Effect of Processing Temperature on Meatballs under Dynamic Storage Condition Using Evaluation of the Arrhenius Model

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ABSTRACT

Temperature treatment during the processing of meatballs is intended to prevent contamination. However, the heat treatment more frequently harms the food structure and loses major nutrients of meatballs. The Arrhenius model has been used to observe the heat-treatment effect on the degradation of food quality. The meatballs samples with edible coating were observed under dynamic temperature storage. The purpose of this study was to use the Arrhenius model to evaluate the heat-treatment relationship on the pH change during dynamic storage conditions: uncontrolled ranging 24 to 31°C, while the studied temperature treatment was ranging between 50 to 90°C for 15 minutes. The results showed an obvious relationship between the heat-treatment aspects for preventing bacteria growth during storage. The evaluation of the Arrhenius model result indicates peak nutrition loss phase transition was found in the temperature treatment range of 83 to 90°C, and the optimum heat-treatment level at 78°C or 148.15 kJ mol^{-1} for developing packaging or preservation methods.

Keywords: Dynamic temperature storage, Food quality, Food storage, Meatballs preservation.

INTRODUCTION

The meatball is a popular food product in southeastern Asia countries. The basic ingredients of meatballs are usually made from various processed meat such as chicken, beef, or fish with the addition of other ingredients (flour, spices) and seasoning (Verma *et al*., 2015; Purnomo and Rahardiyan, 2008). In Indonesia, the meatball, more known as 'bakso', trader has two typical packaging methods: (1) Hygiene packaging put in sterile big pouches located within cool storage which assures long-term storage of products, and (2) More common, usually done during selling, using a rattan basket without a fridge, in the open room. In Indonesia, meatballs as street food, statistically about 68 to 72% of consumers buy from a small trader, vending with traditional wagon strolling from place to place during the sale (BPS, 2020). Therefore, this practice has serious concerns about product safety and becomes questioning necessary to protect customers' health, particularly preventing food poisoning or spoilage. Particularly, food quality control as a function of time under an unstable environment (i.e. temperature, non-chemical hazard) leads to degradations of major food nutrition (Tripathi and Giri, 2014; Møretrø and Langsrud, 2017).

In real-time storage or dynamic condition, fluctuation in temperature could stimulate the instantaneous microbial growth and make it difficult to prevent nutrition loss during storage (Song *et al*., 2016; Wu *et al*., 2018). The high protein content in meatballs is often lead lipid oxidation under 10 hours at room temperature occurring along with changes in pH and acidity and causes

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microbial growth or spoilage spread (Umaraw and Verma, 2017). The rotten meatballs state is at pH 6.0 with 1–1.8% of total acidity (BPOM, 2011; Aberle, 2001). To solve this condition, incorporating the edible with meatballs may be regarded as a common preservation tool. Few published studies have been conducted by Akcan *et al.* (2017) who used edible film during frozen storage and Pakpahan *et al.* (2019) who incorporated edible coating with natural compounds oleoresin. More lately, two meatball study was conducted by Desvita *et al.* (2020) using edible bioactive compound chitosan liquid smoke and Şen and Kılıç (2021) edible coating with matcha tea extracts. However, all these studies used control temperature during storage. There is still no vast study on meatballs with edible under dynamic condition storage (real-time uncontrolled temperature).

For the development of preservation tools in meatballs, hence, a useful predictive model suitable under dynamic conditions is required. Several attempts have been reported for model interaction of temperature and moisture of diffusivity (İsmail and Kocabay, 2018; Voloski, *et al.*, 2016), acidity change with temperature conditions (Jaisan and Lee, 2017), and, recently, Huang (2019) who developed a model to describe thermal inactivation and thermal degradation. Practically, thermal treatment during food processing is the main step to ensure inactivation of certain microorganisms in order to safe food for eat and, usually, getting better shelf-life. In line with this study, but on beef sausage packing with edible, Sukumaran *et al.* (2018) revealed that the thermal treatment preparation before storage have significant influence for inactivating chemical compounds, enzymes, and microorganisms. However, inappropriate thermal temperature procedure on beef adversely affects the breaking of intramolecular bonds and losing intramuscular tissue of the connective fat deposition, resulting in meat becoming very dry, and destruct protein content

(Muhammad *et al.*, 2018; Malva *et al.*, 2018; Aksit *et al.*, 2006).

Therefore, this study aimed to attempt using predictive model of Arrhenius equations for evaluating the thermal process relation, focusing on food quality (chemical and microorganism) degradation process workable to estimate meatballs with edible coating under dynamic storage conditions. A specific first factor is thermal treatment during processing (cooking, and coating) which might contribute to nutrient loss, second factor is pH change, which is more consistent in transition temperatures in storage and is considered susceptible to microbial contamination, while acidity is more affected by seasoning ingredients. Therefore, pH value is more probable than acidity used as factor for modeling estimation of meatballs under dynamic temperature conditions storage.

MATERIALS AND METHODS

The material used for edible coatings in this study was *Xanthosoma sagittifolium* (starch) the common material used among meatball trader obtained 100-mesh starch from the Laboratory of Food Technology at the University of Muhammadiyah Malang. The additive was Potassium sorbate Merck (Darmstadt, Germany), glycerol Merck (Darmstadt, Germany), and all reagents were purchased from Sigma-Aldrich (Missouri, United States). Meatballs ingredients were purchased from a local market.

Processing Meatball

Meatball Preparation

In this study, the meatballs preparation, characteristics, and quality follow Indonesian Standard Food guideline SNI No: 3818:2014 (BSN, 2014). In the initial stages, fresh beef is washed with fresh running water and cleaned, then, mashed using the electric meat grinder (±3.000 rpm)

for 15 minutes (Fomac MGD-G31, Indonesia). Then, mixing with flour (wheat 30%, sago 70%) and the seasoning ingredient (onion powder 15, garlic powder 20%, salt 40%, black paper powder 10%, baking powder 5%) with ratio 70% (beef): 20% (flour): 10% (seasoning) were performed by meat mixer at normal speed (±1.000 rpm) for 10 minutes (Fomac MMX-TQ5A, Indonesia). Later, the dough is stored in the refrigerator (for 30 minutes) (Firahmi *et al*, 2015), then automatic rounding dough in size ±3 cm with 35 g mass each (Henan TMS XZ-605, China). Afterwards, meatballs were boiled at different temperature 50, 60, 70, 80, and 90°C for 15 minutes. In here, 80°C is control temperature based on the Indonesia Standard Food. Each sample was examined on nutrition properties (protein and TVB-N value) and pH before edible coating.

Edible Active Coating Solution Preparation

The edible coating solution was prepared by making 100 mL of edible solution with concentration of starch 2.0% (w/v), distillate water (mL), glycerol 0.8% (b/v), and potassium sorbate 0.60% b/v, stirred at a controlled temperature of 85°C and 1100 rpm for 5 minutes until gelatinization. Then, meatballs were immersed into the edible solution for 2 minutes. After samples seemed fully coated, they were taken out from beaker glass and stored in drying vacuum condition at 15°C for 5 minutes (Binder ED 56, Germany). Finally, meatball were stored in sterile rattan basket on open room to meet dynamic condition storage criteria (real-time uncontrolled temperature) as small meatballs trader practice with traditional wagon strolling. Every multiple 3 hours, meatballs were collected for assay pH, TVB-N and TPC until the meatballs were fully rotten based on Indonesian Standard Food SNI No: 3818:2014.

pH Value

pH value of the meatballs was measured in triplicate with a digital pH meter (SI analytics LAB 875, Germany) and was calibrated with buffers solution at 3.51 and 7.00 (Merck, Germany) at 20°C. The samples containing 10 g of pounded meatballs into the glass electrode was homogenized with 50 mL of distilled water (AOAC, 2000).

Microbial Assay

Total Plate Count (TPC) was applied to estimate microorganism activity and growth. Samples of 10 g were mashed up by stomacher® 400C prepared aseptically. Then, for dilution, 1 mL samples was put into a sterile tube containing 90 ml NaCl substance and homogenized with the vortex. This step was repeated until reaching 10^{-6} (Olsvik *et al*., 2009). Serial dilutions 10^{-4} to 10^{-6} were taken 1 mL using a micropipette poured on Plate Count Agar media (PCA) Merck (Darmstadt, Germany). All plates were incubated at 37 °C for 24 h, only viable colonies were counted using data format colony-forming units (Log CFU/g).

Total Volatile Basic Nitrogen Component (TVB-N)

The Total Volatile Basic Nitrogen component (TVB-N) as an indicator of chemical damage is determined using the method of Mohebi and Marquez (2015) with a little modification. Meatballs (10 g) are finely chopped, soaked in 15 mL of 5% TCA for 5 minutes. The mixture is put into a centrifuge for 10 minutes. The filtrate (5 mL) plus 5 mL of NaOH 2M is transferred to a distillation and then stored in an Erlenmeyer flask containing 15 mL of 0.01 N HCl until the volume reaches 40 mL. Later, 3 drops of phenol red indicator was added before titrating with 0.01N NaOH until it is pink. Determination of the value of

TVB-N based on the volume of NaOH is needed for titration.

Protein

In meatballs, protein is the primary nutrition and main functional properties of meatballs. The protein loss in meatballs during storage will be affected the tenderness, and loss of nutrition (Soladoye *et al*., 2015). To understand the interaction dynamic storage condition effect on meatballs, crude protein is determined using the Kjeldahl micro method (AOAC, 2005). Meatball sample was cut in size of 0.5 g, plus 2 mL H_2SO_4, and 2 g mixture of Na_2SO_4: HgO (20: 1). The mixture of ingredients was heated for 30 minutes, washed and boiled again for 30 minutes. Distilled water of 10 mL, and 15 mL of NaOH: $Na_2S_2O_3$ (40: 5) was added to dilute with distilled water. After distillation, distillate was titrated with 0.02 N HCl. Total N and percentage protein ingredients was then calculated.

Kinetic Calculations

According to Peleg *et al.* (2016), the food quality degradation is calculated from deterioration of quality value (protein, TVB-N, TPC), often expressed by a simple first-order reaction Equation (1), from the Arrhenius equation,

$$\frac{d(\ln k)}{dT} = \frac{E_a}{R\,T^2} \qquad (1)$$

Where, d (ln k) and dT is logarithmic is natural constant toward degradation Temperature (°K convert to °C), then *Ea* is Energy activation and R is the gas constant 1.987 cal °K^{-1} mol^{-1} and T^2 is absolut Temperature in °K following the order reaction (Lin *et al.* 2011; Peleg *et al.* 2012).

Most microorganisms can become inactivate under high heat temperatures, however, there is a negative effect on the food structure and loss of nutrients (Serment-Moreno *et al*., 2014). Therefore, evaluation of the optimum of heat-treatment on food processing has a correlation with the pH value change before storage (Chinma *et al*., 2015). Jaisan and Lee

(2017) models expanded Huang's model (2011) used to describe predicting degradation of physicochemical reactions. In specific, Jasian and Lee model was shown to be used well for acidity change in kimchi under a fluctuating temperature environment that simulates on Arrhenius model Equations (2) and (3), which was adopted for meatballs evaluation of pH results with heat-treatment.

$$\frac{1}{\lambda} = \left(\frac{1}{\lambda_0}\right) \exp\left(\frac{-E_{a,\lambda}}{RT}\right) \qquad (2)$$

$$\mu_{max} = \mu_{max,0} \exp\left(\frac{-E_{a,\mu}}{RT}\right) \qquad (3)$$

Where λ and μ_{max} are the lag time and maximum acidity increase temperature, $(1/\lambda_0)$ and μ_{max0} are the pre-exponential constant for $(1/\lambda)$

As a final step, Huang's model (2019), Equation (4), was adopted and used to analyze the heat-sensitive modeling and heat-sensitive reality data. To calculate the optimum temperature treatment for inactivation of microorganisms and the relation between a certain degree with pH value change, the following equation can be used.

$$ln(D_0) + ln(A) = 0.1987 + \frac{\alpha E_a}{R} \qquad (4)$$

Where $ln(D_0)$ is ideal heat-sensitive modeling and $ln(A)$ is experiment heat data.

Data Analysis

The experiments data were obtained at two-stage (thermal treatment and storage period) out in three series at randomized group design (van Boekel, 2008). As a final step, to figure out the relationship (ln*t* and 1/T) between thermal treatment and acidity, linear regression was performed to analyze the data using Excel® series 2019.

RESULTS AND DISCUSSION

Meatballs Quality Response on Heat-Treatment

Table 1 shows the statistical result of experimental data on heat-treatment of meatballs' quality changes before storage (0

Table 1. Identification of meatballs quality.

Treatment temperature (^{0}C)	t (Hours)	pH	Protein (%)	TPC (Cfu/g)	TVB-N (mgN%)
	0	4.58±0.13a	26.35±2.18a	3.77±0.08a	10.36±0.78a
	3	5.14±1.17b	26.18±2.31c	4.07±0.73c	14.11±0.09a
50	6	5.87±0.06b	24.00±0.07b	4.73±1.98c	17.06±1.96b
	9	6.32±0.91c	21.96±1.32a	5.51±0.16c	22.18±2.77a
	12	6.91±3.13b	17.02±2.31a	6.69±3.07a	26.16±3.03a
	0	4.11±0.08a	25.92±0.81a	3.63±0.21b	9.83±0.48c
	3	4.61±1.41a	25.67±1.73c	3.88±0.74b	12.55±0.22b
60	6	5.09±2.97c	24.13±0.54b	4.64±2.31b	17.40±0.63a
	9	5.63±0.07c	22.96±0.96a	4.97±3.89a	19.06±0.96a
	12	6.59±1.32c	20.03±2.77a	5.91±0.37a	22.11±2.09b
	0	3.57±1.48a	24.76±1.79b	3.55±0.14ba	8.61±0.11a
	3	4.63±0.81a	23.73±1.07c	3.57±0.71c	10.32±0.22a
70	6	4.98±0.01a	21.09±0.01a	4.08±0.90c	14.92±0.91c
	9	5.57±1.91c	21.46±1.39a	4.73±1.36b	17.00±0.39a
	12	6.03±0.56b	19.94±0.74a	5.18±0.34a	19.68±0.05bc
	0	3.55±0.87a	22.17±0.68a	3.55±0.88a	8.53±0.63b
	3	3.61±0.30b	22.44±1.09a	3.90±1.54b	10.01±0.03b
80	6	3.90±1.35a	20.37±0.37b	4.22±0.87c	13.28±1.05c
	9	4.32±0.81c	19.91±2.21b	4.73±0.22a	16.37±0.49c
	12	4.67±0.03c	19.08±0.88b	4.98±1.29a	18.88±0.13c
	0	3.42±0.53a	18.96±1.48a	3.26±0.34b	7.68±0.27b
	3	3.79±0.08b	18.35±0.73c	3.51±0.74b	9.60±0.41c
90	6	3.96±1.33b	17.90±1.12c	3.97±0.33a	10.00±0.33c
	9	4.03±0.01c	17.47±0.64b	4.31±0.81c	12.19±0.96a
	12	4.57±0.05a	16.91±0.05a	4.64±0.03a	14.36±0.08a

hour) and during storage at the dynamic condition. It can be concluded that under dynamic storage conditions, optimal processing temperature of meatballs had more influence than edible coating. At storage time, after a certain period of time, reduction quality (pH, Protein) of meatballs (Table 1) count was followed by significant increase in TPC and TVBN values. As in analysis, scores obtained for the quality change were significant with the lower temperature (50, 60, and 70°C), while at high temperatures (80, and 90°C) displayed almost equal decrease in quality, however, meatballs with better quality index were around the range between 70 to 80°C. For meatballs with higher temperatures,

exposure resulted in substantial changes in uncontrolled enzyme mechanisms benefit impact to lower or more linear production of hydrogen/acid bacteria (England *et al.*, 2017; Heck *et al.*, 2017; Rahman, 2020). This condition also has a positive effect on preventing developing formation of NH$_3$ as TVBN index. In every 3 hours, the rise of value was restrained below ±2.00 during storage. Meanwhile, meatballs with low temperatures had higher pH, the value increased, even edible coating active was not significantly controlled. The increasing trend in pH could be the result of the accumulation of metabolites by bacterial activity in meat, and deamination of proteins. It could be the result of less

bacteria being eliminated, inducing meatballs to have more bacterial metabolite activity. This leads to failed inactivation of bacteria growth and gets spoilage (Umaraw *et al.*, 2020). Thus, it drives the increased availability of NH_3 resulting in bad odor, more loss nutrition, and shorter shelf life. This evident can be related to beef as the main ingredient of meatballs has high protein, and fat enable immediately lipid hydrolysis, This is because meat did not receive suitable heat temperatures during processing. In another study, our finding is in line with Shukla *et al.* (2015) and Kuswandi and Nurfawaidi (2017) studies to the specific hygienic standard of beef quality, shown without heat treatment at 80–90°C beef were decay at 10 hours storage time. However, our results indicated that meatballs at higher temperatures processing had more loss of protein nutritional intake. Therefore, in order to elucidate specific relation of processing temperature

dependency on meatballs quality, the Arrhenius model analysis was carried out to provide information about quality change at dynamic storage time as presented below.

Kinetic Analysis

The temperature during processing variable is known to have a major effect on interaction of protein and pH value (Pereira de Abreu *et al.*, 2012; Sun *et al.*, 2019). Both indexes are commonly used to measure the food quality aspect based on inhibition of microbial activity, nutrition loss, and estimate of shelf-life. In kinetic analysis, food quality loss are specific reactions classified as zero, first, and second order (Heldman *et al.*, 2018). Based on experimental data of the meatballs (Figure 1-A) present the processing temperature effect on pH value and protein content before storage. Figure 1. B are presents
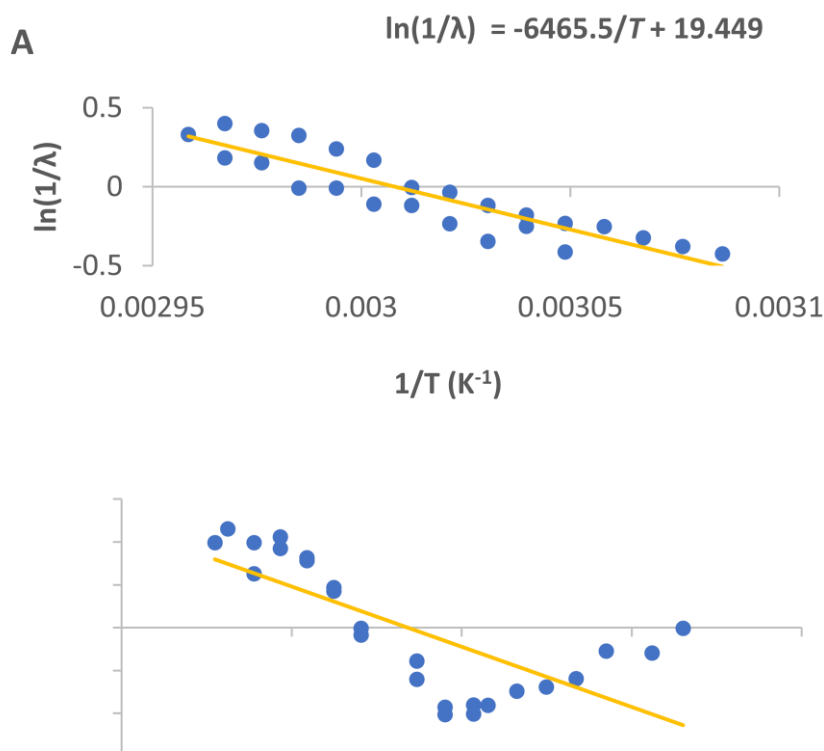

Figure 1. Estimate meatballs pH and protein change of temperature dependence.

dynamic condition storage effect on pH value and protein content. The data presented show the kinetic models fit to a zero-order plot (Equations 2 and 3) response to quality by corresponding temperature. As can be seen, processing temperature $(1/\lambda)$ performance is assumed in a simple linear function as expected with a wide confidence band of kinetic activation energies range 140.15 to 162.86 kJ mol^{-1} and in storage conditions (μ_{max}) at 63.7 to 82.4 kJ mol^{-1}. It shows the rates of reactions temperature dependence are equal to the rate constant k, which typically reactions zero-order kinetics process failed to reach zero rates reaction. This result indicates that relatively good linearity of the kinetic model is obtained between at 70 to 80°C.

Based on the observation, as given in Table 1, results confirmed the heat treatment configurations have the ability to mitigate quality rate change. It describes phase transitions of chemical reaction at storage time are determined by higher-order heat level. In other words, meatballs with exposure to more energy or heat temperatures have a more linear reduction rate change of pH and protein during storage time. It also indicates that there is disorder transition peak during heat-temperature treatment, we assume, that associated with flour as polysaccharides chains (hydrophilic moieties). In this regard, there is equalization change of energy barriers, and could be decomposed at higher temperatures. Hence, the optimal level heat-temperature might control flexibility of the polypeptide chains in order to minimize loss of functional proteins in meatballs. In this way, possible extension would slow down the growth rate of microorganisms without chilling temperature. However, we have an obstacle to compare our result with other cases due to difficulty to find relevant studies. It is interesting to note that interactions average of meatballs' pH change may be contributing to the kinetics capability to be implemented in edible as simple packaging for dynamic storage conditions along distribution chains.

Determination of Thermal Sensitive Point

Activation energy (E_a) is the necessary energy to enable forward chemical reaction, and it is the necessary parameter to evaluate kinetic reaction rate constant. In Yu *et al*. (2020) study, the E_a is a basic principle consistent factor minimum amount of energy when activation energy reaches certain level, it will affect the lag phase of the microorganisms' growth rate and biochemical reaction. In this regard, Huang's model was applied in order to calculate the influence of heat treatment reaction on the rate of pH change and protein denaturing during thermal processing. On model Equation (4), plot of ln D_O+ln A versus 1/T K^{-1} with a slope (E_a/R) which enables comparison of an ideal model of heat with data model obtained from the experimental treatment.

In Figure 2, results from linear lines regression analysis show the E_a ideal model from 50 to 90°C is 344 kJ mol^{-1}, and the analysis of the results indicates experimental E_a optimal heat value is 338 kJ mol^{-1}. Furthermore, in Table 2, β value is 2.160×10^{-9} calculated on 50 to 90°C with the relative error (% difference) is reduced from 1.39 to 0.19%. The R^2 value (0.989) confirms the observed experimental heat-treatment has a significant (P< 0.05) accuracy to the heat ideal model. It means that meatballs with a heating exposure temperature range of 338.15–340.15°K have a close-perfect relationship with linear heat substrate between 1/T and T. Thus, determination heat temperatures that imply a stable reaction bind the quality change rate constants. However, we cannot compare the results with those of other studies, since ln(D$_0$) and ln(A) are not often used to study meat or meatballs quality. In addition to our knowledge, this is the first study that introduces applications of Huang's model (2019) in meatballs.

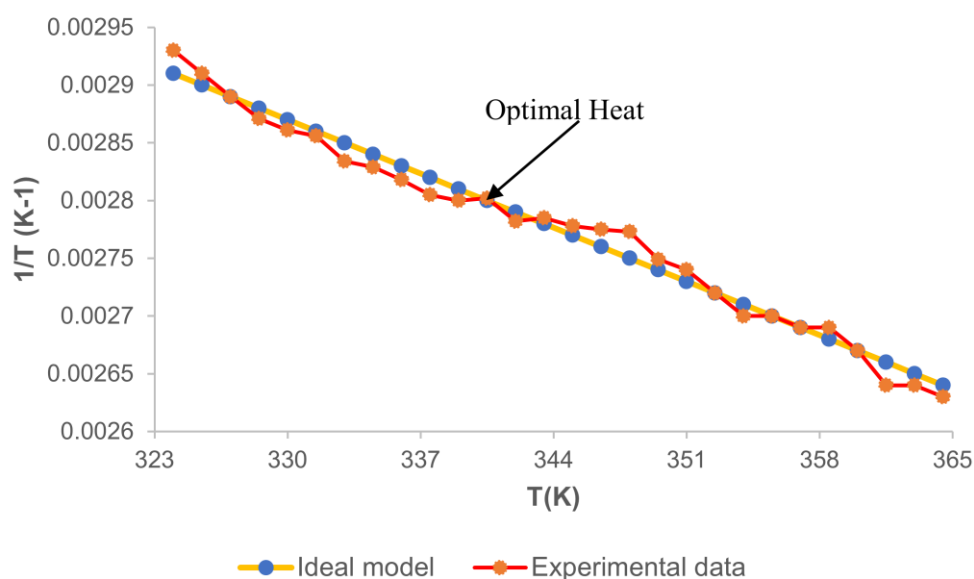However, our study confirmed there is a specific interaction during heat treatment

Figure 2. Comparison of 1/T ideal model linear with experiment data.

Table 2. Result of linear regression comparing ideal model linear with experiment data.

Regression Statistics	
Multiple R	0.9899
R Square	0.9899
Adjusted R square	0.9899
Standard error	1.39E-14
Observations	30

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	5330	2665	1.37E+31	2.30E-11
Residual	29	7.23E-27	1.95E-28		
Total	30	5330			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept (α)	615	5.34E-14	115	1.00E-29	615	615
X Variable (β)	-2.16E-09	1.44E-09	-1.492	2.30E-11	-5.0826E-09	7.7039E-10

processing to prevent developing the rate of quality change (inactivation of microorganisms, nutrient depletion) in meatballs under dynamic storage conditions (Hassoun *et al*., 2020; Stoops *et al*., 2015). As presented in Table 1, it could be assumed

that there is a proven relationship between quality content and the different temperatures of the samples. In this study, our analysis revealed meatballs' endothermic process takes place in the range of 78 and 82°C. At this point, temperature showed no

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significant change with the quality content. It is a unique thermal nature in relation to biological tissue in meat, as meatballs, in which disulfide bonds require mild heating energy (Flores, *et al* 2019).

CONCLUSIONS

This study showed that temperature during meatballs processing can be considered as a pre-reservation treatment to mitigate the quality loss before storage. Results confirmed the correlation of certain levels of heat temperature with varying rate changes in meatballs' quality. The kinetics relationships in Figure 2 may be introduced in practical storage situations. Indeed, some configuration factor needs correction to match with condition and food characteristics. Overall, the set combination model from Jaisan and Lee (2017) integrated with Huang's model (2019) was able to calculate optimal heat temperature for meatballs to maintain quality under uncontrolled temperature conditions. With this capability, the small meatballs producer has a potentially more efficient shelf-life calculation for the delivery period without a fridge in commercial distribution.

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تاثیر دمای فرآوری روی کوفته در شرایط انبارداری پویا با استفاده از ارزیابی مدل آرنیوس (Arrhenius)

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چکیده

هدف از گرم کردن کوفته در طول تهیه آن جلوگیری از آلودگی است. با این حال، عملیات گرم کردن بیشتر به ساختار غذا آسیب می رساند و مواد مغذی اصلی کوفته را از دست می دهد. مدل آرنیوس برای مشاهده اثر عملیات حرارتی بر کاهش کیفیت غذا استفاده شده است. در این پژوهش، نمونه‌های کوفته با پوشش خوراکی تحت دمای انبارداری پویا مشاهده شد. هدف از این مطالعه استفاده از مدل آرنیوس برای ارزیابی رابطه عملیات حرارتی بر روی تغییر pH در شرایط انبارداری و ذخیره سازی پویا بود: محدوده کنترل نشده ۲۴ تا ۳۱ درجه سانتی گراد، در حالی که تیمار حرارتی مورد مطالعه بین ۵۰ تا ۹۰ درجه سانتی گراد برای ۱۵ دقیقه بود. نتایج، رابطه آشکاری بین جنبه های عملیات حرارتی برای جلوگیری از رشد باکتری در طول انبارداری نشان داد. ارزیابی نتایج مدل آرنیوس نشان می‌دهد که فاز اوج از دست دادن مواد مغذی در محدوده عملیات دمایی ۸۳ تا ۹۰ درجه سانتی‌گراد بود و برای روش‌های بسته‌بندی یا نگهداری، دمای بهینه عملیات حرارتی در ۷۸ درجه سانتی‌گراد یا ۱۴۸.۱۵ کیلوژول درمول قراردارد.