

Effect of an Amplitude and Time Duration at Betacyanin Extraction by Ultrasonic-Assisted Extraction (UAE) Method

Aniswatul Khamidah¹, Wenny Bekt Sunarharum² and Widya Dwi Rukmi Putri²

¹ National Research and Innovation Agency

Cibinong Science Center, Bogor, West Java, Indonesia

² Agricultural Product Technology Department, Faculty of Agricultural Technology,

Universitas Brawijaya, Malang City, East Java, Indonesia

*Corresponding author: widya2putri@ub.ac.id

Abstract. Dragon fruit peel is agricultural waste that contains betacyanin as a natural dye. Application of betacyanin as a natural dye obtained by extraction. This study aimed to extract betacyanin from dragon fruit peel using the Ultrasonic-Assisted Extraction (UAE) method and to determine its effect on betacyanin content, redness value and yield. Extraction was carried out by UAE with various amplitude levels (60, 72, 84 and 96%) and extraction time (4, 10, 16, 22 and 28 minutes). The results showed that there was a significant effect on betacyanin content, redness value and yield. Based on the results of the best treatment from Zeleney, it showed that the extraction treatment with an amplitude of 84% for 16 minutes produced the highest response with betacyanin content, redness value and yield respectively of 282,002 mg/L; 48,400 and 64,524%.

1. Introduction

The popularity of natural dyes is currently increasing because consumers are more concern about the health issues of synthetic dyes [1]. Betacyanin is a natural dye that produces a red-violet color [2], has a high solubility in water [3]; It has a stability in a wider pH range (pH 3 to 7) so it making suitable for application to foods that have a low acidity and neutral [4]. Betacyanins are used as food coloring, pharmaceuticals, cosmetic products, have no allergic effect, are anti-radical, antiviral, antimicrobial, antioxidant and protect against oxidative stress [5;2]. One of the plant sources of betacyanin pigment is dragon fruit peel [6].

Betacyanin application as a natural dye is obtained by extraction. Extraction can be done using the Ultrasound Assisted Extraction (UAE) method. The UAE mechanism occurs due to the explosion of cavitation bubbles which causes damage to plant cell walls, a reduction in the size of the extracted particles so it would be resulting in an increase of surface area. This case make the extraction rate increased, as a result the extraction yield increased [7].

The ultrasonic intensity depends on the amount of energy transmitted to the medium. The amount of this energy is influenced by several factors such as the duration of sonication and the percent amplitude generated from the ultrasonic generator [7]. The amplitude of the ultrasonic vibration (with a value of 20-100%) is proportional to the sonication intensity [8]. The higher the amplitude value and the longer extraction time, the ultrasonic intensity will increase [7]. Therefore, this study aimed to extract betacyanin from dragon fruit peel using the Ultrasonic-Assisted Extraction (UAE) method of various

amplitudes and extraction times and to determine its effect on betacyanin content, redness value and yield.

Experimental Section

Materials

Dragon fruit skin is obtained from Banyuwangi Indonesia. The materials used for analysis include aquades, citric acid (Merck), Na₂HPO₄ (Merck), ethanol (Merck), acetate buffer solution pH 4 and 7 and aquades obtained from the Laboratory of Biochemistry and Nutrition, Department of Agricultural Product Technology, Brawijaya University Indonesia.

Instrumentation

Tools for extraction and analysis are: ultrasonic probe (Cole Parmer, 20 kHz), color reader (Konica Minolta CR-10), UV-Vis spectrophotometer (UV mini-1240), Shimadzu, Japan), rotary evaporator (BUCHI Rotavapor R- 205, Germany) and supporting glassware.

Sample Preparation

Dragon fruit peel sorted and discarded the defective part, and the dry scales. The clean dragon fruit peel was placed on a baking sheet and then dried using a cabinet dryer at a temperature of 50°C. Then it was ground using a disk mill and sieved using an 80 mesh sieve to obtain dragon fruit peel powder [10].

Ultrasonic-Assisted Extraction (UAE)

The stages of the extraction process use the modified UAE method [8]. Dragon fruit peel powder was dissolved in distilled water: 96% ethanol (20:80); with a sampel to solvent ratio of 1 : 5 (w/v) [9]. Sonication of the solution using an ultrasonic probe with a frequency of 20 kHz (130 Watt, diameter 13 mm) with a large amplitude and extraction time according to the treatment. The extraction results were filtered using 42 mesh filter paper and then concentrated using a rotary evaporator at 40°C [11]. Furthermore, the results of the extract were analyzed, including analysis of betacyanin content, redness-value (a) and yield.

Statistical Analysis

The experimental design in this study used factorial Randomized Block Design with a various amplitude (60, 72, 84 and 96%) and extraction time (4, 10, 16, 22 and 28 minutes). The data obtained were analyzed using analysis of variance ANOVA (Analysis of variance) followed by the DMRT test (Duncan Multiple Range Test).

Results and Discussion

The difference in amplitude and sonication time give an effect on the betacyanin content, the redness value (a) and the resulting yield response. The following is the average value of betacyanin, redness color (a) and extract yield at various amplitudes and extraction time (**Figure 1,2,3 and Table 1**).

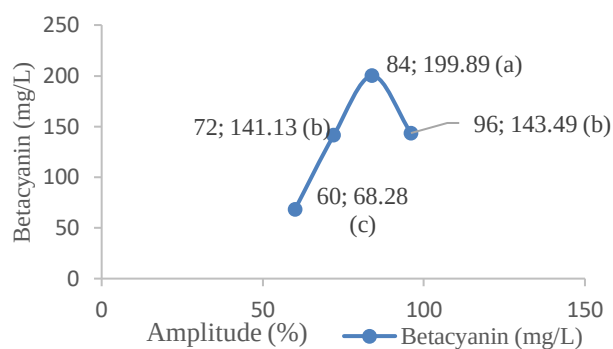


Figure 1. Effect of an Amplitude on Betacyanin (mg/L)

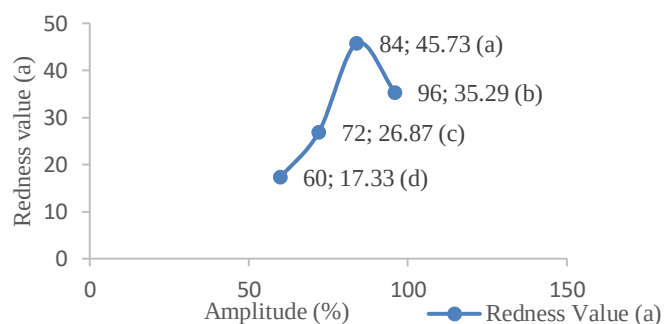


Figure 2. Effect of an Amplitude on Redness Value (a)

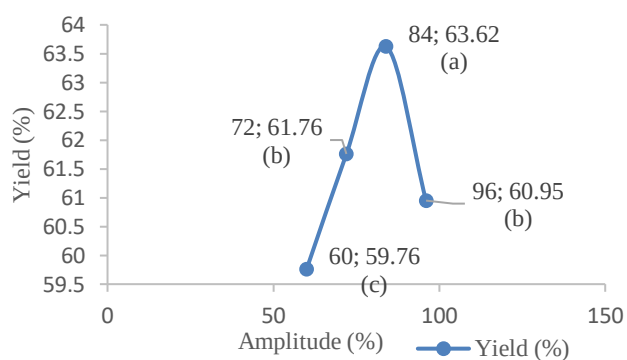


Figure 3. Effect of an Amplitude on Yield (%)

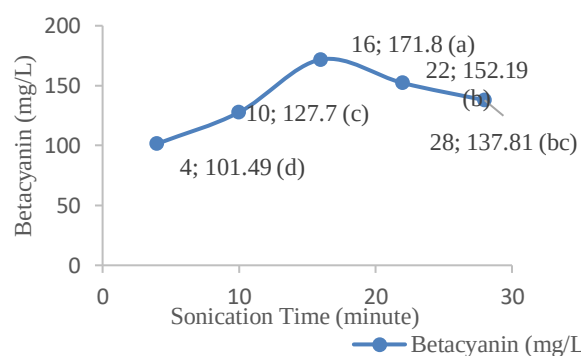


Figure 4. Effect of an Sonication Time on Betacyanin (mg/L)

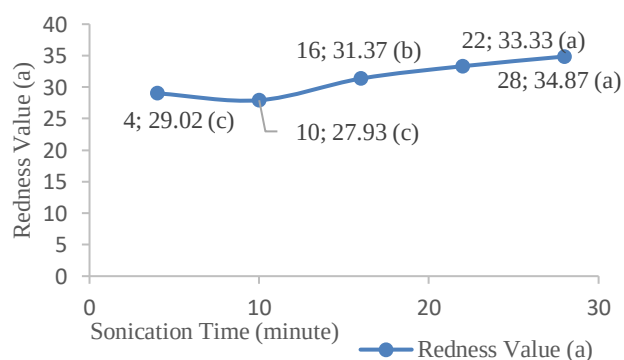


Figure 5. Effect of an Sonication Time on Redness Value

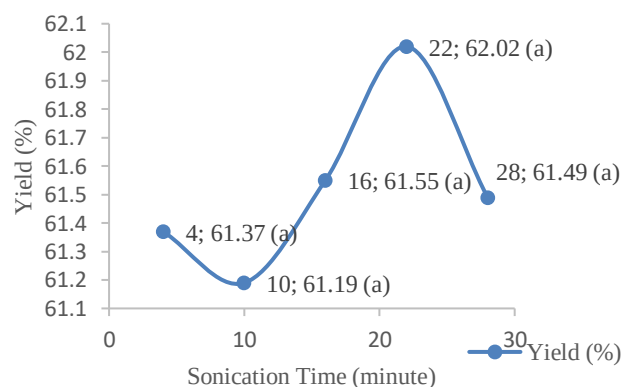


Figure 6. Effect of an Sonication Time on Yield (%)

Table 1. Average value of betacyanin, reddish color (a) and yield of betacyanin extract from various amplitudes and extraction times

Treatment	Response		
	Betacyanin (mg/L)	Redness Value (a)	Yield (%)
• Amplitude 60%, 4 minute	40,129 i	12,033 h	59,762 fg
• Amplitude 60%, 10 minute	59,693 hi	18,233 g	59,762 fg
• Amplitude 60%, 16 minute	79,811 hi	18,133 g	59,286 g
• Amplitude 60%, 22 minute	69,198 hi	17,833 g	59,762 fg
• Amplitude 60%, 28 minute	92,578 fgh	20,400 fg	60,238 efg
• Amplitude 72%, 4 minute	96,454 fgh	19,433 fg	61,191 bcdefg
• Amplitude 72%, 10 minute	155,844 de	24,300 ef	60,952 cdefg
• Amplitude 72%, 16 minute	163,431 cde	22,267 efg	61,905 abcdefg
• Amplitude 72%, 22 minute	153,649 de	32,433 d	62,620 abcde
• Amplitude 72%, 28 minute	136,293 def	35,933 cd	62,143 abcdef
• Amplitude 84%, 4 minute	128,781 efg	40,167 bc	63,333 abc
• Amplitude 84%, 10 minute	152,190 de	43,567 ab	63,095 abcd
• Amplitude 84%, 16 minute	282,002 a	48,400 a	64,524 a
• Amplitude 84%, 22 minute	205,817 bc	48,367 a	63,810 ab
• Amplitude 84%, 28 minute	230,644 b	48,133 a	63,333 abc
• Amplitude 96%, 4 minute	140,606 de	44,433 ab	61,191 bcdefg
• Amplitude 96%, 10 minute	143,076 de	25,633 e	60,952 cdefg
• Amplitude 96%, 16 minute	161,944 cde	36,667 cd	60,476 defg
• Amplitude 96%, 22 minute	180,100 cd	34,700 d	61,905 abcdefg
• Amplitude 96%, 28 minute	91,743 gh	35,033 d	60,238 efg

Note : Letters indicate differences in statistical significance (by DMRT $\alpha = 0,05$)

Figure 1,2,3,4,5,6 and Table 1 shows the effect of amplitude and sonication time on betacyanin content, redness value (a) and yield response. Analysis of variance showed that the amplitude, extraction time and interaction between amplitude and extraction time had a significant effect (p -value < 0.05) on betacyanin content and redness value (a). While on yield response, showing a non-significant effect. Betacyanin content, redness value (a) and the highest yield were 282.002 mg/L, respectively; 48.400 and 64,524%; lies in the 84% amplitude treatment with the extraction time of 16 minutes.

The lowest values of betacyanin and red color were 40.129 mg/L and 12.033 occurred at 60% amplitude with extraction time of 4 minutes. According to [12,13] if the extraction time is lower than the optimal value, the compound may not be able to be extracted completely or, conversely not even degraded.

The increase in betacyanin content occurred to an optimum point of 84% amplitude with an extraction time of 16 minutes which resulted in an optimum betacyanin of 282.002 mg/L. This happens because during the extraction time, it can optimally produce efficient cavitation, so the extracted compound would increase. As a result it will affect the extraction of the resulting betacyanin. However, after exceeding the optimum point, a decrease in betacyanin content occurs. This phenomenon occurs because the higher the amplitude and the longer the time used for extraction, the higher the heat generated so that it can increase the extraction temperature to a very high level, which supports betacyanin degradation.

Ultrasonic is a mechanical wave, the ultrasonic intensity is influenced by the energy generated by the ultrasonic generator. The amount of this energy depends on the magnitude of the amplitude and the length of the extraction time used, so that the magnitude of this amplitude is proportional to the intensity of sonication [7]. The greater the amplitude used, the greater a power (W) would be produced so that the

total energy becomes greater [14]. Vibrating horns immersed in the reactants transmit mechanical energy which is transformed into heat. The higher the amplitude and the longer the time used for extraction, as a result, the higher the intensity of collisions between particles which results in an accumulation of heat formed so that the temperature increases [15].

The resulting effect of ultrasonic waves is disruption of the cell wall which results in the release of the compounds contained in it, resulting in an increase in the diffusion of the extract. Kinetic energy is passed to all parts of the liquid which is followed by the emergence of cavitation bubbles on the wall or surface which results in increased mass transfer between solid-liquid surfaces. Ultrasonic waves cause a mechanical effect that will increase the penetration of the liquid into the cell membrane wall, thus supporting the release of cell components and increasing mass transfer [16]. The greater the amplitude and the longer the extraction time, the higher the cavitation reaction formation will result in an increase in the temperature of the solution. The longer betacyanin is exposed to high temperatures, the lower the stability of the betacyanin pigment. This is in accordance with [17] that the heating process causes a decrease in the red color and the appearance of a light brown color. This is because an increase in high temperature can accelerate the formation of betanin (5-O-glucosides), which is the main structure of betacyanin, which is further degraded to produce betalamic acid and cyclodopa-5-O-glycoside in an unstable form [18].

The degraded betacyanin content is indicated by a decrease in the redness value. Table 1 shows that the higher the amplitude and the longer extraction time, the redness value tends to decrease. This phenomenon is in accordance with the statement of [19] that the redness value indicates the presence of betacyanin pigments contained in it.

While in the yield response, the greater the amplitude value and the longer the sonication time, the yield value tends to increase. When a high ultrasonic wave passes through the solution, it rapidly generates a large number of cavitation bubbles which then collapse and increase intermolecular splits [20]. In addition, there is a decrease in the attachment between particles due to the cavitation effect which can break the bonds between particles [21]. The longer the sonication time is carried out, the more bonds between particles are broken which results in a decrease in particle size so that the surface area is larger, as a result, more compounds will be extracted and the yield will be increased.

The selection of the best treatment using the Zeleney method [22], based on the calculation results of determining the best treatment, which has the minimum L1, L2 and Lmax values is the A3T3 treatment, namely dragon fruit peel extraction with an amplitude of 84% with an extraction time of 16 minutes.

Previous research on the extraction of betacyanin's dragon fruit peel by maceration using ethanol solvent and 1% tartaric acid concentration for 3 days resulted in betacyanin levels of 5,301 mg/L [23] while the extraction of betacyanin on dragon fruit peel using ultrasonic (50 kHz, 30 minutes, temperature 25°C) resulted in a betacyanin content of 176.4 mg/L [24]. In the study of [18], the betacyanin content in dragon fruit peel was 142.7 mg/, while in the study of [25] the extraction of dragon fruit peel betacyanin using the solvent extraction method (ethanol: water = 70:30) resulted in a betacyanin content of 248.1 ± 1.17 mg/L and if using a solvent 100% water produces betacyanin content of 236.6 ± 0.89 mg/L. This difference is thought to be due to differences in the extraction method and pretreatment before the extraction step. The differences in the amount of betacyanin content in each plant tissue also affects the yield produced. The differences in plant matrix structure tissue, due to differences in plant varieties make a result in different levels of impact from the cavitation effect [8].

Conclusion

The magnitude of the amplitude and length of sonication time on the extraction of betacyanin from dragon fruit peel significantly affected the betacyanin content and redness value, while the yield showed an insignificant effect. Based on the results of determining the best treatment, the Zeleney method showed that the extraction treatment with an amplitude of 84% for 16 minutes resulted in the highest response to betacyanin content, redness value and yield of 282.002 mg/L, respectively; 48.400 and 64.524%.

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