Ohmic heating characteristics and degradation kinetics of anthocyanin in mulberry juice

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Abstract. Ohmic heating is considered as a novel technology for pasteurization. Ohmic heating rate is highly influenced by the electrical conductivity of the product. The heating rate and the stability of anthocyanin during ohmic heating need to be investigated in order to analyze the viability of ohmic heating for pasteurization of mulberry juice. Mulberry juice was heated using three different temperatures (80, 85 and 90°C) during 90 minutes. Electrical conductivity of mulberry juice increased from 0.014 Sm⁻¹ at the initial of heating (32°C) to 0.033 Sm⁻¹ at 90°C and the average heating rate was 0.568°C/s. The heating rate and electrical conductivity increased linearly with the increased in temperature. Degradation kinetic of anthocyanin followed the first order kinetic models with $R^2 > 0.9$. The $k$-value of anthocyanin degradation increases along with the increasing of temperature ranging from $8 \times 10^{-3} \text{min}^{-1}$ to $15 \times 10^{-3} \text{min}^{-1}$. Anthocyanin shows relatively high temperature dependence with 135.83 kJ/mol energy activation value. Based on the heating rates obtained from this study, ohmic heating could be used as an alternative pasteurization method for mulberry juice.

1. Introduction

Functional foods such as fruit and their processed products have gained much interest for their beneficial impact on health and the pleasant taste that they provide. Several studies [1-4] have indicated that tropical fruits contain considerable amount of bioactive compounds including Vitamin C, flavonoid and anthocyanin with high antioxidant activity. One of underutilized tropical fruits in Indonesia is Mulberry. Mulberry plants have been cultivated for their leaf as the feed for silk caterpillar, while the fruit itself has not been used. Mulberry cultivated in Indonesia is categorized as black mulberry (Morus nigra. L). It is reported that black mulberry contains 48.4 mg ascorbic acid, around 240 mg QE total flavonoid, and 350 mg total monomeric anthocyanin per 100 gram fruit weight [2]. Therefore mulberry is highly potential as a new material for industrial product such as juice.

One of the obstacles faced by industries in providing high quality juice is the pasteurization technology. Pasteurization is a crucial aspect in processing of beverage, because it could maintain the product safety. Pasteurization is conducted to inactivate enzyme and bacteria that could decrease the quality and shelf life of food products. On the other hands, pasteurization process also affects the quality and nutrient content of the product, specifically for compounds that are prone to degrade at high temperature such as anthocyanin.
Ohmic heating is considered as a novel technology in sterilization and pasteurization process. Ohmic heating adopted the principle of High Temperature Short Time (HTST) method that will rapidly heat the product in short period of time to maintain the quality of the product. The heating process occurred as the impact of electric current that flows through the products with specific resistance value. The current flow creates movement of ions and substances inside the product and generates internal energy that can be used in heating process [5]. Several researchers have identified the advantages of ohmic heating such as uniform and rapid heating, prevent fouling, and minimize the degradation of vitamin and nutrition [6,7]. Ohmic heating had been used to pasteurize many types of fruit juice products such as: orange [8], jaboticaba [9], mango [10], and pomegranate [11]. In addition, ohmic heating has also been used for pulp from acerola ([12], blueberry [13], strawberry [14] and also tomato paste [15]. Several studies showed that electric field generated by ohmic heating does not affect the degradation of beneficial compound in food products [11,12,14,16]. In addition, orange juice that pasteurized by ohmic heating provides longer sensory shelf life compared to conventionally pasteurized juice [8]. Evaluating the stability of nutrient and bioactive compound in juice during heating is critical in order to determine how a technology is viable in pasteurization process and could retain the quality of product. Several studies have analysed the degradation kinetic of active compounds in various type of juice and pulp during ohmic heating, such as; blueberry juice [17], papaya, sapota, and guava pulp [18], jaboticaba juice [9], and acerola pulp [12]. All studies mentioned before indicated that the first order kinetic model was the best fitted model used to describe the degradation kinetics of active compounds in fruit products with varying level of k and Ea values. Information regarding the degradation kinetic of active compounds in mulberry juice was not found. Therefore, the objectives of this study were to investigate electrical conductivity and the degradation kinetic of anthocyanin in mulberry juice during ohmic heating.

2. Materials and Methods

2.1. Sample preparation
Mulberry was collected from a local farmer in Wajo regency in South Sulawesi. Mulberry was sorted based on ripeness and apparent fruit colour. Cloudy juice was produced by crushing the mulberry using a blender and then filtered to remove solid particles. Water was added to the juice with the ratio of 3:2. The juice was freeze to prevent chemical and microbial destruction before used.

2.2. Ohmic heating of mulberry juice
Experiments conducted using a static ohmic heating system. The ohmic heater and data acquisition system used are represented in Figure 1. Juice was placed inside the ohmic heating chamber having a diameter of 4 cm. Two stainless steel electrodes with Teflon pressure caps were placed at each end of the chamber and a thermocouple was placed in the middle of the reactor. The distance between electrodes was 12.1 cm. The chamber was connected to a power supply and data logger to record the real time data of current intensity, voltage, and temperature during the heating process. Juice was heated at three different temperatures (80, 85, and 90°C) with holding times of 90 minutes after the samples reached the desired temperature. 5 ml of sample was taken in every 0, 30, 45, 60, and 90 minutes. The samples were used in further analysis to determine the amount of active compounds in mulberry juice.

2.3. Electrical conductivity and energy consumption measurements
Electrical conductivity (σ, Sm⁻¹) was determined using equation 1 where L is the distance between electrodes (m), A is the cross-section area of the ohmic heating chambers (m²), and R is the electrical resistance of products (Ω).

\[ \sigma = \frac{L}{AR} \]  

The resistance of product (R, Ω) was calculated using equation 2, where V is the voltage applied (V) and I is the current intensity (A).
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$$R = \frac{V}{I} \quad (2)$$

Total energy consumption during experiment were determined using two approximation methods in order to increase the approximation accuracy. The methods used were Simpson 3/8 rule and trapezoid rule that are shown in equation 3 and equation 4, respectively [19].

$$I = \frac{3h}{8} \left[ f(x_0) + 3f(x_1) + 3f(x_i) + f(x_n) \right] \quad (3)$$

$$\int_a^b f(x)dx = (b - a) \left[ \frac{f(a) + f(b)}{2} \right] \quad (4)$$

Figure 1. Schematic system of ohmic heating

2.4. Determination of bioactive compounds in mulberry juice
Bioactive compounds measured were total phenolic, anthocyanin, and vitamin C. The measurements were carried out using UV-Vis Spectrophotometer (Shimadzu, Japan).

2.4.1. Total phenolic content. Total phenolic measurements were done based on the Folin-ciocalteu method described in [20,21] with some modifications. The gallic acid standard was prepared at 5 different concentrations of 2, 5, 6, 8 and 10 ppm. Using this standard, a gallic acid calibration curve will be obtained, and total phenolic content can be calculated from the curve. Dissolved 0.1 mL of mulberry juice in 10 mL mixture of methanol: water (6: 4 v/v) in the test tube. 1 mL of the mixture was reacted with 1.5 mL Folin-Ciocalteu reagent (10 dilution factors) and 1.2 mL Na2C03 (7.5%). The absorbance of samples was measured at a wavelength of 751.5 nm.

2.4.2. Anthocyanin content. Total monomeric anthocyanin was analysed using the pH differential method [22,23] with some modification. Two dilution samples were prepared using a supernatant: one with a pH 1.0 potassium chloride buffer solution; and the other with sodium buffer solution acetate pH 4.5. The diluted sample is placed at room temperature for 20 minutes. The standard used in this measurement is cyanidin-3-glucose. The absorbance values were calculated using equation 5 below:

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5} \quad (5)$$

where $A_{520}$ is the absorbance at the wavelength of 520 nm, and $A_{700}$ is the absorbance at the wavelength of 700 nm. Total anthocyanin was calculated using the following equation:
where A is the absorbance value, MW is the molecular weight of standard (445.2 g/mol), DF is the dilution factor, $\varepsilon$ is the molar absorption capacity (29,600 L/mol.cm), and l is the cuvette length (1 cm).

2.5. Determination of kinetic parameters
The degradation of anthocyanin followed the first-order kinetic form based on the following equation:

$$C = C_0 e^{-kt}$$  \hspace{1cm} (7)

where $C_0$ (mg/L) is the content at 0 s, C is the content at t, and k is the first order kinetic constant.

Decimal reduction time (D) is the time needed to reduce ten times the initial conditions at a certain temperature. D value was calculated by the following equation:

$$D = \frac{\ln(10)}{k}$$  \hspace{1cm} (8)

The half-life ($t_{1/2}$) of degradation was determined by the equation below:

$$t_{1/2} = \frac{\ln(2)}{k}$$  \hspace{1cm} (9)

Temperature-dependence of anthocyanin in mulberry juice was determined by the Arrhenius equation below:

$$k(t) = k_0 \exp \left( -\frac{E_a}{RT} \right)$$  \hspace{1cm} (10)

where k is the degradation rate constant at temperature T (K), $E_a$ is the activation energy (kJ/mol), and R is the universal gas constant (8.314 x 10$^{-3}$ kJ/mol.K).

3. Results and discussion

3.1. Electrical conductivity and power consumption
The results of temperature measurement during ohmic heating of mulberry juice indicate that temperature increase followed a linear model with $R^2 > 0.995$ (figure 2). It is important to note that the ohmic heating rates of mulberry juice were almost identical for the three final temperatures. In the case of heating at the final temperature of 90°C (figure 2), the time required to heat the mulberry juice from initial temperature of 32°C was 102 seconds. Based on these values, the heating rate of the juice was about 0.568°C/s.

Heating rate obtained from the experiment was relatively high considering the low electric field applied to the system: around 19.115 V/cm at the beginning of heating and slightly decreased to 19.045 V/cm at the end of heating. This was due to the significant increase in current flow through the ohmic heating chamber as temperature, and thus electrical conductivity, of mulberry juice inside the ohmic heating chamber increased [24].

Figure 3 illustrates the changes in electrical conductivity of mulberry juice during ohmic heating. Electrical conductivity at the beginning of heating (30°C) was 0.014 S/m and continued to increase with increasing of temperature to 0.033 S/m at 90°C. The trend shows a linear increase in electrical conductivity with temperatures. High coefficients of determination ($R^2 >0.99$) indicates the suitability of the linear model for conductivity variation with temperature. Similar results were shown during ohmic heating of strawberry pulp, tomato paste, and orange juice [14,15,25].
The increase in electrical conductivity due to the increase of temperature can be the result of some physical phenomenon [6]. When a product is heated, there is an increase in ion mobility inside the product which is directly proportional to the increase in electrical conductivity. This phenomenon occurs due to structural changes in the tissue, such as cell wall proproctin breakdown, expulsion of non-conductive gas bubbles, softening, and lowering in aqueous phase viscosity [6].

The electrical conductivity of mulberry juice obtained in this study considered to be lower if compared to the other fruit juices. The electrical conductivity value was reported to be 0.3-0.9 S/m at 26-96°C for tomato paste (6-14 V/cm)[15], 0.2-0.6 S/m for strawberry pulp at 20-80°C (32-70 V/cm)[14], and 6-16 S/m for mango juice with a concentration of 90% at 22-70°C (7-27 V/cm) [10]. As mentioned before, the electrical conductivity depends on the mineral or ionic content of the product. For illustrates, rutin was the highest phenolic compound found in mulberry, averagely contributing to 44.66% of total quantified polyphenolics in mulberry [26]. While ellagic acid, gallic acid, and lycopene were the predominant phenolic compound found in strawberry, mango, and tomato, respectively [27–29]. Different types of compound and their concentration inside a fruit resulting in variety ranges of electrical conductivity depend on the acidity and the chemical reaction during ohmic heating. Total soluble solid and sugar content may affect the electrical conductivity as the drag force increased and limit the movement of ionic content. Similar behaviour was reported in ohmic heating of orange juice. Palaniappan and Sastry in [25] reported that electrical conductivity of juices increased by decreasing the insoluble solid content. Future analysis of active compounds and total soluble content of mulberry juice needs to be done in order to validate this statement.

![Ohmic heating curves of mulberry juice at 90°C](image1)

**Figure 2.** Ohmic heating curves of mulberry juice at 90°C

![Electrical conductivity of mulberry juice at 90°C](image2)

**Figure 3.** Electrical conductivity of mulberry juice at 90°C
Power consumption was calculated using two approximation methods of Simpson 3/8 rule and trapezoid rule. Both methods show similar trend in predicting the power consumed by system. Figure 4 illustrates the accumulation of energy used by the ohmic heating system for 60 minutes. Difference behavior was shown between ohmic heating with a final temperature of 80°C with 85 and 90°C. The energy consumed at 80°C in the beginning of the holding time escalated reaching 852.36979 watt-second in 822 seconds. However, after reaching this point, energy consumption continues to decline. It shows that the temperatures of the product in the ohmic reactor are relatively stable (± 2°C) so that the use of energy can be minimized and energy consumption is lower. Meanwhile at the temperature of 85 and 90°C the energy consumption is relatively constant during 60 minutes of heating for around 300 watts-seconds. This is caused by unsteady product temperature at 80 and 90°C. Temperature inside the product changes rapidly and consequently increased the energy consumption.

![Figure 4](image)

Figure 4. Cumulative power consumption during experiment; (a) Simpson 3/8 rule; (b) trapezoid rule

During the beginning of heating, energy consumption increased along with the increased of temperature. Energy used to raise the sample temperature was similar in the initial heating phase. Changes in trends were spotted when the product reached final temperature due to difference energy needed to maintain the final temperature. The higher the temperature to be maintained, the greater the energy used. Energy consumption during ohmic heating follows the 3rd order polynomial model (table 1.) with R² > 0.98.

| T (°C) | 3rd Order Polynomial Model | R²   |
|-------|---------------------------|------|
| 80    | y = 1E-06x^3 - 0.009x^2 + 22.316x + 767.01 | 0.993 |
| 85    | y = 8E-07x^3 - 0.0051x^2 + 12.749x + 1451 | 0.983 |
| 90    | y = 7E-07x^3 - 0.0054x^2 + 13.549x + 209.9 | 0.992 |

3.2. Total phenolic and anthocyanin content of mulberry juice
The following results are the amount of polyphenol and vitamin C compound on mulberry juice before ohmic heating (fresh juice). Mulberry juice contained 357.03-588.27 mg GAE/100 mL of total phenolic and 25.04-35.38 mg/100 mL anthocyanin. Similar results obtained by Natic et al [26] with highest contain of total phenolic in black mulberry found was 326.29 mg GAE/100 g frozen weight and Ozgen et al [30] reported that the ranges of total phenolic in mulberry were 1766-3488 µg GAE/g fresh weight (176.6-348.8 mg GAE/100 mL). The anthocyanin content found in this study was comparable with the result described in [21] (38.783 mg/100 mL) and [30] (25.3-83.0 mg/100 mL).
Figure 5 illustrates the changes in total phenolic of mulberry juice with the pasteurization temperature. Both temperatures showed that the amount of phenolic content increased slightly between 30-90 minutes of heating.

Similar result was found in [11] which showed that there was a slight increase in total phenolic content of pomegranate juice during heating both ohmic and conventional. Meanwhile, the phenolic content in sajor-caju extract did not increased significantly after heating at 100 and 121°C during 15 and 30 minutes [31]. The increased in total phenolic after heating can be caused by a chemical reaction that occurred when anthocyanin was degraded to phenolic acid [22]. Thermo-labile compounds such as anthocyanin will degrade faster as the temperature used in heating processed increased. Anthocyanin degradation is caused by changes in chemical composition due to the opening of the pyrilium ring and the formation of chalcone glycosides, causing chalcone to be released and degraded to phenolic acids and aldehydes [22]. Long heating period provides a longer reaction time for other compounds to degrade into phenolic acid.

Figure 6. Anthocyanin content ratio over time at 85 and 90°C

The first-order kinetic equations described before were used to determine the kinetic parameters and observed the degradation of anthocyanin with respect to time. The coefficient of determination ($R^2$)
for anthocyanin degradation at 85 and 90°C was 0.9704 \( (y = 27.72e^{0.008}) \) and 0.9958 \( (y = 29.382e^{0.015}) \), respectively. Therefore it can be said that first-order kinetic equation was suitable for predicting degradation of anthocyanin compounds of mulberry juice during ohmic heating at the temperatures of 85 and 90°C. Several other studies also reported that first-order kinetic equation can be used to predict anthocyanin degradation [9,17,22] in several fruit products.

The anthocyanin content of mulberry juice before and after heating was plotted against time to determine the degradation kinetic parameters (figure 6). The linear equations obtained from the graph were used to determine the constant rate \((k)\) value of anthocyanin degradation (table 2). The \(k\) value increased along with the increasing of temperature, ranging from \(8 - 15 \times 10^{-3}/\text{min}\). Other studies reporting the degradation kinetic of anthocyanin in mulberry juice during ohmic heating were not found, but study conducted on acerola pulp during ohmic heating at a temperature of 75-90°C showed similar \(k\) values of 5.9 to 19.7 \( \times 10^{-3}/\text{min} \) [22]. Another study [9] which analyzed the non-thermal effect on anthocyanin degradation of jaboticaba juice (70-90°C) showed a lower \(k\) value of 0.0017 - 0.0075/min, while the study [17] on the thermal treatment of blueberry juice (40-80°C) resulted in a higher \(k\) value of 0.064 - 2.254 \( \times 10^{-3}/\text{min} \). The activation energy \((E_a)\) of anthocyanin in mulberry juice was shown in Table 2. As an illustration, the \(E_a\) value of anthocyanin content in blueberry juice after thermal treatment was 80.42 kJ/mol [17]. The same ranges of \(E_a\) value were found for acerola pulp [22] and jaboticaba juice [9] with \(E_a\) value of 74.8 kJ/mol and 75.5 kJ/mol, respectively. Those results indicated that anthocyanin in mulberry juice have higher temperature dependence among other types of beverage products mentioned, which means that at higher temperature the reaction will occurred faster than the lower temperature [22]. Anthocyanin degradation in mulberry juice during heating can be influenced by the presence of ascorbic acid in high concentration. Several studies have shown that the presence of ascorbic acid has a negative influence on anthocyanin stability which causes degradation in this compound. Some mechanisms that can cause anthocyanin degradation due to ascorbic acid are the presence of hydrogen peroxide formed by oxidation of ascorbic acid which eventually oxidizes anthocyanin pigments, and the presence of ascorbic acid condensation on 4 anthocyanin molecules that cause degradation of both compounds [22]. The amount of solid content also influenced the anthocyanin degradation in a positive correlation. Some studies described by Sarkis [13] implies that the increase in anthocyanin degradation with an increase in solids content, was observed in studies involving strawberries and sour cherries. Increases in solid content will provide higher chance for molecule to react with anthocyanin and resulting higher degradation.

### Table 2. Kinetic parameters of anthocyanin degradation during heating

| T (°C) | k (min⁻¹) | D (min) | t½ (min) | Eₐ (kJ/mol) |
|-------|-----------|---------|----------|-------------|
| 85    | 0.006     | 383.76  | 115.525  | 183.09      |
| 90    | 0.014     | 164.47  | 49.511   |             |

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### 4. Conclusion

It can be concluded that the electrical conductivity of mulberry juice changes with temperature during ohmic heating. Heating rate and electrical conductivity followed the first order polynomial model with \(R^2 > 0.99\) for electrical conductivity. The electrical conductivity of mulberry juice obtained in this study considered to be lower compared to other fruit juice due to low electric field strength applied to the system. Further research needs to be done to evaluate the effect of total soluble solid in electrical conductivity of mulberry juice.

Significant difference in energy consumption is shown during holding time. Ohmic heating at lower temperature (80°C) provides a more stable product’s temperature that resulting lower energy consumption compared to higher temperatures (85 and 90°C). The degradation of anthocyanin follows the form of first-order kinetic equations with \(R^2> 0.9\). Based on the heating rate and electrical conductivity obtained from this study, it can be concluded that ohmic-based technology can be applied to the process of pasteurizing mulberry juice.
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