ K_o = A\ E_a + B \quad (8)$$

where $A = 1/RT_c$

Test of the validity of the compensation effect for the reaction was carried out by comparing the isokinetic temperature (T_c) with the harmonic mean temperature (T_H) [21, 25] described as:

$$T_H = \frac{n}{\sum_{i=1}^{n} 1/T_1} \quad (9)$$

where n = number of experimental temperatures,

T_1 = mean experimental temperatures.

If they are significantly different ($T_1 \neq T_H$), the existence of the compensation is suggested for the heat destruction of *A. hydrophila* in soymilk.

3.2. Statistical Analysis

The tests for significant ($p<0.05$) difference in $\Delta H^{\#}$, $\Delta S^{\#}$, E_a and K_o values among the pH, sugar levels and temperature treatments were calculated using the multiple comparison range method of Kramer and Twigg [26]. The least square linear regression analysis of the Arrhenius, transition state and kinetic compensation parameters were as described by Gupta [27].

4. Result and Discussion

The temperature dependence of heat destruction rate constants (k) for *Aeromonas hydrophila* in soymilk of pH 6.0 to 7.0 and sugar concentrations of 0 to 10% heated at 50 to 65 ℃ are shown in Table 1, along with the regression coefficients. The rate constants were derived on the basis of the Classical thermobacteriology which assumes a first order reaction kinetics [24]. The mean reaction rate values were used to compute E_a, K_o, $\Delta H^{\#}$ and $\Delta S^{\#}$ values.

Table 1. *Thermal destruction rate constants and z-value for Aeromonas hydrophila inactivation in soymilk of varying pH and sugar content.*

				Heating Temperature (℃)						
Soymilk pH	**Sugar level (w/v %)**	**z-value (℃)**	**Regression parameters**	**50**	**52.5**	**55**	**57.5**	**60**	**62.5**	**65**
6.0	0.0	9.598	k	-0.162 ±0.001	-0.220 ±0.002	-0.208 ±0.005	-0.763 ±0.043	-2.016 ±0.012	-2.290 ±0.0173	-3.928 ±0.012
			r^2	0.947 ±0.012	0.989 ±0.005	0.988 ±0.011	0.978 ±0.003	0.976 ±0.009	0.942 ±0.004	0.927 ±0.003
	5.0	9.651	k	-0.150 ±0.001	-0.183 ±0.005	-0.193 ±0.005	-0.695 ±0.038	-1.787 ±0.021	-2.244 ±0.052	-3.712 ±0.013
			r^2	0.950 ±0.003	0.994 ±0.005	0.991 ±0.03	0.997 ±0.010	0.992 ±0.006	0.936 ±0.001	0.974 ±0.001
	10.0	9.572	k	-0.155 ±0.004	-0.195 ±0.007	-0.208 ±0.008	-0.817 ±0.013	-1.918 ±0.164	-2.258 ±0.017	-4.020 ±0.036
			r^2	0.949 ±0.002	0.981 ±0.001	0.988 ±0.004	0.997 ±0.027	0.994 ±0.001	0.976 ±0.010	0.982 ±0.002
6.5	0.0	9.618	k	-0.150 ±0.007	-0.179 ±0.003	-0.202 ±0.005	-0.728 ±0.009	-1.961 ±0.017	-2.273 ±0.036	-3.798 ±0.014
			r^2	0.963 ±0.006	0.986 ±0.001	0.990 ±0.002	0.976 ±0.015	0.957 ±0.017	0.923 ±0.001	0.983 ±0.009
	5.0	9.646	k	-0.140 ±0.012	-0.165 ±0.007	-0.189 ±0.011	-0.649 ±0.033	-1.793 ±0.009	-2.239 ±0.018	-3.646 ±0.232
			r^2	0.967 ±0.008	0.964 ±0.009	0.990 ±0.014	0.993 ±0.011	0.965 ±0.005	0.949 ±0.006	0.978 ±0.009

***Table 1**. Continued.*

Soymilk pH	Sugar level (w/v %)	z-value (℃)	Regression parameters	Heating Temperature (℃) 50	52.5	55	57.5	60	62.5	65
	10.0	9.575	k	-0.129±0.006	-0.152±0.026	-0.179±0.004	-0.556±0.024	-1.665±0.038	-2.210±0.001	-3.379±0.114
			r^2	0.955±0.005	0.938±0.008	0.991±0.007	0.968±0.013	0.936±0.007	0.929±0.002	0.969±0.007
7.0	0.0	9.709	k	-0.144±0.009	-0.175±0.003	-0.201±0.006	-0.691±0.029	-1.935±0.086	-2.261±0.065	-3.686±0.132
			r^2	0.965±0.003	0.968±0.002	0.979±0.004	0.998±0.004	0.974±0.003	0.971±0.003	0.985±0.003
	5.0	9.714	k	-0.136±0.007	-0.155±0.005	-0.188±0.006	-0.614±0.013	-1.734±0.064	-2.229±0.037	-3.444±0.302
			r^2	0.965±0.007	0.968±0.008	0.981±0.020	0.988±0.003	0.976±0.007	0.985±0.005	0.959±0.022
	10.0	9.550	k	-0.127±0.010	-0.147±0.002	-0.175±0.003	-0.535±0.062	-1.703±0.008	-2.199±0.006	-3.289±0.123
			r^2	0.965±0.006	0.963±0.004	0.975±0.017	0.963±0.008	0.916±0.021	0.980±0.036	0.958±0.003

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

4.1. Temperature Dependence Using Arrhenius Model

Using Arrhenius model, the regression parameters obtained (Table 2) revealed a good fit of the model in soymilk studies, with a high coefficient of regression ($r^2 \geq 0.875$). Variations in pH and sucrose content had no significant effects on activation energies for *A. hydrophila* inactivation in soymilk at pH 6.5 and 7.0, but pH 6.0, giving activation energy (E_a) and frequency factor (K_o) values which ranged from 209.21 to 215.28 KJ/mol and 8.09×10^{32} to 8.20×10^{33} min^{-1} respectively, with the highest E_a (215.28 KJ/mol) obtained at pH 6.0 and 10% sugar. This high E_a may be due to the additive effect of the hurdles interacting with soymilk components. According to Sehrawat *et al*., [28], sucrose addition affects the water activity of foods, giving a shielding effect to microorganisms suspended in food. As reported by Hou *et al* [29], soybean seeds (per 100g) used for soymilk production consist of protein (35.3-40g), lipids (19.0-20g), minerals (5g) and carbohydrates (28.2-33g), including sugars such as glucose, fructose, sucrose, raffinose, and stachyose, with sucrose having the highest concentration (41.3–67.5%). Li *et al*. [30] reported activation energies of 205 KJ/mol and 213 KJ/mol for *Listeria monocytogenes* and *Salmonella* serotypes respectively in liquid egg mix of low water activity, similar to that obtained for soymilk with reduced water activity in this study. Thermodynamic parameters including E_a are critical for the development of effective and safe food processing procedures. A knowledge of this ensures adequate amount of heat is applied to destroy food borne organisms while preserving its quality. Large E_a values are usually associated with high temperature dependence of inactivation of the organism in a medium [30-32]. It follows from this study that relatively more heat energy will be required to inactivate *A. hydrophila* in soymilk at pH 6.0 and 10% sucrose concentration. This may require higher temperature and longer exposure times to achieve a significant reduction in the microbial population. Schuman *et al*. [20] reported higher E_a values of 343 to 380 kJ/mol for inactivation of different strains of *A. hydrophila* in liquid whole egg, with the highest E_a values associated with the lowest Z-value. A similar trend of E_a and Z-values was observed in this study. Li *et al*. [30] also reported similar observations.

It was evident in this study that *A. hydrophila* exhibited different responses in soymilk with variations in pH and sugar content. This agrees with the report of Stumbo [33], Peleg and Cole [17], Ray [34], Toledo [24], Barbosa-carnovas *et al*. [35] and Ayeni *et al*. [18] who reported that the response of an organism to thermal inactivation varies and is dependent on the characteristics (composition: fat, protein, carbohydrate; other protective substances, viscosity, pH and water activity among others) of the heating food medium. The range of activation energies obtained in this study falls within the range reported by Peleg *et al*. [17] that the activation energy for inactivation of bacteria is in the range of 100 to 500 kJ/mol.

4.2. Temperature Dependence Using Transition State Model

The temperature dependence of the thermal destruction rate constants and other parameters of fit for *A. hydrophila* in soymilk of varying pH and sugar concentrations estimated using absolute reaction rate or transition state model is presented in Table 3. This showed a high coefficient of regression ($r^2 \geq 0.872$) indicating a good fit of the model. From this study, $\Delta H^{\#}$ and $\Delta S^{\#}$ ranged from 206.52 to 212.56 KJ/mol and 376.04 to 395.30 J/mol deg respectively for soymilk. Generally, $\Delta H^{\#}$ and $\Delta S^{\#}$

reduced with increase in sugar concentration but increased with increase in acidity. On the contrary, Igyor *et al.* [21] reported an increase in $\Delta H^{\#}$ and $\Delta S^{\#}$ with decrease in pH (6.80-6.0) and an increase in sugar content (0.5-10.0% w/v). According to Casolari [32], high $\Delta H^{\#}$ and $\Delta S^{\#}$ imply a higher temperature dependence of the inactivation process. The value of $\Delta H^{\#}$ gives the energy the reactant molecules must possess for the inactivation process to proceed [21]. Therefore, higher energy will be required to inactivate *A. hydrophila* in soymilk of pH 6.0 and 10% sugar content.

Similarly, the highest $\Delta S^{\#}$ was observed with soymilk of pH 6.0 and 10% sugar concentration, signifying a high degree of randomness in the system. $\Delta S^{\#}$ relates to the number of molecules with the appropriate energy to react in the system [21, 36, 37]. A positive $\Delta S^{\#}$ indicates that the transition state is highly disordered compared to the ground state, therefore the activation of *A. hydrophila* proceeds faster [37, 38].

Both the Arrhenius model and Absolute reaction rate or Transition state model revealed similar temperature dependence of thermal inactivation rate for *A. hydrophila* in soymilk of varying pH and sugar concentrations.

Table 2. *Arrhenius model parameters for heat destruction of Aeromonas hydrophila in soymilk of varying initial pH and sugar concentration.*

Sample pH	Regression parameter	Sample initial sugar concentration (%)		
		0.0	**5.0**	**10.0**
6.0	K_o (min^{-1})	9.60×10^{32}	8.09×10^{32}	8.20×10^{33}
	E_a (KJ/mol)	209.40	209.21	215.28
	r^2	0.912	0.911	0.909
6.5	K_o (min^{-1})	2.80×10^{33}	2.40×10^{33}	3.55×10^{33}
	E_a (KJ/mol)	211.90	211.48	212.73
	r^2	0.917	0.917	0.923
7.0	K_o (min^{-1})	5.72×10^{33}	2.77×10^{33}	4.83×10^{33}
	E_a (KJ/mol)	212.40	210.98	212.95
	r^2	0.921	0.922	0.919

Key: K_o =frequency factor; E_a = Activation energy; r^2 = coefficient of regression

Table 3. *Absolute reaction rate parameters for heat destruction of Aeromonas hydrophila in soymilk of varying initial pH and sugar concentration.*

Sample pH	Regression parameter	Sample initial sugar concentration (%)		
		0.0	**5.0**	**10.0**
6.0	$\Delta S^{\#}$ (J/mol deg)	377.34	376.04	395.30
	$\Delta H^{\#}$ (KJ/mol)	206.71	206.52	212.56
	r^2	0.910	0.909	0.907
6.5	$\Delta S^{\#}$ (J/mol deg)	384. 49	382.83	385.91
	$\Delta H^{\#}$ (KJ/mol)	209.24	208.82	210.07
	r^2	0.915	0.915	0.921
7.0	$\Delta S^{\#}$ (J/mol deg)	385.93	380.93	386.54
	$\Delta H^{\#}$ (KJ/mol)	209.73	208.32	210.29
	r^2	0.919	0.920	0.917

Key: K_o =frequency factor; $\Delta S^{\#}$ = Entropy of activation; $\Delta H^{\#}$ = Enthalpy of activation r^2 = coefficient of regression

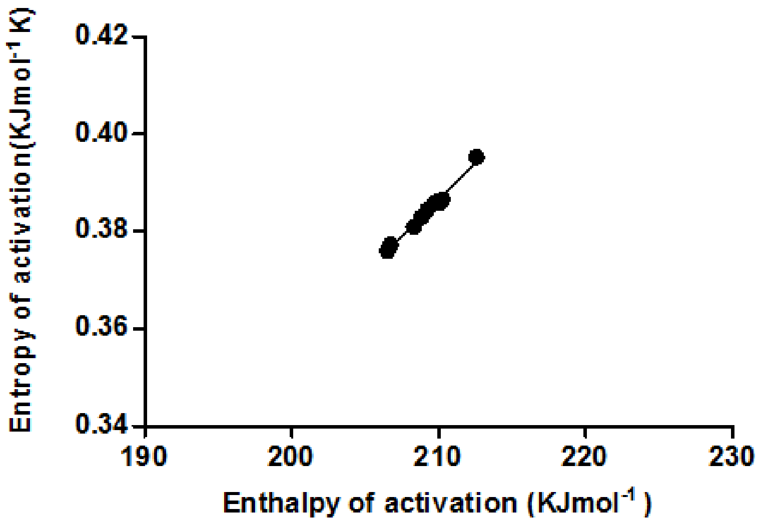


Figure 2. Kinetic compensation effect in heat destruction of Aeromonas hydrophila in soymilk of pH 6.0 – 7.0 with 0, 5 and 10% sugar concentration examined by $\Delta H^{\#}$ /$\Delta S^{\#}$ relationship.

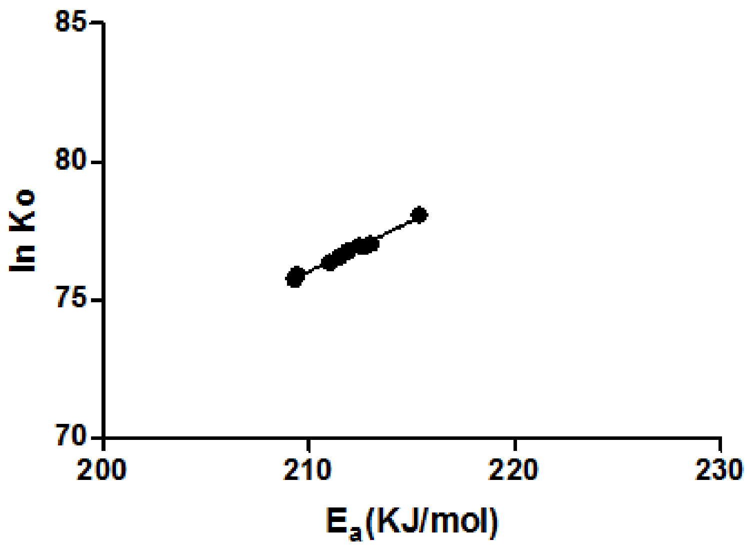


Figure 3. Kinetic compensation effect in heat destruction of Aeromonas hydrophila in soymilk of pH 6.0 – 7.0 with 0, 5 and 10% sugar concentration examined by ln Ko / E_a relationship.

Table 4. Kinetic Compensation effect in heat destruction of Aeromonas hydrophila in soymilk of varying initial pH (6.0-7.0) and sugar contents (0-10.0%) examined by $\Delta S^{\#}$ / $\Delta H^{\#}$ relationship.

Sample	Regression parameter	Value	Parameter	Value
Soymilk	A	0.003040	ΔG^{+} (KJ/mol)	82.86
	b	-0.2519	T_C (°K)	328.95
	r^2	0.9868	T_C (°C)	55.95
	T_H(K)	330.34		
	$T_H > T_C$			

Key: a and b = kinetic compensation parameters; T_H= mean harmonic temperature; T_C = Isokinetic temperature; ΔG^{+} =Gibbs free energy

Table 5. *Kinetic Compensation effect in heat destruction of Aeromonas hydrophila in soymilk of varying initial pH (6.0-7.0) and sugar contents (0-10.0%) examined by In K_o/ E_a relationship.*

Sample	Regression parameter	Value	Parameter	Value
Soymilk	A	0.3649	T_C (°K)	329.62
	B	-0.5916	T_C (°C)	56.62
	r^2	0.9898	Kc (min^{-1})	0.5534
	T_H(K)	333.34		

Note: T_H>T_C.
Key: A and B = kinetic compensation parameters; T_H= mean harmonic temperature; T_C= Isokinetic temperature; K_c =Isokinetic rate constant

4.3. Kinetic Compensation of *Aeromonas hydrophila* in Soymilk of Varying pH and Sugar Concentration

The result in this study follows the compensation law since an increase in the activation energy or activation enthalpy was compensated by an increase in activation entropy [21, 32]. The kinetic or enthalpy- entropy compensation theory states that in order to minimize free energy changes due to the physical and chemical phenomena, compensation arises from the nature of the interaction between reactants causing the reaction, and the relationship between the $\Delta H^{\#}$ and $\Delta S^{\#}$ for a specific reaction is linear [39]. As shown in Figures 2 and 3, there was a positive correlation between $\Delta H^{\#}$ and $\Delta S^{\#}$ and E_a and In k_o signifying an isokinetic relationship. The high activation energy was associated with high activation entropy and vice versa, indicating a true compensation effect.

The kinetic compensation parameters in Table 4 indicates a high coefficient of regression ($r^2 \geq 0.9868$). The isokinetic temperature (T_c) determined from the relationship of $\Delta H^{\#}$ and $\Delta S^{\#}$ for soymilk was 328.95K while that obtained from relating E_a and In k_o was 329.62K. The isokinetic temperature represents the temperature at which all reactions in the series proceed at the same rate [21, 39].

As stated by Klotz *et al*. [40], the rate of reactions is controlled by the rate of formation of the activated complex and is a function of the Gibbs free energy change in moving from normal to the activated state. The reactant molecules must possess sufficient energy to overcome the energy barrier ($\Delta G^{\#}$) to allow inactivation of *A. hydrophila*. At the study conditions used for *A. hydrophila* inactivation in soymilk, the free energy of activation or Gibbs free energy obtained was 82.86 KJ/mol. The Gibbs free energy (ΔG^{+}) obtained for soymilk was positive, which indicates that *A. hydrophila* thermal inactivation in soymilk of varying pH and sugar concentration is a non-spontaneous reaction, driven by entropy [25], being favoured by higher heating temperatures [25, 38]. The magnitude of ΔG^{+} influences the likelihood of the reaction and its rate. It describes the amount of energy needed to drive the reaction at the isokinetic temperature to a completion [39]. From Table 4, it is evident that more free energy will be required to inactivate *A. hydrophila* in soymilk.

The kinetic compensation parameters derived from the relationship of E_a and ln K_o are presented in Table 5, showing a good linear fit with a high coefficient of regression ($r^2 \geq 0.9853$) for soymilk. This reveals isokinetic temperatures slightly different from that obtained from the relationship of $\Delta H^{\#}$ and $\Delta S^{\#}$ with no significant differences ($p \geq 0.05$). Similar observations were reported by Igyor *et al*. [21]. The isokinetic rate constant obtained was 0.5534 min^{-1} for soymilk. It is evident that the rate of *A. hydrophila* inactivation was slow in soymilk. This slow inactivation rate may be due to the composition of soymilk, offering a protective effect to the organism, thereby increasing its resistance to heat [41-43]. According to Murphy *et al*. [44], Toledo [24], van Lieverloo *et al*. [2] and Ayeni *et al*. [18], the kinetic/thermodynamic parameters obtained for an organism may vary for the same environmental conditions in different food products.

As indicated in Table 4, the mean harmonic temperature (T_H) was calculated as 330.34 K (57.34 ℃). For the studied system, Tc ≠ T_H: the mean harmonic temperature is higher than the isokinetic temperature. This shows a true compensation effect for *A. hydrophila* in soymilk.

5. Conclusion

The sensitivity of *Aeromonas hydrophila* in soymilk to inactivation temperature increased with higher initial pH and sugar levels depending on the composition of soymilk. This was evident in the activation energy (E_a), frequency factor (K_o), activation enthalpy ($\Delta H^{\#}$) and activation entropy ($\Delta S^{\#}$) obtained at the experimental conditions. The thermodynamic parameters have revealed that inactivation of *A. hydrophila* in soymilk of varying initial pH and sugar concentration is a non-spontaneous, endothermic reaction being driven by entropy. The study has proven that the intrinsic properties of a food can influence the behaviour of bacteria at similar extrinsic factors (temperature). The data obtained will be useful to optimize thermal processing conditions for soymilk targeting *A. hydrophila*.

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Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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