The effect of solvent type and extraction duration on purple corn anthocyanin compounds (*Zea Mays* L)

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Abstract. The development of the food processing industry and the limited amount and quality of natural dyes cause an increase in the use of synthetic dyes. Since the discovery of synthetic dyes, the use of pigments as dyes has decreased. Therefore the use of natural dyes needs to be raised again. Purple corn (*Zea Mays* L) has the potential as a natural food coloring due to its high anthocyanin content. Anthocyanins can act as natural dyes, antioxidants, antimutagenic, and anti-carcinogens. This study aimed to determine the solvent and extraction duration of purple corn dye on yield and anthocyanin levels. The study was conducted by the maceration extraction method (in which simple filtering was done by soaking purple corn in ethanol 96% and water at room temperature and protected from sunlight) using maceration time (5 hours, 10 hours, 15 hours, 20 hours). The extraction results are then filtered and then analyzed using a UV-Vis spectrophotometer. From this study, it was found that there was an effect of the type of solvent and the length of time of extraction on the yield and levels of anthocyanin obtained. it shows that the best treatment in extracting anthocyanin of a Purple Corn is to use ethanol solution and 20 hours of maceration time, which produces 577.78 mg/l of anthocyanin.

1. Introduction

Food provides not only essential nutrients needed for life but also other bioactive compounds for health and disease prevention. Anthocyanins are one of the bioactive compounds in food sources, especially Purple Corn. Purple corn is one of the new agriculture products in Indonesia, mainly planted in Sulawesi. There have been only a few studies about purple corn. Thus, this commodity needs more study and research. The main characteristic that differs from purple corn compare to other corn is its color.

Anthocyanins are the members of the flavonoid group of phytochemicals. Purple corn is an economic anthocyanin-rich source that could serve as food colorant like beverages, jellies, candies and so on because anthocyanin itself is safe for consumption, non-toxic and does not cause genetic mutations. The benefits of eating anthocyanin-rich foods have been linked to the prevention or delay of the onset of degenerative diseases of aging, cardiovascular disease, diabetes, cognitive dysfunction, cancer, act as an enhancement to the immune system, and as an antihypertensive agent [1].

One method to extract anthocyanin from the plant is to use the extraction method. The extraction method often used is maceration. Maceration extraction is the easiest method by soaking the sample in a

solution [2]. Anthocyanin can be extracted by using polar solvents, such as ethanol and aquadest. Ethanol and aquadest were used because these two solutions are non-toxic and safe for consumption [3,4].

The aim of this study is to find the best solution to extract anthocyanin in Purple Corn (*Zea Mays L.*) through the maceration process and the duration of the extraction process to get the maximum amount of anthocyanin.

2. Methods

2.1. Materials

Materials used were purple corn from farm's faculty agriculture Hasanuddin University, Whatman number 1 filter paper, HCl 2M, NaOH 2M, Ethanol, Aquadest, aluminum foil, Erlenmeyer 100 ml, pipet 10 ml, volumetric flask 100 ml.

2.2. Extraction of anthocyanin

Twenty-five grams of purple corn was put into the Erlenmeyer covered by aluminum foil, followed by adding ethanol, 1% HCl, and 100 ml aquadest. The next step is to extract the Purple Corn by maceration. The solution then kept in a lightless place with room temperature for 5 hours, 10 hours, 15 hours, and 20 hours. After that, anthocyanin extract was filtered by Whatman Paper No. 1.

2.3. Verification test of anthocyanin

HCl 2 M were added into the purple corn extracts, then being heated at 100 ^{o}C for 5 minutes. If the sample turned red, it shows that the sample is positively contained anthocyanin. But if the sample with the addition of some drops of NaOH 2 turned to blueish green that fades slowly, then it is certain that the sample contained anthocyanin.

2.4. Determination of anthocyanin amount

The amount of 0.1 mL of the thick sample from the extraction, were put into 2 test tubes. Then each tube was given 3.9 mL pH 1 and pH 4,5 buffer. Both tubes were put in idle for 15 minutes, then the absorbent measured in wavelength of 510 nm and 700 nm with spectrometer UV-VIS, using aquadest as a blank. And then the concentration counted.

3. Results and discussion

Anthocyanin extraction of Purple Corn in this study done by using two kinds of polar solution, Ethanol mixed by HCl 1% and Aquadest. The result of anthocyanin extraction with aquadest produce the color of brownish purple, while the extraction with ethanol produces the color of thick purple.

Figure 1. Color comparison of extraction process between ethanol (left) and aquadest (right).

After finding the extract that estimated as anthocyanin, the next step is identification test by color-test. In the color-test, solutions with acid characteristic (HCl) and base characteristic (NaOH) were used. This is based on one of the anthocyanin characteristics where it will turn red if mixed by acid pH, and turn blue if mixed by base pH [5].

Figure 2. The result of anthocyanin color-test

From the measurement of anthocyanin amount from both solutions, and by the extraction time for 0 hours, 5 hours, 10 hours, 15 hours, and 20 hours, it indicates that the amount of anthocyanin produced in parallel with the maceration time. This is because the longer the maceration time, the higher the anthocyanin, where the interaction chance between material and solution is bigger, thus the result will increase. The event repeats from time to time until it reaches a balance of concentration between solution outside and inside the cell [6].

Table 1. The amount of anthocyanin with aquadest solution based on maceration time.

Maceration time	Anthocyanin amount (mg/l)
0 hour	25.58
5 hours	34.48
10 hours	41.25
15 hours	43.92
20 hours	65.58

Table 2. The amount of anthocyanin with ethanol solution based on maceration time.

Maceration time	Anthocyanin amount (mg/l)
0 hour	577.72
5 hours	607.57
10 hours	638.91
15 hours	686.38
20 hours	775.87

The comparison of anthocyanin extraction between aquadest and ethanol solution was significant. The lowest amount of anthocyanin was found in the sample with the aquadest solution. The comparison can be seen in figure 3. This is because ethanol can identify metabolite compound more than aquadest. Besides that, the sample with ethanol has the same level of polarity with the compound extracted [7].

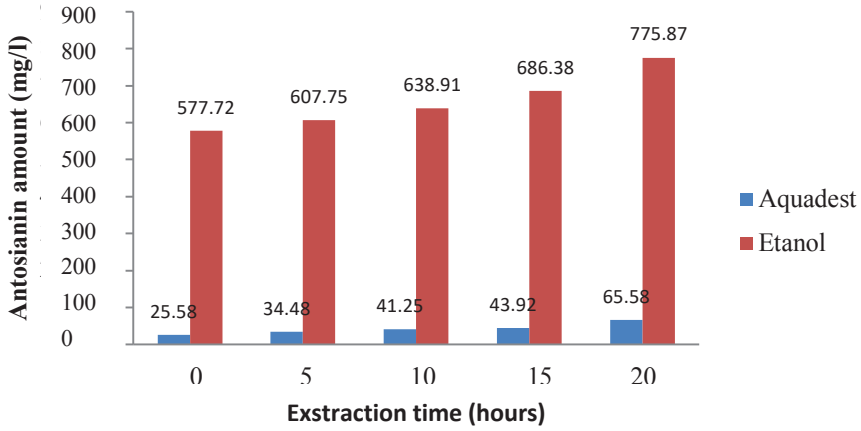

Figure 3. Comparison of anthocyanin amount between aquadest and ethanol solution.

4. Conclusion

Based on the results of observations on the effect of solution and maceration time, it shows that the best treatment in extracting anthocyanin of a Purple Corn is to use ethanol solution and 20 hours of maceration time, which produces 577.78 mg/l of anthocyanin.

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