

Analysis of Flavonoids and Alkaloids on Stick Based on Cham Flour (*Sechium edule*)

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Sticks are one type of snack that is very loved by all people, both children and adults. Sticks made from Siamese pumpkin flour (*Sechium edule*) are a healthy food product that may contain flavonoid compounds and alkaloids. This study aims to determine the levels of flavonoids and alkaloids contained in Siamese pumpkin sticks. Siamese pumpkin sticks were extracted by the maceration method using 96% ethanol as a solvent for 1 day and 24 hours. Analysis of flavonoid and alkaloid content was carried out using the UV-Vis spectrophotometer method at a wavelength of 434.5 nm for flavonoid content and a wavelength of 273 nm for alkaloid content. The flavonoid stick content of Siamese pumpkin was $0,335 \pm 0,025$ g/100g, and the alkaloid content was $1,014 \pm 0,038$ g/100g, according to the results of the analysis. The alkaloid content is higher than the flavonoid content in Siamese pumpkin sticks.

Keywords: Siamese pumpkin stick; flavonoids; alkaloid; UV-Vis spectrophotometer

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Siamese pumpkin is one type of plant cultivated by farmers in Indonesia because it is quite easy to care for and can be grown anywhere, such as in the lowlands and highlands. According to data from the Central Statistics Agency for 2019, in Indonesia, Siamese pumpkin production reached 407,963 tons. In general, people use young Siamese pumpkin as a sour vegetable, a lodeh vegetable, and also stir-fried Siamese pumpkin. There are still many who consider Siamese pumpkin not nutritious, even though it contains vitamins A, B, C, and D and has a good fiber content of 6.2 g per 100 g [1].

Siamese pumpkin is a vegetable that is so widely consumed by the wider community, especially in the city of Palu. The price of Siamese pumpkin is quite cheap, so it has always been the choice of the community in meeting their food needs [2]. Apart from being a vegetable, Siamese pumpkin can cure some diseases, so it can be called a medicinal plant. Siamese pumpkin can be used to lower blood pressure, as a diuretic, to treat fever in children, and to treat gout and diabetes mellitus [3].

Siamese pumpkin plants have two types of metabolite compounds, namely, primary and secondary metabolites. Primary metabolites are used by plants for growth, while secondary metabolites do not play a direct role in plant growth [4]. Siamese pumpkin plants contain secondary metabolite compounds such as flavonoids, alkaloids, and saponins [5].

Flavonoids are the most abundant polyphenol compounds in food intake and are found in many grains, vegetables, and fruits, such as Siamese pumpkin. Flavonoids have antioxidant, anti-aging, and anti-carcinogenic properties. In addition, various flavonoid monomers have been actively developed as drugs that can be used in the fight against degenerative diseases and have a positive effect on the quality of human life [6].

Alkaloids are a type of secondary metabolite compound found in alkaline tissues of plants and animals containing nitrogen (N) atoms with a heterocyclic or aromatic circumference structure [7]. The pharmacologically occurring role of alkaloids can treat diarrhea, diabetes, malaria, and act as antimicrobials [8].

Siamese pumpkin, which contains flavonoid compounds and alkaloids, is a potential food commodity to be used as the main ingredient in product development. The development of Siamese pumpkin food products needs to be improved because it is a source of nutrients in food products. One of the things that can be done is the manufacture of sticks. Sticks are a type of snack that is long in shape and made of flour dough mixed with other ingredients that are fried and have a savory taste. Sticks are generally available in a wide variety of flavors, such as cheese, balado, and others. Stick is loved by many people, be they young children or adults [9].

Research on the content of flavonoid compounds and alkaloids in processed Thai pumpkin products is not yet known in detail. Therefore, the author is interested in conducting research on the analysis of flavonoids and alkaloids on sticks based on Siamese pumpkin flour (*Sechium edule*).

EXPERIMENTAL

This research is a type of laboratory experimental research with repetition (duplo) on sticks based on Siamese pumpkin flour. For the analysis of flavonoid and alkaloid levels using the UV-Vis spectrophotometry method. This research was carried out at the FKIP chemistry laboratory and the FMIPA laboratory of Tadulako University, Palu, Central Sulawesi. The sample used in this study was Siamese pumpkin, which was processed by making sticks with the basic ingredients of Siamese pumpkin flour.

This study used tools, namely: a UV-Vis spectrophotometer, a lumpang and pestle, a digital balance, an Erlenmeyer beaker, a measuring cup, a vacuum buchner, a rotary evaporator (RE), a measuring flask of 10 ml, a drip pipette, a rack tube, a test tube, a separator funnel, a vortex, and aluminum foil.

The materials used in this study were: sticks samples based on Siamese pumpkin flour; 96% ethanol; quercetin standard solution; 10% aluminum chloride (AlCl_3); 1 M potassium acetate; Aquades; caffeine standard solution; PO_4 buffer solution; BCG (Bromocresol Green) solution; chloroform; and filter paper.

Data Collection Techniques

Data Collection Techniques in this study with the following details:

Manufacture of Extracts

A sample of Siamese pumpkin sticks was put into a maceration container, and then 96% ethanol was added. Close the maceration container and leave it for 24 hours. It is further filtered, separating the residue from the filtrate. The filtrate obtained is then concentrated with a rotary evaporator and evaporated until a viscous extract is obtained. The extracts obtained are weighed using an analytical balance sheet.

Analysis of Flavonoid Levels

The process of determining flavonoid levels on Siamese pumpkin sticks begins with the creation of a standard quercetin solution to determine its calibration curve. The procedure for making a standard solution of quercetin begins with weighing the standard solution of quercetin by 10 mg, then putting it in a 10 mL measuring flask. Add ethanol to the limit mark (1000 mg/L of the mother liquor). a series of standard

solutions of 1, 2, 3, 4, and 5 mg/L. Pipette up to 1 mL of quercetin standard solution into the test tube and label it, then add 96% ethanol solution up to 1.5 mL, 10% aluminum chloride (AlCl_3) up to 0.1 mL, potassium acetate 1 M up to 0.1 mL, and aquades up to 2.8 mL. After that, it is incubated for 30 minutes. Measure its absorption at the maximum wavelength obtained using a UV-Vis spectrophotometer.

The process of analyzing the flavonoid content begins by weighing a sample of 0.025 grams. Add ethanol to the limit mark (100 mg/L of the mother liquor). Then pipetting the extract sample as much as 1 mL into a test tube and then labeling it, I added a 96% ethanol solution of 1.5 mL, 10% aluminum chloride (AlCl_3) as much as 0.1 mL, 1 M potassium acetate as much as 0.1 mL, and aquade as much as 2.8 mL, after which it was incubated for 30 minutes. Measure its absorption at the maximum wavelength obtained at 434.5 nm using a UV-Vis spectrophotometer.

Analysis of Alkaloid Levels

The process of determining the alkaloid levels on the Siamese pumpkin stick begins with the creation of a standard solution of caffeine to determine its calibration curve. The procedure for making a standard caffeine solution begins with a standard solution of 100 ppm of caffeine being introduced into a measuring flask of 10 mL each and diluted with aqua dilute to the limit mark, resulting in successive concentrations of the standard solution of 1; 3; 6; 9; 12; and 15 ppm. Then the absorbance is measured at the maximum wavelength obtained, which is 273 nm, using a UV-Vis spectrophotometer.

The process of analyzing the alkaloid content begins by inserting 10 mL of sample extract into the separator funnel. Then add 5 mL of PO_4 buffer solution and 5 mL of BCG (bromocresol green) solution. After that, shake the solution for 5 minutes, then let it stand. After that, add 15 mL of chloroform and homogenize the solution for 15 minutes before allowing it to stand until two layers form. Then the bottom layer is taken out and incorporated into the bottle. After that, the solution is concentrated using a rotary evaporator. Then add 10 mL of aquadis to the evaporator flask. Then homogenize the solution for 15 minutes. Then put the solution in the cuvette. Absorbance was measured at a wavelength of 273 nm using a UV-Vis spectrophotometer.

Data Analysis Techniques

The data analysis technique in this study is divided into two parts: flavonoid content analysis and alkaloid level analysis. Use the formula:

$$\text{Flavonoid Levels: } F = \frac{x \cdot V \times 100}{m}$$

Information:

F = Flavonoid levels (mg/100 g)

C = quercetin concentration (mg/L)

V = volume (L)

m = sample

weight (g)

Alkaloid Levels:

$$A = \frac{C \times V \times 100}{m}$$

Information:

A = Alkaloid levels (mg/100 g)

C = caffeine concentration (mg/L)

V = volume (L)

m = sample

weight (g)

RESULTS AND DISCUSSION

Based on research that has been carried out regarding the analysis of flavonoid and alkaloid compound levels on sticks made from Siamese pumpkin flour (*Sechium edule*), the following research results were obtained:

Analysis of Flavonoid Levels

The results obtained from the analysis of flavonoid levels on sticks based on Siamese pumpkin flour using the UV-Vis spectrophotometry method. Flavonoid levels of sticks "made" from Siamese pumpkin flour, namely quercetin, at the stage of determining flavonoid levels using a raw solution as a comparison.

The use of quercetin as a comparison solution is because quercetin is a flavonoid of the flavonol group, which has a ketone group on the C-4 atom and also a hydroxyl group on neighboring atoms C-3 and C-5 [10].

The total flavonoid content of the Siamese pumpkin stick extract was determined using the aluminum chloride method. The formation of a complex between aluminum chloride and the keto group is the principle of determining the level of flavonoids in the aluminum chloride method. AlCl₃ will react with a keto group on C4 and an OH group on C3 or C5 on a flavonol or flavone compound, forming a stable complex compound [11]. As a result, this method can be used to calculate the amount of flavonoids present in the flavonol and flavone groups. The complex formation reaction between AlCl₃ and flavonols can be seen in Figure 1, and the complex formation reaction between AlCl₃ and flavones can be seen in Figure 2 below.

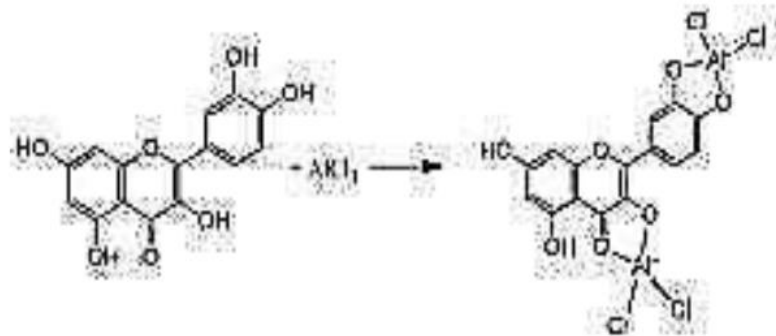


Figure 1. AlCl₃ and Flavonol Complex Formation Reaction [12].

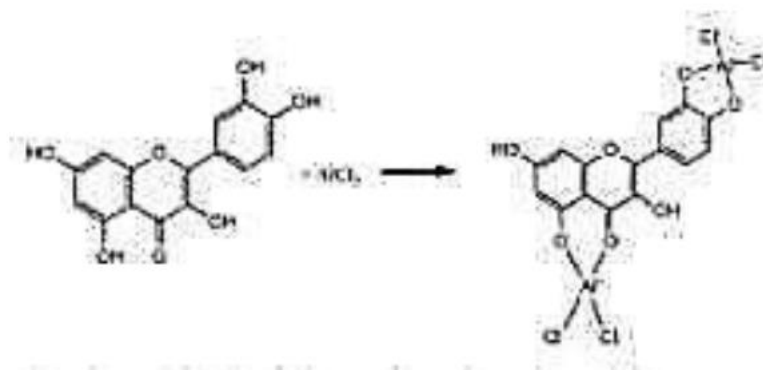


Figure 2. The Complex Formation Reaction of AlCl₃ and Flavones [12].

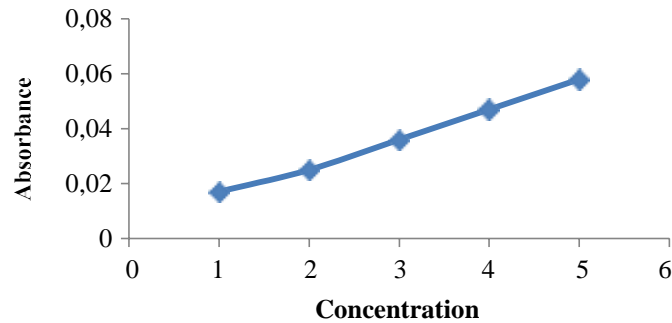


Figure 3. Absorbance of quercetin standard solution.

Table 1. Results of Analysis of Flavonoid Levels on Sticks Based on Siamese Pumpkin Flour and Siamese Pumpkin Flour.

Sample	Flavonoid Levels (Mean±SD) (g/100g)
Siamese Pumpkin Stick	(0.335±0.025)
Siamese Pumpkin Flour*	(0.293±0.022)

Primary data source : *Theresia, OM (2022).

This flavonoid content analysis study used a UV-Vis spectrophotometer at a wavelength of 434.5 nm, which was carried out with two repetitions aimed at improving accuracy in conducting the analysis. The standard curve obtained in this study has the equation $y = 0.0104x + 0.0054$, where y is the absorption and x is the sample concentration, with a square value of the coefficient of correlation (R^2) of 0.9967. Based on the value of the regression coefficient R^2 , which is almost close to 1 and has met the established linearity requirement of 0.99, the relationship between absorbance and concentration becomes very linear and corresponds to the Lambert-Beer law [13].

The results obtained from the flavonoid content test on Siamese pumpkin sticks (*Sechium edule*) can be seen in table 1.

The results of the study of flavonoid content on sticks made from Siamese pumpkin flour when compared to Siamese pumpkin flour obtained different results; the total flavonoid content in Siamese pumpkin flour was 0.293 g/100g while the total flavonoids on Siamese pumpkin sticks were 0.335 g/100 grams.

Flavonoids are actually found in all parts of plants, including leaves, roots, wood, bark, pollen, nectar, flowers, fruits, and seeds. Flavonoids account for about 2% of all carbon photosynthesized by plant tumors [14]. Flavonoids found in Siamese pumpkin

fruit amounted to 0.165±0.022 g/100g [15]. Flavonoids have antioxidant, anti-aging, and anti-carcinogenic properties. In addition, various flavonoid monomers have been actively developed as drugs used in the control of degenerative diseases and have a positive effect on the quality of human life [6].

The results showed that Siamese pumpkin flour processed into Siamese pumpkin stick products experienced an increase in flavonoid levels. The results showed that Siamese pumpkin flour processed into Siamese pumpkin stick products experienced an increase in flavonoid content that was originally present in Siamese pumpkin flour, namely from 0.293 g/100g to 0.335 g/100g.

In this study, flavonoid levels increased when Siamese pumpkin flour was processed into Siamese pumpkin stick products. This can occur because it is influenced by several factors, including the influence of solvents and the extraction method used. This is in accordance with research conducted by Damayanti (2019), who stated that there is an influence of solvent differences on flavonoid content in chickpea extract and obtained flavonoid compound levels of 6,299 mg/g and using water solvents obtained flavonoid compound levels of 4,010 mg/g [16]. Research conducted by Damar et al. (2014) stated that using maceration and sopleation extraction methods in dried lime leaf samples showed the highest total flavonoid levels found in maceration extraction with levels of 6.91 mg/kg [17].

Research conducted by Hayatus Sa'adah (2017) stated that the flavonoid levels contained in the ethanol extract of dayak onion bulbs from the comparison method resulted in a higher maceration extraction method of 1.09% compared to the socleation extraction method of 0.81%. The results of statistical tests show that the extraction method affects flavonoid levels; this is indicated by a sig value of 0.005 smaller than 0.05 with a confidence level of 95%, which means that there is a significant difference between flavonoid levels using the maceration method and socleation extraction [18].

Analysis of Alkaloid Levels

The results obtained from the analysis of alkaloid levels on sticks based on Siamese pumpkin flour using the UV-Vis spectrophotometry method Data on alkaloid levels on sticks made from Siamese pumpkin flour are at the stage of determining flavonoid levels using a standard caffeine solution with the molecular formula $C_8H_{10}N_4O_2$ which is one of the alkaloid compounds from the xanthine group and has a purine core

structure in the form of crystals [19].

To provide optimal results when BCG reacted with alkaloids, total alkaloid levels of Siamese pumpkin stick extract were reacted with pH 4.7 phosphate. The principle of Bromocresol Green (BCG) is the formation of a complex between alkaloids and BCG reagents that will form yellow-colored compounds [20].

This alkaloid content analysis study used a UV-Vis spectrophotometer at a wavelength of 273 nm, which was carried out with two repetitions aimed at improving accuracy in conducting the analysis. The standard curve obtained in this study has the equation: $y \text{ line} = 0.0632x - 0.0064$, where y is the absorption and x is the sample concentration, with a square value of the coefficient of choloration (R^2) of 0.9994. Based on the value of the regression coefficient R^2 , which is almost close to 1 and has met the established linearity requirement of 0.99, the relationship between absorbance and concentration becomes very linear and corresponds to the Lambert-Beer law [13].

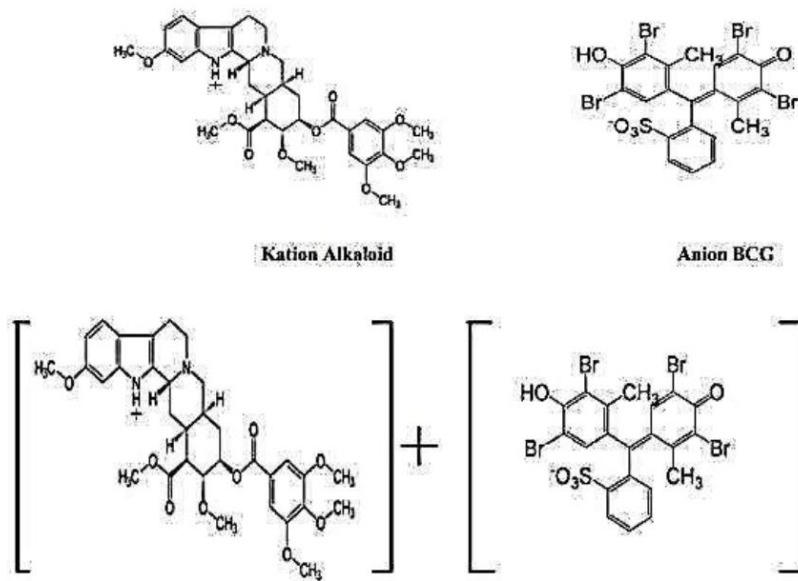


Figure 4. Complex formation reactions between Alkaloids and BCG [21].

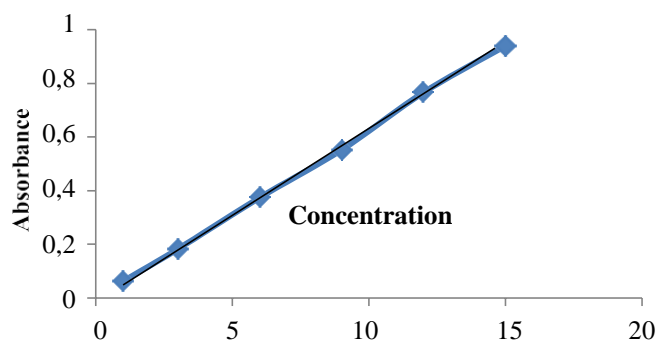


Figure 5. Absorbance of a standard caffeine solution.

Table 2. Results of Analysis of Alkaloid Levels on Sticks Based on Siamese Pumpkin Flour and Siamese Pumpkin Flour

Sample	Alkaloid Levels (Mean±SD) (g/100g)
Siamese Pumpkin Stick	(1.014±0.038)
Siamese Pumpkin Flour	(21.876±0.063)

Primary data source : *Rukmini. (2022).

The results obtained from the alkaloid content test on the Siamese pumpkin stick (*Sechium edule*) can be seen in table 2.

The results of the study on the alkaloid content of the stick made from Siamese pumpkin flour when compared to Siamese pumpkin flour obtained different results; the total alkaloid content in Siamese pumpkin flour was 21,876 g/100 g, while the total alkaloids on the Siamese pumpkin stick were 1,014 g/100 g.

Alkaloids are one of the largest classes of organic compounds found in nature. Almost all alkaloid compounds are of plant origin and are widespread in various types of plants. All alkaloids contain at least one nitrogen atom, which is usually alkaline, and most of these nitrogen atoms are part of the heterocyclic ring [14]. The alkaloids found in Siamese pumpkin fruit amounted to 20,969 g/100g [22]. The pharmacologically occurring role of alkaloids can treat diarrhea, diabetes, malaria, and act as antimicrobials [8].

The results showed that Siamese pumpkin flour processed into Siamese pumpkin stick products experienced a decrease in alkaloid levels. The results showed that Siamese pumpkin flour processed into Siamese pumpkin stick products experienced a decrease in the alkaloid content that was originally present in Siamese pumpkin flour, namely from 21,876 g/100 g to 1,014 g/100 g. In the process of processing Siamese pumpkin into flour, which is then processed into stick products, it can reduce or eliminate secondary metabolite compounds such as alkaloids. Conventional processing, such as heating, can lower alkaloid levels on Siamese pumpkin sticks. This is in accordance with research that has been carried out by Iman Mukhaimin (2018), where an increase in temperature results in a decrease in total alkaloid levels from 0.02981 mg/g at 40 watts to 0.00246 mg/g at 280 watts. This is because the extraction temperature of 67°C, which is 120 watts, exceeds the alkaloid degradation temperature of papaya flowers. This corresponds to the influence of the extraction time on the extraction temperature, which expresses a decrease in the total alkaloid content when the extraction temperature exceeds 37°C [23].

CONCLUSION

Based on the results of the study, the results of the

analysis of Siamese pumpkin stick flavonoids were 0.335±0.025 g/100g and the results of the analysis of alkaloid levels were 1,014±0.038 g/100g.

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