

Comparison of Sonication with Maceration on Antioxidant Potency of Anthocyanin and Karotenoid of Tamarillo (*Solanum betaceum* Cav.)

Gusti Ayu Kadek Diah Puspawati^{1*}, Yustinus Marsono², Supriyadi², Ria Armunanto³

¹Study Program on Food Science and Technology, Faculty of Agricultural Technology, Udayana University, Jl. Kampus Bukit Jimbaran, Badung, Bali 80225, Indonesia

²Food Science Study Program, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia

³Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Jl. Sekip, Yogyakarta 55281, Indonesia

*Email: diahpuspawati@unud.ac.id

Submission: 2 Januari 2018; Acceptance: 22 Agustus 2018

ABSTRACT

Tamarillo is an underutilized fruit in Indonesia, despite the fact that it contains two pigments (anthocyanin and carotenoid) with different properties (polar and non polar). Besides, there is a scarce information about extraction of anthocyanin and carotenoid of Tamarillo using sonication extraction. This study was aimed to investigate the comparison of extraction methods of sonication with maceration on antioxidant potency of anthocyanin and carotenoid of Tamarillo. Two pigmen types (anthocyanin and carotenoid) were extracted using two different methods (sonication and maceration). The parameters of analysis were yield, antioxidant activity (DPPH) with IC₅₀, Ferric Reducing Antioxidant Power (FRAP), anthocyanin total, carotenoid total, phenolic total and scanning electron microscope of cover cell of material. The antioxidant potencies for both anthocyanin and carotenoid by sonication method were higher than those of obtained by maceration method. The results showed that both sonication and maceration were suitable for extraction of anthocyanin and carotenoid from Tamarillo.

Keywords: antioxidant; anthocyanin; carotenoid; sonication; tamarillo

INTRODUCTION

Tamarillo (*Solanum betaceum* Cav) known as tomato tree or terong Belanda, is one of the fruit vegetables that is not yet widely used as food in Indonesia compared with tomatoes or purple eggplants. On the other hand, Tamarillo is an export commodity several countries such as New Zealand, America and Brazil. The breed commonly found in Indonesia is the red one. The red breed has red peel, orange flesh, dark red placenta and hard brownish black seeds (De Rosso and Mercadante, 2007).

According to Osorio *et al.* (2012), red Tamarillo contains both anthocyanin and carotenoid bioactive components, and they have differing characteristics, namely polar and bipolar. Both compounds are natural antioxidants. In Indonesia the availability of fruits which

contain the two components at the same time is still limited.

Anthocyanin belongs to phenolic compound, in the flavonoid group, which has healthy properties, such as lowering blood sugar for type 2 diabete (Sancho and Pastore, 2012). Carotenoid is a non-polar C40 tetraterpenoid compound which has a polydiene structure and health properties as anti-diabetes (Sugiura *et al.*, 2015).

Anthocyanin and carotenoid's potentials as anti-diabetes for type 2 can be caused by antioxidant properties, because in type 2 diabetes an oxidative stress occurs, and oxidative stress can be suppressed by antioxidant. Antioxidant components of a substance (tamarillo) can be taken through extraction process. Tamarillo's anthocyanin and carotenoid compound extraction and identification has been reported by

De Rosso & Mercadante (2007), who stated that anthocyanin and carotenoid compound extraction from the whole tamarillo fruit takes 12 h. Mandal and Ghosal (2012), reported that extraction from several parts of tamarillo fruit could be conducted at a temperature of 50 °C for 8 hours. Tamarillo extraction that has been performed used maceration and soxhlet methods.

Maceration and soxhlet methods are conventional methods which are simple and easy to perform, but it takes a considerable amount of time, between 1 to 24 h, and in soxhlet method, high temperature (50°) is required. Temperature increase during extraction (> 50°) can cause damage to anthocyanin (Pingret *et al.*, 2013).

The sonication method, which is considered as a green extraction method, was first developed to overcome the drawbacks of conventional methods, such as maceration. The sonication method can be performed in much shorter time, less than 1 h, and the damage effects during the process is low or can be minimized (Chemat *et al.*, 2016). Ma *et al.* (2009) reported that degradation of antioxidant compounds as the effect of sonication temperature can be minimized, if the temperature reaches < 40 °C, it can be controlled by applying ice cubes.

The sonication method principle involves ultrasonic wave which creates cavitation. The cavitation phenomenon creates energy, temperature increase and local pressure. This also generates a micro jet, material surface damage towards the inner part, which creates a rapid mass transfer, faster extraction time and more rendement produced (Medina-Torres *et al.*, 2017).

Sonication extraction of tamarillo has been reported by Asmara *et al.* (2013), but on tamarillo peel using ultrasonic wave at 60 °C temperature in 30 minutes. The research used a high temperature above 40 °C, and involved materials not to be eaten. The sonication method for tamarillo anthocyanin and carotenoid extraction involving the edible part of the fruit and using room temperature (≤ 30 °C) has not been reported. In sonication, high temperature increase can cause damage on the compound which has antioxidant characteristics. Therefore, this research aims to evaluate rendement and antioxidant potentials of anthocyanin and carotenoid of tamarillo extract using a sonication method compared with control maceration method.

RESEARCH METHOD

Materials

Red variety of Tamarillo, at age of 6 months after producing flowers, taken from Dieng plateau,



Figure 1. Tamarillo fruit and its cross sections

Wonosobo, Central Java, Indonesia. The form of and the cross section of the tamarillo fruit is shown in Figure 1. Ethanol solvent 70% (technical); acetone (technical) and citric acid (Gajah, technical) obtained from Brataco, Jakarta, Indonesia. Pro analysis chemical substances: Folin Ciocalteau's phenol reagent, $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ and Na_2CO_3 , obtained from Merck, Darmstadt, Germany. Pro analysis chemical substances: ethanol, metanol, acetone, 2,3,5-triphenyl-1,3,4-triaze-2-azoniacyclopenta-1,4-diene chloride (TPTZ) dan 2,2-diphenyl-1-picrylhydrazyl (DPPH), obtained from Sigma Aldrich, St. Louis, MO, USA.

Equipments

The equipments used for extraction included sonicators (Elma T760DH, Singen, Germany), rotary evaporator (IKA WERKE RV06-ML, Staufen, Germany) and shaking water bath (Juloba SW22, Seelbach, Germany). The equipments for analysis were UV-Vis spectrophotometer (Genesys S10, Germany) and Pyrex glass wares (Iwaki, Tokyo, Japan). The tool used for cell surface morphology analysis was a scanning electron microscope (JOEL JSM-6510, Tokyo, Japan)

Tamarillo Anthocyanin Extraction using the maceration method

Tamarillo anthocyanin extraction using the maceration method refers to Atiqah *et al.* (2014). As much as 300 g of tamarillo fruits were peeled, and then they are blended without adding water for 2 minutes, resulting in fruit puree. As much as 200 g of puree was put into a tinted bottle, added with 800 ml ethanol solvent 70% (technical), containing 3% citric acid, and then mixed using a magnetic stirrer for 2 minutes. The mixture was then subjected to maceration extraction in a shaking water bath with a 50 rpm shaking speed, 30 °C temperature for 20 minutes. Filtrate from the extraction was evaporated to rid of its solvent using a rotary evaporator at a heating bath temperature of 35 °C and a 176 mbar initial vacuum pressure, which was gradually lowered to reach 72 mbar, resulting in a thick extract. The extract was a raw tamarillo anthocyanin extract, this was stored in a tinted glass bottle, kept at -20 °C until the time for analysis.

Tamarillo Anthocyanin Extraction using Sonication Method

Tamarillo anthocyanin extraction using sonication method refers to Celli *et al.* (2015). The procedures before and after the extraction process were the same with tamarillo anthocyanin extraction using maceration method. Sonication extraction method was performed in a sonicator at a 40 kHz frequency, 100% power, initial temperature of 27°C and end temperature of 29 °C for 20 minutes.

Tamarillo Carotenoid Extraction using Maceration Method

Tamarillo anthocyanin extraction was conducted using sonication method refers to De Rosso and Mercadante (2007). As much as 350 kg of tamarillo fruit was taken by removing the peel, and then blended without adding water for 5 minutes to get a fruit puree. the puree is then placed in tinted glass bottle, added with 800 mL of acetone solvent 80% (technical), and then mixed using a magnetic stirrer for 5 minutes. The mixture was then extracted using maceration in a shaking water bath at 50 rpm shaking speed and 30 °C temperature for 30 minutes. The filtrate was then retrieved, and then passed through a separator funnel filled with petroleum ether. The next step was fetching the yellow-orange colored solution. The solution was then put into a rotary evaporator at a 30 °C heating bath temperature to evaporate its solvent, applying a 356 mbar initial pressure and 72 mbar end pressure until a rather thick extract was obtained. The extract was called tamarillo raw carotenoid extract.

Tamarillo Carotenoid Extraction using Sonication Method

Tamarillo carotenoid extraction using sonication method refers to Santos *et al.* (2015). The procedures before and after the extraction process were similar with anthocyanin extraction using maceration method. Extraction was conducted in a sonicator under conditions similar with anthocyanin extraction, only the time of extraction is 30 minutes.

Determining Rendemen

Determining rendemen refers to Annegowda *et al.* (2012). Rendemen was determined by weighing the

$$\text{Rendemen(\%)} = \left(\frac{Ba - Be}{Be} \right) \times 100\% \quad (1)$$

Remarks :

Ba (fruit puree weight; Be (extract weight after the solvent is evaporated)

tamarillo fruit puree and its raw extract weight after the solvent was evaporated. The rendemen calculation was determined using equation 1:

Analysis of Antioxidant Activity as Radical Scavenging Antioxidants (RSA) and IC₅₀

Analysis of RSA using DPPH refers to Aadil *et al.* (2014). 600 µL of sample solution (100 µg/mL) was added with 1400 µL of DPPH solution (0,01 M). The mixture was incubated in a dark condition, room temperature, for 30 minutes, and then the absorbance in read using a spectrophotometer at λ 517 nm. A blank was made by changing the sample solution with a solvent. The percentage of RSA is determined with Equation 2.

$$\%RSA = \left(\frac{Ab - As}{Ab} \right) \times 100\% \quad (2)$$

Remarks :

Ab (blank absorbance); As (sample absorbance)

IC₅₀ was performed by making several extract concentrations (20,40,60,80, 100 µg/mL) and by making a linear equation , in which the x axis = extract concentration and the y axis = percentage of inhibition. IC₅₀ is determined using Equation 3.

$$IC_{50} = \frac{50 - a}{b} \quad (3)$$

Analysis of Antioxidant Activity as Ferric Reducing Antioxidant Power (FRAP)

Analysis of FRAP refers to Benzie & Strain (1996). 100 µL of sample solution (100 µg/mL) added with 1200 µL FRAP solution (acetate buffer: TPTZ: FeCl₃.6H₂O; ratio 10:1:1), mixed using a vortex. The mixture was then incubated at 37 °C for 4 minutes, absorbance was read at a spectrophotometer λ 593 nm. Standard solution of FeSO₄.6H₂O was made with several concentrations (10, 20, 30, 40 and 50 mM), and antioxidant activity as Fe³⁺ ion reducer to Fe²⁺ was determined using a standard curve linear equation.

Analysis of Total Anthocyanin

Analysis of total anthocyanin refers to Giusti & Wrolstad (2001). Sample solution (1000 µg/mL) is poured with 500 µL each into two reaction tubes, the first one =was added with 2.5 mL KCl pH 1 buffer, and the second one was added with pH 4.5 acetate buffer. The mixed solutions were incubated in a dark room for 30 minutes, absorbance was read using spectrophotometer at λ 520 nm and λ 700 nm. Anthocyanin extract content

calculation as delphinidin 3 rutinoside was determined using Equations 4 and 5.

$$A = (A520 - A700)_{pH 1} - (A520 - A700)_{pH 4,5} \quad (4)$$

$$TA = \left(\frac{mg}{kg}\right) = \frac{A \times BM \times DF \times V \times 1000}{L \times \varepsilon \times m} \quad (5)$$

Remarks: TA (total anthocyanin); BM (molecule weight of delphinidine 3-rutinoside (611 mol/g)); DF (dilution factor); V (total volume (L)); L (length (1 cm)); ε (extension coefficient (mol/L \times cm)); m (extract weight (kg)); 1000 (conversion from g to mg)

Analysis of Total Carotenoid

Total carotenoid was assessed based on Kurniawan *et al.* (2010). As much as 2 mL of raw carotenoid extract, added with 10 mL acetone solvent 80% pa, and then homogenized using vortex for 5 minutes. The solution was diluted 10 times, and then read for its absorbance using λ 480 nm, λ 645 nm and λ 663 nm spectrophotometer. Total carotenoid was determined using equation 6:

$$TK \left(\frac{\mu mol}{L}\right) = \left((A480 + (0,114 \times A663)) - \frac{0,638 \times A645 \times V \times FP \times 1000}{112,5 \times W} \right) \quad (6)$$

Remarks:

1 μ mol/L (27,25 mg/L (Hendry dan Grime 1993); A480 (absorbance at 480 nm wavelength); A663 (absorbance at 663 nm wavelength); A645 (absorbance at 645 nm wavelength); V (extract volume (mL)); FP (dilution factor); W (sample weight (g)).

Analysis of Total Phenolic

Total phenolic was determined using folin ciocalteu and gallic acid standard refers to Rocha *et al.* (2017). 200 μ L sample solution (1000 μ g/mL), is added with 1000 μ L folin ciocalteu (10%). The mixture was incubated for 5 minutes, and then added with 800 μ L Na₂CO₃ (7,5 %), incubated again for 30 minutes. The mixture was then read for absorbance at λ 763 nm. Gallic acid standard solutions were made at several concentrations (100, 200, 300, 400 and 500 μ g/mL). The total phenolic in the extract was determined using Equation 7.

$$TP (\%) = \frac{a \times FP \times TV \times 100}{sample\ weight(g)} \quad (7)$$

Remarks:

a (x value of gallic acid standard linear equation); FP (dilution factor); TV (total volume).

Material Cell Surface Morphology by means of Scanning Electron Microscope (SEM)

Cell surface morphology was performed on the fruit puree and extraction residue, which had been made into dry powder refers to Altemimi *et al.* (2016).

Tamarillo fruit puree as the basic ingredient for tamarillo anthocyanin and carotenoid extraction and residue of extraction from the maceration and sonication methods are dried using freeze drying, and then refined to become dry powder. The next step, 0.5 g of sample powder was sprinkled evenly on the sample container. Subsequently, it was coated by putting in the chamber for 20 minutes. The sample was observed for its cell surface morphology, with a 5,000 magnification.

Data Analysis

Data analysis was performed using an independent t-test with SPSS 20.0 for Windows. The confidence level was 5% ($p < 0.05$).

RESULTS AND DISCUSSIONS

Tamarillo Raw Anthocyanin Extract (TACE) and Tamarillo Raw Carotenoid Extract (TCCE) by Maceration and Sonication

Rendemen of tamarillo raw anthocyanin extract (EKAT) and tamarillo raw carotenoid extract (EKKT) using sonication method is higher than maceration method ($p < 0.05$). Rendemen of EKAT and EKKT as the results of maceration and sonication were shown in Figure 2.

Rendemen of TACE as the results of maceration and sonication processes were $21.45 \pm 1.25\%$ and $24.70 \pm 1.42\%$ consecutively, the sonication result is 15.14% higher. The results for TACE were $12.37 \pm 0,42\%$ and $15.10 \pm 0,27\%$ consecutively, the sonication result was 22.13% higher. This indicates that tamarillo anthocyanin extraction using the sonication method at a frequency of 40 kHz, 100% power, initial temperature 27 °C and end temperature 29 °C, for 20 minutes and tamarillo carotenoid extraction using the sonication method at the same condition, but for 30

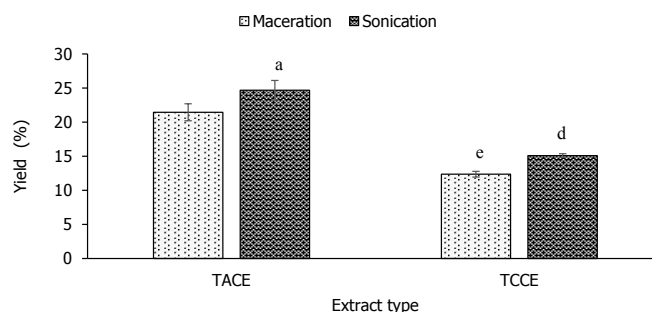


Figure 2. Rendemen of tamarillo raw anthocyanin extract (TACE) and tamarillo raw carotenoid extract (TCCE) using maceration and sonication methods

Note: TACE = Tamarillo athocyanin crude extract; TCCE = Tamarillo carotenoid crude extract

minutes, will produce higher rendement than using the maceration method.

The higher TACE and TCCE rendement from sonication than from maceration is probably caused by the ultrasonic wave which creates a cavitation phenomenon. The cavitation phenomenon can accelerate molecule movement, accelerate solvent penetration into the material, which causes more compounds to be extracted. Medina-torres *et al.* (2017) confirmed that ultrasonic wave which creates the cavitation phenomenon causes energy formation, temperature and local pressure increase which create a micro jet. The condition will accelerate mass transfer process. The results of sonication that was higher than maceration is confirmed by a report from Rombaut *et al.* (2014). It was reported that a temperature increased below 60 °C caused reduction of density and viscosity, increase solubility of the solute with solvents and reduction surface tension. These conditions helped solvents to enter into the material and accelerate mass transfer process. Another reason could be sonication method did not cause degradation of tamarillo anthocyanin and carotenoid. Ma *et al.* (2009), reported that antioxidant component damage can be suppressed if the temperature was below 40 °C and was controlled using ice blocks.

The rendement of TACE and TCCE from sonication which was higher than that of maceration is consistent with the previous research, Ivanovic *et al.* (2014), reported that sonication can increase rendement better than maceration on dragon fruit flesh with 22.94% and blackberry fruit flesh between 5.2 – 6.3%. The previous studies also reported that carotenoid extract rendement from tomato lycopene using the sonication method is 26% higher (He & Teoh, 2012).

Radical Scavenging Antioxidant and IC₅₀ TACE and TCCE from Maceration and Sonication

Radical scavenging antioxidant (RSA) with 2,2-diphenyl-1-picrylhydrazyl (DPPH) was stated as the

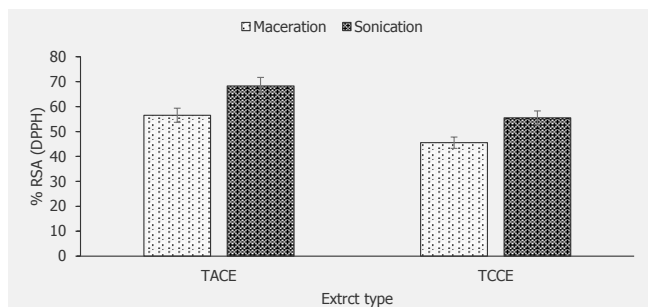


Figure 3. RSA percentage of tamarillo raw anthocyanin extract (TACE) and tamarillo raw carotenoid extract (TCCE) using maceration and sonication methods

percentage of antioxidant activity in scavenging free radicals DPPH (%RSA) and the inhibition concentration is in the form of IC₅₀ value. The IC₅₀ value here is the concentration of TACE and TCCE as scavenging DPPH free radicals by 50%. The concentration of TACE and TCCE is used as the comparison indicator of antioxidant activity with other compounds. The RSA percentage of TACE and TCCE from sonication shows higher results than from maceration ($p < 0.05$) (Figure 3).

RSA percentage of TACE from maceration and sonication were $56.52 \pm 1.27\%$ and $68.28 \pm 3.28\%$, in which the result from sonication was 20.81% higher. Whereas for TCCE, the RSA percentage using maceration and sonication are: $46.40 \pm 2.31\%$ and $55.48 \pm 2.44\%$. The result from sonication is 21.92% higher. This indicates that the sonication method of tamarillo anthocyanin and carotenoid extraction can produce higher RSA percentage than maceration. The RSA percentage of sonication method which is higher than maceration method was probably because of ultrasonic wave with cavitation phenomenon, this accelerates material surface damage so that mass transfer occurred faster and the extraction process of antioxidant compounds that were complexly bound with other compounds will be extracted faster and in higher quantity. Anthocyanin compounds that were bound on the cell walls (cellulose and pectin) and protein create a complex compound on the vacuole and carotenoid compound is bound on the cell wall constituent component and fat forms a complex compound in the inner part of chromoplast membrane can be quickly degraded with the existence of cavitation phenomenon in sonication (Phan *et al.*, 2015). The mechanism of antioxidant activity from the compound is through proton transfer (H⁺) which dampen free radical compounds (DPPH) into non radical (Molyneux, 2004).

The higher RSA percentage in TACE from sonication method was in line with a report from Aadil *et al.* (2014), which stated that sonication in anthocyanin extraction from grapes, in 30 minutes can increase RSA percentage up to 16.67% than maceration method. Whereas the higher result of carotenoid extraction using sonication method compared to using maceration method is similar with the report from Saikia *et al.* (2015), which stated that pineapple carotenoid extraction using sonication for 30 increased antioxidant (DPPH) activity by 40.55% compared to maceration.

Concentration of RSA inhibition percentage at 50% or IC₅₀ value of TACE and TCCE shows lower sonication result compared to maceration result ($p < 0.05$) (data not shown in Figure). The results show lower IC₅₀ therefore, the RSA percentage was higher. IC₅₀ values of TACE using maceration and sonication were 95.50

$\mu\text{g/mL}$ and $85.32 \mu\text{g/mL}$, whereas IC_{50} values of TCCE using maceration and sonication were $99.94 \mu\text{g/mL}$ and $87.71 \mu\text{g/mL}$

IC_{50} values of TACE and TCCE using sonication was lower than using maceration because of cavitation phenomenon which accelerates mass transfer, and therefore it can extract compounds that have antioxidant activity faster and in bigger quantity, which caused lower IC_{50} value. The IC_{50} value of TACE and TCCE was below $100 \mu\text{g/mL}$, therefore, TACE and TCCE belong to strong antioxidants (Molyneux, 2004). The IC_{50} value of TACE and TCCE of Tamarillo obtained in this study were higher than vitamin C ($29.83 \mu\text{g/mL}$) and Trolox ($30.53 \mu\text{g/mL}$) and lower blackberry extract ($96 \pm 0,32 \mu\text{g/mL}$) (Ivanovic *et al.*, 2014). This indicates that TACE and TCCE have antioxidant activity for scavenging free radicals, lower than vitamin C and Trolox, but higher than blackberry extract.

Ferric Reducing Antioxidant Power (FRAP) of TACE and TCCE Using Maceration and Sonication

Antioxidant activity (FRAP) of TACE and TCCE using sonication is higher than maceration ($p < 0,05$). Antioxidant activity (FRAP) of TACE and TCCE using maceration and sonication is shown in Figure 4.

Antioxidant activities (FRAP) of TACE using maceration and sonication were $570.93 \pm 5.62 \text{ mM FeSO}_4 \cdot 6\text{H}_2\text{O}/100 \text{ g extract}$ and $694.45 \pm 25.56 \text{ mM FeSO}_4 \cdot 6\text{H}_2\text{O}/100 \text{ g extract}$. The result from sonication was 17.79% higher. The activities for TCCE were $278.46 \pm 14.77 \text{ mM FeSO}_4 \cdot 6\text{H}_2\text{O}/100 \text{ g extract}$ and $332.95 \pm 19.41 \text{ mM FeSO}_4 \cdot 6\text{H}_2\text{O}/100 \text{ g extract}$, in which the result from sonication was 16.37% higher. This indicated that sonication result with its cavitation phenomenon can increase TACE and TCCE potentials as antioxidants in reducing ferric ion (Fe^{+3}) into ferrous ion (Fe^{+2}).

This is shown by the formation of blue color that gets darker (Benzie and Strain, 1996). The ability to reduce Fe^{3+} into Fe^{2+} from tamarillo anthocyanin and

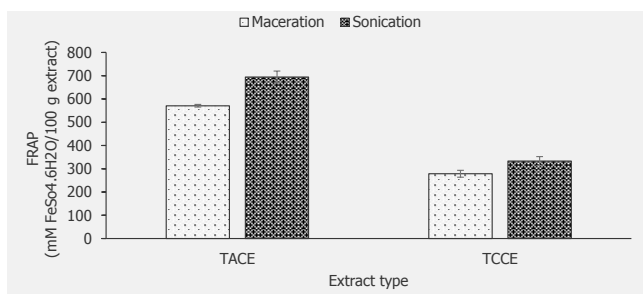


Figure 4. Antioxidant activity (FRAP) of tamarillo raw anthocyanin extract (TACE) and tamarillo raw carotenoid extract (TCCE) using maceration and sonication methods

carotenoid is probably caused by the presence of electron as reducer, or the compound's ability as antioxidant. The antioxidant's property as reducer is due to the presence of hydroxyl cluster in the B ring of anthocyanin, whereas on carotenoid, it is due to the presence of conjugated dienes. The mechanism as antioxidant belongs to SET (single electron transfer) mechanism (Liang and Kitts, 2014).

The result of this study was supported Ramli *et al.* (2014), who reported that red dragon fruit extraction as antioxidant source has higher antioxidant activity (FRAP) using sonication compared with conventional extraction with antioxidant activity of $620 \text{ mM FeSO}_4 \cdot 6\text{H}_2\text{O}/100 \text{ g extract}$ dan $609 \text{ mM FeSO}_4 \cdot 6\text{H}_2\text{O}/100 \text{ g extract}$.

Total TACE Anthocyanin And Total TCCE Carotenoid from Maceration And Sonication

Total anthocyanin in TACE was equivalent with monomeric anthocyanin delphinidine-3 rutinoside (De Rosso & Mercadante, 2007). Total anthocyanin of TACE and total carotenoid in TCCE using sonication was higher than using maceration ($p < 0.05$) as presented in Figure 5.

Total anthocyanin in TACE using maceration and sonication was $286.77 \pm 21.96 \text{ mg}/100 \text{ g extract}$ and $386.48 \pm 19.82 \text{ mg}/100 \text{ g extract}$, respectively indicating that sonication shows 34.77% higher value. Total carotenoids in TCCE using maceration and sonication were $29.93 \pm 1.18 \text{ mg}/100 \text{ g extract}$ and $50.80 \pm 3.02 \text{ mg}/100 \text{ g extract}$, in which sonication shows 64.74% higher. This condition is probably caused by the fact that in sonication there is cavitation phenomenon which can increase anthocyanin or carotenoid mass transfer to the solvent, therefore, anthocyanin and carotenoid compounds extracted increases. In addition, low temperature of sonification did not cause carotenoid damage. According to Ma *et al.* (2009), the sonication temperature at $40 \text{ }^\circ\text{C}$ will cause damage to the bioactive components such as anthocyanin or carotenoid.

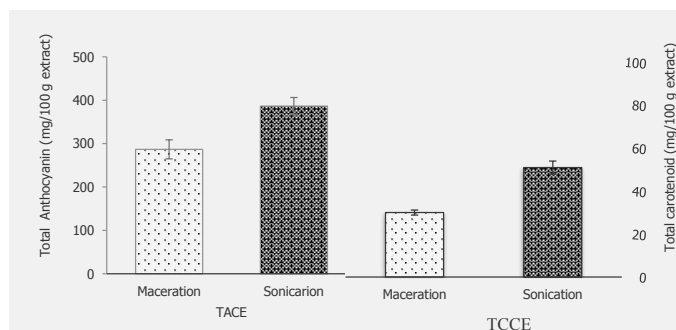


Figure 5. Total of tamarillo raw anthocyanin extract (TACE) and tamarillo raw carotenoid extract (TCCE) using maceration and sonication methods

Total anthocyanin of 386,48 mg/100 g was higher than the total anthocyanin of tamarillo peels using sonication at a temperature of 60 °C for 30 minutes, which is 23.78 mg/100g (Asmara *et al.*, 2013). This difference is caused by the difference of tamarillo parts being used, temperature and duration of sonication extraction. The sonication method that can produce higher amount of anthocyanin and carotenoid is supported by a research on blueberry fruit anthocyanin extraction using sonication, which can increase the total anthocyanin of 58.33% (Rocha *et al.*, 2017). Sonication results can increase the total carotenoid in carrot juice of 3.15% (Jabbar *et al.*, 2014).

Total Phenolic in TACE and TCCE Using Maceration and Sonication

Total phenolic in TACE and TCCE shows that using sonication is higher than using maceration ($p < 0.05$). Total phenolic in TACE and TCCE using maceration and sonication is presented in Figure 6. Total phenolics in TACE using maceration and sonication were 298.55 ± 27.34 mg GE/100 g extract and 395.64 ± 38.93 mg GE/100 g extract. Using sonication produces 24.54% higher. Meanwhile the total phenolic in TCCE using maceration and sonication is as follows were 47.79 ± 4.53 mg GE/100 g extract and 89.28 ± 8.37 mg GE/100 g extract. Using sonication produces 46.48% higher. This was possible because sonication with its cavitation phenomenon can accelerate mass transfer, therefore, can extract more phenolic compound than using maceration. Total phenolic in TACE is higher than in TCCE, it is possibly because the total anthocyanin in TACE is higher than the total carotenoid in TCCE. Anthocyanin is a compound from the flavonoid group which belong to phenolic compounds, whereas carotenoid is from tetraterpenoid group, and does not

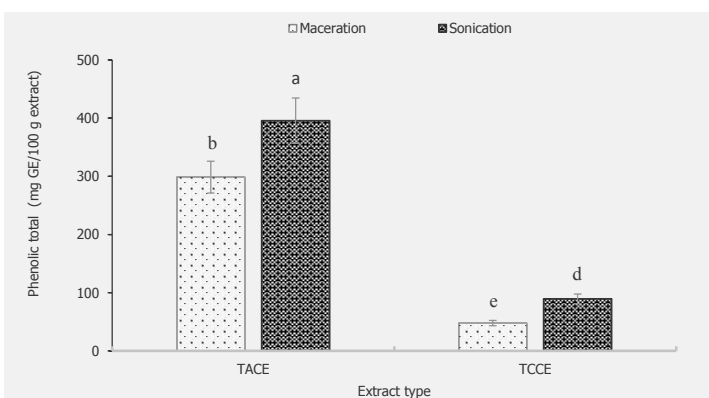


Figure 6. Total phenolic of tamarillo raw anthocyanin extract (TACE) and tamarillo raw carotenoid extract (TCCE) using maceration and sonication methods

belong to phenolic compounds (Sancho and Pastore, 2012; Sugiura *et al.*, 2015).

This result is consistent with what was reported by Rocha *et al.* (2017), that blueberry fruit extraction using sonication method shows 27.87% higher total phenolic than using maceration. In lemon fruit, extraction using sonication method shows 6.5 times higher increase of total phenolic compared to using maceration (Žlabur *et al.*, 2016).

Material Cell Surface Morphology and Residue of Tamarillo Anthocyanin and Carotenoid Extraction Using Maceration and Sonication

Material cell surface morphology and residue of tamarillo anthocyanin and carotenoid extraction using maceration and sonication in the form of dry powder is performed using scanning electron microscope (SEM). The structure of material cell surface experience a change before and after extraction. There is also a difference between the results of extraction using maceration and using sonication. The differences happen both in anthocyanin extraction (Figures 7A-7C) and carotenoid extraction (Figures 7D-7F).

The material's cell surface before anthocyanin and carotenoid extraction showed no damage under 5,000 times magnification. The cell surface damage in maceration residue was less than that in sonication residue. In maceration, no sharp gap was found (black arrow mark), whereas in sonication it is quite the contrary, more intense and deep damage was found (black dotted arrow mark). This is probably because sonication, with its micro jet power, can induce deeper and more damage. This result is in line with what was

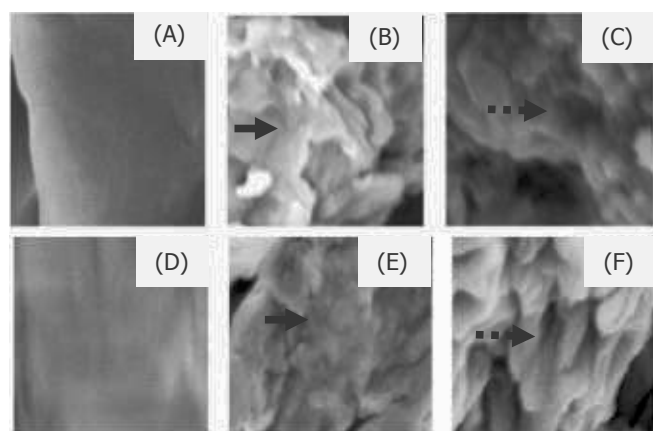


Figure 7. SEM results of material surface, 5,000 x magnification of (A) anthocyanin extraction material; (B) residue of anthocyanin extraction maceration; (C) residue of anthocyanin extraction sonication; (D) carotenoid extraction material; (E) residue of carotenoid extraction maceration; (F) residue of carotenoid extraction sonication

reported by Altemimi *et al.* (2016), results of SEM on chayote after extraction using sonication showed more cell surface damage and more gaps towards the material's inner part than using maceration.

CONCLUSION

Sonication method was able to increase rendement and TACE and TCCE antioxidant potentials than that maceration method. Rendemen increase of TACE and TCCE were 15.14% and 22.13%, respectively. TACE and TCCE's antioxidant potentials using sonication method, including antioxidant activity (DPPH) and IC₅₀ were as follows: 68.28 ± 3.28%; 55.48 ± 2.44% and 85.32 µg/mL; 87.71 µg/mL; antioxidant activity (FRAP) of each: 694.45 ± 25.56 mM FeSO₄.6H₂O/ 100 g extract; 332.95 ± 19.41 mM FeSO₄.6H₂O/ 100 g extract; total anthocyanin of TACE: 386.48±19.28 mg/100 g extract; total carotenoid of TCCE: 50.80 ± 3.02 mg/100 g extract; total phenolic of TACE and TCCE are: 395.64 ± 38.93 mg GE/100 g extract; 89.28 ± 8.37 mg GE/100 g extract. Sonication's better performance than maceration was confirmed by the results of scanning electron microscope, in which more cell surface damage and deeper gaps were found. Sonication extraction method was an alternative extraction method to increase tamarillo extract rendement and antioxidant potentials.

BIBLIOGRAPHY

- Aadil, R. M., Zeng, X.-A., Abbasi, A. M., Khan, M. S., Khalid, S., Jabbar, S., & Abid, M. (2014). Influence of power ultrasound on the quality parameters of grapefruit juice during storage. *Sci Lett.*, 3, 1–8.
- Altemimi, A., Watson, D. G., Choudhary, R., & Dasari, M. R. (2016). Ultrasound Assisted Extraction of Phenolic Compounds from Peaches and Pumpkins. *PLoS ONE*, 17, 1–20.
- Annegowda, H. V, Bhat, R., & Min-tze, L. (2012). Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. *J. Food Sci Technol*, 49(4), 510–514.
- Asmara, Y., Bayu, A. K., Adi, S. G. ., Aini, F., & Pudjihastuti, I. (2013). Rekayasa Proses Pembuatan Serbuk Pewarna Batik Biodegradable Berbahan Antosianin Limbah Kulit Terong Belanda (*Chypomandra betaceae*) dengan Kombinasi Ekstraksi Gelombang Ultrasonik dan Aqua Solvent. *In SNST ke-4 2013*, Semarang, Indonesia.
- Atiqah, N. A. A. K., Maisarah, A. M., & Asah, R. (2014). Comparison of antioxidant properties of tamarillo (*Cyphomandra betacea*), cherry tomato (*Solanum lycopersicum* var. *cerasiform*) and tomato (*Lycopersicon esulentum*). *Intern Food Res J*, 21(6), 2355–2362.
- Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay. *J. Antiox.Anal. Biochem*, 239, 70–76.
- Celli, G. B., Ghanem, A., & Brooks, M. S. (2015). Optimization of ultrasound-assisted extraction of anthocyanins from haskap berries (*Lonicera caerulea* L.) using Response Surface Methodology. *J. Ultsonc*, 27, 449–455.
- Chemat, S., Aissa, A., Boumechhour, A., Arous, O., & Ait-amar, H. (2016). Extraction mechanism of ultrasound assisted extraction and its effect on higher yielding and purity of artemisinin crystals from *Artemisia annua* L. leaves. *J.Ultsonch*, 1–20.
- De Rosso, V. V., & Mercadante, A. Z. (2007). HPLC – PDA – MS / MS of Anthocyanins and Carotenoids from Dovyalis and Tamarillo Fruits AND. *J. Agric. Food Chem*, 55, 9135–9141.
- Eh, A. L. S., & Teoh, S. (2012). Ultrasonics Sonochemistry Novel modified ultrasonication technique for the extraction of lycopene from tomatoes. *J.Ultsonch*, 19(1), 151–159.
- Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and Measurement of Anthocyanins by UV Visible Spectroscopy. *Current Protocols in Food Anal Chem*, F1.2.1-F1.2.13
- Ivanovic, J., Tadic, V., Dimitrijevic, S., Stamenic, M., Petrovic, S., & Zizovic, I. (2014). Antioxidant properties of the anthocyanin-containing ultrasonic extract from blackberry cultivar "Čačanska Bestrna." *Industrial Crops and Prod*, 53, 274–281.
- Jabbar, S., Abid, M., Hu, B., Wu, T., Muhammad, M., Lei, S., ... Zeng, X. (2014). Quality of carrot juice as influenced by blanching and sonication treatments. *LWT - Food Sci Technol*, 55(1), 16–21.
- Kurniawan, M., Izzati, M., Nurhayati, Y., Biologi, L., Tumbuhan, F., Biologi, J., ... Universitas, K. (2010). Kandungan Klorofil, Karotenoid, dan Vitamin C pada Beberapa Spesies Tumbuhan Akuatik. *Buletin Anatom Fisiol*, XVIII(1), 28–40.
- Lianfu, Z., & Zelong, L. (2008). Optimization and comparison of ultrasound / microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. *J.Ultsonch*, 15, 731–737.
- Liang, N., & Kitts, D. D. (2014). Antioxidant property of coffee components: Assessment of methods that define mechanism of action. *Molecules*, 19(11), 19180–19208.
- Ma, Y., Chen, J., Liu, D., & Ye, X. (2009). Simultaneous extraction of phenolic compounds of citrus peel extracts: Effect of ultrasound. *J. Ultsonc*, 16, 57–62.
- Mandal, P., & Ghosal, M. (2012). Antioxidant Activities of Different Parts of Tree Tomato Fruit. *Inter J Pharm Sci Rev Res*, 13(2), 39–47.

- Medina-torres, N., Ayora-talavera, T., Espinosa-andrews, H., Sanchez-Contreras, A., & Pacheco, N. (2017). Ultrasound Assisted Extraction for the Recovery of Phenolic Compounds from Vegetable Sources. *Agronomy*, 7(47), 1–19.
- Molyneux, P. (2004). The use of the stable free radical diphenylpicryl- hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*, 26(2), 212–219.
- Namitha, K. K., & Negi, P. S. (2010). Chemistry and biotechnology of carotenoids. *Critical Rev Food Sci Nutr*, 50(8), 1040–8398.
- Osorio, C., Hurtado, N., Dawid, C., Hofmann, T., Heredia-mira, F. J., & Lucía, A. (2012). Chemical characterisation of anthocyanins in tamarillo (*Solanum betaceum* Cav .) and Andes berry (*Rubus glaucus* Benth .) fruits. *Food Chem*, 132(4), 1915–1921.
- Phan, A. D. T., Netzel, G., Wang, D., Flanagan, B. M., D’Arcy, B. R., & Gidley, M. J. (2015). Binding of dietary polyphenols to cellulose : Structural and nutritional aspects. *Food Chem*, 171, 388–396.
- Ramli, N. S., Ismail, P., & Rahmat, A. (2014). Influence of Conventional and Ultrasonic-Assisted Extraction on Phenolic Contents , Betacyanin Contents , and Antioxidant Capacity of Red Dragon Fruit (*Hylocereus polyrhizus*). *Scient Word J*, 1–7.
- Rocha, J. de C. G., Procopio, F. R., Mendonca, A. C., Vieira, L. M., Perrone, Í. T., Barros, F. A. R. de, & Stringheta, P. C. (2017). Optimization of ultrasound-assisted extraction of phenolic compounds from jussara (*Euterpe edulis* M.) and blueberry (*Vaccinium myrtillus*) fruits. *Food Sci Technol*, 38(1), 45–53.
- Rombaut, N., Tixer, A.-S., Bily, A., & Chemat, F. (2014). Green extraction processes of natural products as tools for biorefinery. *Biofuels Bioprod Bioref*, 1–15.
- Saikia, S., Mahnot, N. K., & Mahanta, C. L. (2015). A comparative study on the effect of conventional thermal pasteurisation , microwave and ultrasound treatments on the antioxidant activity of five fruit juices. *Food Sci Technol Inter*, 22(4), 288–301.
- Sancho, R. A. S., & Pastore, G. M. (2012). Evaluation of the effects of anthocyanins in type 2 diabetes. *Food Res Inter*, 46(1), 378–386.
- Santos, L. E. O., Zarate, L. X. P., & Salcedo, L. O. G. (2015). Optimization of ultrasonic-assisted extraction of total carotenoids from peach palm fruit (*Bactris gasipaes*) by-products with sunflower oil using response surface methodology. *J. Ultsonc*, 1–8.
- Sugiura, M., Nakamura, M., Ogawa, K., Ikoma, Y., & Yano, M. (2015). High-serum carotenoids associated with lower risk for developing type 2 diabetes among Japanese subjects : Mikkabi cohort study. *Diabetes Res Care*, 13–20.
- Žlabur, J. Š., Voća, S., Dobričević, N., Plietić, S., Galić, A., & Boričević, A. (2016). Ultrasound-assisted extraction of bioactive compounds from lemon balm and peppermint leaves. *Int. Agrophys*, 30, 95–104.