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Research

Survey the anthocyanin extraction from crust of shallot (*Allium ascalonicum* L.)

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Highlights

- Studies on the application of disposed shallot skin and the practical applicability of shallot are still scarce.
- Shallot skin is the waste of peeling shallot in processing, which can be used to extract anthocyanins, biologically active substances with theirs high antioxidant property.
- Anthocyanins were extracted from shallot skin with optimal factors such as alcohol content, ratio of solvent to material, temperature and time.

About Author
Pham Hong Hieu (MSc) is a Lecturer at Industrial University of Ho Chi Minh City, Vietnam. His research inter-ests are in biochemistry, post-harvest technology and nutrition.

Abstract

This work studied the influence of factors such as alcohol content, ratios of solvent and material, temperature and time on anthocyanin extraction from the crust of shallot (*Allium ascalonicum* L.). The extracted anthocyanin content was determined by the differential pH method in g/100g dry matter. The results of the experimental were the planning equation and the optimal point determination by using the Response Surface Method (RSM). The result showed that when extracting anthocyanin from the crust of shallot with a 50.5% alcohol solution, solvent and material ratio of 70 ml/g, at an extraction temperature of 60°C for 40 minutes, the highest extracted anthocyanin content was 0.255 ± 0.002 g/100 g dry matter.

Keywords: *Allium ascalonicum* L., crust of shallot, extraction, anthocyanin.

1. Introduction

Shallot, scientifically known as *Allium ascalocium L*, is a small, purple, Asian onion. Shallots are commonly used as a spice for daily meals. Shallots can be stored for a long time by many methods such as fresh preservation, drying or frying. In the Mekong Delta, there are many shallots planted, especially in Vinh Chau Town, Soc Trang Provice.

Shallot contains many components that are good for human health. The color of the shallot is determined to have, in the crust of shallot, a high-ratio anthocyanin composition. However, studies on the application of disposed shallot's crust and the practical applicability of shallot are still scarce. So, we survey the anthocyanin extraction from the shallot's crust (*Allium ascalonicum* L.)

In this research, we are focusing on extracting anthocyanin from shallot's crust with optimal factors such as alcohol content, ratios of solvent and material, temperature and time. The results of the study are the experimental planning equation and the optimal parameters of extraction.

2. Materials and Methods

2.1 Shallot

Shallot is purchased from Vinh Chau Town, Soc Trang Province. Shallot after being purchased is peeled to collect the dry outer crust then washed off. Shallot's crust is then dried at 40 ℃ until the moisture reaches 15%. After that, shallot's crust is grinded by a blender. Finally, after processing the material, the sample is put in a black bag, sealed from exposure to light and air, then stored in the refrigerator freeze.

2.2 Extracting anthocyanin from the crust of shallot

A certain amount of shallot's crust was weighed into an erlen and added solvent with a certain ratio that depended on different experiments. A few drops of HCl were added to adjust pH = 3. The sample was put in the waterbath after preparation. Post-extraction was obtained by vacuum filtration. Then it was vacuum concentrated to evaporate the solvent and collect anthocyanin extraction. After collecting, anthocyanin extraction was kept dark and stored at 4°C.

Note: During the entire experiment process, the sample container must be covered by foil wrapper before and after extraction for avoiding bright.

2.3 Experiments

2.3.1 Determine maximum absorption wavelength λ_{max}

Adjusting the baseline with distilled water at a wavelength of 450 to 700nm.

Determining the maximum absorption spectrum ($\lambda_{vis\ max}$) of the sample solution containing the anthocyanin extracted.

2.3.2 Survey the effect of ethyl alcohol concentration on extraction efficiency

Weighing a certain amount of shallot's crust, then soaking in solution with a solvent and material ratio of 40/1 ml/g and the solvent contains 0%, 30%, 40%, 50%, 60%, 70%, 100% alcohol content in water. Adding a few drops of HCl to adjust pH = 3. Putting the samples into waterbath to keep at 60°C for 60 minutes. Determining the amount of anthocyanin obtained per 100 g of dry matter to select the optimal sample.

2.3.3 Survey the effect of solvent and material ratio on extraction efficiency

Weighing a certain amount of shallot's crust, then soaking in solution with a solvent and material ratio of 30/1, 40/1, 50/1, 60/1, 70/1 ml/g and the solvent contains the certain alcohol content in water which is chosen from experiment 2.3.2. Adding a few drops of HCl to adjust pH = 3. Putting the samples into water-bath to keep at 60°C for 60 minutes. Determining the amount of anthocyanin obtained per 100 g of dry matter to select the optimal sample.

2.3.4 Survey the effect of temperature on extraction efficiency

Weighing a certain amount of shallot's crust, then soaking in solution with the certain solvent and material ratio which is chosen from experiment 2.3.3 and the solvent contains the certain alcohol content in water which is chosen from experiment 2.3.2. Adding a few drops of HCl to adjust pH = 3. Putting the samples into waterbath to keep at 40, 50, 60, 70, 80°C for 60 minutes. Determining the amount of anthocyanin obtained per 100 g of dry matter to select the optimal sample.

2.3.5 Survey the effect of time on extraction efficiency

Weighing a certain amount of shallot's crust, then soaking in solution with the certain solvent and material ratio which is chosen from experiment 2.3.3 and the solvent contains the certain alcohol content in water which is chosen from experiment 2.3.2. Adding a few drops of HCl to adjust pH = 3. Putting the samples into waterbath to keep at the certain temperature is chosen from experiment 2.3.4 for 40, 50, 60, 70, 80 min. Determining the amount of anthocyanin obtained per 100 g of dry matter to select the optimal sample.

2.3.6 Optimizing the factors that affect the anthocyanin extraction process from shallot's crust

After rudimentary surveys have been made including factors such as ethyl alcohol concentration, material and solvent ratio, temperature, time, laying out optimal test according to Response Surface Method (RSM) with the face centered central (CCF) composite design calculating by Modde 5.0 software.

2.4 Analysis methods

2.4.1 Measuring total monomeric anthocyanin by the pH-differential method (Giusti & Wrolstad, 2001; Stanciu et al., 2010)

Principle:

Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra. The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurement of the total anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds.

Materials:

0.025 M potassium chloride buffer, pH 1.0: Mix 1.86 g KCl and 980 ml of distilled water in a beaker. Measure the pH and adjust to 1.0 with concentrated HCl. Transfer to a 1 liter volumetric flask and fill to 1 liter with distilled water.

0.4 M sodium acetate buffer, pH 4.5: Mix 54.43 g CH3CO2Na·3 H2O and ~960 ml distilled water in a beaker. Measure the pH and adjust to 4.5 with concentrated HCl. Transfer to a 1 liter volumetric flask and fill to 1 liter with distilled water.

1. Turn on the spectrophotometer. Allow the instrument to warm up at least 30 min before taking measurements.

2. Determine the appropriate dilution factor for the sample by diluting with potassium chloride buffer, pH 1.0, until the absorbance of the sample at the λvis-max is within the linear range of the spectrophotometer (i.e., for most spectrophotometers the absorbance should be less than 1.2). Divide the final volume of the sample by the initial volume to obtain the dilution factor.

IMPORTANT NOTE: In order to not exceed the buffer's capacity, the sample should not exceed 20% of the total volume.

3. Zero the spectrophotometer with distilled water at all wavelengths that will be used (λ vis-max and 700 nm).

4. Prepare two dilutions of the sample, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, diluting each by the previously determined dilution factor. Let these dilutions equilibrate for 15 min.

5. Measure the absorbance of each dilution at the $\lambda_{vis\text{-}max}$ and at 700 nm (to correct for haze), against a blank cell filled with distilled water.

6. Calculate the absorbance of the diluted sample (A) as follows:

$$A = (A_{\lambda vis\ max} - A_{700nm})_{pH\ 1,0} - (A_{\lambda vis\ max} - A_{700nm})_{pH\ 4,5}$$

7. Calculate the monomeric anthocyanin pigment content in the original sample using the following formula:

Monomeric anthocyanin pigment content: $a = (A \times MW \times DF \times V)/(\varepsilon \times l)$, mg

where MW is the molecular weight (in this study, MW = 449,2 g/mol, the molecular weight of cyanidin-3- glucoside), DF is the dilution factor (for example, if a 0.2 ml sample is diluted to 3 ml, DF = 15), V is the volume of sample (liter), ε is the molar absorptivity (ε = 26900 with cyanidin-3-glucoside), and l is path length (1 cm)

8. Calculate total anthocyanin concentration (anthocyanin content in g obtained per 100 g dry matter) in the original sample using the following formula:

$$TAC = \frac{a \times 100}{m \times (100 - w)}\ ,g/100g$$

where a is monomeric anthocyanin pigment content (mg), m is original sample weight (g), and w is sample moisture (%).

2.4.2 Statistical analysis

Using Excel software to remove erroneous experimental values due to raw errors. Using Statgraphics Centurion XV software to analyze statistical data and to evaluate differences between samples.

Using analysis of variance one factor (ANOVA) to determine whether the differences between the samples are significant. Hypothesis H_0: There is no difference between the analyzed samples (select the significance level of 0.5%). If P-value <0.05, the H_0 hypothesis is rejected, it means there is a significant difference between the analyzed samples. If P-value> 0.05, the H_0 hypothesis is accepted.

Based on the results of preliminary experiments, designed the experiment to optimize the factors that affect the extraction process by RSM with 2 replicates on Modde 5.0 software. The result of the regression equation is:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{44}X_4^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{14}X_1X_4 + a_{23}X_2X_3 + a_{24}X_2X_4 + a_{34}X_3X_4$$

3. Results and discussion

3.1 Determining the maximum absorption wavelength of anthocyanin in the shallot's crust

After scanning the wavelength from 450 nm to 700 nm to find the maximum absorption wavelength of anthocyanin in the shallot's crust and gradually narrowing the steps, the final result was that the maximum absorption wavelength between 490 nm and 540 nm was λ = 510 nm or λ = 515 nm (Fig. 1). There was no significant difference between the absorption at wavelength λ = 510 nm and that at λ = 515 nm ($p>0.05$). This result was nearly identical to λ_{max} (510 nm) of cyanidin-3- glucoside in aqueous buffer, pH = 1 (Giusti & Wrolstad, 2001). So, we chose the maximum absorption wavelength of anthocyanin in the shallot's crust was λ = 510 nm.

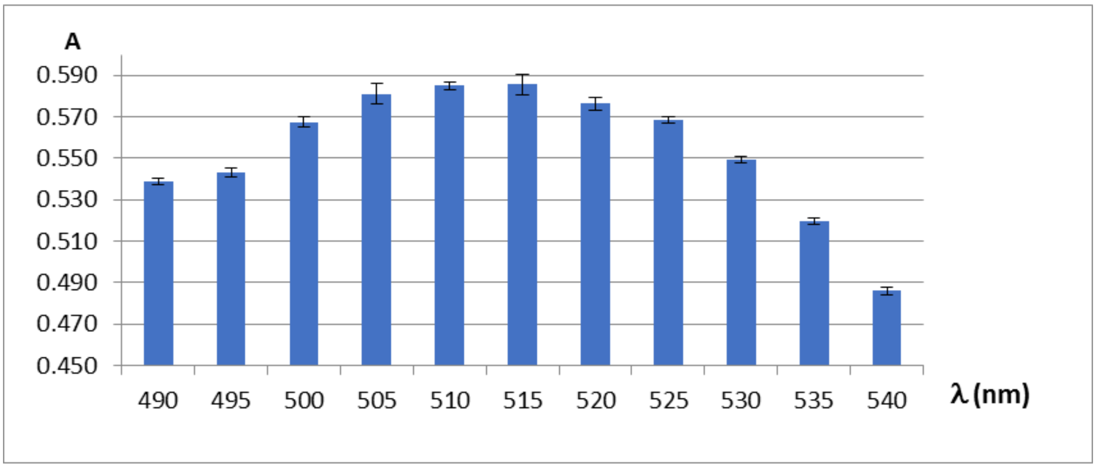

Figure 1. Determining the maximum absorption wavelength of anthocyanin in the shallot's crust

3.2 Survey the effect of ethyl alcohol concentration on extraction efficiency

The result showed that the content of anthocyanin extracted highest when the alcohol concentration in the water was in the range of 40 - 60% (Fig. 2). This suggests that anthocyanin compounds dissolve well in medium-polar solvents.

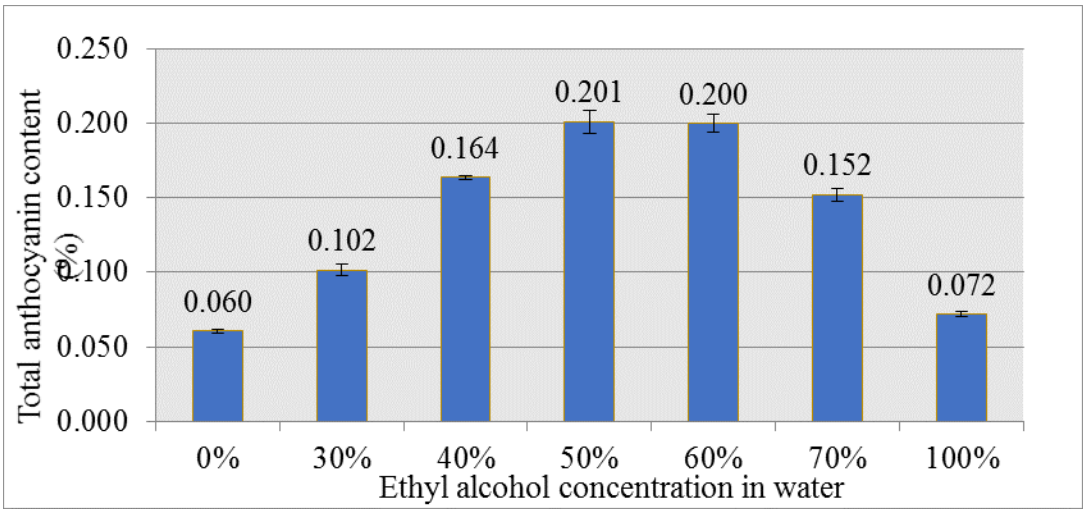

Figure 2. Survey the effect of ethyl alcohol concentration on extraction efficiency (first time)

Narrowing the steps into the range from 40% to 60%, the best solute content was 55% ethyl alcohol in water (Fig 3.).

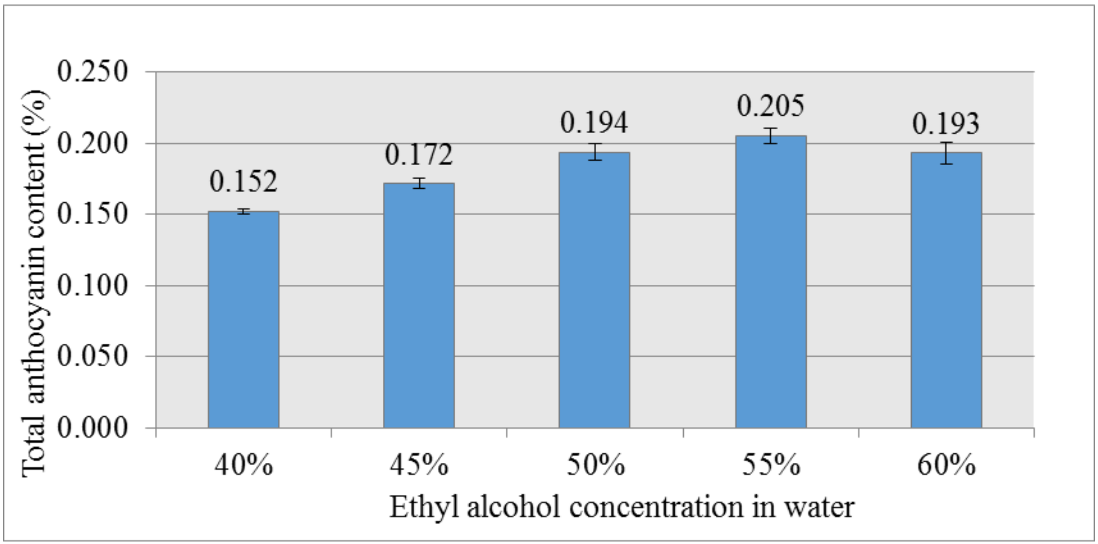

Figure 3. Survey the effect of ethyl alcohol concentration on extraction efficiency (second time)

Anthocyanin is a polarized glycoside that is well soluble in water because it contains polarized radicals of sugars. However, the nonpolar rings of anthocyanidin in anthocyanin partially reduces the anthocyanin polarization (Jackman & Smith, 1996).

3.3 Survey the effect of solvent and material ratio on extraction efficiency

Investigating the effect of solvent and material ratio from 30/1 to 80/1 (ml / g) on extraction efficiency showed that in higher solvent and material ratios, we received higher anthocyanin contents in samples (Fig. 4). That is due to the dynamics of the extraction process is the difference of anthocyanin concentration between in material and that in solvent. However, when increasing solvent and material ratio for a moment, this difference was almost negligible. So there was no significant difference among samples of 60/1, 70/1 and 80/1 ratios ($p>0.05$).

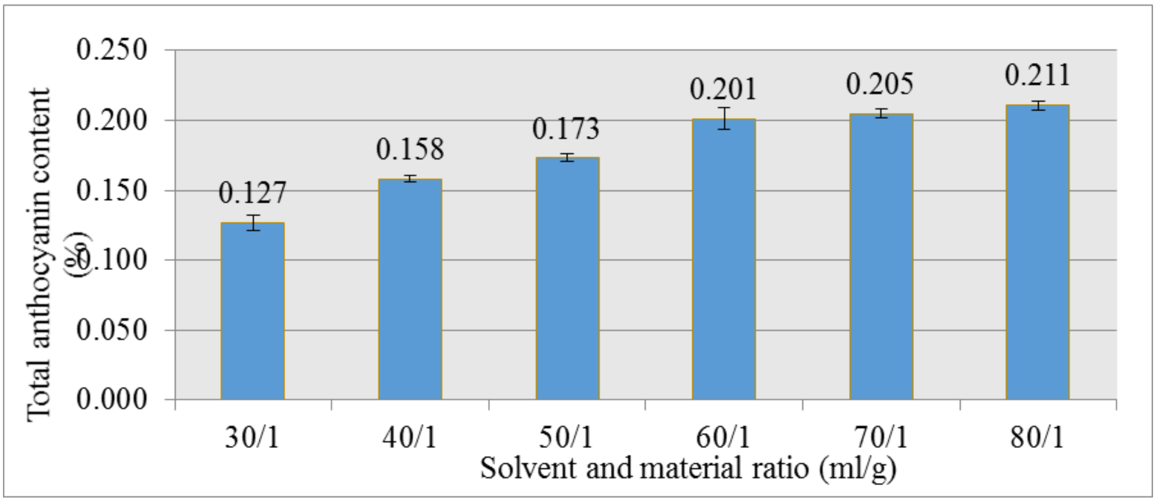

Figure 4. Survey the effect of solvent and material ratio on extraction efficiency (first time)

Narrowing the surveyed area with solvent and material ratios from 60/1 to 80/1 ml/g with 5 ml/g steps, the result showed that when the ratio increased over 65/1 ml/g, there was no significant difference among samples ($p>0.05$). The best sample has 65/1 ml/g solvent and material ratio (Fig. 5).

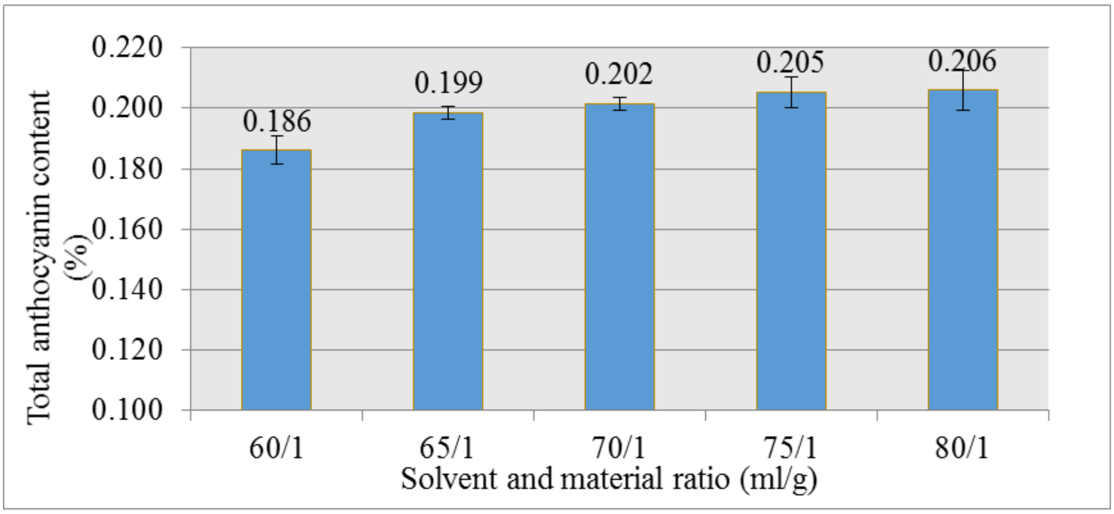

Figure 5. Survey the effect of solvent and material ratio on extraction efficiency (second time)

3.4 Survey the effect of temperature on extraction efficiency

Fig. 6 show the relationship between temperature and anthocyanin extraction capacity from shallot's crust. The best temperature to extract anthocyanin from shallot's crust was 50 ° C. The higher the temperature, the more extracted anthocyanin (Sui, Dong, & Zhou, 2014). That is due to energy promotes the motions of molecular and breaks some bonds as well as activates enzymes that catalyze the hydrolysis of extracting inhibitors. However, elevated temperatures also alter and decompose anthocyanin. This is evident in Fig.6 that the anthocyanin content started to fall sharply as the temperature passes 50°C.

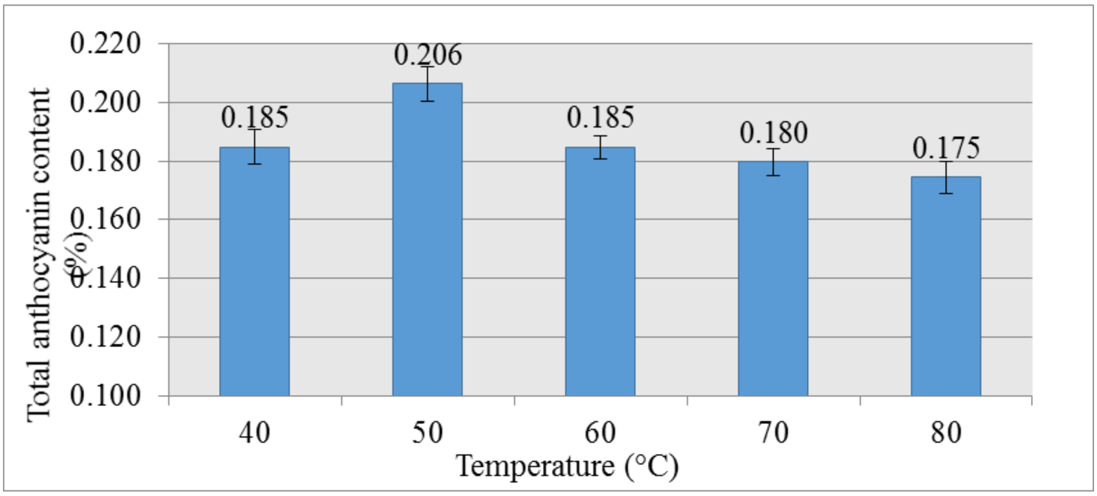

Figure 6. Survey the effect of temperature on extraction efficiency

3.5 Survey the effect of time on extraction efficiency

Fig. 7 showed the relationship between time factor and ability to extract anthocyanin from shallot's crust. In principle, the longer time, the more extracted anthocyanin (Sui, Bary, & Zhou, 2016). However, until a sufficiently long time, the amount of extracted anthocyanin exhausted, the prolonged time does not make a significant difference. In this experiment, the anthocyanin content was almost constant when extracting time was equal and higher than 50 minutes.

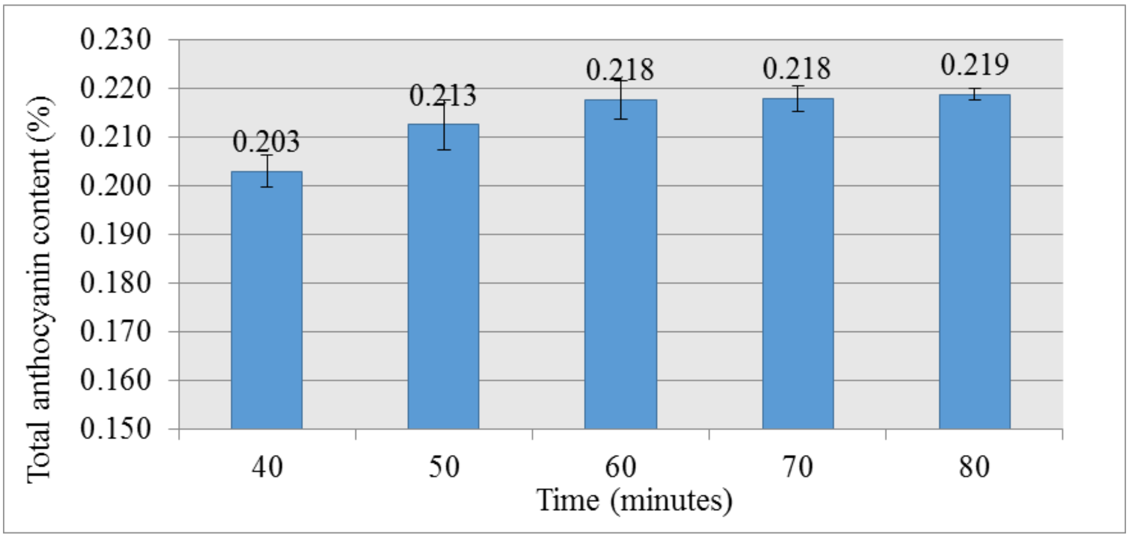

Figure 7. Survey the effect of time on extraction efficiency

3.6 Optimizing the factors that affect the anthocyanin extraction process from shallot's crust

After rudimentary surveys have been made including factors such as ethyl alcohol concentration, material and solvent ratio, temperature, time, laying out optimal test according to Response Surface Method (RSM) with the face centered central (CCF) composite design calculating by Modde 5.0 software. The parameters of the optimization experiment are as follows:

Target function: The highest anthocyanin content (Y, g / 100 g dry matter)

Factors: ethyl alcohol concentration (X_1, %), ratio of solvent and material (X_2, ml / g), temperature (X_3, °C), time (X_4, minutes).

Construct a CCF experiment to find the regression equation with the steps of the factors in table 1.

Table 1. Factors

Factors	Low level, -1	Basic level, 0	High level, +1	Range, ΔX_i
X_1	50	55	60	10
X_2	60	65	70	10
X_3	40	50	60	20
X_4	40	50	60	20

Arranging the experimental matrix and conducting the experiment according to the planned model, the results were shown in Table 2.

The regression equation obtained from that result is:

$$Y = 0.228663 - 0.00420092.X_1 + 0.00473739.X_2 + 0.00753872.X_3$$
$$+ 0.0039881.X_1.X_4 -0.0036074.X_2.X_4 - 0.00300171X_3.X_4$$

Table 2. Influence of variables on the objective function

Total anthocyanin content	Coeff. SC	Std. Err.	P	Conf. int(±)	Choice P<0.05
Constant	0.228663	0.00209582	0	0.00423916	apcept
X_1	-0.00420092	0.00134049	0.00327067	0.00271138	apcept
X_2	0.00473739	0.00134049	0.00107126	0.00271138	apcept
X_3	0.00753872	0.00134049	1.72E-06	0.00271138	apcept
X_4	0.000171342	0.00134049	0.898951	0.00271138	cancel
X_1*X_1	-0.00532125	0.00354661	0.141568	0.00717363	cancel
X_2*X_2	0.000157506	0.00354661	0.964812	0.00717363	cancel
X_3*X_3	0.00114147	0.00354661	0.749287	0.00717363	cancel
X_4*X_4	0.00065952	0.00354661	0.85344	0.00717363	cancel
X_1*X_2	-0.00221841	0.00142181	0.126773	0.00287585	cancel
X_1*X_3	0.000675537	0.00142181	0.637347	0.00287585	cancel
X_1*X_4	0.0039881	0.00142181	0.00780757	0.00287585	apcept
X_2*X_3	0.00139278	0.00142181	0.333327	0.00287585	cancel
X_2*X_4	-0.0036074	0.00142181	0.0152859	0.00287585	apcept
X_3*X_4	-0.00300171	0.00142181	0.041217	0.00287585	apcept
N = 54	Q2 =	0.322	Cond. no. =	6.6122	
DF = 39	R2 =	0.671	Y-miss =	0	
	R2 Adj. =	0.553	RSD =	0.008	
			Conf. lev. =	0.95	

The regression equation revealed that all factors had an effect on the anthocyanin content obtained. Where X_1 (solvent concentration) and X_4 (time) have a negative effect on anthocyanin content while X_2 (solvent and material ratio), X_3 (temperature) has a positive effect on the total anthocyanin content. At the same time there is the interaction between X_4 and other factors. The obtained model was P = 0.000 <0.05. Running optimizations on Modde 5 software obtained the optimal prediction results as follows:

Table 3. Influence of variables on the objective function

X_1	X_2	X_3	X_4	Y
50.0202	69.9995	60	44.5599	0.2499
52.3495	69.9952	59.9996	45.7441	0.2489
50.0002	69.9923	57.1799	40.0001	0.2514
51.6252	69.9999	59.9353	40	0.2548
53.7107	69.994	57.0836	40.0279	0.2489
50.5	70	60	40	0.2552
52.0633	69.9999	58.1543	40.0005	0.2521
50.5	70	60	40	0.2552

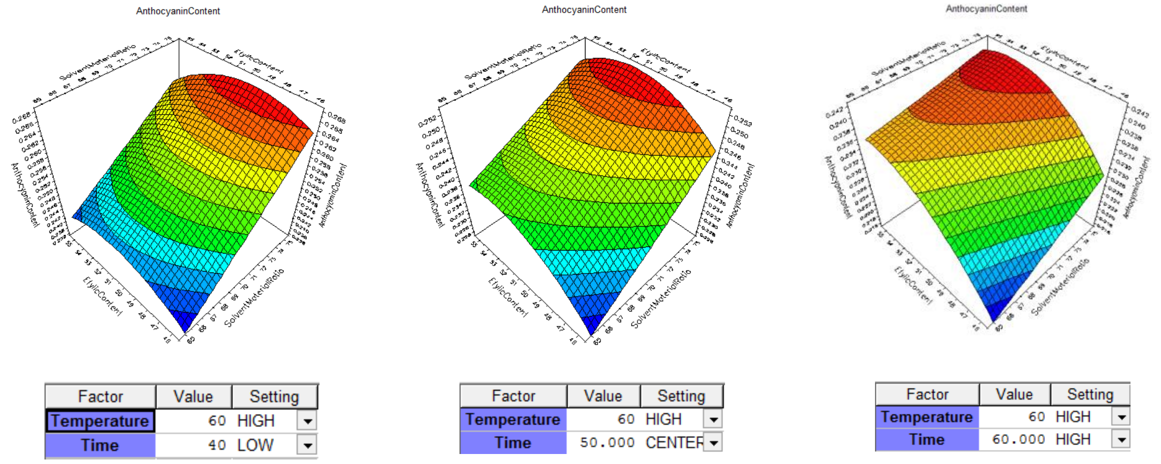

Figure 8. Surface response of experiments investigating anthocyanin extraction from onion crust

As predicted by the software, we conducted experiments at the optimal prediction point: $X_1 = 50.5$, $X_2 = 70$, $X_3 = 60$ and $X_4 = 40$. The result showed that when extracting anthocyanin from the purple onion with alcohol content of 50.5%, the solvent and material ratio was 70 ml / g, at the extraction temperature of 60°C for 40 minutes, the highest extracted anthocyanin content was 0.255 ± 0.002 g / 100 g dry matter.

4. Conclusion

The crust of shallot is the waste of peeling shallot in processing, which can be used to extract anthocyanin, a biologically active substance with its high antioxidant property. The result of the experimental optimization showed that extracting anthocyanin from the crust of shallot with an ethyl alcohol content in water of 50.5%, solvent and material ratio of 70 ml /g, at 60°C for 40 min received the highest anthocyanin content of 0.255 ± 0.002 g /100 g dry matter.

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