ENTHALPY - ENTROPY AND FREQUENCY FACTOR - ACTIVATION ENERGY COMPENSATION RELATIONS FOR DEATH OF *Bacillus coagulans* IN APPLE JUICE

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By

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ABSTRACT

ENTHALPY - ENTROPY AND

FREQUENCY FACTOR - ACTIVATION ENERGY

COMPENSATION RELATIONS FOR DEATH OF

Bacillus coagulans IN APPLE JUICE

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Thermal death kinetics of Bacillus coagulans was studied experimentally in glucose or sucrose added apple juice. The frequency factor and the activation energy of these processes were calculated by using the Arrhenius expression. The activation entropy and the activation enthalpy were calculated with the analogy between the unimolecular chemical reactions and the microbial death kinetics by using the Eyring's theory. No trends were observed in variation of the kinetic parameters with the sugar concentrations, but this function agrees with the kinetic compensation relations. Analysis of the data indicated that, there was no actual isokinetic temperature for the family of related experiments and the thermal death kinetics of B.coagulans has different mechanisms under varying conditions.

Key words: Enthalpy, Entropy, Frequency Factor, Activation Energy

Science Code: 614.02.07

ÖZET

ELMA SUYUNDA Bacillus coagulans' IN

ÖLÜMÜNDE ENTALPİ - ENTROPİ VE

FREKANS FAKTÖRÜ - AKTİVASYON ENERJİSİ

EŞİTLEME İLİŞKİLERİ

ÖZKAN (DURUKAN), Arzu
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Glukoz yada sukroz katılmış elma suyunda Bacillus coagulans'ın ısıl ölüm kinetiği deneysel olarak çalışıldı. Bu işlemlerin frekans faktörü ve aktivasyon enerjileri Arrhenius ifadesi ile hesaplandı. Aktivasyon entropisi ve aktivasyon entalpisi ise unimoleküler kimyasal reaksiyonlar ve mikrobiyel ölüm kinetiği arasında karşılaştırma ile Eyring kuramı yardımıyla hesaplandı. Şeker konsantrasyonu ile kinetic parametrelerin değişimi arasında bir bağlantı gözlenmedi ama bu fonksiyonların kinetik eşitleme ilişkilerine uyduğu görüldü. Verilerin incelenmesi sonucunda gerçek bir isokinetik sıcaklık saptanmadı. Buna göre, değişen koşullarda B. coagulans 'ın ısıl ölüm kinetiğinin farklılık gösterdiği sonucuna varılmıştır.

Anahtar kelimeler: Entalpi, Entropi, Frekans Faktörü, Aktivasyon Enerjisi

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I am grateful to my husband Mr. Tuncay Özkan and to all my friends for their help and encouregement, especially to Mrs. Sibel Özilgen.

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NOMENCLATURE

E : Activation energy (Joules / mol)

$G^{\#}$: Activation Gibbs free energy (Joules / mol)

$H^{\#}$: Activation enthalpy (Joules / mol)

h : Planck constant (Joules sec.)

k_c : Isokinetic reaction rate constant (sec^{-1})

k_d : Death rate constant (sec^{-1})

k_{do} : Frequency factor (sec^{-1})

N_A : Avagadro constant (mol^{-1})

R : Gas constant (Joules / mol K)

$S^{\#}$: Activation entropy (Joules / mol K)

T : Temperature (K or ^{o}C)

t : Time (sec)

T_c : Isokinetic temperature (K)

X : Biomass concentration (cfu / mL)

X_o : Biomass concentration at the beginning of the death process (cfu / mL)

α : Constant in Equation (17)

β : Constant in Equation (17)

δ : Constant in Equation (18)

ϕ : Constant in Equation (18)

κ : Transmission coefficient

CHAPTER - 1

INTRODUCTION

1.1. Apple Juice

Apple juice is produced from mature apples. Immature, overmature or decayed apples are not used in juice production. Washed and sorted apples are reduced to a pulp, suitable for juice extraction and this is performed with special presses. The expected yield of apple juice is from 58 lt. to 70 lt. per 100 kg. of the apples. After extraction, apple juice contains more or less finely divided pomace. To remove these particles the juice is usually screened and filtered. The most important commercial method of apple juice preservation is pasteurization. The main purpose of pasteurization is to destruct the vegetative microorganisms where the usual pasteurization temperatures are between 76.5°C and 87.5°C for treatment times of 25 to 30 seconds. In general pasteurization is applied to kill the majority of the microorganisms, particularly the nonsporulated pathogenic bacteria present in a product. The amount of heat used is much lower than that of sterilization. Therefore, the sensory and nutritional values of pasteurized foods are only slightly changed compared to

the raw material. Preservation by pasteurization has to be combined with other forms of preservation, like refrigeration. In commercial processing some chemical prescrvatives ,i.e. benzoic acid salts, some benzoate derivatives, sulfurous acid or its salts and sorbic acid are also used. Apple juice contains a considerable portion of the soluble constituents of the original apple. The water content of the fruit affects the quality and composition of the juice since it affects the percentage of soluble solids or specific gravity. Apple juice contains sugars, acids, tannin and suspended solids. The quantity of acid and sugar and the kind of sugar varies with variety, condition of fruit, growth conditions and location. Main sugars in apple juice are fructose, glucose and sucrose.

1.2. Apple Juice Microflora

Heat resistant bacteria isolated from apple juice are Bacillus brevis and Bacillus coagulans. B.coagulans is a non-pathogenic, motile, spore forming aerobe having as many as ten flagella per cell. It is a common soil organism with a positive gram stain reaction and grows well at 37-45 °C and at pH values between 5.0 and 7.0 . But it is shown that many cultures in their vegetative form could grow at values as low as pH 3.7 . B.coagulans is a flat sour microorganism which indicates a special type of bacterial spoilage in canned foods. Aspergillus species,

Paecilomyces, Penicillium species are apple juice molds and Aureobasidium, Saccharomyces cerevisiae, Saccharomyces chevalieı apiculata, Pichia vini, Rhodotorula rubra, Saccharomyces rosei, Torulopsis glabrata are the spoilage yeasts of the apple juice (8).

1.3. Transition State Theory

In chemical reactions reactants combine to form an unstable activated complex. Eyring's Activated Complex Theory for unimolecular reactions postulates that the activated complex is in equilibrium with the reactants. This equilibrium is treated by the methods of thermodynamics or statistical mechanics. The greater the activation energy the fewer are the collisions involving sufficient energy to cause reaction at a given temperature; and the slower is the reaction since the activated complex is assumed to be in equilibrium with the reactants ;

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A^{*} \qquad (1)$$

$$K_{eq} = \frac{[A^{*}]}{[A]} \qquad (2)$$

Rate of decomposition of the activated complex into the products determines the reaction rate :

$$A^{*} \xrightarrow{k_3} \text{Products} \qquad (3)$$

$$r_A = k_3 [A^{*}] \qquad (4)$$

where,

$$k_3 = \frac{k'T}{h} \qquad (5)$$

Therefore the reaction rate is ;

$$r_A = \frac{k'T}{h} K_{eq} [A] \qquad (6)$$

since,

$$\frac{k'T}{h} = \frac{R\,T}{N_A\,h} \qquad (7)$$

Equation (6) may be rewritten as :

$$r_A = \frac{R\,T}{N_A\,h} K_{eq} [A] \qquad (8)$$

Activation free energy of a chemical reaction is :

$$\Delta G^{\#} = \Delta H^{\#} - T\,\Delta S^{\#} = -R\,T \ln K_{eq} \qquad (9)$$

since ,

$$k' = \frac{R\,T\,K_{eq}}{N_A\,h} \qquad (10)$$

Reaction rate constant may be expressed by substituting Equation (9) into Equation (10) :

$$k' = \frac{R\,T}{N_A\,h} \exp\left\{\frac{\Delta S^{\#}}{R}\right\} \exp\left\{-\frac{\Delta H^{\#}}{R\,T}\right\} \qquad (11)$$

1.4. Thermal Death Kinetics

Bacterial cells undergo changes when exposed to heat. Every part of the cell will be damaged by some temperature, but to pin point the most heat-sensitive compound, essential for life of the cell, is not feasible today. A bacterial cell has a complex system of enzymes. All these proteins will be denatured by elevated temperatures, but the sensitivity varies greatly. Also, heat destroys the genetic material within the cell. Elevated temperatures damage the cytoplasmic membrane of the bacterial cell and the permeability of the membrane changes. According to recent works, bacterial death is not due to a monomolecular reaction, but rather to a combination of events. Bacteria are said to have resisted a heat treatment if they can be shown to have retained their reproductive capacity. The

conditions under which the cells have been grown and their treatment thercafter will influence their resistance to heat. The better the medium for growth, the more resistant will be the cclls. The temperature for growth of cells influences their heat resistance. The heat resistance of vegetative cells varies with the stage of growth. Bacterial cells show their greatest resistance during the late lag phase, but almost as great resistance during their maximum stationary phase, followed by a decline in resistance. The cells are least resistant during their phase of logarithmic growth. Moist heat is by far the most important one in the thermal processing in the food industry since it is a much more effective killing agent than dry heat. pII is another factor. Cells are most heat resistant in a substrate that is at or near neutrality. An increase in acidity or alkalinity cause faster killing by heat. Constituents of the substrate also affect the heat resistance. Soluble carbohydrates, such as sucrose, generally increase the heat resistance of vegetative cells. This is probably due to a reduction of the water activity. Water activity is also reduced when soluble salts are added to the medium. This might raise the heat resistance, but there is also a chance that the salt in high concentration can cause damage to the cell. As a general rule, vegetative cells show highest heat resistance in complex organic media such as food. This might be due to a low water activity protective substances such as proteins,

peptides, amino acids and fats. Since the heat resistance of microorganisms is based on the cell's viability after heat treatment, the recovery medium becomes extremely important. Death of the microorganisms generally follows first order kinetics (1) :

$$\frac{dx}{dt} = - k_d x \qquad (12)$$

Temperature dependency of the thermal death rate constant k_d may be expressed with the Arrhenius expression as :

$$k_d = k_{do} \exp \{ - \frac{E}{R T} \} \qquad (13)$$

Since the death of the cells may occur due to very large number of causes, numerical values of the constants k_d, k_{do} and E strongly depend on the experimental conditions. Rate constants of a unimolecular chemical reaction may be predicted with the Eyring theory (2). Using the analogy between the unimolecular chemical reactions and the thermal death kinetics as described in Eqn (12), the numerical value of the death rate constant k_d may be estimated as :

$$k_d = \frac{R T}{N_A h} \exp \{ \frac{\Delta S^{\#}}{R} \} \exp \{ - \frac{\Delta H^{\#}}{R T} \} \qquad (14)$$

Comparison of Equation (13) with Equation (14) requires (2):

$$E = \Delta H^{\#} + R T \qquad (15)$$

and

$$k_{do} = 2.72 \frac{R\,T}{N_A\,h} \exp\left\{\frac{\Delta S^{\#}}{R}\right\} \qquad (16)$$

1.5. Kinetic Compensation Relations

Generally linear relations are observed between the activation energy E and the frequency factor k_{do} ; and the activation enthalpy $\Delta H^{\#}$ and the activation entropy $\Delta S^{\#}$ of the family of related reactions :

$$\ln k_{do} = \alpha E + \beta \qquad (17)$$

$$\Delta S^{\#} = \delta \Delta H^{\#} + \phi \qquad (18)$$

A set of chemical reactions occuring in the same medium at different pH values, or a set of chemical reactions occuring in a medium where composition of only one of the components is changing may be referred to as the related family of reactions. The relations expressed by Eqn (6) and Eqn (7) are referred to as the kinetic compensation relations and were widely observed in various areas of chemistry and biology (3-7). The kinetic compensation relations claim that in a family of related reactions the activation energy and the frequency factor; and the activation enthalpy and the activation entropy are not independent from each others and in such reactions any change in the activation energy were

compensated by the changes in the frequency factor; and also any change in the activation entropy were compensated with the changes in the activation enthalpy. Numerical values of parameter E indicates the sensitivity of the reaction rate constant to temperature variations, i.e., larger the numerical value of parameter E, larger is the variation of parameter k_d with temperature changes. In biological systems frequency factor - activation energy compensation relations may serve to reduce the sensitivity of the microorganisms to the temperature effects (4, 5). Numerical values of parameters k_{do}, E, $\Delta S^{\#}$, and $\Delta H^{\#}$, depend on the reaction conditions such as pH, medium composition, etc., however the parameters α, β, ζ and ϕ do not depend on the reaction conditions. Bacillus coagulans species are among the most heat resistant microorganisms isolated from apple juice (9). Information about thermal death kinetics of the microorganisms help in designing better pasteurization or sterilization processes and contribute to the improvement of the quality of the processed beverages. In the presented study, variation of the kinetic parameters associated with death of B. coagulans in glucose or sucrose added apple juice will be analyzed by using the analogy between the rate expressions of the unimolecular chemical reactions and thermal death.

The rate of thermal inactivation of the virus in different concentrations of two different salts were determined. Both families of Arrhenius plots generated from

these data indicate the same temperature of compensation within fairly close limits. Then, it is proposed that the temperature of compensation is a physical constant of biological interest (3).

The death kinetics of a strain of Saccharomyces cerevisiae were studied in an industrial scale spray drier. It was found that during drying, although the rate of death is high, the activation energy is greatly decreased over that of death in aqueous solution. This reduction may be attributed to the thermodynamic compensation phenomenon in which the resulting negative entropy of reaction acts to protect the cells through a water-protein interaction (5).

Sixty eight Arrhenius plots of thermal death in six mesophilic yeast species, tested at various concentrations of NaCl. Linear thermodynamic compensation occured in each of the six strains, when stationary populations of the same strain were tested at various NaCl concentrations. The linear and non-linear thermodynamic compensation in biological rate processes is discussed (6).

Enthalpy/Entropy and Frequency Factor/Activation Energy compensation effects in the heat denaturation of whey proteins were demonstrated (7). Isokinetic temperatures were determined. These values were higher than those associated with thermal death of microorganisms caused by heat denaturation of proteins.

CHAPTER - 2

EXPERIMENTAL METHODS

2.1. Materials

2.1.1. Apples

"Red Delicious" apples (Malus domestica) were purchased in the local market.

2.1.2. Bacteria

Bacillus coagulans, was supplied from the Microbiology Department of Agriculture Faculty of Ankara University.

2.1.3. Growth Media

Solid media used to enumerate the microorganisms was made of 2% peptone (Difco, USA), 1% yeast extract (Difco, USA), 2% glucose (Sigma, USA), and 1% agar (Difco, USA).

2.2. Preparation

2.2.1. Preservation of Bacteria and Preparation of Slants and Petri Plates

Slants and petri plates were prepared with the growth media. Glucose and the rest of the medium were sterilized in separate flasks to prevent excessive browning.

Medium containing flasks were sterilized at 121°C for 15 minutes in an autoclave (Dedeoğlu-TURKEY). It took about 10 minutes to come down to atmospheric pressure after sterilization. The contents of the flasks were cooled down to about 70°C, then mixed under sterile conditions. Petri plates were prepared with about 20 mL of hot growth medium. They were cooled down, solidified, and refrigerated. The slants were prepared in sterile test tubes with about 5 mL of hot growth medium, then cooled down in tilted position and refrigerated. A loop-full of cells from the bacteria slants was transfered to these slants. They were incubated at 37 °C for 24 hours, then refrigerated for further use. Inoculated slants were stored in the refrigerator for less than one month.

2.2.2. Preparation of Apple Juice

Apples were peeled, sliced and juice was obtained by using a typical home juicer (Oster automatic pulp ejector, juicer; USA).

2.2.3. pH and °Brix Adjustment

The natural pH value of filtered apple juice was 3.8 The filtered apple juice had soluble solids content of 14 °Brix. Soluble solids content of the apple juice was adjusted by adding glucose or sucrose. The effect of the

total soluble solids content on kinetic properties of Bacillus coagulans in apple juice was determined at 14 , 16, 18, 20 Brix. The total soluble solids content of apple juice was increased by adding glucose or sucrose.

2.2.4. Inoculum Preparation

Cells were transfered from the slants into the sterile apple juice and cultivated in the shake flasks overnight. Flasks were shaken at low speed on a shaker (Nüve, TURKEY). About 100 mL of culture was grown in 250 mL flask at 37 °C for 24 hours in an incubator (Dedeoğlu-TURKEY).

2.3. Processing

2.3.1. Thermal Processing

About 5 mL portions of the culture were poured into sterile test tubes and capped under sterile conditions. They were thermally processed in a water bath at required temperatures. Regular time intervals were considered for each test tubes and selected temperatures used. 70°C, 80°C, and 90°C were the temperatures used. The selected time interval was 45 seconds.

2.3.2. Plating Procedure

One mL of culture was removed from each thermally processed test tube and placed into 9 mL sterile distilled water. Appropriate dilutions were spread on the sterile solid media containing plates with a sterile glass hockey stick near the open flame. The plates were turned up-side down about 15 minutes after spreading and incubated at 37°C for about 24 hours before the colonies were counted. Sterile glassware were used througout the experiments. The same pipet was never used to handle the suspensions of different concentrations.

CHAPTER - 3

RESULTS AND DISCUSSION

When Equation (12) is integrated, a linear relationship in the form of;

$$\ln X = \ln X_o - k_d t \qquad (19)$$

is obtained. When numerical values of parameter ln X were plotted against time, numerical values of the death rate constant k_d were obtained from the slope of the line. Variation of the death rate constant k_d with temperature was simulated with the Arrhenius expression. When ln k_d was plotted against 1/T , Equation 13 represents a line with slope -E/R and intercept of the line with, 1/T = 0 axis was ln k_{do} . Numerical values of the activation E and the natural logarithm of the frequency factor k_{do} were given in Table - 10 and Table - 11. Numerical values of the activation enthalpy $\Delta H^{\#}$ and the activation entropy $\Delta S^{\#}$ were calculated from Equations (15) and (16) after substituting appropriate values for the activation energy E, temperature T, frequency factor k_{do} , and constants R, h and N_A . It was shown in Table - 10 and Table - 11 that there was no trend in variation of parameters ln k_{do} , E, $\Delta H^{\#}$, $\Delta S^{\#}$ with glucose or sucrose concentrations. Although Equations (15) and (16) imply that parameters $\Delta H^{\#}$ and $\Delta S^{\#}$ were functions of

temperature, within range of the experiments numerical values of these parameters varied less than 1 % in each related set, therefore constant average values were reported in Table - 10 and Table - 11.

In the Eyring's theory, a molecule is first assumed to form an activated complex, then the activated complex is converted into the reaction products. Numerical values of the activation entropy $\Delta S^{\#}$ indicate the amount of the structural changes that the reactants undergo while forming the activated complex. In chemical reactions numerical values of $\Delta S^{\#}$ usually vary between -100 and 100 Joules/K mol (2). If there is an increase in the rotational and vibrational freedom of the activated complex $\Delta S^{\#}$ is positive, if there is a decrease in rotational and vibrational freedom of the activated complex $\Delta S^{\#}$ has a negative value (2). It was shown in Table - 10 and Table - 11 that variation of the numerical values of the activation entropy $\Delta S^{\#}$ was beyond the limits observed in the chemical reactions. This results may imply that activation process in the thermal death phenomena was more complex than those of the chemical reactions.

The compensation reaction between the frequency factor k_{do} and the activation energy E was given in Equation (17). Numerical values of parameters and were determined in Figure - 10 . Numerical values of the parameters of

Equation (18) were determined in Figure - 11 . All the reactions of a related family may have the same rate at a certain temperature. This is referred to as the isokinetic temperature (3-7). When an isokinetic temperature exists, coefficients of Equation (17) and Equation (18) may be cxpressed as $\alpha = 1 / R T_c$, $\beta = \ln k_c$, $\delta = 1 / T_c$ and $\phi = -\Delta G^{\#} / T_c$ (7). From the parameters and numerical values of T_c were calculated as 366 K for both glucose and sucrose added apple juice, however substituting this isokinetic temperature into the Arrhenius expression yielded upto one fold of difference in the numerical values of the death rate constants, implying that there was no actual isokinetic temperature in fruit juice pasteurizations. While studying the kinetics of virus inactivation, Barnes et al. (3) claimed that having an actual isokinetic temperature indicates that all the members of the related family of experiments were actually occuring with the same mechanism. Cellular structure of B. coagulans are much complicated than those of the viruses, therefore slight changes of the medium structure may also change the death mechanism as revealed by the criterion of Barnes et al. (3). The large variations observed in the numerical values of the activation entropy $\Delta S^{\#}$ and the activation energy E within the related sets of experiments also confirmed this conclusion.

The kinetic analysis made in the present study were based on the analogy between the unimolecular chemical reactions and the microbial death. In chemical reactions the term "mol" applies to the actual mols of the chemical species but in the microbial systems the term "mol" is an apperent, i.e., empirical, term and it may be obtained from the elemental analysis of the cell.

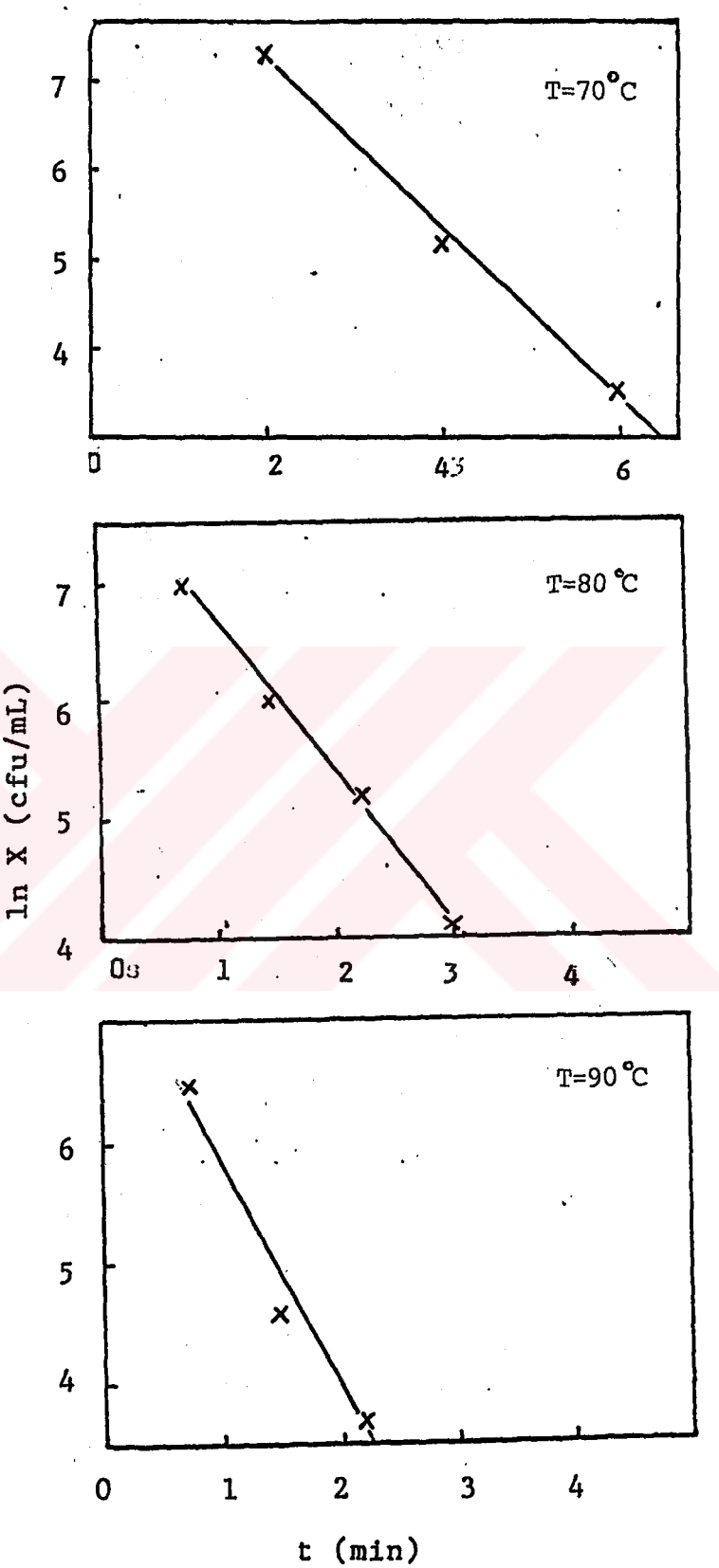

FIGURE 1. Determination of the death rate constant k_d in apple juice (14 ° Brix)

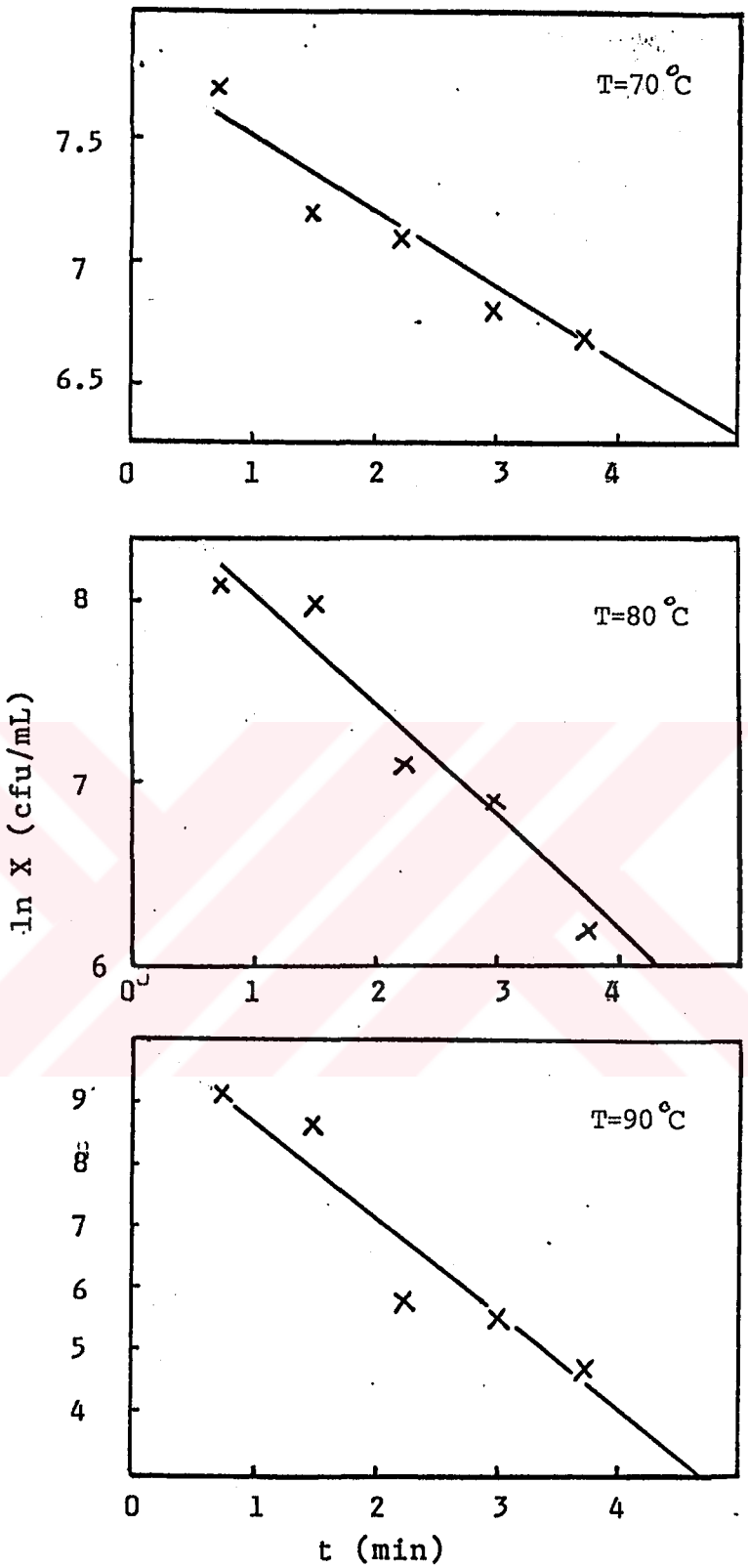

FIGURE 2. Determination of the death rate constant k_d in glucose added apple juice (16 °Brix)

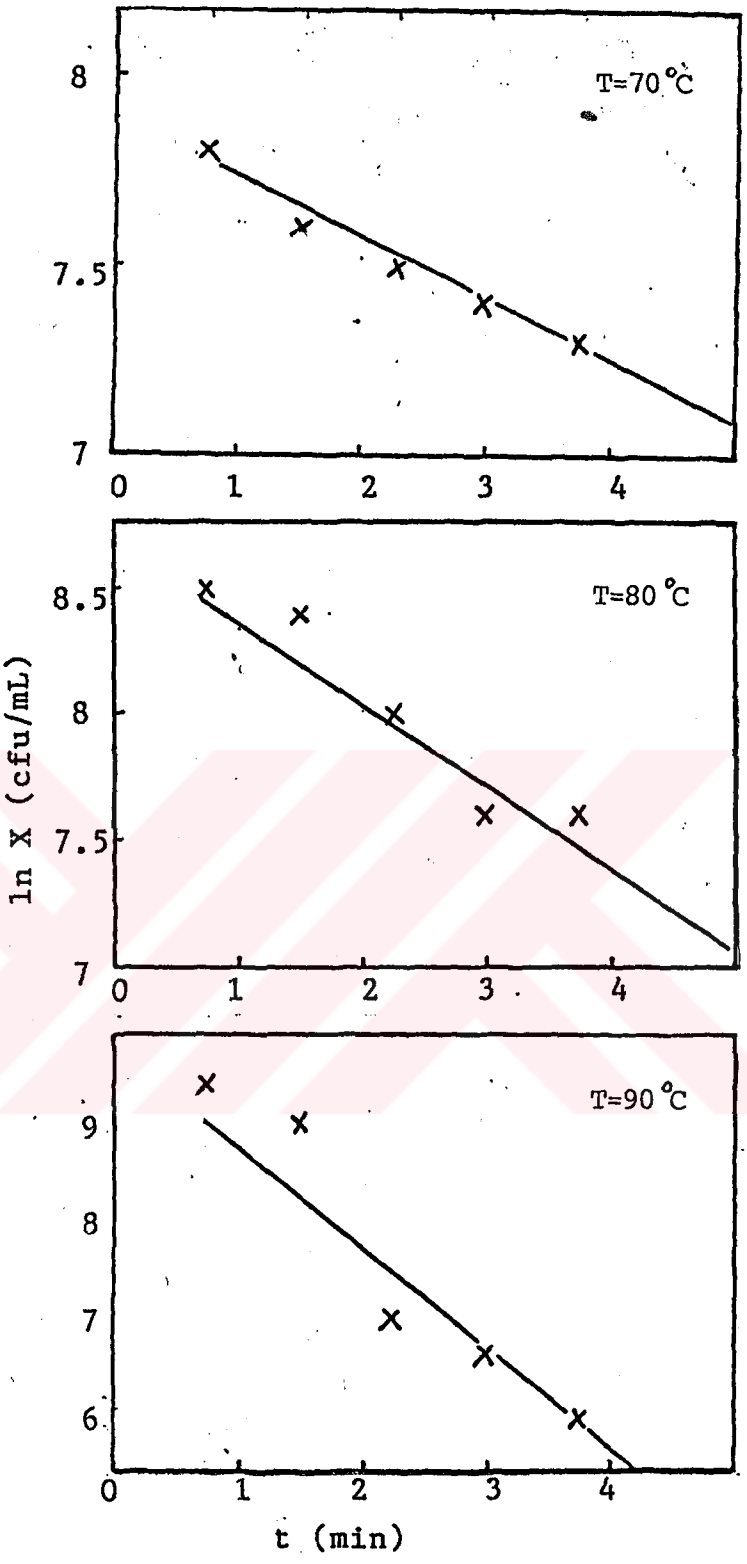

FIGURE 3. Determination of the death rate constant k_d in glucose added apple juice (18 ^{o}Brix)

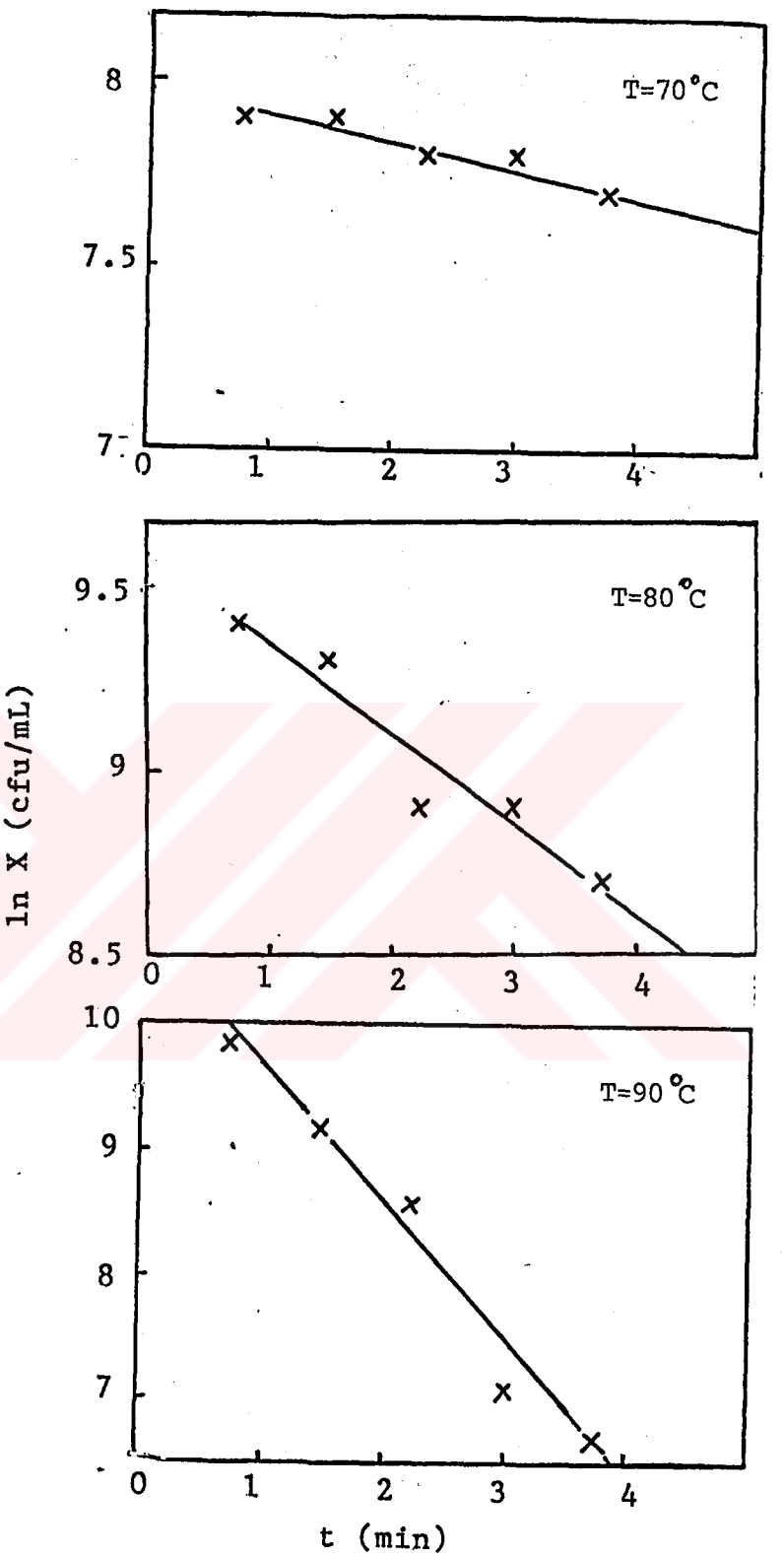

FIGURE 4. Determination of the death rate constant k_d in glucose added apple juice (20 ^{o}Brix)

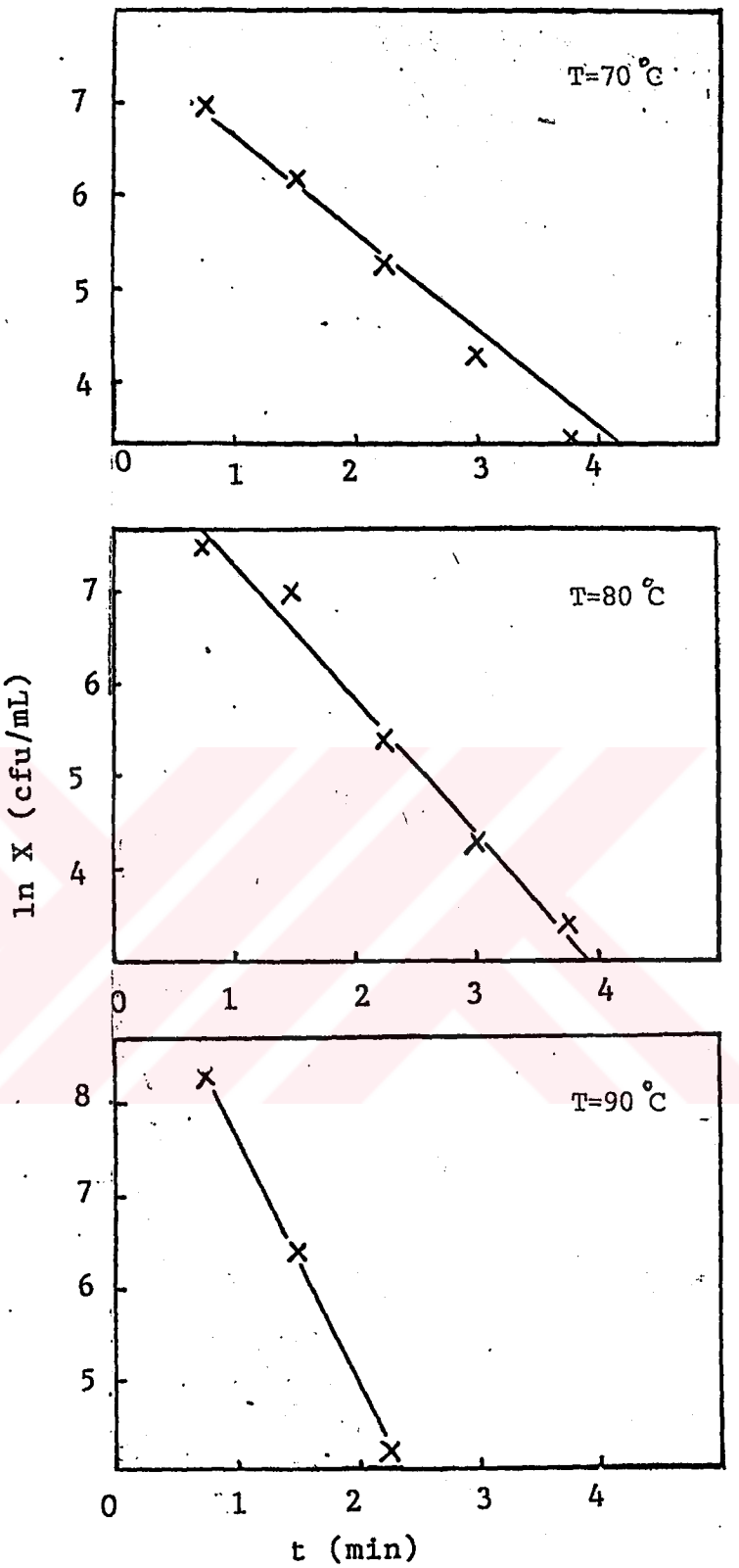

FIGURE 5. Determination of the death rate constant k_d in sucrose added apple juice (16 ^{o}Brix)

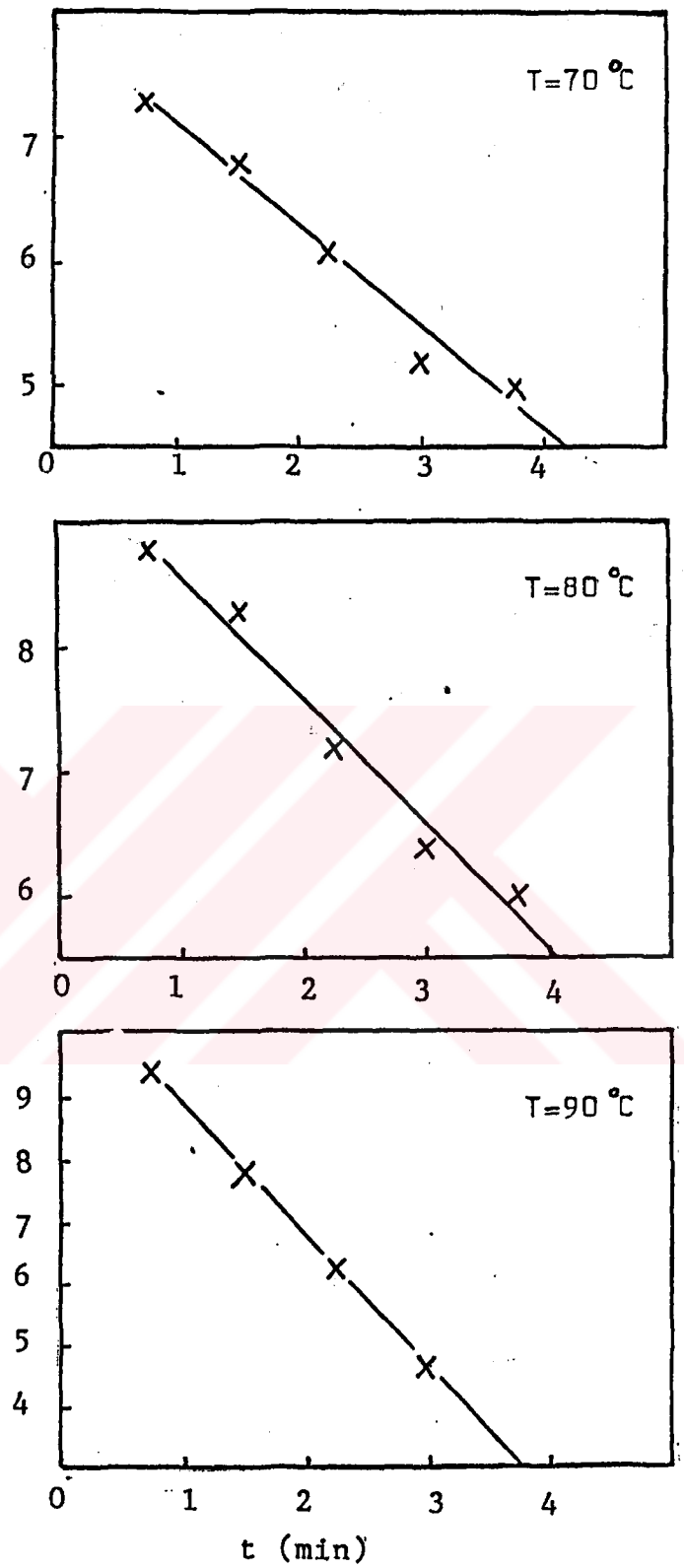

FIGURE 6. Determination of the death rate constant k_d in sucrose added apple juice (18 ^{o}Brix)

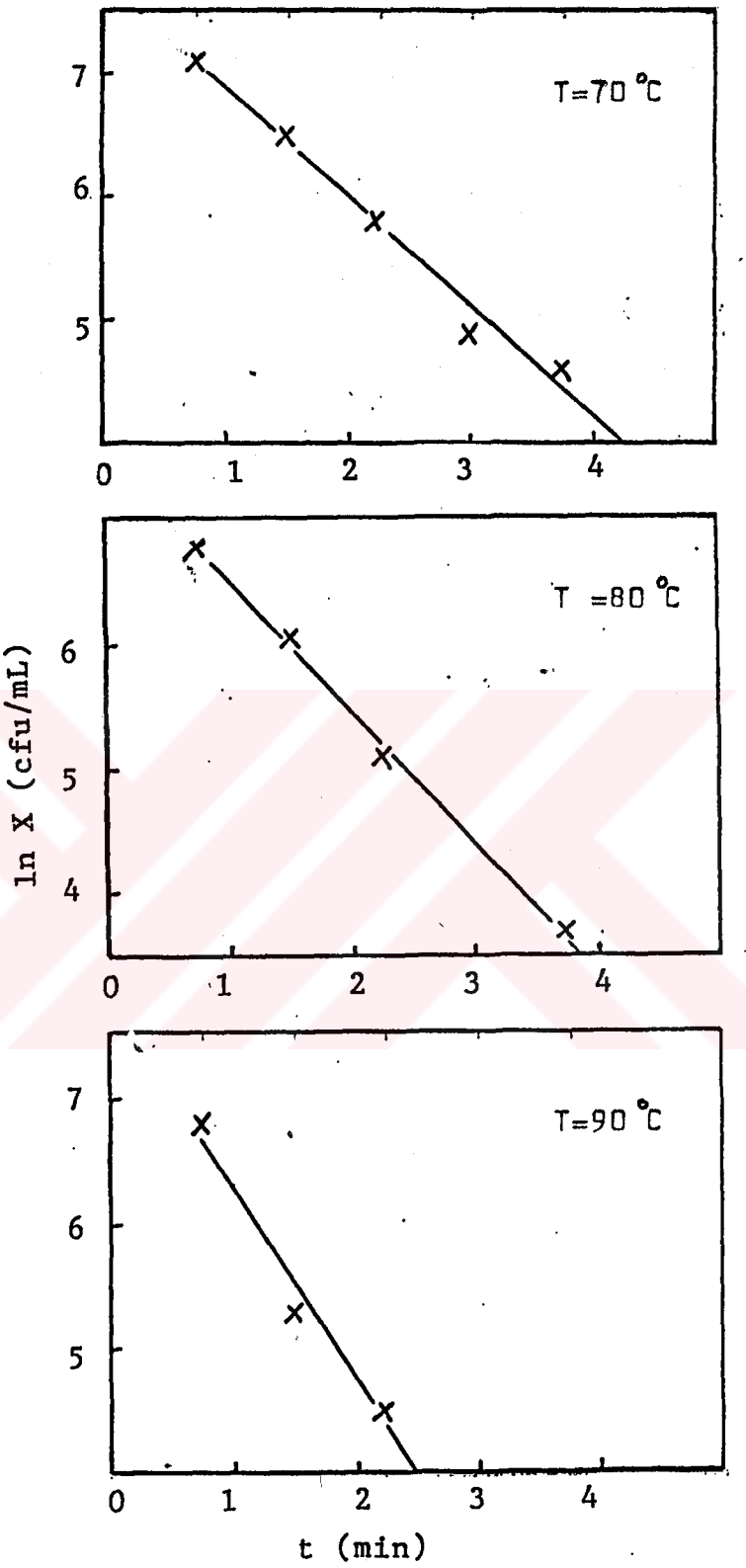

FIGURE 7. Determination of the death rate constant k_d in sucrose added apple juice (20 ^{o}Brix)

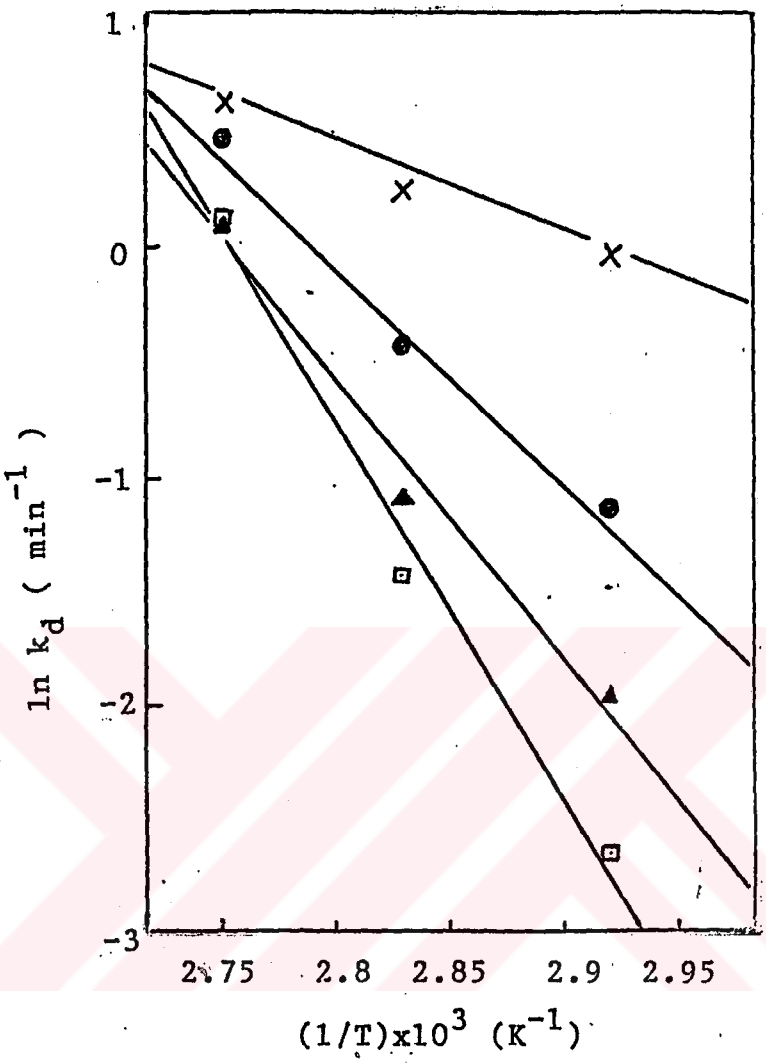

FIGURE 8. Arhenius plots for death of B. coagulans in glucose added apple juice. (x) 14 °Brix, (●) 16 °Brix, (▲) 18 °Brix, (□) 20 °Brix

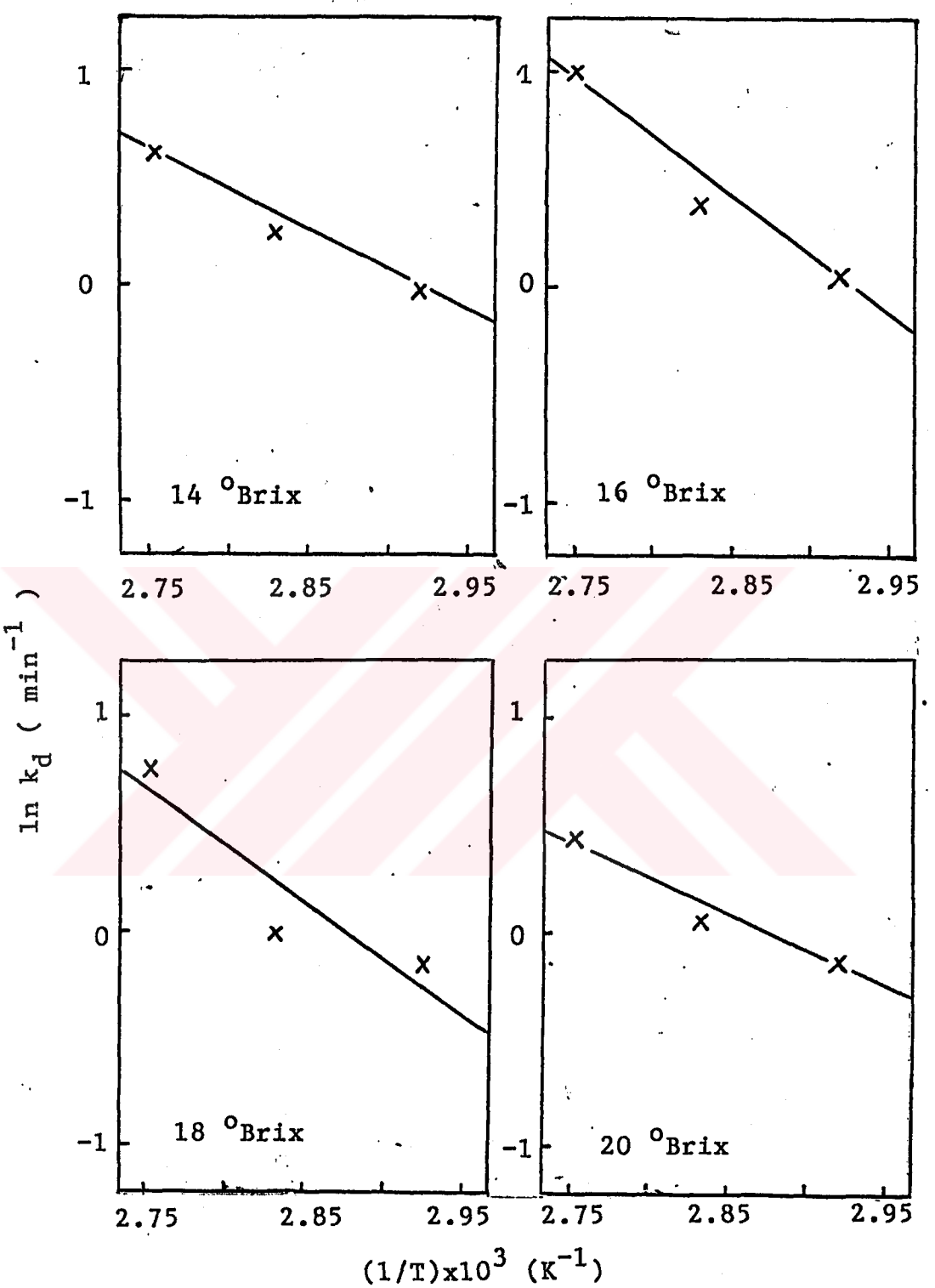

FIGURE 9. Arrhenius plots for death of B. coagulans in sucrose added apple juice.

FIGURE 10. Variation of the frequency factor ln k_{do} with activation energy E under different experimental conditions.

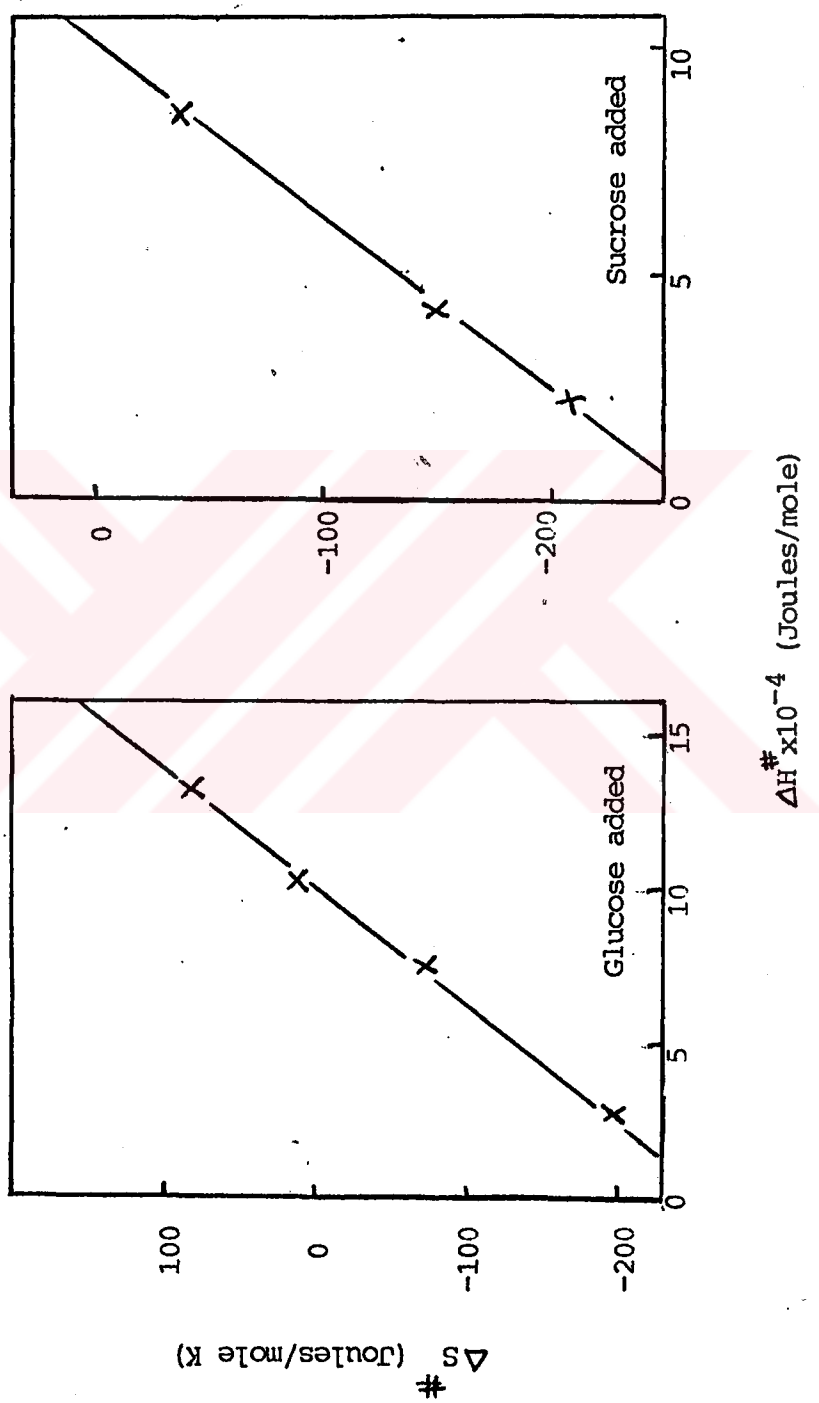

FIGURE 11. Variation of the activation entropy $\Delta S^{\#}$ with the activation enthalpy $\Delta H^{\#}$ under different experimental conditions.

CHAPTER - 4

CONCLUSIONS

Kinetic analysis of the death of B. coagulans in apple juice was made by using the analogy between unimolecular reactions and microbial death. Although the kinetic parameters $\ln k_{do}$, E, $\Delta H^{\#}$ and $\Delta S^{\#}$ did not change systematically with glucose or sucrose content of the apple juice, these parameters agreed with the enthalpy - entropy and the frequency factor - activation energy compensation relations. Analysis of the data indicated that there was no actual isokinetic temperature for thermal death of B. coagulans in apple juice, implying that different death mechanisms were prevailing in each related medium.

CHAPTER - 5

RECOMMENDATIONS

In this research, enthalpy - entropy and frequency factor - activation energy compensation relations for death of B. coagulans in apple juice were studied. Red delicious apples were used in this study. The same study should be repeated with different varieties such as golden apple. Effect of variety growth conditions of fruit should also be studied.

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APPENDIX - A

TABLES :

TABLE - 1 : Results of the experiments done with apple juice at 14 °Brix.

T (°C)	t (sec)	x (cfu/mL)	ln x (cfu/mL)
70	120	1450	7.3
70	240	185	5.2
70	360	30	3.4
80	45	1143	7.0
80	90	390	6.0
80	135	180	5.2
80	180	60	4.1
80	225	-	-
90	45	648	6.5
90	90	105	4.6
90	135	40	3.7
90	180	-	-
90	225	-	-

TABLE - 2 : Results of the experiments done with glucose at 16 °Brix.

T (°C)	t (sec)	x (cfu/mL)	ln x (cfu/mL)
70	45	2246	7.7
70	90	1364	7.2
70	135	1204	7.1
70	180	900	6.8
70	225	838	6.7
80	45	3333	8.1
80	90	3050	8.0
80	135	1193	7.1
80	180	977	6.9
80	225	495	6.2
90	45	8567	9.1
90	90	5700	8.7
90	135	317	5.8
90	180	245	5.5
90	225	110	4.7

TABLE - 3 : Results of the experiments done with glucose at 18 °Brix.

T(°C)	t (sec)	x (cfu/mL)	ln x (cfu/mL)
70	45	2415	7.8
70	90	1920	7.6
70	135	1860	7.5
70	180	1720	7.4
70	225	1540	7.3
80	45	5050	8.5
80	90	4675	8.4
80	135	3058	8.0
80	180	2056	7.6
80	225	2000	7.6
90	45	13167	9.5
90	90	9133	9.1
90	135	1100	7.0
90	180	757	6.6
90	225	360	5.9

TABLE - 4 : Results of the experiments done with glucose at 20 °Brix.

T(°C)	t (sec)	x (cfu/mL)	ln x (cfu/mL)
70	45	2820	7.9
70	90	2690	7.9
70	135	2455	7.8
70	180	2345	7.8
70	225	2290	7.7
80	45	12475	9.4
80	90	10750	9.3
80	135	7700	8.9
80	180	7433	8.9
80	225	6067	8.7
90	45	19475	9.9
90	90	9475	9.2
90	135	5400	8.6
90	180	1260	7.1
90	225	775	6.7

TABLE - 5 : Result of the experiments done with sucrose at 16 °Brix.

T (°C)	t (sec)	x (cfu/mL)	ln x (cfu/mL)
70	45	1150	7.0
70	90	477	6.2
70	135	210	5.3
70	180	75	4.3
70	225	55	4.0
80	45	1758	7.5
80	90	1093	7.0
80	135	218	5.4
80	180	75	4.3
80	225	30	3.4
90	45	3967	8.3
90	90	583	6.4
90	135	70	4.2
90	180	-	-
90	225	-	-

TABLE - 6 : Results of the experiments done with sucrose at 18 °Brix.

T (°C)	t (sec)	x (cfu/mL)	ln x (cfu/mL)
70	45	1443	7.3
70	90	943	6.8
70	135	445	6.1
70	180	183	5.2
70	225	145	5.0
80	45	6475	8.8
80	90	3867	8.3
80	135	1397	7.2
80	180	593	6.4
80	225	403	6.0
90	45	12700	9.4
90	90	2520	7.8
90	135	503	6.2
90	180	104	4.6
90	225	-	-

TABLE - 7 : Results of the experiments done with sucrose at 20 °Brix.

T (°C)	t (sec)	x (cfu/mL)	ln x (cfu/mL)
70	45	1200	7.1
70	90	670	6.5
70	135	330	5.8
70	180	130	4.9
70	225	100	4.6
80	45	887	6.8
80	90	430	6.1
80	135	160	5.1
80	180	-	-
80	225	40	3.7
90	45	857	6.8
90	90	193	5.3
90	135	87	4.5
90	180	-	-
90	225	-	-

TABLE - 8 : Numerical values of the kinetic parameters with glucose added apple juice.

°Brix (%)	T (°C)	k $(min^{-1})^{d}$	ln k $(min^{-1})^{d}$	$1/T*10^{3}$ (K^{-1})
14	70	0.97	- 0.030	2.92
	80	1.28	0.247	2.83
	90	1.85	0.615	2.75
16	70	0.32	- 1.140	2.92
	80	0.66	- 0.415	2.83
	90	1.58	0.457	2.75
18	70	0.14	- 1.966	2.92
	80	0.33	- 1.110	2.83
	90	1.10	0.095	2.75
20	70	0.07	- 2.660	2.92
	80	0.24	- 1.430	2.83
	90	1.13	0.120	2.75

TABLE - 9 : Numerical values of the kinetic parameters with sucrose added apple juice.

°Brix (%)	T (°C)	k (min^{-1})	ln k (min^{-1})	$1/T*10^3$ (K^{-1})
14	70	0.97	- 0.030	2.92
	80	1.28	0.247	2.83
	90	1.85	0.615	2.75
16	70	1.05	0.050	2.92
	80	1.45	0.370	2.83
	90	2.73	1.000	2.75
18	70	0.86	- 0.150	2.92
	80	1.00	0.000	2.83
	90	2.13	0.760	2.75
20	70	0.88	- 0.128	2.92
	80	1.05	0.050	2.83
	90	1.53	0.430	2.75

TABLE - 10 : Variation of the numerical values of the kinetic parameters with glucose added apple juice.

^{o}Brix (%)	ln k_{do} (1/min)	E (Joules/mol)	$\Delta H^{\#}$ (Joules/mol)	$\Delta S^{\#}$ (Joules/mol.K)
14	11.1	$3.16*10^{4}$	$2.87*10^{4}$	- 197
16	26.1	$7.78*10^{4}$	$7.49*10^{4}$	- 71
18	34.1	$1.1*10^{5}$	$9.9*10^{4}$	14
20	44.85	$1.35*10^{5}$	$1.32*10^{5}$	83

TABLE - 11 : Variation of the numerical values of the kinetic parameters with sucrose added apple juice

°Brix (%)	ln k$_{do}$ (1/min)	E (Joules/mol)	$\Delta H^{\#}$ (Joules/mol)	$\Delta S^{\#}$ (Joules/mol.K)
14	11.1	$3.16*10^{4}$	$2.87*10^{4}$	- 197
16	16.19	$4.6*10^{4}$	$4.30*10^{4}$	- 156
18	30.1	$8.3*10^{4}$	$9.7*10^{4}$	- 41
20	9.34	$2.70*10^{4}$	$2.30*10^{4}$	- 207