Reaktor

p-ISSN 0852 – 0798
e-ISSN 2407 – 5973

Website: http://ejournal.undip.ac.id/index.php/reaktor/

Reaktor, Vol. 22 No. 3, December Year 2022, pp. 86-91

The Effect of Drying on Anthocyanin Content and Antioxidant Activity in Red Cabbage and White Cabbage

Rahmat Efendi[1)], Rahmandika Irfan Pradana[1)], Chandrawati Cahyani[1)], and Luthfi Kurnia Dewi[1,*)]

[1)]Department of Chemical Engineering, Faculty of Engineering, Brawijaya University
Jl. Jl. MT. Haryono No. 167, Malang, 6541.

[*)] Corresponding author: luthfikurnia@ub.ac.id

(Received: 8 June 2022; Published: 1 December 2022)

Abstract

Red cabbage and white cabbage contain several beneficial compounds, especially the anthocyanins that can scavenge free radicals. This study aimed to examine the effects of drying on anthocyanin content and antioxidant activity in red cabbage and white cabbage. Each cabbage was subjected to two pretreatments, namely: (1) drying, and (2) without drying. Maceration extraction was performed using a 50:50 (v/v) mixture of water and methanol acidified with 7% citric acid employing a liquid to solid mass ratio of 1:4 (w/v) at a constant temperature of 60°C for 1 h. Cabbage extract was examined for its anthocyanin content by differential pH method, while its antioxidant activity was tested using the DPPH method. The results showed that the anthocyanin content in crude fresh red cabbage extract, crude dried red cabbage extract, crude fresh white cabbage extract and crude dried white cabbage extract were 64.124 mg/L, 210.74 mg/L, 0.445 mg/L, and 0.584 mg/L, respectively. Meanwhile, the antioxidant activity (IC50) of crude fresh red cabbage extract, crude dried red cabbage extract, crude fresh white cabbage extract, and crude dried white cabbage extract were 54,317 ppm, 49,464 ppm, 131,878 ppm, and 107,069 ppm, respectively. The best result was a 25.2% yield of crude dried red cabbage extract with an anthocyanin content of 210.74 mg/L and an IC50 value of 49.464 ppm.

Keywords: antioxidant; anthocyanin; cabbage; DPPH; extraction; maceration

How to Cite This Article: Efendi, R., Pradana, R.I., Cahyani, C., and Dewi, L.K., (2022), The Effect of Drying on Anthocyanin Content and Antioxidant Activity in Red Cabbage and White Cabbage, Reaktor, 22(3), 86-91, https://doi.org/10.14710/reaktor. 22.3.86-91

INTRODUCTION

Cabbage (*Brassica oleracea L.*) is a vegetable that is commonly consumed and cultivated in Indonesia for its beneficial health promoting vitamin and mineral content (Putri, et al. 2017). Based on data uploaded by Statistics Indonesia (Badan Pusat Statistik) in 2019, cabbage production in Indonesia reached 1,413,060 2 tons in 2019 (BPS, 2019).

The types of cabbage that are easily found in Indonesia are white cabbage and red cabbage. White cabbage (*Brassica oleracea L. var. capitata f. alba*) contains vitamins (A, C, E, and K), anthocyanins and other phytochemical compounds (Nosek et al. 2015). The anthocyanin content in white cabbage is 0.01-0.02 mg/100 grams (Ali and Atwaa, 2013). Red cabbage (*Brassica oleracea L. var. capitata f. rubra*) contains

vitamins A, B, C and E, potassium, calcium, and anthocyanins (Lukitasari et al. 2017). The anthocyanin content in red cabbage measured against the effect of several types of solvents is between 0.77 to 53.82 mg/100 grams of its edible portion (Galvao et al. 2020).

Anthocyanins are organic compounds that provide pigments in plant tissues and are highly soluble in polar solvents. In addition to giving color to plants, anthocyanins also function as free radical scavengers or commonly referred to as natural antioxidant compounds because they eliminate free radicals (Priska et al. 2018). Anthocyanins are generally obtained from plants through an extraction process. One of them being the maceration extraction method, which is a process of immersing raw materials with a solvent and accompanied by several times of stirring either with or without heating to avoid the deterioration of thermolabile compounds (Depkes RI, 2000). Anthocyanin extraction can be affected by various factors, such as solvent type, temperature, duration, and raw material preparation/pretreatment (Saona and Wrolstad, 2001).

Raw materials for the extraction process can be either fresh or dried materials. The degree of the effect depends on their physical characteristics that could affect both solute and solvent diffusion process during the extraction (Pham et al. 2017 in Hazmi et al. 2019). The dry raw material is commonly obtained through a drying process to reduce the moisture content of material by providing heat to allow the vaporization of water molecules from the material (Asgar and Musaddad, 2006).

Oven drying is one of the most widely applied drying methods, which can result in a constant dry weight of the material much faster than sun drying (Winangsih et al. 2013). Drying provides the advantage that the material will not easily damage and leads to prolong the shelf life of the material before further processing as well as ease distribution and reduces distribution cost (Depkes RI, 2000).

There have been a few researches of the potential of anthocyanin content in cabbage as a natural compound with remarkable health benefits. However, the effect of drying on anthocyanin content and antioxidant activity in red cabbage and white cabbage has yet to be reported. Therefore, this study was conducted to determine the effect of drying on anthocyanin content and antioxidant activity in red cabbage and white cabbage.

MATERIALS AND METHOD

Materials

Red cabbage and white cabbage were obtained from Oro Oro Dowo Market, Malang City. The chemicals used in this work were analytical grade methanol, citric acid, hydrochloride, potassium chloride, and sodium acetate. In addition, distilled water was also utilized as the main solvent in this research.

Maceration Method Without Drying

A carefully weighed 250 grams of fresh cabbage was extracted by a mixture of water and methanol 50:50 (v/v) acidified with 7% citric acid with a liquid to solid mass ratio of 1:4 (w/v) and with a controlled temperature of 60°C for a period of 1 h. After being filtered with filter paper, the extract was transferred into vacuum filtration apparatus to separate the precipitate from filtrate. Then, the filtrate was concentrated using a vacuum evaporation apparatus at 50°C.

Maceration Method Without Drying

A thoroughly prepared 250 grams of fresh cabbage was dried in an oven at 100°C to obtain cabbage with a constant mass. The dried cabbage was extracted by a mixture of water and methanol 50:50 (v/v) acidified with 7% citric acid with a liquid to solid mass ratio of 1:4 (w/v) and with a controlled temperature of 60°C for a period of 1. After being filtered with filter paper, the extract was transferred into vacuum filtration apparatus to allow the separation of the precipitate from filtrate. Then, the filtrate was concentrated using a vacuum evaporation equipment at 50°C.

Determination of Anthocyanin Content

The total monomeric anthocyanin (TMA) was determined using pH-differential method by Giusti and Wrolstad (2001). Cabbage extract was diluted with potassium chloride with pH 1 and sodium acetate pH 4.5 buffer solutions, respectively by considering the dilution factor. After that, absorbance was measured at 510 nm and 700 nm.

The relative absorbance of cabbage extract was computed by equation (1):

$$A = (A\lambda_{510} - A\lambda_{700})\lambda_{pH\ 1.0} - (A\lambda_{510} - A\lambda_{700})\lambda_{pH\ 4.5} \quad (1)$$

The total monomeric anthocyanin (TMA) contained in cabbage extract was then calculated using equation (2):

$$TMA\left(\frac{mg}{L}\right) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L} \quad (2)$$

where MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF = dilution factor, ε = molar absorptivity of cyanidin-3-glucoside (26,900 L/(mol.cm)) and L = cuvette Width (1 cm)

Determination of Antioxidant Activity (DPPH Method)

The antioxidant activity of the red and white cabbage extracts was carried out at the Nutrition Laboratory of the Department of Health Nutrition, Faculty of Public Health, Airlangga University - Surabaya.

RESULTS AND DISCUSSION

Phytochemical Test Results

Table 1. Phytochemical test result

Material	Treatment	pH 1	pH 4,5
Red cabbage	Without drying	Red	Bluish Green
	With Drying	Red	Bluish Green
White cabbage	Without Drying	Clear	Brownish Yellow
	With Drying	Light Brown	Yellow

Table 1 presents that both raw and dried red cabbage ethanolic extracts contained anthocyanins, as indicated by the facts that at pH 1 the color of the extract was red and it was bluish green at pH 9.5 (Harborne, 2003). This is because under acidic conditions (pH < 7), anthocyanins generally tend to appear reddish. Accordingly, anthocyanins will turn to bluish under alkaline pH conditions (pH> 7) (Nassour et al. 2020).

Meanwhile, the crude ethanolic extracts of raw and dried white cabbage did not indicate the presence of anthocyanins. The results could be due to the extremely low anthocyanin content of white cabbage that its anthocyanins were not identified using the pH-differential method. This is in accordance with research conducted by Nassour et al. (2020), it is known that the color of anthocyanins is influenced by the concentration of pigments. Furthermore, a possible reason for the color change that occurs during the phytochemical test is caused by the content of flavones and flavonols in white cabbage, which will give the color fading to become colorless in acidic conditions and give a yellowish color when alkaline conditions (Chang, 2016).

Effect of Drying on Yield of Crude Extract

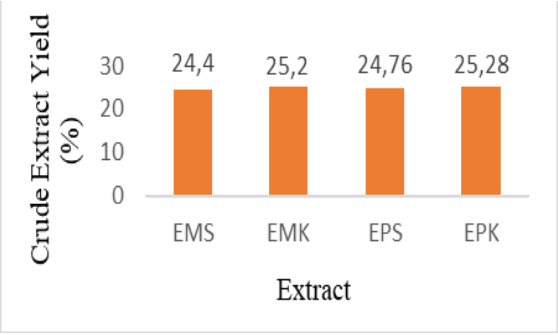

Figure 1. Effect of Drying on Yield of Crude Extract

Information:
EMS = Crude extract of fresh red cabbage
EMK = Crude extract of dried red cabbage
EPS = Crude extract of fresh white cabbage
EPK = Crude extract of dried white cabbage

Figure 1 clearly shows that the crude extract of fresh red cabbage obtained a yield of 24.4% while the crude extract of dried red cabbage with a yield of 25.2%. Meanwhile, crude extract of fresh white cabbage obtained a yield of 24.76%, and crude extract of dried white cabbage with a yield of 25.28%. The results show that extraction of dried red cabbage and white cabbage using aqueous ethanol resulted in a slightly higher yield than that of raw red cabbage and white cabbage. However, the difference in those results of the starting raw materials was not significant.

There are two processes that occur during drying operation, namely the heat and the mass transfer processes. There is a difference in temperature in the oven with the temperature of the cabbage will cause a heat transfer. Hot air circulated in the oven will increase the temperature of the cabbage so that the water content in the cabbage becomes water vapor. The temperature of the cabbage that increases due to the heat transfer process will cause the water vapor pressure of the cabbage to be higher than the water vapor pressure in the oven. This situation results in a mass transfer of water vapor inside cabbage tissue to the air in the oven, which commonly occurs via an evaporation mechanism. The evaporation process continues until the achievement of equilibrium of water vapor pressure between the cabbage and the air oven (Mercer, 2014).

The extract volumes obtained were crude fresh red cabbage extract, crude dried red cabbage extract, crude fresh white cabbage extract, and crude dried white cabbage extract, were respectively 700 mL, 650 mL, 720 mL, and 650 mL. The difference in extract volume can be caused by the water content in fresh cabbage, it is known that the moisture content in fresh cabbage weighed with moisture balance in white cabbage is 95% and in red cabbage is 91%, so the volume of fresh cabbage extract obtained is greater than the volume of extract from dried cabbage and it caused the solvent separation of extract took a longer time. However, after being vacuum separated from the solvent, which the concentrated extracts mass obtained were not much different. The mass of concentrated extract of crude fresh red cabbage extract, crude dried red cabbage extract, crude fresh white cabbage extract, and crude dried white cabbage extract were 61 grams, respectively. 63 grams, 61.9 grams, and 63.2 grams. These data indicated that the dried cabbage extract produced a slightly larger mass of viscous extract, which could be due to the extract containing more anthocyanin compounds compared to the fresh cabbage extract. Thus, it can be concluded that there is no significant effect of drying on the yield of crude extract. However, drying was observed to affect the volume of the extract.

Effect of Drying on Anthocyanin Content

Table 2 shows the crude extracts of the two cabbages contain anthocyanin compounds. Crude fresh red cabbage extract produced TMA content of

64.124 mg/L (6.4124 mg/100 gram) and crude dried red cabbage extract 210.74 mg/L (21.074 mg/100 gram). In accordance with research conducted by Galvao et al. (2020), it is known that red cabbage contains anthocyanins between 0.77 mg/100 grams to 53.82 mg/100 grams. In addition, from these results it is known that the highest anthocyanin contents are shown in crude dried red cabbage extract, this difference is caused by the drying process which can increase the rate of solvent diffusion at the time of extraction (Pham et al. 2017 in Hazmi et al. 2019).

Table 2. Anthocyanin Content Results

Extract	TMA (mg/L)
EMS	64.124
EMK	210.74
EPS	0.445
EPK	0.584

Information:
EMS = Crude extract of fresh red cabbage
EMK = Crude extract of dried red cabbage
EPS = Crude extract of fresh white cabbage
EPK = Crude extract of dried white cabbage

Meanwhile, crude white cabbage extract had a TMA content of 0.445 mg/L (0.0445 mg/100 gram) and crude dried white cabbage extract had a value of 0.584 mg/L (0.0584 mg/100). Based on research conducted by Ali and Atwaa (2013), the anthocyanin content of white cabbage extract is 0.01-0.02 mg/gram white cabbage. The difference in these results could be caused by differences in the initial treatment method. Then, the yield of the crude white cabbage extract was slightly higher than that of the fresh white cabbage extract, the variation in yield from fellow white cabbage is caused by the drying process of the sample which causes the damage of the cell walls that promotes the dissolution of the solute (Arcan, 2009 in Hazmi, et al. 2019). Due to the relatively low anthocyanin content in white cabbage, the anthocyanin levels produced were not much different from dried white cabbage extract or fresh white cabbage extract. The low levels of anthocyanins can be seen in the color shown in the phytochemical assay, which is the color that does not match the reference. The color change that occurs during the phytochemical test is thought to be caused by the content of flavones and flavonols in white cabbage, which will give the color fading to become colorless in acidic conditions and give a yellowish color when alkaline conditions (Chang, 2016).

Hence, it can be concluded that drying has an effect on anthocyanin content in red cabbage extract and there is no effect on drying on anthocyanin content in white cabbage extract. In addition, it is also revealed that the highest anthocyanin content was obtained from crude red cabbage extract, which was 210.74 mg/L, and the lowest anthocyanin content was obtained from crude fresh white cabbage extract with a value of 0.445 mg/L.

Effect of Drying on Antioxidant Activity

The DPPH test is an antioxidant test based on electron transfer which produces a violet solution in an antioxidant solution that has been mixed with DPPH (2,2-diphenyl-1-picrylhydrazyl) (Garcia et al. 2012). One of the parameters that is useful for showing the results of the DPPH method is "efficient concentration" or EC50 value (or IC50 value). This parameter is defined as the ability of the substrate concentration to cause loss or reduce 50% of DPPH activity or color change shown by DPPH. In addition, it is known that the higher the antioxidant activity, the lower the IC50 value (Molyneux, 2004). Based on the IC50 value of the sample, antioxidant activity can be classified into several antioxidant characteristics which are shown in Table 3. And the results of the antioxidant activity test of our research sample are shown in Table 4.

Table 3. Antioxidant Characteristic based on IC50 Value

Value of IC50 (ppm)	Characteristics
200-150	Low
150-100	Medium
100-50	Strong
<50	Very Strong

Source: Molyneux (2004)

Table 4. Antioxidant Activity Test Results

Extracts	IC50 (ppm)
EMS	54.317
EMK	49.464
EPS	131.878
EPK	107.069
Vit C	22.4

Information:
EMS = Crude extract of fresh red cabbage
EMK = Crude extract of dried red cabbage
EPS = Crude extract of fresh white cabbage
EPK = Crude extract of dried white cabbage

Based on Table 3 and Table 4, it is clearly observed that Vitamin C is a very strong antioxidant with an IC50 value of 22.4 ppm. This means that to reduce 50% of DPPH radical activity, 22.4 ppm of Vitamin C is needed. Vitamin C was tested as a standard solution or a positive control, aiming to check that the procedure was working properly and to compare the antioxidant abilities of the samples (Molyneux, 2004). Vitamin C was also chosen because it is one of the secondary antioxidants that work by capturing free radicals and preventing chain reactions (Sayuti and Yenrina, 2015).

In addition, it is also proven that crude fresh red cabbage extract is a strong antioxidant with an IC50 value of 54.317 ppm and crude dried red cabbage

extract is a very strong antioxidant with an IC50 value of 49.464 ppm. This means that to reduce 50% of DPPH radical activity, crude red cabbage extract is needed at 54.317 ppm and crude red cabbage extract at 49.464 ppm. Based on these data, it is known that the crude dried red cabbage extract has a better IC50 value than the crude fresh red cabbage extract. This is because the drying process will damage the cell walls of the extraction raw material and the compounds to be extracted will more easily diffuse out of the cells so that more anthocyanin compounds are extracted (Arcan, 2009 in Hazmi, et al. 2019). More anthocyanin levels extracted in the crude dried red cabbage extract will lead to a better IC50 value. This is shown in the crude extract of fresh red cabbage obtained a total monomeric anthocyanin content of 64,124 mg/L and the crude extract of dried red cabbage obtained a total monomeric anthocyanin content of 210.74 mg/L. In accordance with research conducted by Podsedek (2006), it is known that anthocyanins are the main source of antioxidant activity in red cabbage. In addition, based on data on the total monomeric anthocyanin content, it was shown that there was a significant difference between the two pretreatments of the raw material but the antioxidant activity was not much different. This could be due to the role of other compounds in the antioxidant activity of red cabbage. The result is in accordance with data obtained by Podsedek (2006), which shows that red cabbage contains total phenol (134-171 mg/100 g) and ascorbic acid (62-73 mg/100g). Based on data on total monomeric anthocyanin levels and antioxidant activity, the greater the anthocyanin content, the higher the antioxidant activity of red cabbage extract. Hence it can be said that there is a linear relationship between anthocyanin content and antioxidant activity in red cabbage extract.

Meanwhile, it is observed that both crude white cabbage extracts demonstrated moderate antioxidant activity with the fresh extract having an IC50 value of 131.878 ppm and the dried extract produces an IC50 value of 107.069 ppm. This means that to reduce 50% of DPPH radical activity, crude fresh white cabbage extract of 131.878 ppm is required and crude extract of dried white cabbage is 107.069 ppm. Based on these data, it is known that crude dried white cabbage extract has a better IC50 value than crude fresh white cabbage extract. This is because the drying process can increase the rate of solvent diffusion at the time of extraction (Pham et al. 2017 in Hazmi et al. 2019) and can increase the efficiency of the extraction process because the water content in the raw materials that can interfere with the extraction process has been evaporated (Omusuli et al. 2017 in Hazmi et al. 2019). Based on the research, it was clearly observed that the anthocyanin content in white cabbage extract was low, crude fresh white cabbage extract obtained a total monomeric anthocyanin content of 0.445 mg/L and crude dried white cabbage extract obtained a total monomeric anthocyanin content of 0.584 mg/L. From these data it can be said that anthocyanin compounds are not the main compounds that play a role in antioxidant activity in white cabbage and are thought to be due to the role of other compounds that are also extracted during the extraction process. In accordance with the research of Podsedek (2006), it is known that anthocyanins are not the main compounds that play a role in the antioxidant activity of white cabbage, but total phenol compounds (20-29 mg/100 g). However, the content of these compounds is also lower than the total phenol content) in red cabbage (134-171 mg/100 g. Although the difference in anthocyanin content in the two initial treatments of raw materials was small, it can be said that there was a linear relationship between anthocyanin content and antioxidant activity in white cabbage extract.

Thus, it can be said that drying has a significant effect on the antioxidant activity of red cabbage and white cabbage extracts. In addition, there is a linear relationship between anthocyanin content and antioxidant activity in red cabbage and white cabbage extracts. The best antioxidant activity value was obtained by Vitamin C as a standard solution (22.4 ppm) and when compared with other crude extracts, the crude dried red cabbage extract had the best antioxidant activity value (49.464 ppm) and the lowest antioxidant activity value was obtained from fresh white cabbage crude extract (131.878 ppm). Although the antioxidant activity of white cabbage extract was lower than that of red cabbage, its antioxidant activity was still categorized as moderate antioxidant.

CONCLUSION

The results proved that drying tends to increase the anthocyanin content and antioxidant activity of the cabbage extract samples. The highest yield (25.2%) was obtained from aqueous ethanol maceration of dried red cabbage with anthocyanin content and IC50 value were 210.74 mg/L and of 49.464 ppm, respectively.

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