Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.64020

Degradation kinetics of anthocyanin, flavonoid, and total phenol in bignay (*Antidesma bunius*) fruit juice during ohmic heating

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Abstract

The effect of ohmic heating on bioactive compounds in bignay (*Antidesma bunius*) fruit juice during ohmic heating were evaluated. The parameters measured were total phenol, anthocyanin, flavonoid, and antioxidant activity. Ohmic heating was conducted at 70, 90, and 110 °C, and samples were collected at heating times of 0, 15, 30, and 45 minutes. Electrical conductivity of bignay fruit juice increased linearly with temperature with values ranged from 0.012 S/m at 32 °C to 0.039 S/m at 110 °C. Insignificant change in total phenol was observed, while anthocyanin and flavonoid showed significant degradation and the degradation kinetics followed the first-order kinetic model. The degradation rate constants for anthocyanin ranged from 0.0016 to 0.0213 min^{-1} with activation energy (E_a) of 63.880 kJ/mol and the degradation rate constants for flavonoid were in the range of 0.0107 to 0.0209 min^{-1} with activation energy of 18.210 kJ/mol. Antioxidant activities (IC_{50}) obtained from DPPH method ranged from 0.106-0.168 mg/mL while those obtained from ABTS method ranged from 0.131-0.161 mg/mL. The results indicate that anthocyanin and total phenol in bignay fruit juice is much more stable during heating compared to flavonoid.

Keywords: bignay fruit juice; ohmic heating; bioactive compounds; antioxidant activity.

Practical Application: Production of antioxidant rich bignay fruit juice using ohmic heating technology.

1 Introduction

During the past decade, fruit juice has gained remarkable interests in beverage market sector. Processing of fruits into juices has been a commercial way to diversify the usage of the fruits and to fulfill demands beyond harvest season. In addition, fruit juice has been viewed as a more convenient way to obtain comparable health benefits from the fruits to those from direct consumption. As the markets for fruit juice are approaching saturation, however, competitions among industries to attract consumers with their juice products are escalating. This condition requires industries to constantly innovate and introduce new products to the market. One approach that the beverage industry can use to win the competition is by introducing exotic fruit juice to the market.

Exotic fruits from tropical countries such as bignay (*Antidesma bunius*), Indian black plum or jamun (*Syzygium cumini* L), red mulberry (*Morus rubra* L.), and black mulberry (*Morus nigra* L.) have gained interests from researchers for their potential as sources of bioactive compounds and natural antioxidants. Studies on bignay fruits (Butkhup & Samappito, 2008; Hardinasinta et al., 2020; Jorjong et al., 2015; Lim, 2012; Ngamlerst et al., 2019), black plum (Aqil et al., 2012; Banerjee et al., 2005; Singh et al., 2018), and mulberry (Isabelle et al., 2008; Kim et al., 2010; Zhang et al., 2008) indicate that these fruits are rich in phenolic compounds such as flavonoids and athocyanins which have the potential to provide health benefits as reported by numerous authors (Aiyer et al., 2008; Basli et al., 2017; Chowtivannakul et al., 2016; Mazza, 2007; Ngamlerst et al., 2019; Stoner et al., 2007; Timmers et al., 2015; Wang & Stoner, 2008). Bignay fruit is an exotic fruit which is mostly found in Southeast Asian countries such as Thailand and Indonesia. This fruit resembles berries with a purplish-black color and a sweet-sour taste when fully ripe. In addition, due to its chemical contents, this fruit can be used as a raw material for production of antioxidant-rich beverages (Chaikham et al., 2016; Sripakdee et al., 2015).

In production of drinks and beverages, microbial safety of the products is of paramount importance. This is usually achieved through thermal treatments such as pasteurization and sterilization or non-thermal treatments such as pulsed electric field processing and high-pressure processing. One of the negative impacts of heat treatments of foods is the degradation of quality attributes such as nutrient contents, texture, color, and bioactive compounds. To minimize these effects, thermal technologies which can provide rapid and uniform heating such as ohmic and microwave heating technologies and non-thermal technologies have been developed. In the case of ohmic heating, rapid and uniform heating have been shown both experimentally and through mathematical simulations (Cokgezme & Icier, 2019; Icier & Ilicali, 2005; Kaur et al., 2016; Lascorz et al., 2016; Petruzzi et al., 2017; Priyadarshini et al., 2019; Qihua et al., 1993; Salengke & Sastry, 2007a, b; Sastry & Salengke, 1998; Varghese et al., 2014).

The use of ohmic technology in heating and processing of various types of solid and liquid foods has been studied extensively (Abedelmaks et al., 2018; Achir et al., 2016; Athmaselvi et al., 2017; Cappato et al., 2018a,b; Castro et al., 2004; Cokgezme & Icier, 2019; Darvishi et al., 2011, 2013; Farahnaky et al., 2012; Fattahi & Zamindar, 2020; Icier et al., 2017; Lascorz et al., 2016;

Received 20 Nov., 2020
Accepted 05 Jan., 2021
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Liu et al., 2017; Olivera et al., 2013; Poojitha & Athmaselvi, 2018; Sabanci & Icier, 2017; Salengke & Sastry, 2007c; Sarang et al., 2008; Sarkis et al., 2013; Torkian Boldaji et al., 2015; Yildiz et al., 2010; Zell et al., 2009). Many of these studies were mainly conducted to evaluate the possibility of using ohmic heating in thermal treatments of food products.

In recent years, there are several new applications of ohmic heating that have recently been reported such as application in carrageenan extraction (Hasizah et al., 2018), cocoa and coffee beans fermentations (Supratomo et al., 2019; Salengke et al., 2019), cheese production (Rocha et al., 2020a), whey beverages production (Ferreira et al., 2019a, b; Coimbra et al., 2020), dairy dessert and milk product production (Kuriya et al., 2020; Silva et al., 2020), and paraprobiotics production (Barros et al., 2021). These studies indicate that ohmic heating can provide faster extraction and fermentation process (Hasizah et al., 2018; Supratomo et al., 2019), better and consistent coffee bean quality (Salengke et al., 2019), better sensory attributes and consumer acceptance (Coimbra et al., 2020; Rocha et al., 2020a; Silva et al., 2020), and improved health benefits (Rocha et al., 2020a, b; Barros et al., 2021). In addition, physical properties of beverage produced via ohmic heating can be tailored by varying ohmic heating parameters such as frequency and filed strength (Ferreira et al., 2019b).

Many studies on the effects of ohmic heating on degradation of phenolic compound and anthocyanin in processed foods have been reported (Brochier et al., 2019; Ferreira et al., 2019a; Hardinasinta et al., 2019; Loypimai et al., 2015; Makroo et al., 2017; Mercali et al., 2015, 2013; Sarkis et al., 2013, 2019; Yildiz et al., 2009). Salari & Jafari (2020) conducted a review on the results of various studies and concluded that the effects of ohmic heating on the degradation of phenolic compounds and anthocyanin are not consistent since some researchers reported that ohmic heating resulted in higher degradation compared to conventional heating, while others reported that ohmic heating resulted in lower phenolic and anthocyanin degradation or similar to that of conventional heating.

Castro et al. (2004) reported that the magnitude of the electric field applied did not affect ascorbic acid degradation in the strawberry product. The same trend was reported by Mercali et al. (2015) for their study on jaboticaba juice. However, other studies reported that higher electric field resulted in higher degradation of anthocyanin in blackberry pulp (Sarkis et al., 2019) and ascorbic acid in tropical fruit pulp (Athmaselvi et al., 2017). The degradation kinetics of some bioactive compounds during ohmic heating also differ among products. The rate constant for anthocyanin from acerola pulp (Mercali et al., 2013) showed higher value compared to that of jaboticaba juice (Mercali et al., 2015). These results indicate that the stability of anthocyanin is relatively different in different products. Therefore, it is important to determine the effect of ohmic heating process on the stability of bioactive compounds in the scarcely studied exotic fruits such as bignay fruit. This kind of study can form an important step towards the development of commercial processes at industrial scale. Therefore, the objectives of this study were: (1) to evaluate the effects of ohmic heating on antioxidant activity and degradation kinetics of phenolic, anthocyanin, and flavonoid compounds in bignay juice, and (2) to obtain electrical conductivity of bignay fruit juice during ohmic heating.

2 Materials and methods

2.1 Sample preparation

Freshly harvested bignay fruits were obtained from a local market in Makassar, South Sulawesi, Indonesia. The fruits were washed and sorted based on maturity level and fruits with good maturity, distinguished by their black-purplish color, were used in this study. The selected fruits were crushed using a commercial juicer (Philips HR1832) and then filtered to separate the pulp from the juice. The single strength juice obtained was stored at -20 °C until used.

2.2 Ohmic heating experiments

Ohmic heating experiment was conducted using a laboratory-scale static ohmic heater as illustrated in our previous study (Hardinasinta et al., 2019). The experiment was conducted at three levels of temperature (70, 90, 110 °C) and 2 mL of sample was drawn from the ohmic heater at different heating time (0, 15, 30, 45 min). The prolonged heating time was used to bring about appreciable changes in the bioactive compounds in the juice samples so as to obtain an appropriate fit for the degradation kinetics model. During ohmic heating, temperature, electric field strength, and electric current were recorded using a data logger (CR1000 Campbell Scientific, Inc., Logan - Utah,).

2.3 Electrical conductivity measurement

The electrical conductivity of bignay juice during ohmic heating was determined using Equation 1,

$$\sigma = \frac{L}{A} \cdot \frac{I}{V} \quad (1)$$

where L is the distance between electrodes (m), A is the cross-section area of the ohmic heating chamber (m^2), I is current consumption (A), and V is applied voltage (V).

2.4 Chemical analysis

Chemical analysis were carried out to determine total phenolic, anthocyanin, and flavonoid contents as well as antioxidant activity. Chemicals used in the analysis were analytical grade and all measurements were carried out in triplicate.

2.5 Total phenolic content

Prior to analysis, stock solutions of control and ohmically treated juice samples were prepared by dilution of 0.1 mL of the samples with 10 mL of methanol/water (6:4, v/v) solution. The Folin-Ciocalteu method was used to determine total phenolic content of each sample based on the procedure described by Tezcan et al. (2009) with some modification. From the stock solution, 0.5 mL of aliquot was mixed with 1.5 mL of Folin Ciocalteu reagent (7.5%) and 1.2 mL of Na2CO3 (7.5%). Distilled water was then added to the mixture to reach a total

volume of 5 mL. The mixture was allowed to rest for 90 min at room temperature before measurement of absorbance was carried out. The absorbance of the samples was measured using UV-Vis Spectrophotometer (UV-1800, Shimadzu, Japan) at 760 nm and the results of measurements were expressed in gallic acid equivalent (GAE). Standard curve was prepared by diluting gallic acid in methanol/water (6:4, v/v) with five different concentrations ranging from 2-10 ppm and the absorbance of each solution was measured using the same wavelength (760 nm).

2.6 Anthocyanin content

Total monomeric anthocyanin was determined using the pH differential assay as described by Jiang et al. (2013). This method measures color difference of two samples obtained by reacting samples with a buffer solution at two different pH levels. The preparation of the samples was carried out as follow. Each of the control and ohmically treated juice sample was pipetted 50 µL into two glass vials. A 100-fold dilution was carried out for each sample by adding potassium chloride (0.025 M) buffer solution at pH 1.0 to one of the vial and sodium acetate (0.4 M) buffer solution at pH 4.5 to the other vial. The preparation of the buffer solution was based on the standard method by (Association of Official Analytical Chemists, 2005). The diluted samples were placed in a dark room for 20 min and the absorbance of each sample was measured using 520 and 700 nm wavelength. Total absorbance of the samples was calculated using Equation 2:

$$A = \left(A_{520} - A_{700}\right)_{pH\,1,0} - \left(A_{520} - A_{700}\right)_{pH\,4,5} \quad (2)$$

where A520 is the absorbance at the wavelength of 520 nm, and A700 is the absorbance at the wavelength of 700 nm. Total anthocyanin content was determined using Equation 3,

$$Anthocyanin\left(\frac{mg}{L}\right) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (3)$$

where A is total absorbance, MW is the molecular weight of cyanidin-3-glucoside (445.2 g/mol) as the standard, DF is the dilution factor, ε is the molar absorption capacity (29,600 l/mol.cm), and l is the cuvette length (1 cm).

2.7 Flavonoid content

Analysis of flavonoid content was carried out using Thin Layer Chromatography (TLC) assay (Altemimi et al., 2015). A 20 × 20 cm TLC plates pre-coated with silica gel 60 F254 (Merck, Germany) was divided into four pieces of equal size (5 × 20 cm) and then dried in an oven at 110 °C for 20-30 min before use. Standard solution was prepared using rutin (250 ppm). The mixture of ethyl acetate: formic acid: water (10:2:3 v/v/v) was used as solvent for the separation process. The chromatography chamber was filled with 10 mL solvent and allowed to saturate for 30-40 min before used. The standard solution was dotted on the TLC plate in five different volumes of 0.1, 0.5, 1.0, 2.0, and 4.0 µL followed by 2 µL of samples with a distance of 1 cm each. The plate was placed inside the chamber in a straight horizontal position and then covered to allow the separation process. The reading of peak area was performed using a densitometer (Camag TLC 3 scanner, Switzerland) at the wavelength of 245 nm according to the spot reference from the standard solution. From the peak area of the standard spot, the calibrating curve of rutin was acquired and used to determine the flavonoid content of the juice samples.

2.8 Antioxidant activity

Antioxidant activity measurements were carried out using two different approaches, i.e. 2,2 Diphenyl-1-picrylhydrazyl (DPPH) assay and 2,20-azino-bis (3-ethylbenzthiazonline-6-sulfonic acid) (ABTS) assay.

2.9 DPPH method

The DPPH radical-scavenging activity was determined based on the method described in Kenny et al. (2013) with several modifications. The DPPH solution was prepared by the dilution of 8 mg DPPH (Sigma-Aldrich) in 50 mL of methanol. Juice samples (5 mg) were diluted in 5 mL of methanol to obtain stock solution of the sample. A series of five different sample concentrations ranging from 75-375 ppm were prepared by pipetting 15, 30, 45, 60, and 75 µL of the stock solution across the 96-well plate, followed by the addition of 75 µL DPPH solutions. Methanol was then added into the well to reach the final volume of 200 µL. The mixture was kept in the dark for 30 minutes at room temperature to allow optimum reaction to occur. The absorbance of each sample was measured at the wavelength of 515 nm using ELISA plate reader (ELx808 BioTek, Vermont, USA). The results were expressed in IC_{50} value.

2.10 ABTS method

The ABTS assay was performed based on the method illustrated in Jorjong et al. (2015) with modifications. To obtain the ABTS reagent, a 7.4 mM ABTS solution and a 2.6 mM potassium persulfate solution were required. ABTS solution was prepared by diluting 0.018 g of ABTS in 5 mL of methanol while potassium persulfate solution was obtained by mixing 4.6 mg of potassium persulfate with 5 ml methanol. Both solutions were mixed with the ratio of 1:1 (v/v) and the final volume was made up to 25 mL with methanol. The reagent was incubated in the dark for 12-16 hours before use. Sample preparation procedure was similar to the DPPH method mentioned above, except for the volume of ABTS used (125 µL) and the wavelength at which the absorbance was measured (650 nm).

2.11 Degradation kinetic measurement

The rate constant for degradation of a compound due to heat treatments can be determined using first-order kinetic as represented in Equation 4.

$$C = C_o e^{-kt} \quad (4)$$

In the above equation, C0 (mg/L) is initial concentration of the compound, C is concertation at time t, and k is the rate constant. The time required to reduce the concentration of a compound to one-tenth of its initial concentration is denoted

as decimal reduction time (D-value). This value was determined using Equation 5. Another parameter that is often used to describe the rate of degradation of a compound is the half-time ($t_{1/2}$) which represents the time needed to reduce the concentration of the compound to one-half of its initial concentration (Equation 6).

$$D = \frac{ln(10)}{k} \quad (5)$$

$$t_{1/2} = \frac{ln(2)}{k} \quad (6)$$

Temperature dependence of degradation rate of compounds can be derived from the Arrhenius equation as shown in Equation 7.

$$k(T) = k_0 exp\left(-\frac{E_a}{RT}\right) \quad (7)$$

In the above equation, k is the degradation rate constant at temperature T (in K), Ea is the activation energy (kJ/mol), and R is the universal gas constant (8.314 x 10^{-3} kJ/mol.K).

2.12 Statistical analysis

The IC_{50} value was analyzed using the trial version of Prism 8.0 (GraphPad Software, San Diego, CA-USA). Other statistical analyses were performed using R Studio software (RStudio PBC, Boston, MA-USA).

3 Results and discussions

3.1 Electrical conductivity of bignay juice

Electrical conductivity is a substantial aspect affecting the heat generation inside the product and consequently it can significantly influence the design process. Figure 1 shows the change in electrical conductivity of bignay fruit juice during ohmic heating. The electrical conductivity of bignay juice increased linearly from 0.128 to 0.390 S/m as temperature increased from 32 °C to 110 °C. The relationship between temperature and electrical conductivity is given in Equation 8 with $R^2 > 0.9986$.

$$\sigma = 0.0034T + 0.0111 \quad (8)$$

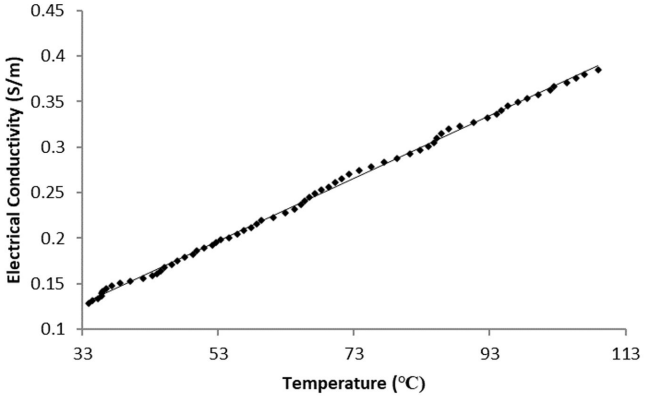

Figure 1. Electrical conductivity of bignay fruit juice during ohmic heating.

This result is in accordance with the results reported by other researches which indicated that electrical conductivity increased linearly with temperature (Palaniappan & Sastry, 1991; Srivastav & Roy, 2014).

The result obtained in this study is comparable to the results of previous studies conducted on blueberry and strawberry pulp with the electrical conductivity of 0.79-3.86 mS/cm (0.079-0.386 S/m) at 30-82 °C and 0.001-0.004 S/cm (0.1-0.4 S/m) at 20-100 °C, respectively (Castro et al., 2004; Mercali et al., 2011). The electrical conductivity of bignay juice can be considered optimum for ohmic heating processing since electrical conductivity in the range of 0.1-5 S/m is considered optimum for ohmic heating by several researchers (Proctor, 2018; Salari & Jafari, 2020). Beside temperature and ionic compounds, electrical conductivity also depends on the applied electric field, free water content, and solid content of the product (Castro et al., 2003; Icier & Ilicali, 2004; Varghese et al., 2014).

3.2 Change in bioactive compounds during heating

Fresh bignay juice contained 1202.5 mg GAE/100 mL total phenolic, 426.6 mg/100 mL anthocyanin, and 3.78 mg/100 mL flavonoid. The total phenolic content obtained was comparable to the results reported in Butkhup & Samappito (2008), while anthocyanin content in this study was higher than the anthocyanin content of bignay juice described in Chaikham et al. (2016). On the contrary, a study conducted by Jorjong et al. (2015) reported higher flavonoid content compared to the result found in this study. The influence of temperature and time during ohmic heating was shown in Figure 2 below. Each type of compound possessed different behavior when exposed to both treatments. Statistical analysis was used to further determine the interaction between the observed bioactive compounds and the treatment used.

Total phenolic in bignay juice decreased during ohmic heating at all temperatures with the highest reduction occurred at 110 °C. Significant degradation was observed during heating from ambient temperature to the targeted treatment temperatures. During constant temperature period (up to 45 minutes holding time at treatment temperatures), we found insignificant change in phenolic content. Previous studies reported that degradation of phenolic content occurred during ohmic heating of watermelon juice (Makroo et al., 2017) and sugarcane juice (Brochier et al., 2016). Ohmic heating of sugarcane juice at varying frequencies demonstrated no further degradation that occurred after time zero. The phenolic content of watermelon juice decreased significantly during the first 30 seconds of heating while the reduction of this compound became insignificant afterward. Another study which evaluate the effect of heating method on fruit juice indicated no significant difference between ohmic and conventional heating in terms of total phenolic content (Brochier et al., 2016; Yildiz et al., 2009). However, the study conducted for pomegranate juice reported that phenolic content increased after processing both with ohmic and conventional heating (Yildiz et al., 2009). An increase in phenolic compounds after thermal treatment was also observed in sajor-caju extract (Saad et al., 2014) and jambolana pulp (Branco et al., 2016). The different trends reported by various researchers can be due to varying processing conditions applied to the products. For

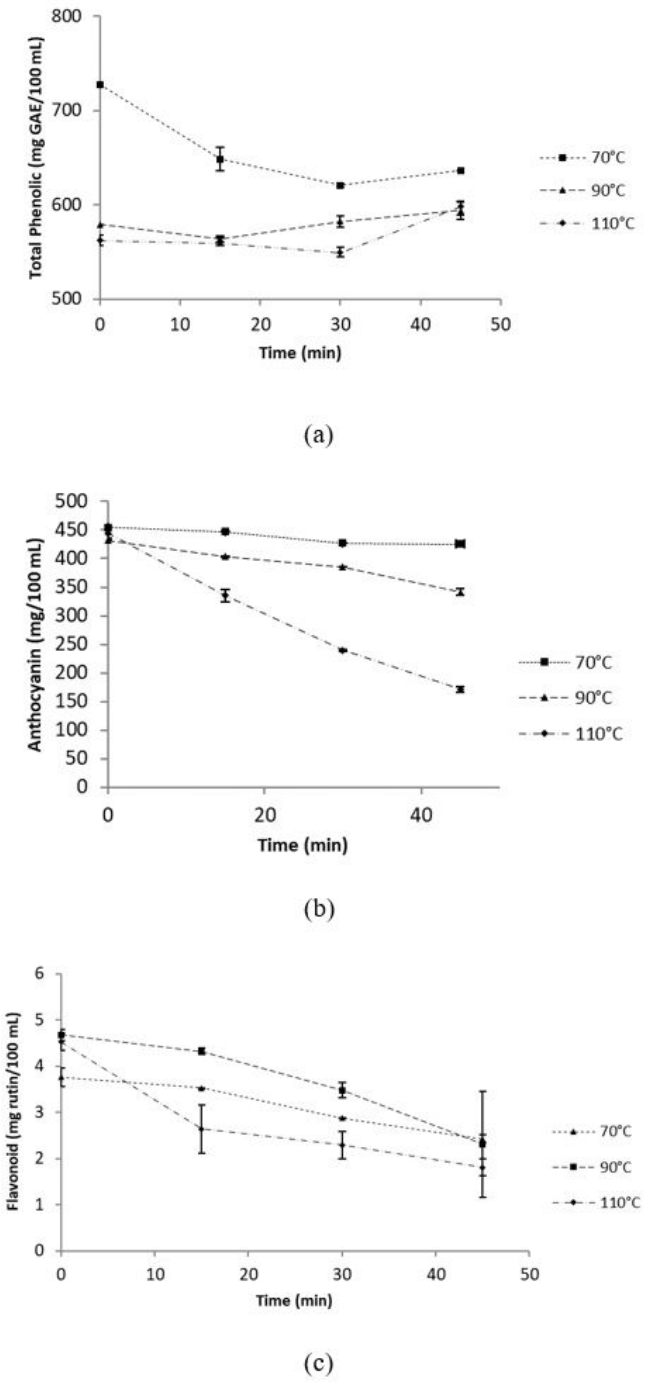

Figure 2. Degradation of bioactive compounds during ohmic heating; (a) Phenolic content; (b) Anthocyanin content; (c) Flavonoid content

instance, Cappato et al. (2018a, b) described that the quality of whey acerola-flavored drink was highly affected by the voltage and electric frequency used during ohmic heating. In regard to phenolic compound, the worst operating condition was reported at 60 V - 60 Hz combination, while the highest retention was reported at 25 V - 1000 Hz combination (Cappato et al., 2018a). The types of phenolic compounds contained in the product also affect the change in the phenolic compound during heating. Xu et al. (2007), described that free phenolic acid fraction in citrus peel extract increased after thermal treatment while the phenolic acid found in the form of ester, glycoside, and ester-bound fractions decreased during heating.

Anthocyanin is known to be susceptible to several environmental factors such as temperature, pH, light, and oxygen (Loypimai et al., 2016; Moreno et al., 2016). The same trend was observed in this study where anthocyanin in the treated bignay juice degraded significantly ($p < 0.05$). Ohmic heating at 70 °C for 45 minutes retained 93.7% of anthocyanin in the treated bignay juice, while heating at 90 °C and 110 °C for 45 minutes caused degradation in the order of 21% and 62% respectively. Similar trend was reported for blackberry pulp, where anthocyanin showed better retention at lower temperatures (70-75°C) compared to that at higher temperatures (80-90 °C) which showed degradation for up to 40% (Sarkis et al., 2019). Although anthocyanin content of bignay juice decreased during ohmic heating for 45 minutes, the reduction was statistically insignificant ($p > 0.05$). Degradation of anthocyanin also occurred during thermal treatment of plump juice (Turturică et al., 2018), sour cherry concentrate (Zoric et al., 2014), and jamun fruit juice (Shaheer et al., 2014). Sarkis et al. (2013) compared anthocyanin degradation due to ohmic and conventional heating of blueberry pulp and reported that ohmic heating at high voltage (200 and 240 V) caused higher degradation than conventional heating. Similar trend was also reported by Sarkis et al. (2019) who studied anthocyanin degradation in blackberry pulp. Meanwhile, ohmic heating conducted at lower voltages (25, 45, and 60 V) provided no significant difference in anthocyanin degradation compared to that of conventional heating (Ferreira et al., 2019a; Mercali et al., 2015).

In contrast to the phenolic and anthocyanin degradation, the degradation of flavonoid in bignay juice is more time-dependent. The degradation of flavonoid was 36%, 51%, and 60% after 45 min of heating at 70, 90, and 110°C, respectively. Prolong heating time significantly affected the destruction of flavonoid content ($p<0.05$), while the influence of temperature was not significant ($p>0.05$). Thermal treatment applied to two types of flavonoids (fisetin and quercetin) showed similar behavior where the concentration of both compounds decreased in a time-dependent manner (Wang & Zhao, 2016). The same time-dependent behavior was also reported from a study on flavonoid in white grape juice as affected by High Voltage Atmospheric Cold Plasma (Pankaj et al., 2017) except that the flavonoid content increased during the treatment.

3.3 Degradation kinetics of anthocyanin and flavonoid

The first-order kinetic models were used to describe the degradation kinetics of anthocyanin and flavonoid in bignay juice in terms of the k, $t_{1/5}$, D, and Ea values and the results are shown in Table 1. These models have been applied widely in defining the kinetics of degradation of compounds for their mathematical simplicity. These models are widely used to obtain the degradation rate constants which are the main parameters that describe the time and temperature dependence of compounds' stability during heating (Sarkis et al., 2019). Applications of first-order kinetics model to determine the thermal degradation of anthocyanin have been reported for black mulberry juice (Fazaeli et al., 2013), purple potato (Nayak et al., 2011), juçara and "Italia" grapes (Peron et al., 2017), while modeling of flavonoid degradation with first-order

Table 1. Kinetics parameters of anthocyanin and flavonoid degradation in bignay juice during ohmic heating.

Temperature (°C)	Degradation models	k (min^{-1})	D (min)	Ea (kJ/mol)
	Anthocyanin			
70	$y = 454.3e^{-0.002t}$; $R^2 = 0.904$	0.0016	1151.29	
90	$y = 435.63e^{-0.005t}$; $R^2 = 0.959$	0.005	460.517	63.88
110	$y = 452.01e^{-0.021t}$; $R^2 = 0.998$	0.0213	109.647	
	Flavonoid			
70	$y = 3.9686e^{-0.0107t}$; $R^2 = 0.980$	0.0107	215.195	
90	$y = 4.9925e^{-0.0142t}$; $R^2 = 0.975$	0.0142	162.154	18.21
110	$y = 4.5207e^{-0.0209t}$; $R^2 = 0.992$	0.0209	110.172	

Note: k = degradation rate constant; D= decimal reduction time; Ea = activation energy.

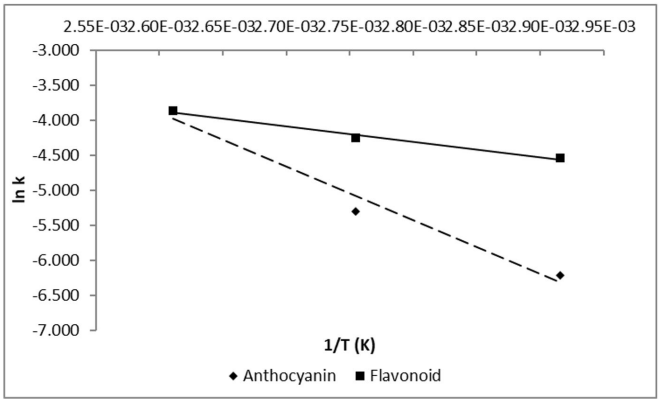

Figure 3. Arrhenius plot of ln k vs. 1/T for the kinetic degradation of anthocyanin and flavonoid in bignay fruit juice.

kinetics equation can be found in studies on the effects of heat on plump extract (Turturică et al., 2016), black rice flour extract (Bolea et al., 2016), and mandarin slices (Akdaş & Başlar, 2015).

The results shown in Table 1 indicate that the degradation rate constant (k-value) for anthocyanin in bignay fruit juice ranged from 0.0016 to 0.0213. Comparable values were reported from studies on anthocyanin degradation in blackberry (Sarkis et al., 2019), jaboticaba (Mercali et al., 2015), and acerola fruit juice (Mercali et al., 2013). The k-values reported in these studies ranged from 0.00155-0.0051 min^{-1} for blackberry pulp at 70-90 °C (Sarkis et al., 2019), from 0.0017-00075 min^{-1} for jaboticaba juice at 70-90 °C (Mercali et al., 2015), and from 0.0059-0.0197 min^{-1} for acerola pulp processed at 75-90°C (Mercali et al., 2013). Contrary to anthocyanin, research evaluating the degradation kinetics of flavonoid compounds during ohmic heating is still limited. However, compared to the degradation rate constant of flavonoid in other products using other heating methods, the results obtained in this study provide lower degradation rate constant. For instance, microwave processing of York cabbage at 400-800 W resulted in k-values of 0.144-0.197 (Jaiswal & Abu-Ghannam, 2013) while the k-value for catechin (included in flavonoid group) under ultrasonic treatment at 28-135 kHz were in the range of 0.0099-0.0182 min^{-1} (Zhu et al., 2018). Comparable k-values (0.012 to 0.025 min^{-1}) were reported for flavonoid in plump extract heated thermally at 70-110°C (Turturică et al., 2016). The results shown in Table 1 also indicate that flavonoid degradation rates were relatively higher than those of anthocyanin. This finding was validated by the significantly smaller half time ($t_{1/2}$) value for flavonoid which indicate that the time required to degrade 50% of flavonoid in bignay fruit juice is significantly shorter than that of anthocyanin. Comparable $t_{1/2}$ values for anthocyanin (129-447 min) were reported in blackberry pulp treated by ohmic heating (Sarkis et al., 2019).

The effect of temperature on the degradation rate constant was significant as shown in Table 1. It can be observed that the k-value for anthocyanin degradation at 110 °C was more than 10 times the k-value at 70 °C. On the other hand, the k-value for flavonoid degradation was only double as temperature was increased from 70 °C to 110 °C. Consequently, the activation energy (E_a) of anthocyanin degradation was significantly higher than that of flavonoid (Figure 3). Based on the Ea values, anthocyanin is much more thermally stable than flavonoid since its Ea value was about 3.5 times the Ea value of flavonoid. A closer examination to the data in Table 1 indicates that at 70°C, the degradation rate constant for flavonoid was about 6.7 times that of anthocyanin, while at 90 °C the value was about 2.8. On the other hand, at 110°C, the degradation rate constant for flavonoid was about 0.98 that of anthocyanin. These values indicate that at the lower treatment temperature (70 and 90 °C), anthocyanin was much more stable that flavonoid while at the highest treatment temperature (110 °C) the degradation rate constants of the two compounds were almost identical. Therefore, overall, anthocyanin in bignay fruit juice is much more stable during ohmic heating compared to flavonoid. This trend can also be seen from the slopes of the curves in Figure 2. For the effect of temperature on anthocyanin degradation, results of statistical analysis indicate that degradation of anthocyanin in bignay fruit juice was significantly higher at 90 and 110 °C than that at 70 °C ($p<0.05$). The activation energy for degradation of anthocyanin found in this study was comparable to those reported for blackberry pulp (67 kJ/mol) and acerola pulp (74.83 kJ/mol) (Mercali et al., 2013; Sarkis et al., 2019).

It is interesting to note that the Ea value for degradation of flavonoid in bignay fruit juice during ohmic heating as found in this study as comparable to those reported previously for sour cherry marasca paste with Ea value of 18.1 kJ/mol (Zoric et al., 2014), plump extract with Ea value of 18.0 ± 2.0 kJ/mol and black rice flour with Ea value of 15.80 ± 1.50 kJ/mol (Bolea et al., 2016; Turturică et al., 2016). Another important bioactive compound that has been reported to have comparable E_a value was ascorbic acid in pineapple juice with Ea vaalues ranged from 14.22 to 29.78 kJ/mol (Dhakal et al., 2018) and ascorbic acid in strawberry pulp with Ea value of 21.36 kJ/mol (Castro et al., 2004).

3.4 Changes in antioxidant activity

The antioxidant activity of bignay fruit juice is expressed as IC_{50} with lower value implies higher antioxidant activity. The IC_{50} value of bignay fruit juice ranged from 0.106-0.168 mg/mL for DPPH method and 0.131-0.161 mg/mL for ABTS method. Comparable range of antioxidant activity was reported for black plump (71.30 - 114.69 µg/mL) and *Litchi chinensis* fruit pulp (0.102 mg/mL) (Prakash et al., 2011; Baliga et al., 2011).

Antioxidant activity of bignay fruit juice obtained from both DPPH and ABTS methods slightly decreased after undergoing heating for 45 min (Figure 4). However, the overall effect of ohmic heating duration on the reduction of antioxidant activity measured using both DPPH and ABTS methods was statistically insignificant ($p > 0.05$). Results of statistical analysis indicate that the effect of temperature on antioxidant activity was significant ($p < 0.05$) based on the measurement results using the DPPH method but insignificant based on measurement results from the ABTS method. It is important to note that based on the results obtained from the DPPH method, bignay fruit juice heated by ohmic heating at 90 and 110 °C provided higher antioxidant activity than that at 70 °C ($p < 0.05$). In fact, the antioxidant activity of ohmically heated bignay juice at 90 °C exhibited no significant difference from that of fresh bignay fruit juice in our previous study (Hardinasinta et al., 2020). This implies that higher temperature showed a positive effect on antioxidant activity. Increased in antioxidant activity of food product at higher temperature or after thermal treatment has been reported by other researchers (Alizadeh & Aliakbarlu, 2020; Jaramillo-Flores et al., 2003; Jeong et al., 2004; Keenan et al., 2010; Kusznierewicz et al., 2008; Mannozzi et al., 2019; Nayik & Nanda, 2016; Sharma et al., 2015). Mannozzi et al. (2019) reported that antioxidant activity of carrot and apple juice preheated at 80 °C was higher than those preheated at 40 and 60 °C. There are several postulates have been proposed for this trend such as the disruption of chemical bonds, formation of chemical reaction products that provide scavenging activity, and inactivation of oxidative enzymes (Keenan et al., 2010; Kusznierewicz et al., 2008; Mannozzi et al., 2019; Nayik & Nanda, 2016; Sharma et al., 2015).

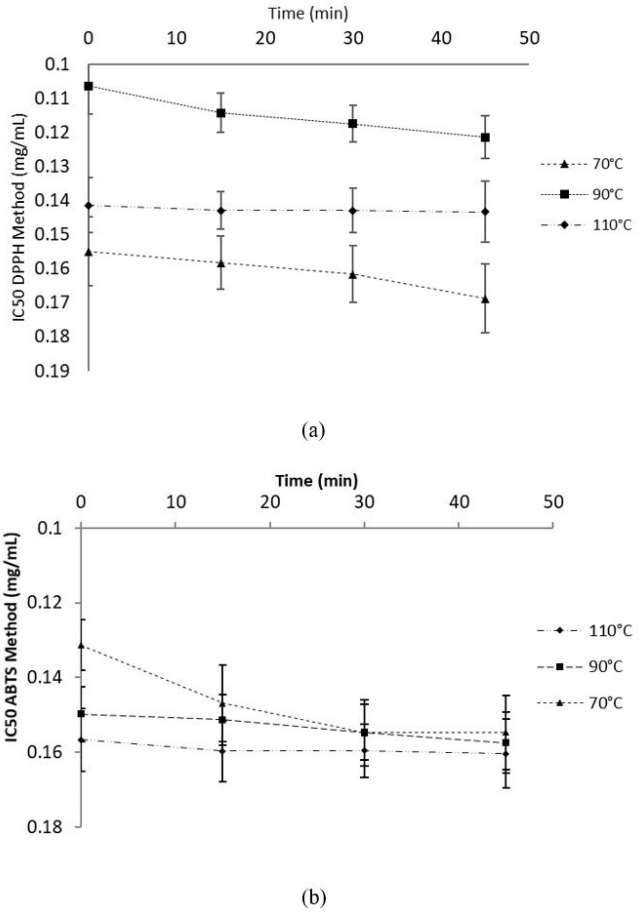

Figure 4. Antioxidant activity of bignay juice during ohmic heating represented as IC_{50} value; (a) DPPH method; and (b) ABTS method.

4 Conclusions

The present study demonstrates that an insignificant change in total phenol occurred during ohmic heating of bignay juice, while anthocyanin and flavonoid contents tended to decrease. Activation energy for the degradation of anthocyanin was much higher than that of flavonoid which suggests that anthocyanin in bignay fruit juice is much more stable during heating compared to flavonoid. The degradation rate constants at 70 and 90 °C for the two compounds indicate that anthocyanin is much more stable at the lower temperature treatment (70 and 90 °C) but these compounds have almost the same destruction rate constant at 110 °C. Antioxidant activity of bignay fruit juice showed a significant increase after undergoing ohmic heating even though the natural bioactive compounds contained in the juice decreased during ohmic heating.

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