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A synergistic of pectinase, cellulase, and glucoamylase on anthocyanin content and extraction yield of roselle petals (*Hibiscus sabdariffa* L.)

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ABSTRACT

The Roselle petals contain anthocyanin pigment which as acts as an antioxidant and a natural food colorant. The objective of this research is to study the effect of three enzymes; pectinase, cellulase, and glucoamylase, on the quality of the extract of the Roselle petals. The Roselle petals were extracted using distilled water in a ratio of 1:4, and divided into four parts. Pectinase (P) of 1000ppm; pectinase and cellulase (PC) of 500:500ppm; pectinase and glucoamylase (PG) of 500:500ppm; and pectinase, cellulase and glucoamylase (PCG) of 333:333:333ppm were added into each part, 1% of citric acid was added to all treatments. Determination of the chosen treatment used is based on residue extract, anthocyanin analysis, and the pH value. The results show that fresh Rosella extract with PC has a yield value of 7.60% and it was slightly different from the extract with PCG which yielded 7.37%. Dried Rosella extract with PCG has the highest yield of 22.10% compared to the control (without enzyme) of 12.96%. However, the PCG treatment procedure generates a sticky product. Both fresh and dried Roselle extracts with PC have the highest anthocyanin content of 156.64±1.30 mgL^{-1} and 35.09±0.04 mgL^{-1}, respectively. The pH values of fresh and dried Roselle extracts were 2.65 and 2.24 respectively. This research shows that treatment of fresh and dried Roselle petals with P, PC or PCG can greatly increase the extraction yield value. In addition, these enzymes can also increase the anthocyanin content of the extracts.

Keywords: anthocyanin content, enzyme treatment, extraction yield, pH value, roselle petal

Introduction

Roselle (*Hibiscus sadbariffa* Linn) is a member of the Malvaceae family. The Rosella plant thrives well in subtropical and tropical climates and contains a high content of anthocyanin which is a natural red pigment and an antioxidant. Furthermore, Roselle petals contain cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, cyanidin-3-O-sambubioside, and delphinidin-3-O-sambubioside (Kouakou et al., 2015). Roselle is very effective in lowering blood pressure and sugar levels (diabetes) (Mardiah et al., 2014; Riaz & Chopra, 2018; Yusni & Meutia, 2020), protecting the liver from damage (Halim et al., 2019), enhancing antioxidant enzyme activity in the liver, anti-inflammation, analgesics (Mardiah et al., 2015; Izquierdo-Vega et al., 2020) as well as lowering uric acid (Yuanta, 2019). Roselle petals can be processed into a variety of products such as tea, jam, jelly, juice, natural colorant, and extract powder which can be applied in foods, supplements, and drugs. According to Mardiah et al. (2014), Roselle extract was dried using spray dryers yields lower extraction result. This is due to the residues (pectins) which are normally attached to the walls of the spray dryer that leads to a decline in extraction yield.

Numerous researches have proven that pectinase greatly increases the extract yield of rosella petals (Kumar, 2015; Mardiah et al., 2018). During the extraction of the grape's red pigments, pectin can also shorten the time involved for macerating, settling, and the filter processes a lot faster than just using ethanol (Lotfi et al., 2015). According to Hanafi (2009), the combination of both pectinase and cellulase simultaneously, can greatly increase the yield. the enzyme

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combination has a synergistic effect of increasing the yield of banana juice extraction (Handique et al., 2019) and oil extraction from the pulp of *Euterpe oleracea* fruit (de Ferreira et al., 2018). In this research, combination of pectinase, cellulase and glucoamylase enzymes were conducted to increase the extraction yield, quality and anthocyanin content for studying the synergistic effect of this combination.

Materials and methods

Materials

The objects of the study were fresh Roselle petals obtained from West Palimanan, Cirebon, Indonesia. Dried Roselle was obtained from drying fresh Rosella under the sun for three days. Pectinase, cellulase, and glucoamylase (Jiangsu Boli Bioproducts Co., Ltd, China), distilled water, citric acid (PT Budi Acid Jaya Tbk., Indonesia), maltodextrin (Shandong Xiwang GroupCo., Ltd, China), and other chemicals were used for this analysis. This research consists of two stages namely; extraction and analysis.

The anthocyanin extraction

Anthocyanin extraction was conducted using distilled water in a 1:4 combination ratio (Roselle petal (100 g fresh or dried): distilled water (400 ml)). Then the addition of pectinase (P) of 1000 ppm, pectinase and cellulase (PC) of 500:500 ppm, pectinase and glucoamylase (PG) of 500:500 ppm, pectinase, cellulase and glucoamylase (PCG) of 333:333:333 ppm, and without enzyme (TE) as a control. Furthermore, there was an addition of 1% (w/w) of citric acid to all the treatments. The extraction was conducted at 50°C for 60 minutes. The extract was filtered and dried using a spray dryer with 10% maltodextrin (w/w) as filler, and inlet and outlet temperatures of 150°C and 80°C respectively.

Analysis stages

The stages consisted of chemical analysis of the pH values using a pH meter (WTW, Xylem Inc., Germany) and a total determination of anthocyanin content with a differential pH method using UV-VIS spectrophotometry (WTW, Xylem Inc., Germany). Anthocyanin content was determined with two replications and quantified in cyaniding 3-glucoside by the following formula

$$A = [(A520 - A700)pH\ 1.0 - (A520 - A700)pH\ 4.5] \quad (1)$$

$$\text{Anthocyanin concentration (mg/L)} = (A \times MV \times DF \times 1000)/(\varepsilon \times L) \quad (2)$$

A520 is the absorbance of wavelength of 520 nm, A700 is the absorbance of wavelength of 700 nm, A is optional density, MV and € is the molecular weight (449.2) and molar absorbance of cyaniding 3 glucoside respectively, DF is the dilution factor, L is the cuvette thickness (cm), and ε is molar absorbance. Measurement of the yield was calculated based on the weight of the Roselle extract after drying & divided by the amount of initial Roselle weight. The amount of sample used was 1 ml of rosella extract.

Data analysis

This research using a descriptive statistics in data analysis with one factor and two replication via SPSS® software version 21 (IBM Co., NY, USA).

Results and discussion

Extraction yield

The extraction yield of fresh and dried Roselle petals using several enzymes can be seen in Table 1.

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Table 1. Extraction yield of fresh and dried Roselle petals

Enzyme	Extraction yield (%)	
	Fresh Roselle petals	Dried Roselle petals
P	7.60±0.35	18.54±0.45
PC	5.84±0.34	14.79±0.42
PG	6.56±0.18	17.30±0.42
PCG	7.37±0.57	22.10±0.36
TE	5.80±0.13	12.96±0.48

P = Pectinase; P:C = Pectinase : Cellulase; P:G= Pectinase : Glucoamylase; P:C:G = Pectinase : Cellulase: Glucoamylase; T.E = control (without enzyme)

Table 1 shows the extraction yields of fresh Roselle petals by adding P(1000ppm), PG, PC, and PCG are 7.60%,6.56%, 5.84%, and 7.37%, respectively. Descriptive analysis shows the extraction yield of the Roselle petals by adding P is slighttly different from the treatment of PCG. However, it is considerably quite different from the others. The extraction of dried Roselle petals by adding PCG produces the highest yield of 22.10%, while the extraction yields by adding P, PG, and PC are 18.54%, 17.3%, and 14.79% respectively. In addition, the extraction yield of dried Roselle petals by adding PCG is the highest than other treatments. In addition, Table 1 also shows that the addition of pectinase can increase the yield compared to control (without enzyme) on the treatment of fresh and dried Rosella. These results are in accordance to Mardiah et al. (2018) which stated that pectinase enzyme can degrade pectin compounds further into simpler forms to increase the yield. Pectinase can break the pectin bonds on the cell and increase procyanidin extract. Other studies (Kumar, 2015; Sudeep et al., 2020; Nguyen & Nguyen, 2018) also explained that pectinase could increase the extraction yield, total soluble solid, and titratable acidity as well as decrease the turbidity & viscosity level. Nguyen & Nguyen (2018) stated that pectinase could degrade pectin led to a reduction in the water holding capacity of the pectin, which released more juice in the mixture, and consequently, more juice yield.

The combination of PC, PG, and PCG enzymes can also increase the yield in both dried and fresh Rosellas. The combination of carbohydrase enzyme as pectinase, pectinesterase, hemicellulase, and cellulase may increase enzyme activity because the enzymes work synergistically. According to Oumer (2017), the structure of plant cell walls consists of cellulose, hemicellulose and pectic compounds. Cellulose compounds play a role in giving strength to cell walls while hemicellulose and pectic compounds function as cementing substances for cellulose tissue. Pectic compounds contribute to complex physiological processes such as cell growth and cell differentiation including determining the integrity and rigidity of plant tissue. Pectinase can effectively degrade cell wall structure through hydrolysis of pectic substances. while cellulase works to reduce the molecular size of cellulose particles and substrate viscosity.

Hanafi (2009) also explained that the mechanism of pectinase and cellulase combination would increase the yield due to their synergistic effect. Cellulase degrades the cell wall of cellulose while pectinase degrades pectin molecules which are bound to cellulose compounds. Handique et al., (2019) and de Ferreira et al. (2018) also stated that the extraction using more than one enzyme could produce a higher yield.

Anthocyanin content

The anthocyanin content from fresh and dried Roselle petals can be seen in Table 2.

Table 2. Anthocyanin content of fresh and dried Roselle petals

Enzyme	Anthocyanin content ($mg.L^{-1}$)	
	Fresh Roselle petals	Dried Roselle petals
P	101.29±0.53	26.40±0.46

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PC	184.02±0.15	35.09±0.04
PG	156.64±1.30	33.20±0.80
PCG	141.05±0.56	23.51±0.53
TE	131.89±0.76	23.25±0.08

P = Pectinase; P:C = Pectinase : Cellulase; P:G= Pectinase : Glucoamylase; P:C:G = Pectinase : Cellulase: Glucoamylase; T.E = control (without enzyme)

Table 2 shows that fresh Rosella extract has more anthocyanin content than the dried extract. This is caused by the high-temperature drying process which is applied to dry Rosella petals which decreases the anthocyanin levels (Mardiah et al., 2015). The analysis shows that the anthocyanin contents of all enzyme treatments are tend to higher than the TE. The anthocyanin content of fresh Roselle petals with P is the lowest in content. According to Vukoja et al. (2019), pectinase can decrease anthocyanin stability due to its glucosidase activity which can hydrolyze glycoside β1-2 cyanidin-3-sophoroside and cyanidin-3-glucosyl-rutinoside bonds into cyanidin-3-glycoside and cyanidin-3-rutinoside. Furthermore, the extraction with the PC addition has the highest anthocyanin content in both fresh and dried Roselle extraction. According to Lotfi et al. (2015), pectinase and cellulase have a synergistic effect in breaking down the cell walls and making presence pigment (anthocyanin) in the cell wall easily extractable. The same thing was stated by Ranveer et al. (2020) that the addition of cellulase and pectinase enzymes could increase the concentration of anthocyanins in the kokum fruit's peel (*Garcinia indica* Choisy). Jia et al. (2019) also informed that the combination use of cellulose, hemicellulose, and pectinase enzymes could increase anthocyanins, as well as shorten the processing time, increase efficiency and reduce processing temperatures in cherry winemaking than the use of a single enzyme. This was occurred due to the hemicellulase enzymes could decompose cellulases and hemicellulases, dissolving the plant cell walls and releasing more intracellular solutions. In contrast, lower anthocyanin content is obtained when glucoamylase is added (PG and PCG) into the treatment. This occurred because glucoamylase can decrease anthocyanin pigment and make it unstable by hydrolyzing the bond between aglycone and glycone. This hydrolysis resulted in the aromatic ring on anthocyanin turning into a cation compound that is colorless.

The glucoamylase can increase the sugar level in the extract. The presence of sugar can induce an increase in the intensity of anthocyanin colors in acidic conditions. However, a higher sugar level in the extraction leads to lower anthocyanin stability. The presence of sugar can accelerate the degradation of anthocyanin and its ability to condense with anthocyanin and generate brown compounds. The glucoamylase can hydrolyze the bonds of α-1, 6 glycosidic linkage so that dextrin products will be converted into glucose units, the dextrin products cause a high degree of sweetness and sugar yield (Tiwari et al., 2015). Also, the extraction treatment with PCG produces a sticky extract which is due to the high levels of glucose. Kumar (2015) also reported that the glucoamylase reacts with carbohydrates that leading to a sticky extract.

pH value

Table 3. pH value of fresh and dried Roselle petals

Enzyme	pH	
	Fresh Roselle petals	Dried Roselle petals
P	2.56±0.10	2.79±0.04
PC	2.74±0.33	2.46±0.09
PG	2.38±0.09	2.21±0.13
PCG	2.65±0.07	2.24±0.28
TE	2.65±0.10	2.03±0.04

P = Pectinase; P:C = Pectinase : Cellulase; P:G= Pectinase : Glucoamylase; P:C:G = Pectinase : Cellulase: Glucoamylase; T.E = control (without enzyme)

Table 3 shows the pH value of all treatments are in the range of 2.3-2.7, which means that the pH value of Roselle petals extract is stable. The enzyme addition on fresh or dried Roselle petals does not affect the pH value. Also, the analysis shows that all enzyme combinations do not affect the pH value of fresh Roselle petals; however, they have an effect on pH of dried Roselle petals extract. However, the pH condition also shows the optimum working conditions for the enzyme, where the pectinase has an optimum pH of 5.8 (Sudeep et al., 2020). Li et al. (2020) reported that the best pH to produce the most anthocyanins in mulberry juice extract using the pectinase was in the range of 3-6. At higher pH, the pectinase has a lower ability to decompose the cell wall, and this impacts the lower the extracted anthocyanins.

The anthocyanin stability is affected by several factors, one of which is the pH value. Low pH values from 2-4 affects the anthocyanin stability because at this condition, anthocyanin is reddish in color and in the form of cyanidin-3 glucoside and cyanid-3 on the outside. Decreasing pH will shift the equilibrium condition to the red flavilium cation. Conversely, increasing pH will form carbinol and colorless balcony (Nurtiana, 2019). Khoo et al. (2017) reported that β glucosidase enzyme can cause discoloration of anthocyanins into anthocyanidins and sugars that spontaneously form aglycone (anthocyanidin) to become colorless. The pH measurement shows a more stable cyanidin content at pH below 3 (Lotfi et al., 2015), and Jia et al. (2019) stated that at pH 3, the anthocyanin extract reached the maximum value. There is a pH effect on anthocyanin discoloration, where pH less than 3 can control the color changes that correlated with anthocyanin content in fermented beverage from red sweet (Khoo et al., 2017). The addition of acids such as citric and lactic acid checkmated the damage on anthocyanin. In this study, 1% of citric acid was used in all treatments of the extraction of Roselle petals. It maintains a pH below 3, so the powder color of Roselle extract is in excellent stability. Lotfi et al. (2015) reported the color of the anthocyanin extract produced using solvents with an enzyme addition (pectinase, cellulose, protease) resulting in better chroma value and a more stable lightness than those produced from acidified ethanol solvents.

Conclusion

The data in this work confirmed that the addition of PCG enzymes can increase the extraction yield on both fresh and dried Roselle petals, followed by the addition of P, PG and PC, however, PCG produces a sticky extract. The highest anthocyanin level was obtained from the extract with PC addition on both fresh and dry Roselle petals. The addition of enzymes on fresh or dried Roselle petals tend not to affect the pH value range (2.3-2.7).

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