



Formulation and potential of purple cabbage (*Brassica oleracea L.*) as a nutraceutical product in maintaining cardiovascular health

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Introduction

Purple cabbage (*Brassica oleracea L.*) belongs to the Brassicaceae family which contains lots of vitamin A, B, and C. In the antioxidant potential of cabbage varieties, the highest antioxidant is obtained from purple cabbage (*Brassica oleracea*

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Abstract

Purple cabbage (*Brassica oleracea L.*) is one of the agricultural products that contain anthocyanins which have the potential as antioxidants. The amount of production in Indonesia is still relatively low due to a lack of consumer demand and a lack of innovation in its utilization. So it is necessary to make new innovations, one of which is by processing it into flour to make Almond Crispy which is then given the name "Ceamond Crispy". The reason Almond Crispy is made, because it is easy to process and can be consumed for a long time. This study aims to determine the anthocyanin compounds contained in processed food "Ceamond Crispy". Almond Crispy is made with a ratio of purple cabbage flour and moderate protein flour in 3 formulations namely formulation 1 (1:2), formulation 2 (1:1), and formulation 3 (2:1). This research was conducted by organoleptic test, anthocyanin, nutritional content. Therefore, it can be concluded that the organoleptic test results obtained brown color, taste like chocolate, vanilla aroma, and crunchy texture. The anthocyanin test results obtained positive results in all three formulations. The results of the nutritional content test showed that the 3 formulations contained the same protein, carbohydrates (starch), and carbohydrates (glucose), but did not contain fat. The results of the preference test showed that formulation 3 was the most preferred formulation. Thus, proving that "Ceamond Crispy" can be received with a positive response by the community and has the potential to be developed into an innovative food that is beneficial for the heart. Thus, proving that "Ceamond Crispy" is safe for consumption by the public and can be developed into innovative foods that are beneficial for heart health.

Keywords: purple cabbage cookies, nutraceutical, cardiovascular health, anthocyanin

L.), and according to Wahyuni²⁶ the ethanol extract of purple cabbage (*Brassica oleracea L.*) has an IC50 value of 44.64 ppm which shows very strong antioxidant activity.

Purple cabbage (*Brassica oleracea L.*) is one of the agricultural products whose existence is quite well known by the public. The amount of purple cabbage production in Indonesia is relatively low, this is due to the lack of consumer demand for this commodity. The use of purple cabbage in Indonesia is only limited to making salted vegetables and as a mixture in salads.¹ The

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nutritional content of purple cabbage can be seen in **Table 1**.

Purple cabbage is a vegetable rich in anthocyanins. Anthocyanins in red cabbage have a

good level of stability. purple cabbage has an anthocyanin content of 113mg/100g.² Along with the times, many people still do not know the benefits of purple cabbage, one of which is as an antioxidant.

Table 1. Nutritional content in every 100 grams of purple cabbage (*Brassica oleracea L.*).²⁷

Nutrient	Amount
Proteins	1.4 g
Carbohydrate	5.3 g
Fat	0.2 g
Calcium	46 mg
Phosphor	31 mg
Iron	1 mg
Vitamin A	80 IU
Vitamin B1	0.06 mg
Vitamin C	50 mg

Antioxidants are compounds that can withstand, eliminate, and clean up the effects of free radicals which can cause various degenerative diseases such as premature aging, heart disease, stroke, cataracts, osteoarthritis, and cancer. One type of antioxidant that can be found in purple cabbage is anthocyanin.³

Anthocyanins are pigments composed of flavonoids (anthocyanidins) with an aromatic ring in ring B, a C6-C3-C6 cyclic ring, and sugar groups in different positions. Anthocyanins include water-soluble pigments found in epidermal cells in flowers or mesophyll cells in leaves. The types of anthocyanins contained in Purple cabbage are cyanidins with sugar groups either acylated with caffeic, ferulic, synapic acid, and p-coumarate or not.⁴ Many studies have shown that anthocyanins have a positive effect on health. There is also a lot of evidence that anthocyanins are non-toxic but also have positive therapeutic and pharmacological properties.⁵

Anthocyanins have many health benefits, namely as anti-diabetic, anticancer, anti-inflammatory, antimicrobial, anti-obesity and help prevent cardiovascular disease. In the context of

cardiovascular pathology, anthocyanins have been reported to have cardioprotective effects against isoproterenol-induced myocardial infarction damage.³

Cardiovascular disease is one of the non-communicable diseases that causes the number 1 death in the world, which is around 80%. Cardiovascular disease is widely known as a disease with disorders of the heart and blood vessels.⁶ Cardiovascular disease can develop due to hypertension, platelet aggregation, high blood Low-Density Lipoprotein (LDL), and dysfunction of endothelial cells in the blood vessels. Epidemiological studies show that consumption of vegetable and fruit-based foods can increase protection against heart disease due to the presence of bioactive compounds, one of which is anthocyanin. The mechanism of the cardioprotective effect of anthocyanins is by preventing LDL oxidation, protecting the integrity of the endothelial cells lining the blood vessel walls so that damage does not occur, and preventing platelet aggregation. One way that anthocyanins protect endothelial cells is by inhibiting the

induction of tumor necrosis factor alpha (TNF α) which triggers inflammation through monocytes.⁷ Functional or nutraceutical foods are natural ingredients that are processed into food products, so they have the potential to have a higher health value than nutritious foods in general.²⁸ Development of purple cabbage nutraceutical products to become one of the foods suitable for consumption by the public. One of the snacks known by the public is Almond Crispy. Almond Crispy is a variation of cookies or pastries that are flat and have a crunchy texture.⁸

Crispy Almonds were chosen because they are easy to process, namely through the mixing and roasting process, the texture resembles biscuits, can be stored in a closed container, and can be consumed for a long time.² This research obtained a cookie product namely Almond Crispy Purple cabbage (*Brassica oleracea L.*) which contains anthocyanin which can maintain heart health. This product was created to innovate purple cabbage (*Brassica oleracea L.*) food products into Crispy Almond products. The utilization of purple cabbage (*Brassica oleracea L.*) here is expected to be a snack that can provide added value and can maintain heart health. Therefore, this study was conducted to determine the presence of anthocyanins contained in purple cabbage Crispy Almonds (*Brassica oleracea L.*) using various comparisons of purple cabbage flour (*Brassica oleracea L.*) and other ingredients by setting the right drying temperature to get the taste and consistency appropriate.

Methods

Research design

The research design used was a qualitative analysis method by observing the presence of anthocyanins and nutritional content (protein, carbohydrates, and fats) in purple cabbage flour (*Brassica oleracea L.*) and "Ceamond Crispy" products.

Time and place

This research was conducted in the Phytochemistry Laboratory, Department of Pharmacy, Faculty of

Medicine and Health Science, Maulana Malik Ibrahim State Islamic University Malang, on 8th December 2022.

Tools and materials

The tools used in the manufacture of this research are a mixer (Miyako HM-620), digital scales (SF 400), sieve, baking sheet, baking paper, spoon, and oven (Hakashima Electric Oven 23 Lt).

The tools used for testing in this study were analytical balances (Ohaus CP 214), oven (Mettler Universal Oven Original UN 30), test tubes (Herma), test tube racks, watch glass, gauze, bunsen, tripod, beaker glass 100 ml (Iwaki), 250 ml beaker glass (Iwaki), stirring rod, 5 ml measuring pipette (Iwaki), 10 ml measuring pipette (Iwaki), pushball, spatula, 100 ml measuring cup (Iwaki), spoon horn, wooden tongs, and a dropper (Pyrex).

The materials used in the manufacture of this research include purple cabbage, egg whites, powdered sugar, salt, vanilla powder, medium protein flour, butter, almonds, and grated cheese. All ingredients are obtained from the cake ingredients shop.

The materials used for testing in this study were NaOH, HCl, Lugol's reagent, Fehling A, Fehling B, CuSO₄, and KHSO₄. All ingredients are obtained from pharmacy stores.

Research methods

Large purple cabbage is cut into small pieces and washed thoroughly with water then drained. Slices of Purple cabbage were dried in an oven (MEMMRT Universal Oven Original UN 30) at 50°C for 24 hours. Then grind it into powder.⁹

The mixing of dry ingredients in the production of "Ceamond Crispy" includes medium protein flour, purple cabbage flour, vanilla powder, and salt, mixed using a mixer (Miyako HM-620) until well blended. The purpose of mixing the dry ingredients is to do it separately first so that the dry ingredients get even conditions so that when stirring is done, the dough will be more even and there will be no accumulation or clumping of the dough.¹⁰

The product "Ceamond Crispy" is made by weighing all the ingredients first. Next, mix the egg whites and sugar using a mixer (Miyako HM-620) until stiff peaks (swell). Sift the dry ingredients that have been mixed and put them into the egg-white mixture in three stages, stirring until evenly distributed. Pour in the melted butter and stir again until smooth. Prepare a baking sheet lined with baking paper. Take enough dough, about ¼ tsp, put it on baking paper, and flatten it with the back of a

spoon to form a circle. Sprinkle with almond chips and grated cheese on top of the dough. In the oven (Hakashima Electric Oven 23 Lt) at 50°C for 1 hour. In the last step, Ceamond Crispy is weighed as much as 20 grams and put into the package. The product formulation can be seen in **Table 2**.

Table 2. Product formulation of “Ceamond Crispy”

Ingredients	F1 (%)	F2 (%)	F3 (%)
Purple Cabbage Flour	20 g	30 g	40 g
Medium protein flour	40 g	30 g	20 g
Egg whites	2	2	2
Fine granulated sugar	50 g	50 g	50 g
Salt	1/8 tsp	1/8 tsp	1/8 tsp
Powdered vanilla	1/4 tsp	1/4 tsp	1/4 tsp
Butter	45 g	45 g	45 g
Almond Chips	qs	qs	qs
Grated cheese	qs	qs	qs

Organoleptic test

Organoleptic is a food ingredient test based on preferences and desires for a product. Organoleptic test, also known as sensory test or sensory test, is a method of testing using the human senses as the main tool for measuring product acceptance.¹¹ The product “Ceamond Crispy” which was tested organoleptic consisted of 3 formulations added with Purple cabbage flour (*Brassica oleracea* L.) according to the formulation of the addition of flour respectively (20 grams, 30 grams and 40 grams). Organoleptic testing of "Ceamond Crispy" includes color, taste, aroma, and texture. The senses used in this test are sight, taste, smell, and touch. Organoleptic testing was carried out

simultaneously with hedonic testing, involving 35 panelists living in the Malang districts.

Hedonic test

The hedonic test is a test in organoleptic sensory analysis which is used to determine the magnitude of the difference in quality between several similar products by providing an assessment or score of certain properties of a product and to determine the level of preference of a product.²⁹ The parameters of the "Ceamond Crispy" hedonic test included color, aroma, taste, texture, and overall. The selected panellists were untrained panellists who were randomly selected according to the age group of 35 panellists who live in Malang district.

Anthocyanin test

The anthocyanin test was carried out to determine the anthocyanin content in purple cabbage and “Almond Crispy” products. The first step, the

sample is put into a test tube, then the sample is heated above Bunsen with a drop of 2M HCl solution for 2 minutes using a temperature of 100 °C, the color of the sample is observed. If the red color in the sample does not change (constant), it indicates a positive anthocyanin. In the second stage, the samples were mixed by adding 2M NaOH drop by drop. If the sample from red turns to blue-green and fades slowly, it indicates that the sample is positive for anthocyanin.¹²

Nutritional content test

Carbohydrate test

1. Put the sample solution into 2 test tubes, 2 mL each.
2. Test tube 1 added 2-3 drops of Lugol's reagent, and a positive reaction for starch shows a blue or purple-black color.
3. In test tube 2, 2 mL of Fehling A and Fehling B solutions are added each, then add 4 drops of 10% NaOH solution. Positive for glucose if there is a brick-red precipitate.

Protein test

1. Make a 2% sample solution in distilled water, then take 1 mL to put it in a test tube.
2. Add 1 mL of 10% NaOH, and 1 mL of 0.1% CuSO₄ solution and shake. The positive reaction of the protein occurs purplish-blue.

Fat test

1. Put 3 drops of the sample solution into the test tube.
2. Add 1 tablespoon of KHSO₄ and then heat it. A positive reaction of grease has the smell of burnt fat with white smoke.

Results

Organoleptic test results

An organoleptic test is used to see how the appearance of the product "Ceamond Crispy" is

assessed by the five senses. Organoleptic results can be seen in **Table 3**.

Table 3. Organoleptic test results for “Ceamond Crispy” products

Parameter	F1	F2	F3
Color	Chocolate	Chocolate	Purplish brown
Flavor	Like chocolate	Like chocolate	Like chocolate and slightly bitter
Aroma	vanilla	vanilla	vanilla
Texture	crispy	crispy	crispy

This "Ceamond Crispy" product has the same texture in the three formulations because in the manufacture it is formed flat and thin. In addition, the aroma is the same because when making the dough, vanilla powder is added. This "Ceamond Crispy" product is made with powdered sugar which makes it taste slightly sweeter than the original taste of purple cabbage flour (*Brassica oleracea L.*). The color of the product “Ceamond Crispy” is influenced by the presence of anthocyanin which is a color pigment in Purple cabbage (*Brassica oleracea L.*) and can act as a natural dye.

Hedonic test results

The hedonic test is a test used to determine panelist preferences for the "Ceamond Crispy" product. The population in this hedonic test were men and women ranging in age from children around 6 years to parents aged 72 years with a total of 35 untrained panellists. The percentage of male panellists was 49% and 51% female. Data on the number of panellists by age and gender can be seen in **Figure 1** and **Figure 2**.

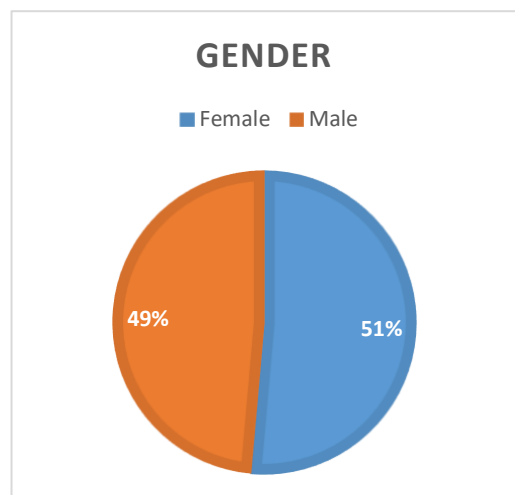


Figure 1. Respondent's gender

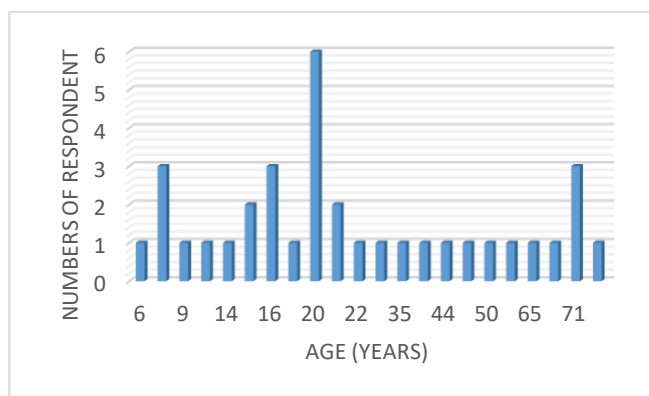


Figure 2. Respondents' ages

The hedonic test was conducted to assess the level of preference for the "Ceamond Crispy" product in the people of Greater Malang with a total of 35 panellists of various ages with hedonic test parameters including texture, color, smell, taste, and an overall assessment of the "Ceamond Crispy" product with a score 1-5 through a questionnaire using the google form media. The hedonic test results are shown in **Table 4**.

Table 4. Hedonic test results of "Almond Crispy"

Question	1	2	3	4	5
Do you like the texture of Crispy Almond products?	0 (0%)	0 (0%)	2 (5.7%)	11 (31.4%)	22 (62.9%)
Do you like the smell of Crispy Almond products?	0 (0%)	5 (14.3%)	14 (40%)	11 (31.4%)	5 (14.3%)
Do you like the color of Crispy Almond products?	0 (0%)	2 (5.7%)	12 (34.3%)	12 (34.3%)	9 (25.7%)
Do you like the taste of Almond Crispy products?	0 (0%)	0 (0%)	0 (0%)	13 (37.1%)	22 (62.9%)

Based on the results of testing the filling out of the questionnaire on question number 1, there were 22 panellists who chose option 5 which means they really like the texture of "Ceamond Crispy", 11

panellists who chose option 4 which means they like it, and 2 panellists who chose option 3 which means neutral. The results of test number 2 on the questionnaire, 5 panellists chose option number 2

which means dislike, 14 panellists chose option 3 which means neutral, 11 panellists chose option number 4 which means like, and 5 panellists chose number 5 which means really like the smell of "Ceamond Crispy" product. The results of test number 3 on the questionnaire, 2 panellists chose option 2 which means they don't like it, 12 panellists chose option 3 which is neutral, 12 panellists chose option 4 which means they like it, and 9 panellists chose option 5 which means they really like the smell of the product "Ceamond Crispy". The results of test number 4 on the questionnaire, 13 panellists who chose option 3, namely neutral, and 22 panellists who chose option 5, which means they really like the taste of the "Ceamond Crispy" product.

Processed product of Red Cabbage (*Brassica oleracea* L.) "Ceamond Crispy" has good properties in terms of texture, smell, color and taste so that the product can be accepted and enjoyed by people of all ages. Based on the questionnaire that has been given, the community tends to prefer formula 3 with a percentage of 54% (Figure 3).

Table 7. Anthocyanin test results

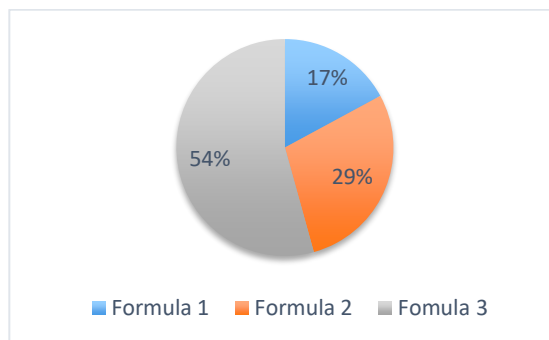


Figure 3. Respondent's formula preference

This proves that the product "Ceamond Crispy" is received with a positive response by the public and can be developed into an innovative food that is beneficial for cardiovascular health.

Anthocyanin test results

This test was carried out to identify anthocyanin compounds in the form of a color test using 2M NaOH and 2M HCl reagents. The results of the anthocyanin test on purple cabbage Flour and "Ceamond Crispy" Products can be seen in Table 7.

Sample	Results		Color	
	2M HCl	2M NaOH	2M HCl	2M NaOH
Purple Cabbage Flour	Positive (+)	Red Green Blue	Red Green Blue	Blue-green
F1	Positive (+)	Red Green Blue	Red Green Blue	Blue-green
F2	Positive (+)	Red Green Blue	Red Green Blue	Blue-green
F3	Positive (+)	Red Green Blue	Red Green Blue	Blue-green

This test was conducted to determine the presence of anthocyanins in purple cabbage Flour (*Brassica oleracea L.*) and the product “*Ceamond Crispy*”. The results of the anthocyanin test on all samples were positive (+) indicated when the sample solution was dropped with 2M HCl it produced a constant red color and when 2M NaOH was dropped the red color changed to blue-green and faded slowly. These results indicate the characteristics of anthocyanins, where the color change is caused by changes in the anthocyanin structure caused by the influence of H⁺ and OH⁻ ions.¹³

Anthocyanins belong to phenolic substances which are widely found in plants and give them blue, red, or purple colors.³ One of the factors that affect the color of anthocyanin is a change in pH.⁴ In acidic media, anthocyanins are red, purple in neutral solutions, and green-blue in alkaline solutions.³ Apart from changes in pH, pigment concentration, presence of mixtures with other compounds, the number of hydroxy and methoxy groups also affect the color of anthocyanins. The dominant hydroxy group causes the color to tend to be blue and is relatively unstable, while the dominant methoxy group causes a red color and is relatively more stable.¹⁴

Purple cabbage (*Brassica oleracea L.*) anthocyanins are preferred because of their good solubility and easy extraction.⁵ Anthocyanin is a natural pigment in purple cabbage which is water soluble and has antioxidant properties.¹⁵ The antioxidant activity of purple cabbage (*Brassica oleracea L.*) extract powder was 84.69%. These results are to the research of Karoui et al. (30) which stated that the antioxidant activity of purple cabbage extract powder ranged from 80-93.89%. The antioxidant activity of purple cabbage (*Brassica oleracea L.*) extract powder can be derived from phenolic compounds, α -tocopherol, ascorbic acid, and carotenoids present in purple cabbage. In addition, the anthocyanins in purple cabbage are also quite high, ranging from 40.53-76.16 mg/100 g of purple cabbage (*Brassica oleracea L.*).³

Anthocyanin is a plant pigment that has a wide range of colors, namely purple, blue, orange, and red to slightly greenish blue.¹⁶ Anthocyanins can be

used as alternative dyes in food and medicinal products.¹⁷ Anthocyanin stability is strongly influenced by temperature, both during processing and storage. The higher the temperature, the greater the possibility of anthocyanin color degradation.¹⁸ Much evidence shows that anthocyanins are not only non-toxic but also have positive pharmacological and therapeutic properties. Therefore, this pigment can be consumed without showing negative effects on health.¹⁹

Nutritional content test results

The nutritional content test was carried out to determine the levels of protein, carbohydrates, and fat in the product “*Ceamond Crispy*” Formula 1, Formula 2, and Formula 3. The results can be seen in **Table 8**.

Carbohydrate test results

Carbohydrates are the main source of calories for almost the entire population in the world. Although the number of calories that can be produced by 1 gram of carbohydrates is only 4 kcal when compared to protein and fat, carbohydrates are a cheap source of calories. In the body, carbohydrates are useful for preventing ketosis, excessive breakdown of body protein, and loss of minerals, and are useful for helping fat and protein metabolism.²⁰

The carbohydrate test was carried out using two test tubes for each formulation, where the Lugol test was carried out in the first tube and the Fehling test in the second tube. Lugol's test or iodine test is a qualitative test of carbohydrates to determine the presence of complex carbohydrates. The iodine test is one of the test methods used to differentiate polysaccharides from disaccharides and monosaccharides. The color change of the solution occurs because, in the starch solution, there are glucose units that form. The principle of the iodine test is that starch or starch which reacts with iodine will form a blue color, dextrin will form a purplish-red color, and glycogen will form a brownish-red color.²¹ This research was conducted by dripping 2 ml of the sample with 2-3 drops of Lugol's solution, samples containing complex carbohydrates will

cause a discoloration to blue or dark purple. The Lugol carbohydrate test experiment using “*Ceamond Crispy*” showed positive test results for each formulation marked by the formation of a purple color.

Fehling’s test is used to test the reduced sugar content in a sample. This test is based on the presence of a free aldehyde or ketone group. Fehling’s reagent is divided into two, namely Fehling A (copper (II) sulfate) and Fehling B (KOH and sodium potassium tartrate). Fehling’s test is used to show the special properties of carbohydrates in the presence of reducing

carbohydrates. The test results show that glucose and sucrose are sugars that can reduce Fehling’s solution and as reducing carbohydrates. Fehling’s reagent plus carbohydrates are then heated, then a brick-red precipitate will form in the final result.²² In this “*Ceamond Crispy*” carbohydrate test experiment, positive test results were indicated for each formulation marked by the formation of a brick red precipitate.

Table 8. The nutritional content of “*Ceamond Crispy*” products test results

Sample	Test	Smell	Color	Result
F1	Proteins	-	Purple	+
	Carbohydrates (starch)	-	Purple	+
	Carbohydrates (glucose)	-	Brick Red Precipitation	+
	Fat	-	-	-
F2	Proteins	-	Purple	+
	Carbohydrates (starch)	-	Purple	+
	Carbohydrates (glucose)	-	Brick Red Precipitation	+
	Fat	-	-	-
F3	Proteins	-	Purple	+
	Carbohydrates (starch)	-	Purple	+
	Carbohydrates (glucose)	-	Brick Red Precipitation	+
	Fat	-	-	-

Protein test results

Proteins are polymers of amino acid monomers connected by peptide bonds. A peptide bond is formed between the carboxyl groups or the amino groups of adjacent amino acids. Protein is needed for growth and an ideal protein must have an amino acid arrangement that suits the needs of humans and animals.²³

Qualitative protein analysis is an analysis that aims to determine the presence or absence of protein in a food ingredient. In the protein content test study using copper sulfate (CuSO₄) reagent which produces Cu²⁺ ions that in alkaline conditions will react with polypeptides or peptide bonds that form proteins to form purple complex compounds and use sodium hydroxide (NaOH) as a base medium.²⁴ In this “*Ceamond Crispy*” protein content test experiment, positive test results were

indicated by the dormation of purple color in each “*Ceamond Crispy*” product formulation.

Fat test results

Fats and oils are triglycerides or triacylglycerols, both of which mean “triesters (of) glycerol”. The difference between a fat and an oil is arbitrary: at room temperature fats are solid and oils are liquid. Most of the glyceride in animals is in the form of fat, while the glyceride in plants tends to be oil. Fats are classified based on the saturation of bonds in their fatty acids. The classification is saturated and unsaturated fatty acids. Fats contain saturated fatty acids, namely fatty acids that do not have double bonds.²⁵ Due to the relationship between intake of saturated fatty acids and HDL cholesterol levels in CHD patients, this study uses very little fat so that it does not affect heart health.

As for this study, 3 drops of the solution were put into a test tube, the sample was added with 1 spoon of KHSO₄, and samples containing fat would smell burnt fat and cause white smoke. The fat test experiment using "*Ceamond Crispy*," showed negative test results for each formulation, which was indicated by the absence of a burnt fat odor and the absence of white smoke appearing in each formulation.

The addition of purple cabbage extract powder to the "*Ceamond Crispy*" product causes the fat content of the product to tend to decrease with increasing concentration of the extract powder. This could be because the purple cabbage extract powder does not contain fat, so the addition of purple cabbage extract powder to the "*Ceamond Crispy*" dough will cause the weight of the dough to increase. However, this does not increase the fat content of the product, so the "*Ceamond Crispy*" fat content will be lower with the addition of purple cabbage extract powder.³

Conclusions

Based on the research that has been done, it can be concluded that the results of laboratory tests showed that the *Ceamond Crispy* product positively contained anthocyanins, protein, carbohydrates, and negative results for fat. The product "*Ceamond Crispy*" has a taste like chocolate on F1, F2 and a slightly bitter chocolate taste on F3, and smells like vanilla. The product "*Ceamond Crispy*" is brown on F1, F2 and purplish brown on F3, and has a crunchy texture. Based on the results of the preference test conducted on 35 panellists for each formula. The most preferred "*Ceamond Crispy*" product is Formula 3 with a percentage of 54%. The results of the panelist questionnaire stated that they liked the product "*Ceamond Crispy*". This proves that the product "*Ceamond Crispy*" is safe for consumption by people of all ages and has the potential to be a healthy food for cardiovascular health.

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Conflict of Interest

All authors declare there was no conflict of interest regarding this research.

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