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Optimization of Temperature and Extraction Time for the Nutritional And Phytochemical Contents of Corn Bran With Ultrasonic-Method

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A B S T R A C T

One of the natural antioxidants sources that have the potential to be utilized as raw material for functional food is corn bran. Corn bran is a by-product of the milling process of corn that is gaining attention as a functional food is increasing in recent years. This study aimed to optimize temperature and extraction time for the nutritional and phytochemical contents of corn bran with ultrasonic-method. Optimized Custom Design was applied to investigate the effect of experimental factors on the nutritional and phytochemical contents. This study used Randomized Block Design (RBD) arranged in-factorial with 3 treatments, namely temperature and time of extraction. Temperature: A1=50^{0}C, A2=55^{0}C, A3=60^{0}C, and A4=65^{0}C, and time: B1=10 minutes, B2=15 minutes, B3=20 minutes, and B4=25 minutes. The data obtained were analyzed statistically using ANOVA with a significance level of 95% and then processed with Software DX13.0 ® Program. The results of the research show the formula of the experiment which is optimal at a temperature of 50^{0}C and 10 minutes. In this condition, the result is the yield at 38.34%, nutritional contents (water at 9.17%; ash at 0.33%; fat at 1.33%, protein at 4.40%, carbohydrates at 85.47%; and crude fiber at 1.88%. produce yield 38.34%, and phytochemical contents (total phenols at 1778.07 µg GAE/g. flavonoids at 92.11 µg GAE/g, vitamin C at 5.84 mg, antioxidant activity at 43.33%, and tannins at 0.11%). This study implies that there is an increase in added value from the conversion of corn bran into nutrient-rich products and has a promising phytochemical content.

1. INTRODUCTION

1.1. Research Background

Zea comes from ancient Greek and is a generic name for cereal and grains. Corn (Zea mays L.) is the world's third-leading cereal crop, after wheat and rice [1]. Corn (Zea mays L.) is a potential source of carbohydrates like flour and corn starch. The demand for corn is constantly increasing for food, fuel, and feed [2]. Corn is a plant that is resistant to and adaptable to environmental conditions. The growth and development of corn depend on the ecological characteristics of the external conditions, the longevity of the species, water demand, soil temperature, and air temperature, light, and nutrient supply [3].

Rice bran is a by-product of the milling of rice paddy, which is a brown fine powder from the outermost layer of the cracked rice paddy [1]. The results of the rice milling are to produce rice (70%), rice bran (8%), and embryo (2%) [2]. Corn bran is a mixture of the aleurone layer and pericarp that regardless in the process of grinding corn. The process of grinding and corn milling will produce 16 to 28% husks (hulls), 6-11% bran, 2-4% polish, and about 60 percent of the endosperm. Corn bran is also rich in nutrients. As the nutritional content consists water at 1%; calories at 356; protein at 9%; fat at 8.5%; carbohydrate at 64.5%; Ca at 200 mg; Fe at 10 mg; P at 500 mg; vitamin A at 51 mg; vitamin B at 1.2 mg, and vitamin C at 89% [3]. During this time, they are used as cattle feed because not many people know the health benefits. When the level of rice bran is about 10% [4], then the potential of rice bran that can be utilized is about 347 tons. This amount is quite large and has the potential to be used as one of the raw materials of the food industry. Bran is reported to contain some phenolic compounds, antioxidants, and is rich in dietary fiber, vitamins, and minerals [5], [6]. In addition, it is a source of dietary fiber, especially do not dissolve, contribute to the increased levels of iron and zinc, as a product of low-calorie and low-phytic, negative effecton the bioavailability of iron in rats, and can be used for the enrichment of food products [7]. Some research on the functionality of bran for health, among others: anticancer, antihypocholesterolemic, and antiatherogenic [5], [8]–[11].

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1.2. Literature Review

Bioactive components contained in a material can be obtained by the method of extraction. There are various extraction methods, namely maceration, percolation, ultrasonic, and soxhlet. The extraction method used in this research is the method of extraction with ultrasonic waves. The method of extraction with the use of ultrasonic waves that acoustic waves with a frequency greater than 16 kHz. The method of extraction with ultrasonic waves is known to have advantages compared with the method of maceration. One of the advantages of the method of extraction ultrasonic is the speed of extraction, compared with extraction by thermal or conventional. The method of extraction with the ultrasonic waves is safer, shorter, and increases the yield of coarse [12], [13]. Factors that can affect the extraction temperature and the extraction time used. Research reported an increase in the temperature in the extraction process needs to be considered, extractiontemperature is too high, and the extraction time is too long and exceeds the optimum limit can lead to loss of compounds in solution because of the occurring process of oxidation [14]. Bioactive components such as flavonoids are not resistant to a high temperature above 50ºC, which changes the structure as well as produces an extract that low. Extraction temperature is too low, and extraction time is too short will cause the bioactive components extracted from the material is not the maximum so that the bioactive components obtained low [15]. A study about the influence of temperature and time of extraction of the leaves of the soursop using the ultrasonic that the temperature of 45ºC with a time of 20 minutes gives the best result with the value of the yield, total flavonoids, and high antioxidant activity [15].

One of the solvents that are often used for the extraction of flavonoid compounds is ethanol. Ethanol including a polar solvent is safe, easily available, and environmentally friendly. Ethanol also called ethyl alcohol is a chemical substance that belongs to the class of alcohol [16]. Ethanol has a chemical structure CH_3CH_2OH, has the contents of a volatile, colorless, and are polar so it is used as a solvent for many compounds [17]. According to Ref. [18], several contributing factors affect the rate and quality of the extraction on the components of the bioactive compounds phenol, namely the type of extraction solvent, solvent concentration, particle size, temperature, pH, and extraction time. Meanwhile, according to Ref. [19], the factors that affect the rate of extraction are the surface area, a comparison of solute and solvent, speed, and time of stirring.

1.3. Research Objective

This study aimed to optimize temperature and extraction time for the nutritional and phytochemical content of corn bran with ultrasonic-method.

2. MATERIALS AND METHODS

2.1. Materials

The raw material in this study is corn bran varieties NK212, age 120 days, obtained from corn farmers in Mranggen, Demak Regency, Central Java, Indonesia, and the chemical materials used to test the nutritional and phytochemical content obtained from the Laboratory of Chemistry and Biochemistry the Department of Agricultural Technology, Semarang University, Indonesia.

2.2. Equipment

The tools used for producing corn barn powder include a knife, pan, blender, dry (National PBL-104), cabinet dryer automatic (OVG-12), sieve 60 mesh (ATE-126, 0.250 mm), thermometer, plastic, and brush. The tools used for the extraction of corn bran powder is the ultrasonic vibrating device (Cole Palmer/CPX 130), stirrer glassware (Pyrex), smooth filter paper, rotary evaporator (Buchi B-490), freezer, vacuum filter (Butchi V500), shaker water bath, analytical balance, pipette drops, dark bottle, nitrogen spray equipment, and aluminum foil. The tools used for the analysis include the stative, oven electric, pH meter, analytical balance, color reader, becker glass, petri dish, desiccator, suction ball, pipette, lamp, thermometers, racks, aluminum foil, wooden tube, cuvette, spectrophotometer UV-Vis, Folin-Ciocalteu colorimetric, centrifuge, GC-MS, and some glassware for analysis.

2.3. Preparation of Materials

Fresh corn bran washed with distilled water, dried by ovenat a temperature of 60ºC for 24 hours [20] the final water content is 10 %, pounded into powder using a grinder, then vacuum packaged and stored <-20ºC until it is used for analysis.

2.4. Extraction of Corn Bran

The powder of corn bran weighed as much as 15 grams with an analytical balance, put into an Erlenmeyer flask. Added ethanol 70% as much as 150 ml (1:10), then extracted with a combination of a temperature of 50ºC, 55ºC, 60ºC, and 65ºC with a time of 10, 15, 20, and 25 min using an ultrasonic bath. Powder bran corn that has been extracted with ultrasonic bath and then filtered using Whatman paper no.1. The filtrate obtained was then performed evaporation. Evaporation is done by a rotary vacuum evaporator with a pressure of 100 bar, a temperature of 40ºC, and 100 rounds rpm [21] that have been modified). The condensed extract obtained was weighed and calculated the yield of the extract was then placed in a bottle, for further analysis.

2.5. Analysis

Analysis of the nutritional and phytochemical contents carried out on water content and ash using the oven method [22], the protein content using the method of Micro-Kjeldahl [22], the fat content using Soxhlet method [22], and the carbohydrate content using carbohydrate by difference method [22], and the crude fibers [22], as well as the phytochemicals contents (total phenol [23], total flavonoids [24], vitamin C [25], antioxidant activity[23], tannins [24], and yield.

2.6. Experimental Design

The experimental design used in this study is a Randomized Block Design (RBD) factorial with 3 replications. The treatment used is the temperature of extraction (A1=50ºC, A2=55ºC, A3=60ºC, and A4=65ºC). The time of extraction (B1=10 minutes, B2=15 minutes, B3=20 minutes, and B4=25 minutes). Furthermore, the data obtained were analyzed statistically using ANOVA with a significance level of 95 % then processed with the Software Design Expert 13 ® program with Response

Surface Methodology (RSM) to determine the optimum concentration of the extract powder of corn bran. After a combination of 2 treatments which is the temperature and the time of extraction were shown in Table 1.

Table 1. Independent variables

Component	Independent variable	Category	Minimal	Maximal
X1	Temperature	50ºC, 55ºC, 60ºC, and 65ºC	50	65
X2	Time	10, 15, 20, and 25 minutes	10	25

The data are expressed as mean and standard deviation (SD). Furthermore, the data obtained were analyzed statistically using ANOVA with a significance level of 95% and if there is a difference between the treatment was continued with Duncan New Multiple Range Test (DNMRT) at the level of 5%. The results of the research were processed with the software Design's DX 13 ® to determine the optimum concentration of temperature and time extraction from corn bran.

3. RESULT AND DISCUSSION

3.1. Description of nutritional content

Table 2. Description of nutritional content

Treatment	Components						
	Yield (%)	Water (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Crude fibers (%)
A1B1	38.40 ±0.46	9.77 ±0.10	0.33 ±0.00	1.38 ±0.02	4.41 ±0.05	84.16 ±0.17	1.88 ±0.02
A1B2	37.88 ±0.26	7.47 ±0.52	0.32 ±0.00	1.40 ±0.01	4.35 ±0.03	86.46 ±0.47	1.84 ±0.01
A1B3	37.73 ±0.45	6.97 ±0.13	0.32 ±0.00	1.44 ±0.02	4.33 ±0.05	86.95 ±0.20	1.85 ±0.02
A1B4	37.07 ±0.06	6.23 ±0.16	0.30 ±0.00	1.46 ±0.00	4.26 ±0.01	87.75 ±0.17	1.85 ±0.00
A2B1	37.07 ±0.44	7.34 ±0.38	0.32 ±0.00	1.25 ±0.01	4.18 ±0.05	86.92 ±0.31	1.82 ±0.02
A2B2	36.29 ±0.25	5.91 ±0.18	0.31 ±0.00	1.35 ±0.01	4.09 ±0.03	88.35 ±0.15	1.75 ±0.01
A2B3	35.90 ±0.43	5.68 ±0.31	0.30 ±0.00	1.42 ±0.02	4.04 ±0.05	88.57 ±0.24	1.74 ±0.02
A2B4	34.76 ±0.05	5.26 ±0.17	0.28 ±0.00	1.44 ±0.00	3.92 ±0.01	89.11 ±0.18	1.70 ±0.00
A3B1	35.14 ±0.42	7.22 ±0.15	0.31 ±0.00	1.25 ±0.01	4.04 ±0.05	87.18 ±0.21	1.80 ±0.02
A3B2	34.64 ±0.24	5.53 ±0.08	0.31 ±0.00	1.32 ±0.01	3.98 ±0.03	88.87 ±0.04	1.74 ±0.01
A3B3	33.98 ±0.41	5.35 ±0.22	0.30 ±0.00	1.37 ±0.02	3.90 ±0.05	89.07 ±0.16	1.76 ±0.02
A3B4	32.81 ±0.05	4.54 ±0.06	0.28 ±0.00	1.41 ±0.00	3.77 ±0.01	90.01 ±0.07	1.72 ±0.00
A4B1	30.83 ±0.37	5.51 ±0.11	0.31 ±0.00	1.20 ±0.01	3.44 ±0.04	89.55 ±0.16	1.78 ±0.02
A4B2	30.32 ±0.21	5.10 ±0.23	0.30 ±0.00	1.25 ±0.01	3.40 ±0.02	89.96 ±0.20	1.75 ±0.01
A4B3	29.75 ±0.35	4.45 ±0.48	0.29 ±0.00	1.28 ±0.02	3.33 ±0.04	90.65 ±0.43	1.72 ±0.02
A4B4	28.17 ±0.04	4.51 ±0.12	0.26 ±0.00	1.29 ±0.00	3.14 ±0.01	90.80 ±0.12	1.63 ±0.00

Description:
Temperatur: A1 (50ºC), A2 (55ºC), A3 (60^0C), A4 (65^0C)
Time: B1 (10 minutes), B2 (15 minutes), B3 (20 minutes), B4 (25 minutes)

3.1.1 Yield

Fig. 1 the contour plot of the yield of a dark blue color indicates the response of the lowest yield at 28.17% and the dark red color shows the response of the highest yield at 38.40%. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of yield. Table 2 shows that the yield obtained in A4B4 (65°C and the temperature-time 25 minutes) at 28.17%, while the highest average soluble fiber is achieved on A1B1 (50°C and the temperature is 10 minutes) at 38.40%.

The lowest average yield was obtained in A4B4 (65°C and the temperature 25 minutes) at 28.17%, while the highest average soluble fiber was achieved on A1B1 (50°C and the temperature is 10 minutes) at 38.40%. The different treatment causes different yield. The difference in the suspected presence of evaporation leads to reduced ability of the yield of corn bran. From Table 2 it is known that the higher the temperature and the longer the extraction time, the yield of the extract produced will be lower. This is by following per under research conducted by [26], [27]. The higher the temperature and the longer the time of extraction, the lower the yield obtained. This is due to too high temperatures with a long time cause more water content evaporated, resulting in yield decrease, and vice versa, the lower the temperature used, then the less water evaporated so it can obtain the high yield [28]–[31].

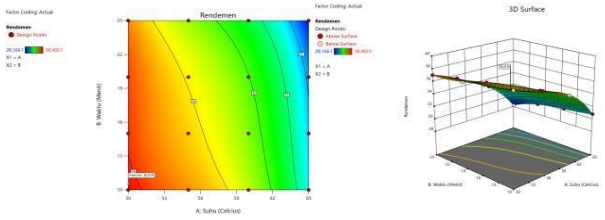

Fig. 1. Contour plot chart and 3D surface yield

3.1.2 Water content

Fig. 2 chart contour plot of the value of the water content of the dark blue color indicates the value of the response of the lowest water content at 4.45% and a dark red color shows the response of the highest water content at 9.77%. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of the water content. Table 2

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shows that, the lowest average of water content obtained on the parameters of A4B4 (65ºC and the temperature-time 25 minutes) at 4.51%, while the highest average of water content is obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 9.77%. Fig. 2 shows that with the high temperature and long extraction time, then the water content in the corn bran will decrease.

The lowest average of water content is obtained on the parameters of A4B4 (65ºC and the temperature-time 25 minutes) at 4.51%, while the highest average of water content is obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 9.77%. Figure 2 shows that the high temperature and long extraction time, then the water content in the corn bran will decrease. These results are by following per with the results of previous research conducted by [32]. According to [33], the longer the cooking time, the more water content will decrease, causing the evaporation of more water, so the water content in the material is vanishingly small. This is due to the higher extraction temperature and the longer the presence of heat in the extraction process, the greater the heat energy that will carry the air, so that the more the amount of mass of water evaporated from the surface of the material [34]. Evaporation is also caused due to the occurrence of the difference in vapor pressure between the water on the material with water vapor in the air. The water vapor pressure on the material, in general, is greater than the vapor pressure of water in the air, resulting in the displacement of the water mass of material into the air [35].

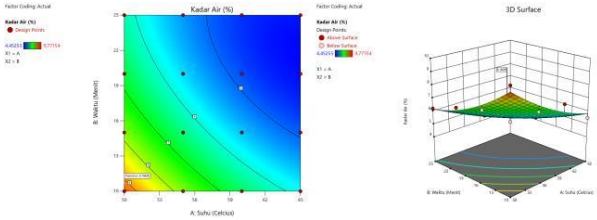

Fig. 2. The chart contour plot and 3D surface water content

3.1.3 *Ash content*

Fig. 3 chart contour plot of the value of the ash content of the dark blue color indicates the value of the response of the lowest ash content at 0.26% and a dark red color shows the response of the highest ash content at 0.33%. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of ash content. The lowest average of ash content was obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 0.26%, while the highest average ash content is obtained in the A1B1 (50°C and the temperature is 10 minutes) at 0.33%. From table 2 it is known that the higher the temperature and the longer the extraction time, the more ash content will decrease.

The lowest average of ash content was obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 0.26%, while the highest average ash content was obtained in the A1B1 (50°C and the temperature is 10 minutes) at 0.33%. From table 2 it is known that the higher the temperature and the longer the extraction time, the more ash content will decrease. According to [36], this is presumably due to the longer and higher the temperature of extraction used will make the lower ash content, due to the higher the water that out of the material surface. The value of ash content in the corn bran can be derived from the content of mineral material [37], [38]. The greater the ash content of a food material showed higher mineral that was conceived by the food [39].

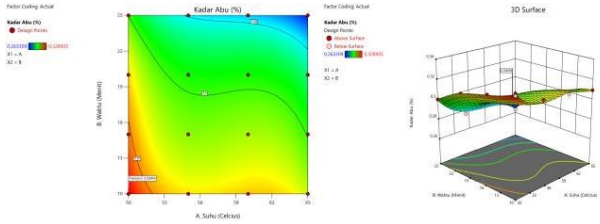

Fig. 3. Contour plot chart and 3D surface ash content

3.1.4 *Fat content*

Fig. 4 chart of the contour plot of the value of the fat content of dark blue color indicates the value of the response of the lowest average of fat content at1.20% and a dark red color shows the response of the highest average of fat content at 1.46% The lines consisting of dots on the chart contour plot shows the combination of these two components with a different number that produces the same response of the fat content. The lowest average of fat content was obtained in A4B1 at 1.20%, while the highest average of fat content was obtained on A1B4 at 1.46%. From Table 2 it can be known that the fat content increased. The lowest average protein content obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 3.14%, while the highest average of protein content is obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 4.41%.

The lowest average of fat content was obtained in A4B1 at 1.20%, while the highest average of fat content was obtained on A1B4 at 1.46%. From Table 2 it can be known that the fat content increased. The fat content will increase directly proportional with the increase of the time and temperature of extraction. This means that the longer the time of extraction with the higher temperature, will cause the contact between the solute and solvent gets long so that the fat content obtained will be higher [40]. Decreased levels of fat in the material allegedly because it contains free fatty acids, in which the free fatty acid has a solubility pretty good in ethanol [41]. Low-fat content can be caused by the high water content so that the fat content is proportionally decreased [42].

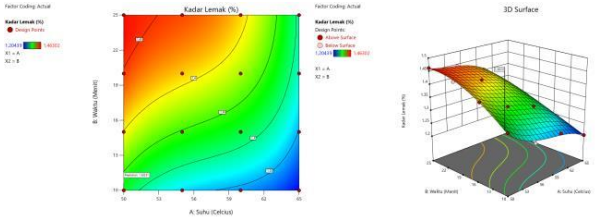

Fig. 4. Contour plot chart and 3D surface fat content

3.1.5 *Protein content*

Fig. 5 chart contour plot of the value of protein content of the dark blue color indicates the value of the response of the lowest average of protein content at 3.14% and a dark red color shows the response of the highest average of protein content at 4.41%. The lines consisting of dots on the chart contour plot show the combination of these two components with the

different amounts that produce the same response of the protein content.

The lowest average protein content obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 3,14%, while the highest average of protein content is obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 4.41%. With the increase of temperature and time extraction, the protein content in corn bran will decrease. This is because the higher the temperature used causes the protein in the extract to decrease. After all, because protein is a nutrient that is easily damaged when it is exposed to high heat [43], [44]. According to [45], the solubility of the protein generally increased when the temperature rises 0-40ºC, and the longer the time of the dissolution, then the more contact between the solute and solvent is getting long, so the solute that is taken will be higher.

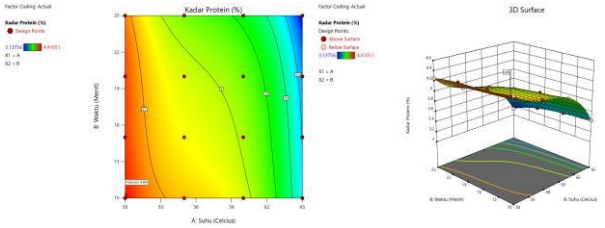

Fig. 5. Contour plot chart and 3D surface protein content

3.1.6 Carbohydrate content

Fig. 6 chart of the contour plot of the value of the carbohydrates content, dark blue color indicates the value of the response of the lowest average of carbohydrate content at 84.16%, and a dark red color shows the response of the highest average of carbohydrate content at 90.80%. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of the carbohydrates content. The lowest average of carbohydrate content was obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 84.16%, while the highest average of carbohydrate content was obtained on A4B4 (65ºC and the temperature-time 25 minutes) at 90.80%.

The lowest average of carbohydrate content was obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 84.16%, while the highest average of carbohydrate content was obtained on A4B4 (65ºC and the temperature-time 25 minutes) at 90.80%. The longer the extraction time, the carbohydrate content will be higher, this is presumably due to the higher the total sugar content in the extract [46]. It is also supported by a statement from [47], the presence of water evaporation during heating, causing the water content to decrease and the concentration of solids will increase. The decline in water content will also make the higher levels of nutrients that are left behind, including carbohydrates [48].

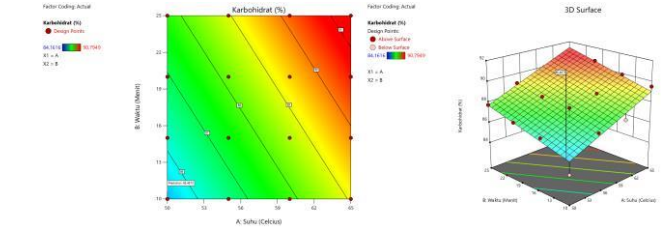

Fig. 6. Contour plot chart and 3D surface carbohydrate content

3.1.7 Crude fibers

Fig. 7 chart contour plot of the value of crude fiber, dark blue color indicates the value of the response of the lowest average of crude fiber at 1.63% and a dark red color shows the response of the highest average of crude fiber at 1.88%. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of the crude fiber. The lowest average of crude fiber obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 1.63%, while the highest average of crude fiber is obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 1.88%. From Table 1. it can be seen that the higher the temperature of extraction and the longer the extraction time, the result in the crude fiber content of the extract will be lower.

The lowest average of crude fiber obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 1.63%, while the highest average of crude fiber is obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 1.88%. From Table 1. it can be seen that the higher the temperature of extraction and the longer the extraction time, the result in the crude fiber content of the extract will be lower. It is alleged due to a temperature of 40ºC and 50ºC enzyme amylase contained in the materials is still actively hydrolyzing carbohydrates, as a result, the content of crude fiber will decrease [49], [50]. A decrease in the content of crude fiber in this study, allegedly due to the breakdown of hemicellulose, resulted in the content of crude fiber because hemicellulose is part of the crude fiber.

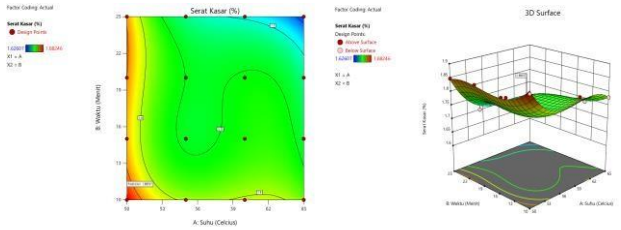

Fig. 7. Contour plot chart and 3D Surface Crude Fibers

3.2. Description of phytochemical content

3.2.1 Total phenol

Fig. 8 chart contour plot of the value of total phenol, dark blue color indicates the value of the response of the lowest average of total phenols at 1043.18 µg GAE/g and a dark red color shows the response of the highest average of total phenol at 1796.94 µg GAE/g. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of total phenol. Table 3 indicated that the lowest average of total phenol obtained in the A1B1 of (50°C and the obtained in A4B4 at 1043.18 µg GAE/g (65°C and the temperature-time 25 minutes), while the highest average of temperature is 10 minutes) at 1796.94 µg GAE/g.

The lowest average of total phenol was obtained in the A1B1 of (50°C and the obtained in A4B4 at 1043.18 µg GAE/g (65°C and the temperature-time 25 minutes), while the highest average of temperature is 10 minutes) at 1796.94 µg GAE/g. From Table 3 it is known that the higher the temperature and the longer the extraction time resulted in the decline of total phenols. This is because after passing a temperature of 45ºC which is the optimal temperature of total phenol for extraction,

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the content of total phenols will decrease [51]. However, at a temperature below 45ºC, when the temperature and extraction time is increasing, it will be able to release phenolic compounds of the cell wall or phenolic compounds that are bound, thus causing more and more compounds to be phenol extracted. The use of high temperatures to perform the extraction increases the solubility of phenol. High temperature can release phenol cell wall or phenolic compound that is bound to that caused by the breakdown of the elements of the cell, thus causing more and more compounds are phenol extracted [52].

Table 3. Description of phytochemical content

Treatment	Components				
	Total phenol (µg GAE/g)	Flavonoid (µg GAE/g)	Vitamin C (mg)	Antioxidant activity (%)	Tannins (%)
A1B1	1796.94±21.42	92.42 ±1.10	5.82 ±0.07	41.96 ±0.50	0.11 ±0.00
A1B2	1686.12±11.51	91.02 ±0.62	5.77 ±0.04	40.81 ±0.28	0.11 ±0.00
A1B3	1642.38±19.58	90.71 ±0.62	5.69 ±0.07	39.20 ±0.47	0.11 ±0.00
A1B4	1617.37 ±2.40	87.98 ±0.13	5.58 ±0.01	37.20 ±0.06	0.10 ±0.00
A2B1	1576.06 ±18.79	88.25 ±1.05	5.77 ±0.07	41.90 ±0.50	0.10 ±0.00
A2B2	1486.95 ±10.15	87.26 ±0.60	5.72 ±0.04	36.15 ±0.25	0.10 ±0.00
A2B3	1444.98 ±17.22	86.85 ±1.04	5.52 ±0.07	34.69 ±0.41	0.10 ±0.00
A2B4	1386.07 ±2.06	85.54 ±0.13	5.29 ±0.01	33.27 ±0.05	0.10 ±0.00
A3B1	1501.35 ±17.90	85.45 ±1.02	5.57 ±0.07	40.43 ±0.48	0.10 ±0.00
A3B2	1378.40 ±9.41	84.63 ±0.58	5.50 ±0.04	34.89 ±0.24	0.10 ±0.00
A3B3	1236.14 ±14.73	83.40 ±0.99	5.47 ±0.07	34.22 ±0.41	0.10 ±0.00
A3B4	1217.32 ±1.81	82.23 ±0.12	5.19 ±0.01	31.37 ±0.05	0.10 ±0.00
A4B1	1288.27 ±15.36	83.45 ±0.99	5.34 ±0.06	37.60 ±0.45	0.10 ±0.00
A4B2	1178.66 ±8.05	80.82 ±0.55	5.30 ±0.04	30.27 ±0.21	0.10 ±0.00
A4B3	1087.72 ± 12.97	80.06 ±0.95	5.14 ±0.06	29.75 ±0.35	0.10 ±0.00
A4B4	1043.18 ±1.55	79.02 ±0.12	5.01 ±0.01	27.18 ±0.04	0.10 ±0.00

Description :
Temperatur: A1 (50ºC), A2 (55ºC), A3 (60ºC), A4 (65ºC)
Time: B1 (10 minutes), B2 (15 minutes), B3 (20 minutes), B4 (25 minutes)

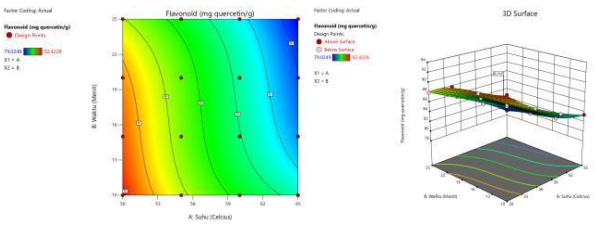

Fig. 8. Contour plot chart and 3D surface total phenol

3.2.2 *Flavonoids*

Fig. 9 chart contour plot of the value of flavonoids dark blue color indicates the value of the lowest average of flavonoids at 79.02 µg GAE/g and a dark red color shows the response of the highest average of flavonoids, which is at 92.42 µg GAE/g. The lines consisting of dots on the chart the contour plot shows the combination of these two components with a different number that produces the same response of the flavonoids. The lowest average of flavonoids was obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 79.02 µg GAE/g, while the highest average of flavonoids was obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 92.42 µg GAE/g.

The lowest average of flavonoids was obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 79.02 µg GAE/g, while the highest average of flavonoids was obtained in the A1B1 (50ºC and the temperature is 10 minutes) at 92.42 µg GAE/g. The value of flavonoids in the extract powder of corn bran is caused by the temperature and time of extraction used. From Table 3 it can be seen that the higher the temperature of extraction and the longer the extraction will make a decline in the content of flavonoids in the extract. The higher the temperature of extraction until it reaches a temperature of 45ºC will produce the higher total flavonoids. However, extraction temperature beyond the optimum temperature which is 45ºC will cause the total flavonoids to decrease, this is due to the occurrence of the process of oxidation of flavonoid compounds [53]. According to [54], flavonoid compounds are not heat resistant and it is easily oxidized at the high temperature. Flavonoids will be degraded at temperatures above 100ºC. Flavonoids are sensitive to heat, because of the hydroxyl groups and ketone, as well as the double bonds of unsaturated [55].

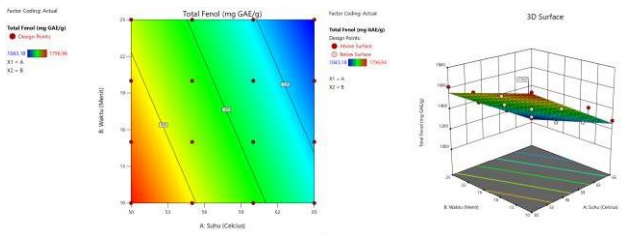

Fig. 9. Contour plot chart and 3D surface flavonoids

3.2.3 *Vitamin C*

Fig. 10 chart contour plot of the value of vitamin C dark blue color indicates the response of the lowest average of vitamin C at 5.01 mg and dark red color shows the response of

the highest average vitamin C, which is at 5.82 mg. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of vitamines C. The lowest average of vitamines C obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 5.01 mg, while the highest average of vitamin C is obtained on A1B1 (50ºC and the temperature is 10 minutes) at 5.82 mg. From Table 3 it can be seen that the higher the temperature of extraction and the longer the extraction time will make the vitamin C in the extract is decreasing.

The lowest average of vitamin C was obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 5.01 mg, while the highest average of vitamin C is obtained on A1B1 (50ºC and the temperature is 10 minutes) at 5.82 mg. From Table 3 it can be seen that the higher the temperature of extraction and the longer the extraction time will make the vitamin C in the extract is decreasing. According to [56], this is because vitamin C is a vitamin that is easily oxidized by heat. Vitamine C is easily damaged when in contact with air (oxidation) especially when it gets heat [57], [58].

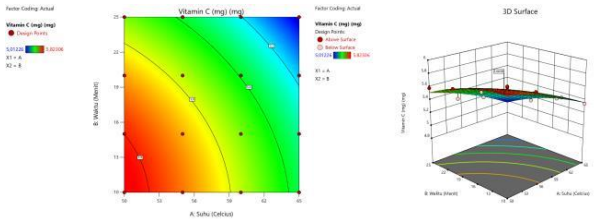

Fig. 10. Contour plot chart and 3D surface vitamin C

3.2.4 Antioxidant activity

Fig. 11 chart of the contour plot of the value of the total antioxidant activity, the dark blue color indicates the response of the lowest average of the total antioxidant activity at 27.18%, and a dark red color shows the response of the highest average of total antioxidant activity 41.96%. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number that produces the same response of the antioxidant activity. The lowest average of antioxidants was obtained in A4B4 (60ºC and the temperature-time 25 minutes) at 27.18%, while the average of the highest average of antioxidant activity was obtained on the A1B1 (50ºC and the temperature is 10 minutes) at 41.96%. It is known that the higher the temperature and the longer the extraction time, the antioxidant activity will decrease (Table 3).

The lowest average of antioxidants was obtained in A4B4 (65ºC and the temperature-time 25 minutes) at 27.18%, while the average of the highest average of antioxidant activity was obtained on the A1B1 (50ºC and the temperature is 10 minutes) of 41.96%. From Table 3 it is known that the higher the temperature and the longer the extraction time, the antioxidant activity will decrease. The antioxidant activity decreased along with the compounds that are categorized as antioxidants, such as phenolic compounds, flavonoids, and tannins. Phenolic and flavonoids have high antioxidant activity. But after reaching the optimum conditions, then the antioxidant activity will be decreased in the harmony with the decline of compounds that are antioxidants [59]. According to [60] stated that the heating process can extract more antioxidant compounds, but the process of excessive heating will damage the antioxidant activity. The value of total phenols and flavonoids in the corn bran showed high antioxidant activity. The values of phenol are influenced by its ability to ward off the free radicals [61]. The configuration and the total hydroxyl group are the very basis that affects the mechanism of its activity as an antioxidant. There is a positive correlation between the antioxidant activity with the content of polyphenolic compounds [62].

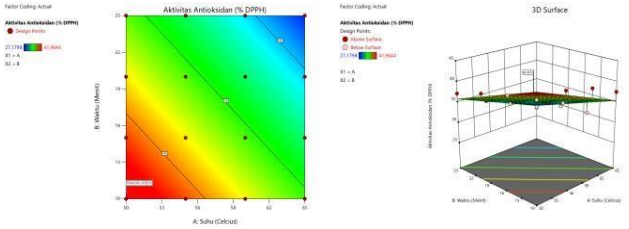

Fig. 11. Contour plot chart and 3D surface antioxidant activity

3.2.5 Tannins

Fig. 12 of chart contour plot of the value of the tannins dark blue color indicates the response of the lowest average of tannins at 0.09% and a dark red color shows the response of the highest average of tannins at 0.11%. The lines consisting of dots on the chart contour plot show the combination of these two components with a different number than the same response of tannins. The lowest average of tannins is obtained in A4B4 (65°C and the temperature-time 25 minutes) at 0.09%, while the highest average of tannins is obtained in the A1B1 (50°C and the temperature is 10 minutes) at 0.11%. From Table 3 it can be seen that the content of tannins decreased.

The lowest average of tannins is obtained in A4B4 (65ºC and the temperature-time 25 minutes) by 0.09%, while the highest average of tannins is obtained in the A1B1 (50ºC and the temperature is 10 minutes) of 0.11%. From Table 3 it can be seen that the content of tannins decreased. The temperature has already reached the optimal point, i.e. the temperature of more than 35ºC, tannins will decline. This is because the tannins are damaged from the hydrolysis process during the extraction process and the heating that takes place continuously. Tannins are not resistant to high heat. On the temperature and time that has reached the optimum point then the tannins will decrease, this is because the diffusion process is already not in progress [63].

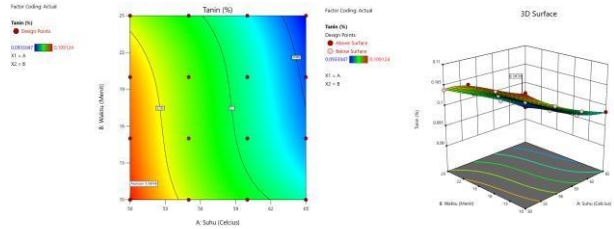

Fig. 12. Contour plot chart and 3D surface tannins

Table 4 shows that the results of the Duncan Test on the difference in the treatment produce the significance on the ANOVA of each of the response levels of water content, ash content, fat content, protein content, carbohydrate, crude fiber, total phenols, flavonoids, vitamin C, antioxidant activity, and tannins in each response no significant difference for each of the factors with a p-value of<0.05 thus there are differences in the

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results of the phytochemical testing as a result of the different treatment, which is temperature and time of extraction.

Table 4 shows that the regression coefficient on each response, there is no significant difference for each of the factors with a p-value of<0.05. The p-value was used to determine the significant presence or absence of each factor. The smaller the p-value, the more significant the price of the coefficient, and the more it contributes to the obtained result. Meanwhile, based on the results of the equations shown, there are no significant differences for all factors with a Predicted R2 value is more than 0.05. The value of the coefficient price of determination (R2) in the model equation was obtained more than 0.05

Table 4. Model analysis for nutritional and phytochemical testing in corn bran

Response	Model	Equation	Significance (p<0.05)	Adjusted R2	Predicted R2
Nutritional Content Yield	Cubic	Y = 35.41 -2.49A - 0.7149B - 0.3060 AB - 1.47A2 - 0.3166B2 + 0.1876A2B - 0.2636AB2 - 1.36A3 - 0.4687B3	< 0.0001	0.9992	0.9972
Water Content	Quadratic	Y = 5.50 - 1.28A - 1.10B + 0.4678AB + 0.4463A2 + 0.5493B2	< 0.0333	0.9180	0.8419
Ash Content	Cubic	Y = 0.3016 + 0.0029A - 0.0128B - 0.0035 AB + 0.0049A2 - 0.0057B2 - 0.0003A2B - 0.0022AB2 - 0.0148A3 - 0.0058B3	< 0.0243	0.9784	0.9092
Fat Content	Cubic	Y = 1.37 -0.0452A + 0.0955B - 0.0124AB - 0.0218A2 - 0.0266B2 - 0.0347A2B - 0.0081AB2 - 0.0349A3 - 0.0047B3	< 0.0037	0.9917	0.9703
Protein Content	Cubic	Y = 4.03 -0.1405A - 0.0848B - 0.0341AB - 0.1760A2 - 0.0410B2 + 0.0220A2B - 0.0389AB2 - 0.3442A3 - 0.0496B3	< 0.0001	0.9993	0.9972
Carbohydrates	Linear	Y = 88.40 + 1.84A + 1.17B	< 0.0001	0.8999	0.8562
Crude Fibers	Cubic	Y = 1.75 + 0.0238A - 0.0018B - 0.0244AB + 0.0384A2 + 0.0016B2 - 0.0018A2B - 0.0259AB2 - 0.0780A3 - 0.0483B3	< 0.0390	0.9280	0.7126
Phytochemical Content	Linear	Y = 1410.49 – 262.34A – 113.06B	< 0.0001	0.9746	0.9663
Total Phenol Flavonoid	Cubic	Y = 85.45 - 4.95A - 0.5254B - 0.0793AB + 0.2632A2 - 0.0565B2 - 0.6959A2B + 0.7383AB2 - 0.3087A3 - 0.9389B3	< 0.0363	0.9927	0.9738
Vitamin C	Quadratic	Y = 5.55 – 0.2540A – 0.1778B – 0.0128AB - 0.0538A2 – 0.0759B2	< 0.0360	0.9524	0.9047
Antioxidant Activity	Linear	Y = 35.68 - 4.06A - 3.86B	< 0.0001	0.8819	0.8392
Tannins	Cubic	Y = 0.1009 -0.0058A - 0.0006B - 0.0001AB + 0.0003A2 - 0.0001B2 - 0.0008A2B + 0.0009AB2 - 0.0004A3 - 0.0011B3	< 0.0363	0.9927	0.9738

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Table 5. Optimized response components, targets, limits, and interests in the formula optimization stage

Response Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Temperature	In Range	50	65	1	1	3
B: Time	In Range	10	25	1	1	3
Yield	Maximize	28.17	38.40	1	1	3
Water	Maximize	4.45	9.77	1	1	5
Ash	Maximize	0.26	0.33	1	1	5
Fat	Maximize	1.20	1.46	1	1	5
Protein	Maximize	3.14	4.41	1	1	5
Carbohydrates	Maximize	84.16	90.80	1	1	5
Crude Fibers	Maximize	1.63	1.88	1	1	5
Total Phenol	Maximize	1043.18	1796.94	1	1	5
Flavonoid	Maximize	79.03	92.42	1	1	5
Vitamin C	Maximize	5.01	5.82	1	1	5
Antioxidant Activity	Maximize	27.18	41.96	1	1	5
Tannins	Maximize	0.09	0.11	1	1	5

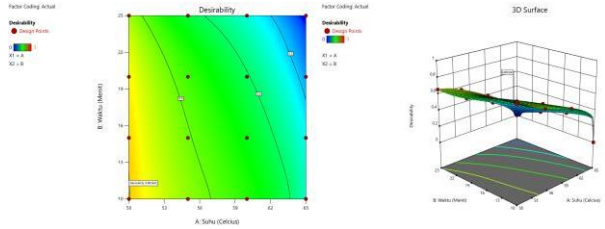

Fig. 13 Contour plot chart and response of optimum surface of corn bran

3.3. Model analysis for chemical and phytochemical testing in corn bran

Table 5 shows the optimized components, their targets, minimum and maximum, and the level of importance at the stage of the optimization formula. Optimization is then carried out by the method of optimal design. This method will optimize the corresponding variable data and the measurement data of the response is entered. The output of stage optimization is some recommended a new formula that is optimal according to the program Design Expert.Optimization is done by determining the limits (goal) criteria, the response is desired with a range that allows it to be achieved. The most optimal formula is the formula with the value of the maximum desirability. The value of desirability is the value function for optimization purposes, which shows the ability of the program to meet the desires based on the criteria outlined in the final product. The value of the desirability that is closer to a value of 1 indicates the ability of the program to produce the more desired product. In addition, the optimization is done after the quadratic mathematical model for each response, and the optimization is done to get the desired response (desirability).

Testing response and phytochemical is the component that is optimized with maximum target with 5 interest rate (+++++). Testing response and phytochemical is the response that determines the efficiency of the process. Time and temperature are optimized with a range of 3 interest rates (+++). The different levels of importance, essentially because of the maximized level of optimization.

Time and temperature will affect the quality of corn bran produced. This condition has a value of high desirability of 0.80 as shown in Figure 13. After the analysis of the model, performed the determination of the optimum temperature and time to produce the optimum conditions for testing and phytochemicals. The value of desirability that is produced is 0.80 (Montgomery, 2001). The optimum solution was obtained at a temperature of 50ºC and 10 minutes. From the optimum solution, it is expected to produce yield at 38.34%, nutritional contents (water at 9.17%, ash at 0.33%, fat at 1.33%. protein at 4.40%, carbohydrate at 85.47%, and crude fiber at 1.88%) and phytochemical contents (total phenols at 1778.07 µg GAE/g. flavonoids at 92.11 µg GAE/g. vitamin C at 5.84 mg, antioxidant activity at 43.33%, and tannins at 0.11%).

4. CONCLUSION

The optimum conditions were obtained on the value of the desirability of 0.80 at a temperature of 50ºC and 10 minutes. From the optimum solution, it is expected to produce yield at 38.34%, nutritional contents (water at 9.17%. ash at 0.33%. fat at 1.332%. protein at 4.40%. carbohydrate at 85.47%. and crude fibers at 1.88%), and phytochemical contents (total phenols at 1778.07 µg GAE/g. flavonoids at 92.11 µg GAE/g. vitamin C at 5.84 mg, antioxidant activity at 43.33%, and tannins at 0.11%).

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