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Optimization of Carotenoid Extraction from Yellow Passion Fruit Pomace Using Ultrasound-assisted Extraction and Coconut Oil as a Solvent

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ABSTRACT: Passion fruit pomace, a by-product of passion fruit juice processing, is a source of carotenoids that can be utilized for food products as an antioxidant. The purpose of this study was to determine the optimum condition of carotenoid extraction process from passion fruit pomace using ultrasonic waves with coconut oil as a solvent, to determine the physicochemical properties of carotene-enriched coconut oil and the physicochemical properties of carotene-enriched coconut oil blended with sesame oil. The variables studied were extraction time (X_1) and extraction temperature (X_2). Response Surface Methodology (RSM) with the design of Central Composite Face-centered Design (CCF) is used to obtain a mathematical model that corresponds to the relationship between total carotenoid and the variables that influence it. After that, coconut oil will be evaluated on its quality including total carotenoids and antioxidant activity, compared to extraction with conventional methods (maceration). Sesame oil was blended with the carotene-enriched coconut oil extract to improve its antioxidant stability. The results showed that the optimum condition of the yellow passion fruit pomace carotenoid extraction (9.47 µg of carotenoids/g oil) and had higher antioxidant activity (80% RSA). Characteristics of mixed oils between carotenoid-enriched coconut oil and sesame oil have higher peroxide, lower Oxidative Stability Index, and higher antioxidant activity.

Keywords: carotenoid, central composite design (CCF), coconut oil, response surface methodology (RSM), yellow passion fruit pomace

INTRODUCTION

Carotenoids are dyes or pigments that are naturally contained in plants and animals. In addition to its important role as a pigment, carotenoids play a role in the protection of plants against oxidative stress due to their activity to scavenge the oxidative compounds (Stahl & Sies, 2003), increasing antioxidant activity (Foo et al., 2017), provitamin A activity (Vicario et al., 2007), and preventing liver disease, cancer and cardiovascular disease (Tapiero et al., 2004). One of the fruits that is also rich in carotenoids is yellow passion fruit. Carotenoid compounds identified in yellow passion fruit include phytoene, phytofluene, ζ-carotene (carotenoid dominant), neurosporen, β -carotene, lycopene, prolicopene, monoepoxy- β -carotene, β -kriptoxanthin, β -citraurin, antheraxanthin, violaxanthin and neoxanthin (Mercadante et al., 1998).

According to Azad *et al.* (2014), the fruit processing industry produces for almost 5-20% by-products of total fruit. The by-product of juice is called pomace (Yates *et al.*, 2017). Carotenoid compounds such as β -carotene are antioxidant compounds that are reported to be contained in yellow passion fruit pomace (Da Silva *et al.*, 2014). Thus, the yellow passion fruit pomace has high antioxidant, antimicrobial and dietary fiber (pectin)

properties (López-Vargas, et. Al., 2013). Then to take full advantage of yellow passion fruit pomace that contains some bioactive compounds, it can be used as an ingredient in making edible films or ingredients of functional foods (Munhoz *et al.*, 2018). However, the optimum extraction method should be developed to obtain the maximum carotenoid compound to be utilized.

The conventional method such as maceration has been widely used to extract carotenoid compounds, but the conventional methods have disadvantages, such as requiring many organic solvents, long extraction times, and low selectivity of compound targets (Santos *et al.*, 2013). Therefore, an advanced method is needed. Ultrasound-assisted extraction method could be used as an advanced method that has a high level of efficiency and extraction rate compared to conventional (Oliveira *et al.*, 2016). The extraction efficiency can be caused by several factors, such as ultrasonic power, intensity, temperature, density, and time, which can be optimized by Response Surface Methodology (RSM) trial design (Saini & Keum, 2018; Goula *et al.*, 2017).

One of the most influential factors of carotenoid extraction is solvent. Carotenoids are a group of oilsoluble pigments. Several studies on the extraction of carotenoids using oil as a solvent have been carried out,

including the extraction of carotenoids (astaxanthin) from shrimp shells with various types of vegetable oils (Sachindra & Mahendrakar, 2005). Li *et al.*, (2013) developed an ultrasonic carotenoid extraction from carrots with sunflower oil used as a substitute for organic solvents. Likewise, Goula *et al.* (2017) use sunflower oil and soybean oil as solvents to extract carotenoids from pomegranate peel.

One of the oils that can be used as a substitute for organic solvents is coconut oil. Sachindra & Mahendrakar (2005) reported that the yield of carotenoids (astaxanthin) from shrimp waste that was obtained by coconut oil solvents was quite high, which was not significantly different from soybean oil and bran oil but was significantly higher compared to the use of sunflower oil, peanut oil, sesame oil, and mustard oil. By using coconut oil as a solvent, the separation of carotenoids from the oil can be ignored because carotenoid-rich oils will contribute to the amounts of antioxidants and can be used as carotenoid sources for different products (Goula *et al.*, 2017).

Further, the repetition of using oil at high temperatures in food processing will change the oil physically and chemically. Oxidative stability is also an important indicator for maintaining oil quality. Oxidative stability can be improved by blending oil that is rich in polyunsaturated fatty acids with more saturated fatty acids or monounsaturated fatty acids (Hammouda *et al.*, 2018). Coconut oil is an oil that is rich in saturated fatty acids, such as sesame oil. Besides containing a lot of polyunsaturated fatty acids (linoleic acid) (Handajani *et al.*, 2010), sesame oil also contains natural antioxidants, namely lignans (sesamine, sesamole, sesamoline) which contribute to oil stability (Lee *et al.*, 2008).

The yellow passion fruit pomace powder will be added to coconut oil and extracted using an ultrasound-assisted extraction method. The application of carotenoid extraction assisted by ultrasonic waves and the use of oil as a solvent is said to be an innovative, fast, and more environmentally friendly method. A Central Composite Face-centered Design (CCF) simultaneously with RSM was used to obtain the optimum extraction condition of the Ultrasound-assisted Extraction (UAE) to extract the carotenoids.

Furthermore, the carotenoids extracted by ultrasoundassisted method under optimum conditions blending with sesame oil which are expected to improve oil stability when used at high temperatures and prevent oxidative damage that can occur in the heating process, are also evaluated using peroxide value, fatty acid profiles, Oxidative Stability Index (OSI), and antioxidant activity. The purpose of this study was to determine the optimum condition of the carotenoid extraction process from passion fruit pomace using ultrasonic waves with coconut oil as a solvent and to determine the physicochemical properties of carotene-enriched coconut oil and the physicochemical properties of carotene-enriched coconut oil blended with sesame oil.

MATERIALS AND METHODS

Materials

The main materials that used were yellow passion fruit (*Passiflora edulis* var. *Flavicarpa*) obtained from Lintang Panglipuran Farm, Karangasem Village, Ngemplak, Sleman Regency. Then the vegetable oil used is commercial coconut oil and commercial sesame oil found in the local market. All the chemical such as hexane, acetone, and petroleum ether (HPLC grades) was from Merck, Germany.

Preparation of yellow passion fruit pomace powder

The first step is the preparation of yellow passion fruit pomace powder. Passion fruit pomace preparation begins by washing the passion fruit, draining it, and then separating it between the peel and the pulp, which still contains seeds. The pulp (100 grams) was crushed by a juicer, then filtered which would produce pomace. Pomace was drained for 15 hours at 50 °C on a cabinet dryer. After that, the size reduction was carried out and sieved with a 60 mesh sieve to obtain yellow passion fruit pomace powder. The powder then were analyzed for total yield (%), moisture content (%) by the AOAC method (2006), total carotenoid (μ g/g Dry weight) (AOAC, 2006), and identified carotenoid compounds by HPLC-PDA (Shimadzu DGU-20A5R, Japan) (Ningrum *et al.*, 2015).

Extraction of yellow passion fruit pomace carotenoid powder

Yellow passion fruit pomace carotenoid extraction is done by a UAE and coconut oil as a solvent. Then as a comparison or control, extraction of conventional methods is also done by maceration method using hexane. The total of carotenoids (AOAC, 2006) and antioxidant activity (DPPH) from carotenoid-enriched coconut oil extract were then evaluated (Banerjee, 2005).

Extraction of yellow passion fruit pomace carotenoid using UAE and coconut oil as solvent

This extraction method has been done by Li *et al.* (2013) with several modifications. A total of 5 g of passion fruit pomace powder was mixed with 25 mL of oil in a flask to be extracted in an ultrasonic waterbath (SIBATA SU-2TH, 38 kHz, Japan). For optimization, variations in extraction time were carried out for 10, 20, 30, and 40 minutes with temperature variations of 20, 30, 40, and 50 °C. The extracted samples were then filtered by vacuum filter through filter paper to remove the residue before the total carotenoid was analyzed.

Std Order	Run Order	PtType	Blocks	<i>X</i> 1	X_2
8	1	-1	1	0	1
4	2	1	1	1	1
12	3	0	1	0	0
1	4	1	1	-1	-1
2	5	1	1	1	-1
3	6	1	1	-1	1
13	7	0	1	0	0
9	8	0	1	0	0
6	9	-1	1	1	0
5	10	-1	1	-1	0
10	11	0	1	0	0
11	12	0	1	0	0
7	13	-1	1	0	-1

Table 1. Design Experiment using Response Surface Methodology (RSM)

Note: X_1 = Extraction time (minute); X_2 = extraction temperature (°C)

Extraction of yellow passion fruit pomace carotenoid using conventional method

This conventional method modifies the method carried out by Li, *et al.*, (2013). Samples of passion fruit pomace powder were weighed as much as 5 grams and added to 25 mL hexane, then stirred for one hour at room temperature. The mixture is filtered through filter paper assisted by a vacuum filter, while the solid residues are collected and re-extracted with hexane under the same conditions. After extraction, hexane was evaporated in a Rotary Vacuum Evaporator (IKA RV 10B, Germany), and then extracted carotenoids were mixed with 25 mL of coconut oil until dissolved.

The blending of carotene-enriched coconut oil with sesame oil

Coconut oil enriched with carotenoid under the optimal extraction conditions then mixed with sesame oil with a ratio of 80:20 (v/v) of coconut oil to sesame oil. The ratio of this mixture is based on the optimal ratio from Tiwari *et al.*, (2014), which blends palm oil with sesame oil. Blending coconut oil with sesame oil is done with a magnetic stirrer at room temperature for 5 minutes. Blending oils then would be characterized including peroxide value (AOCS, 2009), fatty acid profiles (Park & Goins, 1994), Oxidative Stability Index (OSI) (Kivavele *et al.*, 2011), and antioxidant activity (Banerjee, 2005).

Design Experiment and Data Analysis

Central Composite Design (CCD) of RSM is used to get the optimum extraction condition as an experimental design. The type of CCD used in this study is the experimental design of Central composite face-centered (CCF). Two factors were tested, namely extraction time and extraction temperature with three levels (-1, 0, and +1) for each factor. The experimental design obtained 13 treatment combinations as presented in Table 1. The optimization results were based on total carotenoid levels.

For another analysis of data, the results of descriptive statistics are used to determine the mean and standard deviation (SD). Analysis data are presented as mean \pm SD. The comparison of the mean of each group was analyzed statistically using One-Way Analysis of Variance (ANOVA). All data analysis was performed using Minitab17 software (Minitab Ltd., UK).

RESULT AND DISCUSSION

Characteristics of yellow passion fruit pomace powder

In this study, the yield of passion fruit pomace powder was calculated from three repetitions resulting in $1.61 \pm 0.41\%$ as yield value (Table 2). The yield of this passion fruit pomace is lower than the yield of passion fruit pomace from the research of de Oliveira *et al.* (2009), which is 2.5%.

Table 2. Characteristics of yellow passion fruit pomace powder

Parameter	Value
Yield (%)	1.61 ± 0.41 [A1]
Moisture Content (%)	10.79 ± 1.78
Total of Carotenoids (µg/g dry weight)	49.12 ± 5.84

The moisture content of yellow passion fruit pomace powder that was obtained is $10.79 \pm 1.78\%$. Some

	Carotenoids	Concentrations (µg/g dry weight)	λmax			λref*		
RT			Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
24.236	β-cryptoxantin	3.94	427	451	478	(428)	450	478
26.847	Lutein-5,6-epoxide	7.58	424	442	468	420	440	470
27.432	Neoxanthin	10.17	414	436	466	416	438	467
28.194	β-carotene	36.11	427	452	478	(429)	452	478
30.099	ζ-carotene	1.58	378	402	426	378	400	425

Table 3. Identified carotenoids of yellow passion fruit pomace by HPLC

Note: (*) Three peaks are adjusted to three maximum absorbances for each carotenoid absorbance in acetone (Rodriguez-Amaya, 2001)

previous studies stated that the moisture content of passion fruit seeds was $8.50 \pm 0.09\%$ (de Oliveira *et al.*, 2016) and $7.38 \pm 0.07\%$ (Malacrida & Jorge, 2012). The difference in the results of the analysis can be caused by the harvesting time of passion fruit used, and the drying method used is also different (Ansar *et al.*, 2019).

The total carotenoid content in yellow passion fruit pomace powder was $49.12 \pm 5.84 \ \mu\text{g/g}$ dry weight. The previous study found that the total carotenoid content of yellow passion fruit pomace was $13.62 \pm 1.36 \ \mu\text{g/g}$ dry weight (Da Silva *et al.*, 2014). This difference can be caused due to the differences in fruit varieties, cultivation methods, and extraction methods (Pertuzzati *et al.*, 2015)

Identification of carotenoids compound on yellow passion fruit pomace

Carotenoids in yellow passion fruit pomace powder were identified by comparing the retention time and absorption spectrum. The maximum absorbance of each compound is compared with the data in the previous study. The approach from retention time and spectrum can show the correct identification results because each compound will have the same spectrum.

Based on the results of HPLC analysis, the major carotenoid contained in yellow passion fruit pomace powder is β -carotene (36.11 µg/g dry weight). Then other fewer amounts of carotenoids were neoxanthin (10.17

 μ g/g dry weight), lutein-5.6-epoxide (7.58 μ g/g dry weight), β -cryptoxanthin (3.94 μ g/g dry weight), and ζ -carotene (1.58 μ g/g dry weight). Data on retention time, maximum absorbance, and semi-quantification of carotenoids identified are presented in Table 3.

Mercadante et al., (1998) identified carotenoid compounds in mature yellow passion fruit, namely phytoena, phytofluene, ζ-carotene (main carotenoid), neuropore, β -carotene, lycopene, prolicopene, monoepoxy- β -carotene, β -cryptoxanthin, β -citraurin, antheraxanthin, violaxanthin, and neoxanthin. These differences can be caused by differences in varieties, the level of fruit maturity, methods or instruments used for different identification, and the parts of the samples analyzed are different. In that study, the sample that was used was fresh fruit, while in this study, the part that was analyzed was pomace powder.0020

Extraction of yellow passion fruit pomace carotenoid powder

Response Surface Optimization

The RSM optimization model for total carotenoids is shown in Figure 1. It can be seen that the optimum results are in the experimental area, but the time factor has no significant effect on the results because it does not form a peak. The optimum area is shown by the white color spot in the response surface plot and dark green in the contour plot. The optimum conditions were obtained at 30





minutes at 35 °C. The R^2 of the prediction models for total carotenoids was 91.98% and the validity of the model was statistically assessed using a lack-of-fit test (0.714) that showed the model was suitable to extract carotenoid in yellow passion fruit pomace at the confidence level of 95%.

The linear regression equation for real variables in RSM is:

$$Y = -24,17 + 0,353 X_1 + 1,822 X_2 - 0,00236 X_1^2 - 0,02319 X_2^2 - 0,00605 X_1 X_2$$

Note:

 X_1 = extraction time (minutes) X_2 = extraction temperature (°C)

Y =total carotenoids (µg/g Dry weight)

Based on the Response Optimizer feature in the Minitab 17 software, the optimum conditions for the desired target were obtained at 29.49 minutes and a temperature of 35.45 °C as shown in Figure 2.



Figure 2. Optimization of extraction conditions against total carotenoids

Goula *et al.* (2017) state that carotenoid extraction assisted by ultrasonic waves from pomegranate peels with sunflower oil solvents and soybean oil can be carried out for 30 minutes to obtain maximum carotenoid yield. In the study of carotenoid extraction assisted by ultrasonic waves from carrots, optimum conditions were obtained at 40 °C for 20 minutes to produce maximum yield (Li *et al.*, 2013). So, it can be said that the results of this study indicate the acquisition of optimum conditions for ultrasonic wave-assisted extraction was not significantly different from previous studies. The differences can be caused by different types of samples, solvents, and types of ultrasonic instruments used.

Characteristics of Carotene-Enriched Coconut Oil at Optimum Condition

Characteristics that were evaluated included total carotenoids and antioxidant activity. The results of the characterization were then compared to conventional extracted (macerated) carotenoid oil and coconut oil. The total carotenoids of several types of oil are shown in Figure 3.

The total carotenoid in carotene-enriched coconut oil, which is produced by ultrasound-assisted extraction increases, compared to total carotenoids in coconut oil. The results also show that ultrasound-assisted extraction can significantly increase the total carotenoid level with the highest value (15.79 µg carotenoids/g oil) in just 30 minutes, whereas the maceration method that uses hexane as a solvent gives a smaller total carotenoid (9.47 μ g/g oil) after one-hour extraction. This can be caused by the presence of ultrasonic cavitation forces that can accelerate the heat transfer rate and mass which can damage the cell wall and cause the release of compounds to be extracted (Chemat et al., 2011). Several studies related to the extraction of ultrasonic-wave carotenoid compounds with vegetable oil solvents also produced a total carotenoid yield that increased significantly compared to extraction with conventional methods (Li et al., 2013; Goula et al., 2017).

Then from its antioxidant activity, carotenoid-enriched oil from ultrasound-assisted extraction showed an increase compared to coconut oil and carotenoid-enriched oil as a result of conventional method extraction. The antioxidant activity of the sample is shown in Figure 4.

At the same concentration, carotenoid-enriched oil by ultrasound-assisted extraction showed higher antioxidant activity compared to conventional method extraction results. This can be caused by carotenoids extracted by ultrasound-assisted extraction, which is more than conventional extraction methods. Carotenoids influence antioxidant activity because it is an efficient scavenger of free radicals (Nishino *et al.*, 2017). Handajani *et al.*, (2010) in their research also stated that the high content of carotenoids and tocopherol in oil was followed by increasing antioxidant activity. therefore, in this study, it can be seen that the higher the total carotenoids produced also affect the high antioxidant activity in the carotenoidenriched oil.

Characteristics of Blending Oil

Peroxide value

The amount of peroxide in several types of oil samples in this study is shown in Figure 5. Coconut oil has a peroxide number of 2.86 ± 0.07 mEq/100g. After adding carotene from pomace, the peroxide value decreased to 1.02 ± 0.02 mEq/100g, and after blending with sesame oil, the peroxide value was obtained to 1.42 ± 0.01 mEq/100g. Those peroxide values are higher than on sesame oil, which equals 0.15 ± 0.02 mEq/100g.

Decreasing the peroxide value of carotene-enriched oil can be caused by the increase in carotenoid content. Carotenoids, especially β -carotene, can provide health benefits and oil stability because of their role as antioxidants (Sunil *et al.*, 2015). With the addition of 20% sesame oil from the total mixed oil, it shows an increase in peroxide value. This can be due to the increase in



Note: The different letter means significant differences based on Duncan (p < 0.05)





Figure 4. Antioxidant activity of coconut oil and carotene-enriched oil



Figure 1. Peroxide value in various oil samples

unsaturated fatty acid content in the oil. Unsaturated fatty acids found in oil can bind oxygen to their double bonds

to form peroxide (Ketaren, 2008). However, the peroxide value produced from the blending oil is still below the

Fatty Acid	Coconut Oil+Pomace	Coconut Oil+Pomace:Sesame Oil (80:20)
Caprylic Acid(C _{8:0})	10.10	8.41
Capric Acid (C _{10:0})	7.98	6.90
Lauric Acid(C _{12:0})	53.26	49.29
Myristic Acid(C _{14:0})	16.03	14.23
Palmitic $Acid(C_{16:0})$	5.39	6.32
Stearic Acid($C_{18:0}$)	0.84	1.37
Oleic Acid(C _{18:1})	2.51	6.24
Linoleic Acid(C _{18:2})	1.40	5.50
Linolenic Acid(C _{18:3})	0.02	0.04
Saturated Fatty Acid	95.97	88.02
Mono-Unsaturated Fatty Acid	2.57	6.35
Poly-Unsaturated Fatty Acid	1.46	5.64

Table 4. Composition of fatty acids (%) in coconut oil enriched wit	h carotenoids and with the additi	on
of sesame oil			

limit of peroxide value according to SNI 01-2902-1992, which is a maximum of 5 mEq/100g sample.

Fatty acid profile

The fatty acid profile of oils in this study is shown in Table 4. The most dominant fatty acid in caroteneenriched coconut oil is lauric acid (53.26%). This is in accordance with several studies that state that the major fatty acid in coconut oil is lauric acid (Chandrashekar *et al.*, 2010; Ketaren, 2008). While based on the literature, the dominant fatty acid in sesame oil is oleic acid and linoleic acid (Handajani *et al.*, 2010; Abdulkarim *et al.*, 2010).

Based on the dominant fatty acid content, the blending of carotenoid-enriched coconut oil with sesame oil causes the greatest decrease in lauric acid and an increase in oleic acid and linoleic acid. Besides that, it can be seen that in blending oil, there is an increase in levels of monounsaturated fatty acids and polyunsaturated fatty acids. Blending various vegetable oils can alter the composition of fatty acids, such as by increasing the content of unsaturated fatty acids and enhancing the levels of natural antioxidants. This, in turn, can enhance the nutritional value and stability of the oil (Aladedunye & Przybylski, 2013).

Oil Stability Index (OSI)

The Oil Stability Index (OSI) can also be called as induction time. The OSI value of coconut oil enriched with carotenoid and sesame oil can be seen in Table 5.

Table 5. Induction time of rancimat (hours) of coconut oilenriched with carotenoids and added withsesame oil at a temperature of $110 \ ^{\circ}C$

Oils	Induction Time
Coconut Oil+Pomace	23.66 hours
Coconut Oil+Pomace:Sesame Oil (80:20)	21.45 hours

At the same temperature, Tan *et al.* (2002) tested the induction period in coconut oil and obtained the induction time at 11.25 hours. In comparison, the sesame oil obtained an induction time of approximately 5 hours (Metrohm, 2013) at a temperature of 120 °C. When compared to the induction time of coconut oil in the previous studies. It can be assumed that there is an increase in induction time in carotene-enriched oil by pomace. This can be caused by the addition of pomace, which increases the carotenoid content as a natural antioxidant, thus increasing its oxidative stability.

While a decrease in the induction time after blending can be caused by increasing oil unsaturation. The trend of induction time in this study can be said to have a direct correlation with the percentage of saturated fatty acids (Kivevele *et al.*, 2011). This is due to the addition of sesame oil. reducing the induction period in blending oils that have saturated fatty acid levels of 88.02%. Coconut oil enriched with carotenoids with saturated fatty acids of 95.97% showed higher oxidation stability than blending oils.

Antioxidant activity

Based on its antioxidant activity, carotenoid-enriched oil mixed with sesame oil shows higher antioxidant activity than before blending and sesame oil. The antioxidant activity is shown in Figure 7.

Sesame oil contains natural antioxidants, namely sesamin, sesamol, and sesamolin (Lee *et al.*, 2008), which will increase the content of natural antioxidants in mixed oils. In the research of Serjouie *et al.*, (2010) report that the mixture of palm oil with sesame oil can produce a stable cooking oil because of the increase in the content of natural antioxidants from sesame oil.

CONCLUSION

The optimum condition for the ultrasound-assisted extraction of carotenoid from yellow passion fruit pomace

is 30 minutes at 35 °C. Characteristics of carotenoidenriched coconut oil from ultrasound-assisted extraction has higher total carotenoid levels (15.79 μ g of carotenoids/g oil) compared to conventional extraction (9.47 μ g of carotenoids/g oil) and higher antioxidant activity. The characteristics of blending oils between coconut oil enriched with carotenoids and sesame oil have higher peroxide value, lower Oxidative Stability Index, and higher antioxidant activity than carotenoid-enriched coconut oil before blending.

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