International Journal on Advanced Science Engineering Information Technology

Vol.13 (2023) No. 1
ISSN: 2088-5334

The Effect of Ratio of Methanol and Concentration of Methanol in Corn Silk Extracts with Ultrasonic-assisted Extraction

Haslina [a], Dewi Larasati [a], Iswoyo [a], Ni Luh Suriani [b], Novizar Nazir [c]

[a] *Faculty of Agricultural Technology, Semarang University, Semarang 50196, Central Java, Indonesia*
[b] *Department of Biology, Mathematics and Natural Science Faculty, Udayana University, Bali, Indonesia*
[c] *Faculty of Agricultural Technology, Andalas University, Padang 26123, Indonesia*
Corresponding author: [*]chana_panca@yahoo.com

***Abstract*—Cornsilk refers to stigmas of female maize flowers. Corn silk contains abundant flavonoids. Flavonoids show a variety of biological activities, such as antioxidants. Several methods, such as microwave, ultrasonic, supercritical fluid extraction, and multiple extraction technologies, were used to extract flavonoids from corn silk. The ultrasonic-assisted extraction method (UAE) seems to be very reliable among these methods. UAE has the advantage of simplicity and can be easily implemented with other extraction techniques. Hence, the current study aimed to determine the effect of the ratio (material: methanol) and concentration of methanol corn silk extracts in UAE. The experimental design used was a factorial Randomized Completely Block Design (RCBD) with two factors and three replications for each treatment. The two treatments were: A1=methanol (1:4) (w/v), A2=methanol (1:6) (w/v), A3=methanol (1:8) (w/v) and methanol concentration (60, 70 and 80%). Results showed that the ratio of methanol and concentration had a significant effect ($p<0.05$) on nutritional and phytochemical content in corn silk extracts. The best treatment interaction on A3B3 (1:8 (w/v) and methanol concentration 80% with nutritional content analysis (water 6.38%, ash 1.11%, fat 0.44%, protein 4.70%, carbohydrates 87.23%, and dietary fiber 2.72% and the analysis of phytochemicals content (total phenol 2.91 µg GAE/g, flavonoids 2.32 µg GAE/g, beta carotene 1.07 ppm, vitamins C 12.47 mg, and antioxidant activity 27.08%). The implication of this study is that corn silk extracts could be developed as a source of nutrients and bioactive compounds, and corn silk waste can be converted into value-added products.**

***Keywords*—Corn silk extract; methanol; nutritional; phytochemical; UAE.**

Manuscript received 28 Oct. 2021; revised 3 Jun. 2022; accepted 20 Nov. 2022. Date of publication 28 Feb. 2023.

I. INTRODUCTION

Indonesian people widely used traditional medicines derived from nature because they were cheap, easy, and relatively safe compared to synthetic medicines [1]. This condition triggers research of chemical compounds of secondary metabolites contained in plants, such as in parts of the corn plant that corn silk [2]. Corn silk was a typical substance used in traditional medicine as a laxative and blood pressure-lowering agent [3] containing secondary metabolite compounds such as main, β-carotene, beta-sitosterol, geraniol, hordenine, limonene, menthol, site skin, oil volatile, alkaloid, saponin, tannin, flavonoid, flobotanin, alcohol, terpenoid, glycoside, protein, carbohydrate, fiber, B vitamin, vitamin C, vitamin K, Zn, Ka, Ca, Mg and P, steroids such as stigmasterol, anthocyanin, protocatechin, vanillic acid, derivatives hesperidin, quercetin, chlorogenic acid, and phenolic compounds other [4]–[11]. The content of nutritional and functional properties of corn silk was potential functional food and food ingredient.

According to Nurhanan, Rosli, and Mohsin. [12], extract the antioxidant components of corn silk using methanol and water, with the total polyphenol content in the methanol and water extracts being 272.81 mgGAE/100g and 256.36 mgGAE/100 mg, respectively (dry). The extract of sweet corn silk with the solvent combination (methanol: water) (85:15) was higher than the extract with the solvent combinations (ethanol: water), (acetone: water), and (ethyl acetate: water) [13]. The research that was conducted in the extract of soursop leaves best using the ultrasonic method of long extraction 20 minutes [14]. The results of the research by Sholihah, Ahmad, and Budiastra [15] using an ultrasonic method with the amplitude of 65% yield value yield, can produce the best antioxidant activity and anthocyanin levels.

The weakness of the extraction with the conventional way takes a long time and the high temperature in the extraction and the results of the extracts were low. Ultrasonic-Assisted Extraction was one of the extraction methods available.

According to Keil [16], the ultrasonic method was an effective and efficient method of extraction of non-thermal to improve the liquid's penetration towards the cell wall membrane, support the release of cell components, and increase the transfer of mass. This study aims to determine the effect of the ratio (material: methanol) and concentration of methanol corn silk extracts with the Ultrasonic-Assisted Extraction method (UAE).

II. Materials and Method

A. Material and Tools

Fresh cornsilk varieties were used as raw materials in this study. Bisma was obtained from Pati Regency, Central Java, at the age of 80 days (ripe). Methanol was used as the solvent, and pro-quality analysis was performed (Sigma, or E-Merck). Knife, pan, blender, dry (National PBL-104), cabinet dryer automatic (OVG-12), sieve 60 mesh (ATE-126, 0.250 mm), thermometer, plastic, and brush were used to make the powder corn silk. Corn silk was extracted using ultrasonic horn shakes (Cole Palmer/CPX 130), stirrer glassware (Pyrex), smooth filter paper, a rotary evaporator (Buchi B-490), a freezer, vacuum filter (Butchi V500), a shaker water bath, analytical balance, pipette drops, dark bottle, spray equipment nitrogen, 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, lights, thermometers, racks, aluminum foil, wooden tube, cuvette, spectrophotometer UV-Vis, Folin-Ciocalteu colorimetric, centrifuge, GC - MS, and some glassware for analysis.

B. Research Procedure

Fresh corn silk was sorted, drained, and crushed with a blender to produce corn silk powder and weighed 20 g of corn silk powders. Corn silk powders extract with ratio (material: methanol): 1:4, 1:6, 1:8 w/v, and concentration of methanol: 60%, 70%, 80%, and ultrasonic: 20 minutes, amplitude 65%, temperature 60^{0}C. Furthermore, filtering and separation were carried out using a rotary flash evaporator to produce corn silk powder extracts. Next, each sample was filtered and stored at 4ºC in an amber flask, which was capped and sealed for the next analysis [17].

C. Experimental Design

The design of the experiment used is Factorial Randomized Completely Block Design (RCBD) with two factors and three replications. The treatments are the ratio (material:methanol) consist of A1=(material:methanol) (1:4) (w/v), A2=(material:methanol) (1:6) (w/v), and A3= (material:methanol) (1:8) (w/v) and concentration of methanol, B1=60%, B2=70%, and B3=80%.

The information was presented in the form of an average±standard deviation (SD). Furthermore, the data obtained were analyzed using ANOVA at a significance level of 95%, and if there was a difference between the treatments, the Duncan Multiple Range Test (DMRT) was used at a level of 5%.

D. Parameters

The parameters were observed in corn silk extracts include nutritional content analysis (water, ash, fat, protein, carbohydrates, and dietary fiber) [18] and the analysis of phytochemical content (total phenols [19], total flavonoids [20], beta carotene [21], vitamins C [18], and antioxidant activity [20].

III. Results and Discussions

A. Nutritional Content

Table 1 shows the water content of extracts of corn silk was ranged between 6.38-8.07%. Ash content of corn silk extracts was ranged between 1.07-1.11%. The fat content of the extract of corn silk was ranged between 0.44-0.47%. The protein content of corn silk extracts was ranged between 4.52-4.70%. The carbohydrate content of corn silk extracts was ranged between 84.59-87.23%. Dietary fiber corn silk extracts were ranged between 2.30-2.72%. Table 1 shows p-value of water (p-value 0.001), ash (p-value 0.003), fat (p-value 0.001), carbohydrates (p-value 0.015), and dietary fiber (p-value 0.005). There was a difference between treatments after the DMRT at the level of 5% with a p value<0.05.

TABLE I
THE DESCRIPTION OF NUTRITIONAL CONTENT

Treatment	Nutritional content					
	Water (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Dietary Fiber (%)
A1B1	8.07 ± 0.01	1.07 ± 0.00	0.47 ± 0.00	4.52 ± 0.01	85.39 ± 0.70	2.30 ± 0.00
A1B2	8.06 ± 0.01	1.09 ± 0.00	0.47 ± 0.00	4.53 ± 0.01	85.85 ± 0.13	2.32 ± 0.00
A1B3	7.60 ± 0.13	1.09 ± 0.00	0.46 ± 0.00	4.56 ± 0.01	85.95 ± 0.10	2.34 ± 0.02
A2B1	7.51 ± 0.08	1.09 ± 0.00	0.46 ± 0.00	4.60 ± 0.01	86.12 ± 0.10	2.37 ± 0.00
A2B2	7.41 ± 0.06	1.09 ± 0.01	0.46 ± 0.00	4.61 ± 0.04	86.22 ± 0.28	2.37 ± 0.02
A2B3	6.76 ± 0.01	1.10 ± 0.01	0.45 ± 0.00	4.65 ± 0.01	86.27 ± 0.24	2.51 ± 0.02
A3B1	6.73 ± 0.05	1.10 ± 0.00	0.45 ± 0.00	4.66 ± 0.01	86.28 ± 0.32	2.56 ± 0.00
A3B2	6.68 ± 0.01	1.11 ± 0.01	0.45 ± 0.01	4.67 ± 0.04	87.03 ± 0.03	2.67 ± 0.00
A3B3	6.38 ± 0.05	1.11 ± 0.01	0.44 ± 0.00	4.70 ± 0.04	87.23 ± 0.04	2.72 ± 0.00
p value	0.001	0.003	0.001	0.000	0.015	0.005

Ratio: A1 (material:methanol (1:4)(w/v)), A2 (material:methanol(1:6)(w/v)), A3 (material:methanol(1:8)(w/v)).
The concentration of methanol: B1 (60%), B2 (70%), B3 (80%)

Table 1 shows that the water content of extracts of corn silk was ranged between 6.38-8.07%. The principle of the water content analysis was to know and determine the water content contained in the material [22]. From Table 1, it was known the smaller the ratio (material: methanol) and the concentration of methanol, the water content in the extract would decrease. The decline in water levels correlated with increased levels of total phenol, in which the lower the water content, the content of total phenols higher. The total phenol in an extract decreased along with increased water in the extract [23]. In addition, the water in food material was also closely related to the product's durability. The reduction of water either in cooking or the addition of other materials was to food preservation so that it can be resistant to chemical damage and microbe [24].

Ash content of corn silk extracts ranged between 1.07-1.11%. Ash content was an organic substance from the residual combustion of organic material. The higher the ash content of food material revealed, the more mineral that was conceived in the food [25]. The content of mineral material can explain the high and low levels of ash content in corn silk [26], [27]. Table 1 shows that the concentration of methanol will increase when the ash content in corn silk extract also increases. So, it means the higher the concentration of methanol, the higher the ash content obtained. This is because methanol is a polar compound called a universal solvent because it can extract polar components and nonpolar components, including ash [28]. Ash content contained in corn silk extracts was polar and non-polar. Polar minerals will dissolve insolvent in the liberation of the fat and the extraction process [29]. Based on polarity and solubility, polar substances will easily be soluble in polar solvents, while the substances that were non-polar will easily soluble in the non-polar solvents [30].

Table 1 shows the fat content of the extract of corn silk was ranged between 0.44-0.47%. Fat levels decrease when the ratio (material: methanol) and concentration of methanol increase. From Table 1, it was known that the smaller the number ratio (material: methanol) and the concentration of methanol were added, the fat content in the extract of corn silk will be smaller. This was due to the lower concentration of methanol. Then, the contact between the corn silk and methanol will be smaller so that the fat extracted will be less. This result was consistent with the research findings that methanol was a polar solvent, while the fats were non-polar molecules [31]. Fat reduction is due to the methanol solvent because fats can not be extracted by the methanol, because they have different polarities. From like dissolve-like principle, it is known n that the polar compounds will only dissolve in polar solvents and non-polar compounds will only dissolve in non-polar solvents [32]. The reduction of fat levels in the material is also due to the presence of free fatty acids, which have fairly good solubility in methanol [33]. The water content can cause low-fat content was high enough, so the fat content will proportionally decrease [34].

The protein content of corn silk extracts was ranged between 4.52-4.70% (Table 1). Table 1 shows that the higher the solvent concentration and the ratio of the material: solvent, the higher the protein content. This is because protein is an organic component that can be taken with a universal solvent, which is methanol [35]. Proteins are high molecular weight complex organic compounds that are polymers of amino acid monomers linked to each other by peptide bonds [36]. Protein was a macromolecule polypeptide composed of several L-amino acids and connected beyond a peptide bond [37]. The high and low protein values measured can be influenced by the magnitude of the material's water loss (dehydration) [38]. Methanol as a solvent increases the protein content of corn silk extract. It was claimed that the protein was dissolved due to the presence of methanol in both. Protein in food has a bond complex with other components such as carbohydrates, fats, minerals, and water [39]. However, after removing components of the nonprotein, causing the protein was free, so that was easily measurable at the time of analysis. The protein content in the protein concentrate was increased since most carbohydrates have been dissolved in the methanol solvent.

Table 1 shows that corn silk extracts' carbohydrate content ranged between 84.59-87.23%. Carbohydrates are the main energy source for the human body, and one gram of carbohydrates equals four calories of energy food. The carbohydrate content also has an important role in determining the characteristics of food, such as taste, color, texture, and others [40]. The increase in methanol concentration causes high carbohydrate content. This is because the higher the solvent concentration, the more compound sugar levels contained in the powder corn silk will be extracted [41], [42].

The difference was expected because the extract's total sugar content was increasing [41]. A statement also supported that water evaporation during heating resulted in a decrease in water content and an increase in solids concentration [43]. The drop in water levels will exacerbate the high levels of nutrients left behind, and the increased methanol concentration causes high carbohydrate content. This was because the higher the concentration of the solvent, the more sugar levels contained in powder corn silk will be extracted. The good solvent should have the properties of solubility and selectivity against glycosides that do not react (damaging) the desired compound and can be separated easily after the extraction process [41].

Table 1 shows that dietary fiber corn silk extracts ranged between 2.30-2.72%. Dietary fiber consists of a form of carbohydrate that cannot be digested, and there was usually in the form of polysaccharides derived from plant foods [44]. From the data of Table 1, the smaller the proportion/ratio (material: methanol) and the concentration of methanol added, the higher the fiber content in corn silk extract will be. Dietary fiber can dissolve in polar water [45]. Because it was soluble in a polar solvent, the higher the concentration of methanol added to the extract, the greater the increase in dietary fiber. The higher the concentration of solvent and the ratio of ingredients and solvents, the higher the fiber content in the food. This is because the higher the dietary fiber contained in the extract [46], [47].

B. Phytochemical Content

Table 2 shows that total phenol corn silk extracts ranged between 2.46-2.91 μg GAE/g. The total flavonoids of corn silk extracts ranged between 1.96-2.32 μg GAE/g. Beta carotene content corn silk extracts ranged between 0.96-1.07 ppm. Vitamins C content in corn silk extracts ranged between 10.54-12.47 mg. The antioxidant activity of corn silk extracts ranged between 22.88-27.08%. Table 2 shows p-value of total phenol (p-value 0.005), flavonoid (p-value 0.005), beta carotene (p-value 0.003), vitamins C (p-value 0.000), and antioxidant activity (p-value 0.000). There was a difference between treatments after the DMRT at the level of 5% with a $p\text{-value}<0.05$.

TABLE II
THE DESCRIPTION OF PHYTOCHEMICAL CONTENT

Treatment	Phytochemical content				
	Total Phenol (µg GAE/g)	Flavonoid (µg GAE/g)	Beta Carotene (ppm)	Vitamins C (mg)	Antioxidant activity (%)
A1B1	2.46 ± 0.00	1.96 ± 0.00	0.96 ± 0.01	10.54 ± 0.08	22.88 ± 0.17
A1B2	2.48 ± 0.00	1.98 ± 0.00	0.96 ± 0.00	10.65 ± 0.01	23.12 ± 0.03
A1B3	2.50 ± 0.02	2.00 ± 0.02	0.98 ± 0.01	10.67 ± 0.01	23.17 ± 0.03
A2B1	2.53 ± 0.00	2.02 ± 0.00	0.99 ± 0.00	10.70 ± 0.08	23.22 ± 0.18
A2B2	2.53 ± 0.02	2.02 ± 0.02	1.00 ± 0.00	10.80 ± 0.01	23.45 ± 0.03
A2B3	2.69 ± 0.02	2.14 ± 0.02	1.00 ± 0.01	10.83 ± 0.08	23.51 ± 0.18
A3B1	2.73 ± 0.00	2.18 ± 0.00	1.02 ± 0.00	12.34 ± 0.02	26.79 ± 0.04
A3B2	2.86 ± 0.00	2.28 ± 0.00	1.04 ± 0.00	12.43 ± 0.02	26.98 ± 0.04
A3B3	2.91 ± 0.00	2.32 ± 0.00	1.07 ± 0.00	12.47 ± 0.02	27.08 ± 0.04
p value	0.005	0.005	0.003	0.000	0.000

Ratio: A1 (material:methanol(1:4)(w/v)), A2 (material:methanol(1:6)(w/v)), A3 (material:methanol(1:8)(w/v))
The concentration of methanol: B1 (60%), B2 (70%), B3 (80%)

Table 2 shows total phenol corn silk extracts ranged between 2.46-2.91 µg GAE/g. This shows that the ratio of the solvent was 1:8 (w/v) was more effective in generating the content of phenolic compounds in the extract of corn silk compared to the other treatments. This was likely caused because of the nature of the phenol compound which was soluble in the organic solvent or solvents that were polar, where methanol was an organic solvent with the polar nature that can dissolve with better content of phenol compounds in the material, so that the greater the ratio, the greater the occurrence of contact between the particles of the powder with the solvent, so the phenol will be more extracted. It means the higher the concentration of methanol solvent, the higher the total phenol content in the extract. This result is in line with the previous study conducted by Ref. [48] and [49]. The small ratio of the solvent can be possible to evaporate perfectly during the process of separation of the solvent. However, this does not occur when the ratio is increased. There might have been still solvent in the extract, which could result in the hydrolysis of phenol compounds, so it was not analyzed as phenol compounds; thus, the higher the solvent ratio, the lower the total phenol [50]. It was not by following per the statement from [51] which states that the increase in the total phenol increased with the increase of the ratio of material and solvent. The total phenol obtained in the extract was directly proportional to the amount of methanol added and the concentration of methanol. The possibility of complexes of several phenolic compounds forming in the extracts dissolved in methanol may also contribute to the increase in total phenol. These phenolic compounds have more cluster phenol or have a molecular weight higher than the phenolics in the water extract [52]. The higher the ratio (material: methanol) leads to the average total phenol getting higher and higher. The more solvent that was added, the greater the solvent's ability to dissolve the material, and thus the greater the number of material components that could be extracted by the solvent. Because the material-to-solvent ratio and solvent concentration were both high, the solubility of phenolic compounds in the solvent was increased. With the increasing ratio of material and solvent as well as solvent concentration, the diffusion process can be large, so that the extraction process will also run quickly [53]. Material components that were extracted will continue to increase until the solution becomes saturated, after passing through the saturated solution will not increase the extraction yield with the addition of solvent [54].

Table 2 shows the total flavonoids of corn silk extracts was ranged between 1.96-2.32 µg GAE/g. The ratio of (material: methanol) and the concentration of methanol were responsible for the high and low flavonoid levels in corn silk extracts. Antioxidant compounds include phenolic compounds and total flavonoids. Antioxidant properties of phenolic compounds play an important role in preventing and controlling degenerative diseases [55]. According to Ref. [56], a compound of phenolic or polyphenolic which can be a class of total flavonoids, derivative of saint, coumarin, tocopherol, and polyfunctional acids can serve as an antioxidant compound. The phenolic components can inhibit lipid oxidation by donating hydrogen atoms to free radicals [57], as a result, the solvent can extract more material components. Because of the high material-to-solvent ratio and solvent concentration, phenolic compounds will be more soluble in the solvent. The diffusion process can be expanded as the material/solvent ratio and solvent concentration increase, allowing the extraction process to run more quickly [53]. Material components that were extracted will continue to increase until the solution becomes saturated, after passing through the saturated solution will not increase the extraction yield with the addition of solvent [54]. So it means the higher the concentration of methanol causes the total flavonoid increases. This is by following per the previous study conducted by [58] and [59].

Beta carotene content corn silk extracts were ranged between 0.96-1.07 ppm (Table 2). Carotenoids play an important role in cell protection and can also act as powerful antioxidants [60]. Carotene included in the class of carotenoids can act as antioxidants [61]. Increasing the ratio of the material: methanol and increasing the solvent's concentration will increase the levels of total carotene and it was followed by the value of antioxidant activity IC50, where the smaller the value of the IC50 the greater the activity of the antioxidant [61]. An increase in beta carotene in the extract is due to an increase in the concentration of methanol as a solvent and will cause more beta carotene to be extracted [62].

Table 2 shows vitamins C content corn silk extracts was ranged between 10.54-12.47 mg. Vitamins C also called ascorbic acid, was a vitamin that was easily changed due to oxidation, but very useful for humans. It was chemical structure consists of a chain of 6 atoms of C, and its position was not stable ($C_6H_8O_6$) because it was easy to react with O_2 in the air to be dehydroascorbic acid [63]. Table 2 shows that the higher the ratio and concentration of the solvent, the more vitamin C content will increase. This was because vitamins C were vitamins that have water-soluble properties, where water was the polar compound that was the same as the methanol solvent used in this study. The research results [64] said that the vitamin C content was usually increased with the amount and the concentration of solvent added.

The antioxidant activity of corn silk extracts ranged between 22.88-27.08% (Table 2). There was a difference between treatments after the DMRT at the level of 5% with a p-value <0.05. The ability of phenolic compounds to act as antioxidants was related to their chemical structure [65]. The configuration and the total hydroxyl group of the very base were influenced by the mechanism of its antioxidant activity [66]. There was a positive relationship between antioxidant activity and polyphenolic compound content [67]. The higher the ratio between samples with solvents, the antioxidant activity from the extract will be lower [68]. This can be caused by the ratio of large samples, the concentration of the solution nearing the point of equilibrium. The phenolic compounds contained in the gambier surface will be extracted with difficulty. When the sample-to-solvent ratio is small, the time required to reach the point of equilibrium is long, and the concentration of phenolic in the liquid becomes very dilute, producing low antioxidants (large IC50) [66]. The higher the ratio and the concentration of the solvents, the antioxidant activity from the extract will be higher [68]. From Table 2, the phenolic and flavonoid compounds also increased. Antioxidant activity increases along with antioxidant compounds, such as phenolic and flavonoids. High phenolic and flavonoids have high antioxidant activity [69]. The increase in antioxidant activity in extracts of corn silk is in Table 2. Due to more and more corn silk extracted with an increase in methanol solvent, then the results of the extract will be higher anyway. The high results of the extract will also cause high antioxidant activity in the extract. This result follows the previous study conducted by Ref. [70]. According to Ref. [71], corn silk has the potential to be used as a natural antioxidant because it has high antioxidant activity.

IV. Conclusion

The best treatment interaction on the A3B3 (1:8) (w/v) and the concentration of methanol 80% with nutritional content analysis (water at 6.38%, ash at 1.11%, fat at 0.44%, protein at 4.70%, carbohydrates at 87.23%, and dietary fiber at 2.72%) and the analysis of phytochemicals content (total phenol at 2.91 µg GAE/g, flavonoids at 2.32 µg GAE/g, beta carotene at 1.07 ppm, vitamins C at 12.47 mg, and antioxidant activity at 27.08%).

Acknowledgment

We were grateful to the Directorate of Resources of the Directorate General of Higher Education (DIKTI), Jakarta Indonesia with contract number: 90/LL6/PG/SP2H/JG/2021.

References

[1] Dachriyanus, D. O. Katrin, R. Oktarina, O. Ernas, M. Suhatri, and H. Mukhtar, "Uji Efek α-Mangostin Terhadap Kadar Kolesterol Total, Trigliserida, Kolesterol Hdl, Dan Kolestrol Ldl Darah Mencit Putih Jantan Serta Penentuan Lethal Dosis 50 (LD50)," *J. Sains dan Teknol. Farm.*, vol. 12, no. 2, pp. 64–81, 2014.

[2] P. V. Darsono and E. M. Kuntorini, "Gambaran Struktur Anatomis Dan Uji Aktivitas Antioksidan Daun Serta Batang Hydroleaspinosa," *Bioscentia. J*, vol. 9, no. 2, pp. 63–73, 2012.

[3] E. F. T. Nuridayanti, "Uji Toksisitas Akut Ekstrak Air Rambut Jagung (Zea mays L.) Ditinjau dari Nilai LD50 dan Pengaruhnya terhadap Fungsi Hati dan Ginjal pada Mencit." Jakarta, 2011.

[4] B. Bushman, "Daya Tabir Surya dan Antioksidan Formula Krim Ekstrak Rimpang Kencur (Kaempferia galanga L) dan Rimpang Temu Kunci (Boesenbergia pandurata (Roxb.)," Universitas Muhammadiyah Purwokerto, 2002.

[5] M. A. Ebrahimzadeh, F. Pourmorad, and A. R. Bekhradnia, "Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran," *African J. Biotechnol.*, vol. 7, no. 18, pp. 3188–3192, 2008, doi: 10.4314/ajb.v7i18.59257.

[6] J. Guo, T. Liu, L. Han, and Y. Liu, "The effects of corn silk on glycaemic metabolism," *Nutr. Metab.*, vol. 6, no. 47, 2009, doi: 10.1186/1743-7075-6-47.

[7] J. Liu, C. Wang, Z. Wang, C. Zhang, S. Lu, and J. Liu, "The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (Zea mays L.) and related flavone glycosides," *Food Chem.*, vol. 126, no. 1, pp. 261–269, 2011, doi: 10.1016/j.foodchem.2010.11.014.

[8] A. Rahmayani, *Telaah Kandungan Kimia Rambut Jagung (Zea mays L.)*, vol. 37, no. 1. Bogor: Bogor, 2012.

[9] S. C. Ren, Z. L. Liu, and X. L. Ding, "Isolation and identification of two novel flavone glycosides from corn silk (Stigma maydis)," *J. Med. Plants Res.*, vol. 3, no. 12, pp. 1009–1015, 2009.

[10] S. Sarfare, S. Menon, and S. Shailajan, "Cornsilk as a bioavailable source of betasitosterol: A pharmacokinetic study using HPTLC," *Asian J. Plant Sci.*, vol. 9, no. 1, pp. 44–50, 2010, doi: 10.3923/ajps.2010.44.50.

[11] M. A. Sholihah, W. W. I. Rosli, and A. R. Nurhanan, "Phytochemicals screening and total phenolic content of Malaysian Zea mays hair extracts," *Int. Food Res. J.*, vol. 19, no. 4, pp. 1533–1538, 2012.

[12] A. R. Nurhanan, W. I. Wan Rosli, and S. S. J. Mohsin, "Total polyphenol content and free radical scavenging activity of cornsilk (Zea mays hairs)," *Sains Malaysiana*, vol. 41, no. 10, pp. 1217–1221, 2012.

[13] Haslina and M. Eva, "Extract Corn Silk with Variation of Solvents on Yield, Total Phenolics, Total Flavonoids and Antioxidant Activity," *Indones. Food Nutr. Prog.*, vol. 14, no. 1, p. 21, 2017, doi: 10.22146/ifnp.24280.

[14] H. Handayani, F. H. Sriherfyna, and Yunianta, "Ekstraksi Antioksidan Daun Sirsak Metode Ultrasonic Bath (Kajian Rasio Bahan : Pelarut Dan Lama Ekstraksi)," *J. Pangan dan Agroindustri*, vol. 4, no. 1, pp. 262–272, 2016.

[15] M. Sholihah, U. Ahmad, and I. W. Budiastra, "Aplikasi Gelombang Ultrasonik untuk Meningkatkan Rendemen Ekstraksi dan Efektivitas Antioksi dan Kulit Manggis," *J. Keteknikan Pertan.*, vol. 5, no. 2, pp. 161–168, 2017.

[16] F. J. Keil, "Modeling of Process Intensification," *Modeling of Process Intensification*. pp. 1–405, 2007, doi: 10.1002/9783527610600.

[17] A. J. Borrás-Enríquez, E. Reyes-Ventura, S. J. Villanueva-Rodríguez, and L. Moreno-Vilet, "Effect of Ultrasound-Assisted Extraction Parameters on Total Polyphenols and Its Antioxidant Activity from Mango Residues (Mangifera indica L. var. Manililla)," *Separations*, vol. 8, no. 7, p. 94, 2021, doi: 10.3390/separations8070094.

[18] Association of Official Analytical Chemist (AOAC), *Official Method 980.17 Preservatives in Ground Beef. Spectrophotometric Method*. USA: Association Of Official Analytical Chemist (AOAC), 2007.

[19] E. Candesa and L. Parker, *Handbook of Antioxidant*. New York: Marcell Dekker Inc, 2007.

[20] J. Liu *et al.*, "Supercritical fluid extraction of flavonoids from Maydis stigma and its nitrite-scavenging ability," *Food Bioprod. Process.*, vol. 89, no. 4, pp. 333–339, 2011, doi: 10.1016/j.fbp.2010.08.004.

[21] I. Yinusa, N. I. George, R. G. Ayo, and Y. Rufai, "Vacuum Liquid Chromatographic Isolation and Characterization of Beta-Sitosterol from the Bark of Sarcocephalus Latifolius (Smith Bruce)," *An Int. Res. Journal), www.chemistry-journal.org Sept.*, vol. 5, no. 9, pp. 480–488, 2015, [Online]. Available: www.chemistry-journal.org.

[22] J. da S. Carneiro, R. M. Nogueira, M. A. Martins, D. M. de S. Valladão, and E. M. Pires, "The oven-drying method for determination of water content in Brazil nut," *Biosci. J.*, vol. 34, no. 3, pp. 595–602, 2018, doi: 10.14393/BJ-v34n3a2018-37726.

[23] N. Elboughdiri, "Effect of Time, Solvent-Solid Ratio, Ethanol Concentration and Temperature on Extraction Yield of Phenolic Compounds From Olive Leaves," *Eng. Technol. Appl. Sci. Res.*, vol. 8, no. 2, pp. 2805–2808, 2018, doi: 10.48084/etasr.1983.

[24] O. O. Adekoyeni and A. S. Adeboye, "Effect of Storage Duration and Processing Parameters on Some Cooking Properties of Ofada Rice," *Turkish J. Agric. - Food Sci. Technol.*, vol. 6, no. 11, pp. 1550–1555, 2018, doi: 10.24925/turjaf.v6i11.1550-1555.1900.

[25] T. Czaja, A. Sobota, and R. Szostak, "Quantification of ash and moisture in wheat flour by Raman spectroscopy," *Foods*, vol. 9, no. 3, p. 280, 2020, doi: 10.3390/foods9030280.

[26] M. Darmawan, Syamdidi, and E. Hastarini, "Pengolahan Bakto Agar dari Rumput Laut Merah (Rhodymenia ciliata) dengan Pra Perlakuan Alkali," *J. Pascapanen dan Bioteknol. Kelaut. dan Perikan.*, vol. 1, no. 1, p. 9, 2006, doi: 10.15578/jpbkp.v1i1.83.

[27] S. Kumala, R. Sumarni, R. Rachmani, and A. Ruswita, "Alga Merah (Gracilaria verrucosa) sebagai Bahan Bakto Agar," *J. Farm. Indones.*, vol. 6 No. 3, no. 3, pp. 166–171, 2013.

[28] D. H. Truong, D. H. Nguyen, N. T. A. Ta, A. V. Bui, T. H. Do, and H. C. Nguyen, "Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of severinia buxifolia," *J. Food Qual.*, vol. 2019, pp. 1–9, 2019, doi: 10.1155/2019/8178294.

[29] H. H. S. Abdel-Naeem, K. I. Sallam, and H. M. B. A. Zaki, "Effect of different cooking methods of rabbit meat on topographical changes, physicochemical characteristics, fatty acids profile, microbial quality and sensory attributes," *Meat Sci.*, vol. 181, no. November 2021, 2021, doi: 10.1016/j.meatsci.2021.108612.

[30] S. Yuliani and S. Rusli, *Prosedur ekstraksi. Balai Penelitian Tanaman Rempah dan Obat*. Bogor: Balai Penelitian Tanaman Rempah dan Obat, 2003.

[31] G. G. Hewavitharana, D. N. Perera, S. B. Navaratne, and I. Wickramasinghe, "Extraction methods of fat from food samples and preparation of fatty acid methyl esters for gas chromatography: A review," *Arab. J. Chem.*, vol. 13, no. 8, pp. 6865–6875, 2020, doi: 10.1016/j.arabjc.2020.06.039.

[32] B. Zhuang, G. Ramanauskaite, Z. Y. Koa, and Z. G. Wang, "Like dissolves like: A first-principles theory for predicting liquid miscibility and mixture dielectric constant," *Sci. Adv.*, vol. 7, no. 7, pp. 1–7, 2021, doi: 10.1126/sciadv.abe7275.

[33] Ismiyarto, S. A. Halim, and P. J. Wibawa, "Identifikasi Komposisi Asam Lemak dari Minyak Benih," *J. Kim. Sains Apl.*, vol. 9, no. 1, pp. 1–5, 2006.

[34] S. P. S. Dia, Nurjanah, and A. M. Jacoeb, "Komposisi Kimia Dan Aktivitas Antioksidan Akar, Kulit Batang Dan Daun Lindur. Chemical Composition, Bioactive Components and Antioxidant Activities from Root, Bark and Leaf Lindur," *Pengolah. Has. Perikan. Indones.*, vol. 18, no. 2, pp. 205–219, 2015, [Online]. Available: https://core.ac.uk/download/pdf/291863573.pdf.

[35] A. Della Malva *et al.*, "Methods for Extraction of Muscle Proteins from Meat and Fish Using Denaturing and Nondenaturing Solutions," *J. Food Qual.*, vol. 2018, pp. 1–10, 2018, doi: 10.1155/2018/8478471.

[36] Q. Zhao, J. Lin, C. wang, L. Yousaf, Y. Xue, and Q. Shen, "Protein structural properties and proteomic analysis of rice during storage at different temperatures," *Food Chem.*, vol. 361, 2021, doi: 10.1016/j.foodchem.2021.130028.

[37] D. Tomé, "Protein: what's on in research on clinical nutrition," *Eur. J. Clin. Nutr.*, vol. 72, no. 9, pp. 1215–1220, 2018, doi: 10.1038/s41430-018-0240-9.

[38] R. I. Pratama, I. Rostini, and E. Liviawaty, "Karakteristik Biskuit dengan Penambahan Tepung Tulang Ikan Jangilus (Istiophorus Sp.)," *J. Akuatika*, vol. 5, no. 1, pp. 30–39, 2014.

[39] A. Rahmawati, *Kandungan Fenol Total Ekstrak Buah mengkudu (Morinda citrifolia)*. Fakultas Kedokteran Universitas Indonesia, 2009.

[40] X. Li, T. Lowary, P. A. Driguez, N. L. Pohl, and J. Zhu, "A new era of discovery in carbohydrate chemistry," *J. Org. Chem.*, vol. 85, no. 24, pp. 15770–15772, 2020, doi: 10.1021/acs.joc.0c02548.

[41] Yulianti, B. Susilo, and R. Yulianingsih, "Pengaruh lama ekstraksi dan konsesntrasi pelarut etanol terhadap difat fisika-kimia ekstrak daun stevia (Stevia rebaudiana bertoni M.) dengan metode microwave assisted extraction (MAE)," *J. Bioproses Komod. Trop.*, vol. 2, no. 1, pp. 35–41, 2014.

[42] R. Zhu, J. Tolu, L. Deng, A. Fiskal, L. H. E. Winkel, and M. A. Lever, "Improving the extraction efficiency of sedimentary carbohydrates by sequential hydrolysis," *Org. Geochem.*, vol. 141, no. 2020, pp. 1–12, 2020, doi: 10.1016/j.orggeochem.2019.103963.

[43] A. M. Ibrahim, F. H. Sriherfyna, and Yunianta, "Pengaruh Suhu dan Lama Waktu Ekstraksi Terhadap Sifat Kimia dan Fisik Pada Pembuatan Minuman Sari Jahe Merah (Zingiber officinale var. Rubrum) Dengan Kombinasi Penambahan Madu Sebagai Pemanis," *J. Pangan dan Agroindustri*, vol. 3, no. 2, pp. 530–541, 2015.

[44] T. M. Barber, S. Kabisch, A. F. H. Pfeiffer, and M. O. Weickert, "The health benefits of dietary fibre," *Nutrients*, vol. 12, no. 10, pp. 1–17, 2020, doi: 10.3390/nu12103209.

[45] U. Złotek, S. Mikulska, M. Nagajek, and M. Świeca, "The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimum basilicum L.) extracts," *Saudi J. Biol. Sci.*, vol. 23, no. 5, pp. 628–633, 2016, doi: 10.1016/j.sjbs.2015.08.002.

[46] X. Du *et al.*, "Effects of different extraction methods on structure and properties of soluble dietary fiber from defatted coconut flour," *Lwt*, vol. 143, 2021, doi: 10.1016/j.lwt.2021.111031.

[47] C. L. Li, S. Y. Yu, and Y. Lu, "Study on Extraction of Dietary Fiber from Potato Peel by Acid-base Chemical Method," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 267, no. 5, pp. 1–6, 2019, doi: 10.1088/1755-1315/267/5/052028.

[48] S. Felhi, A. Daoud, H. Hajlaoui, K. Mnafgui, N. Gharsallah, and A. Kadri, "Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits," *Food Sci. Technol.*, vol. 37, no. 3, pp. 483–492, 2017, doi: 10.1590/1678-457x.23516.

[49] H. Nawaz, M. A. Shad, N. Rehman, H. Andaleeb, and N. Ullah, "Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds," *Brazilian J. Pharm. Sci.*, vol. 56, p. 1–9, 2020, doi: 10.1590/s2175-97902019000417129.

[50] J. Maligan, V. Idayanti, and E. Zubaidah, "Identifikasi senyawa mikroalga laut Tetraselmis chuii (Kajian metode ekstraksi maserasi, jenis pelarut dan waktu ekstraksi)," *J. Teknol. Pertan.*, vol. 16, no. 3, pp. 195–206, 2015.

[51] L. Aulia and S. Widjanarko, "Optimasi Proses Ekstraksi Daun Sirsak (Annona Muricata L) Metode Mae (Microwave Assisted Extraction) Dengan Respon Aktivitas Antioksidan Dan Total Fenol," *J. Agroindustri Halal*, vol. 4, no. April, pp. 79–87, 2018.

[52] O. R. Alara, N. H. Abdurahman, and C. I. Ukaegbu, "Extraction of phenolic compounds: A review," *Curr. Res. Food Sci.*, vol. 4, pp. 200–214, 2021, doi: 10.1016/j.crfs.2021.03.011.

[53] S. Margaretta, S. D. Handayani, N. Indraswati, and H. Hindarso, *Ekstraksi Senyawa Phenolic Pandanus Amaryllifolius Roxb. Sebagai Antioksidan Alami*, vol. 10, no. 1. No.1: WIDYA TEKNIK Vol.10, 2011.

[54] E. D. M. Siregar, "Perlakuan Jenis Pakan Alami pada Daun Sirsak dan Teh," Institut Pertanian Bogor, Bogor, 2005.

[55] O. Kadiri, "A review on the status of the phenolic compounds and antioxidant capacity of the flour: Effects of cereal processing," *Int. J. Food Prop.*, vol. 20, no. 1, pp. S798–S809, 2017, doi: 10.1080/10942912.2017.1315130.

[56] M. K. Rafsanjani and W. D. R. Putri, "Karakterisasi Ekstrak Kulit Jeruk Bali Menggunakan Metode Ultrasonic Bath (Kajian Perbedaan Pelarut dan Lama Ekstraksi)," *J. Pangan Dan Agroindustri*, vol. 3, no. 4, pp. 1473–1480, 2015.

[57] A. T. Septiana and A. Asnani, "Kajian Sifat Fisikokimia Ekstrak Rumput Laut Coklat Sargassum Duplicatum menggunakan Berbagai Pelarut dan Metode Ekstraksi," *Agrointek J. Teknol. Ind. Pertan.*, vol. 6, no. 1, pp. 22–28, 2012, doi: 10.21107/AGROINTEK.V6I1.1950.

[58] I. See, G. C. L. Ee, S. H. Mah, V. Y. M. Jong, and S. S. Teh, "Effect of Solvents on Phytochemical Concentrations and Antioxidant Activity of Garcinia benthamiana Stem Bark Extracts," *J. Herbs, Spices Med. Plants*, vol. 23, no. 2, pp. 117–127, 2017, doi: 10.1080/10496475.2016.1272523.

[59] M. R. R. Rahardhian, B. T. Murti, D. Wigati, R. Suharsanti, and C. N. Putri, "Solvent concentration effect on total flavonoid and total phenolic contents of Averrhoa bilimbi leaf extract," *Pharmaciana*, vol. 9, no. 1, pp. 137–144, 2019, doi: 10.12928/pharmaciana.v9i1.8793.

[60] S. Jeyakodi, A. Krishnakumar, and D. Chellappan, "Beta Carotene - Therapeutic Potential and Strategies to Enhance Its Bioavailability," *Int. J. Nutr. Food Sci.*, vol. 7, no. 4, pp. 1–7, 2018, [Online]. Available: https://juniperpublishers.com/nfsij/pdf/NFSIJ.MS.ID.555716.pdf.

[61] A. Manasika and S. B. Widjanarko, "Ekstraksi Pigmen Karotenoid Labu Kabocha menggunakan Metode Ultrasonik (Kajian Rasio Bahan Pelarut dan Lama Ekstraksi)," *J. Pangan dan Agroindustri*, vol. 3, no. 3, pp. 928–938, 2015.

[62] P. Adadi, N. V. Barakova, and E. F. Krivoshapkina, "Selected Methods of Extracting Carotenoids, Characterization, and Health Concerns: A Review," *J. Agric. Food Chem.*, vol. 66, no. 24, pp. 5925–5947, 2018, doi: 10.1021/acs.jafc.8b01407.

[63] H. Sharma, H. prasad Sapkota, A. Khanal, O. Dhakal, and R. Gurung, "a Comparative Analysis of Vitamin-C Concentration in Commercial Fruit Juices and Fresh Fruits of Nepal With Effect of Temperature," *Int. J. Pharm. Pharm. Sci.*, vol. 11, no. 8, pp. 46–51, 2019, doi: 10.22159/ijpps.2019v11i8.33408.

[64] R. R. Singh and A. Harshal, "Effects of Cooking on Content of Vitamin C in Green Leafy Vegetables," *Sch. J. Agricltural Vet. Sci.*, vol. 3, no. 6, pp. 416–423, 2016.

[65] Y. A. Miryanti, L. Sapei, K. Budiono, and S. Indra, "Ekstraksi Antioksidan Dari Kulit Buah Manggis (Garcinia mangostana L.)," Universitas Katolik Parahyangan, Bandung, 2011. doi: Bandung: Universitas Katolik Parahyangan.

[66] G. Yeni, E. Gumbira-Sa'id, K. Syamsu, and E. Mardliyati, "Penentuan Kondisi Terbaik Ekstraksi Antioksidan dari Gambir Menggunakan Metode Permukaan Respon," *J. Litbang Ind.*, vol. 4, no. 1, pp. 39–48, 2014, doi: 10.24960/jli.v4i1.637.39-48.

[67] S. Ogawa, "Studies on Antioxidant Activity in Japanese Edible Seaweeds," Tokyo University of Fisheries, Tokyo, 2003.

[68] A. Noviyanty and C. A. Salingkat, "Pengaruh Rasio Pelarut Terhadap Ekstraksi Dari Kulit Buah Naga Merah (Hylocereus polyrhizus)," *Kovalen J. Ris. Kim.*, vol. 5, no. 3, pp. 280–289, 2019.

[69] G. Hao *et al.*, "Effect of temperature on chemical properties and antioxidant activities of abalone viscera subcritical water extract," *J. Supercrit. Fluids*, vol. 147, no. 2019, pp. 17–23, 2019, doi: 10.1016/j.supflu.2019.02.007.

[70] N. Hashim, A. R. Shaari, A. S. Mamat, and S. Ahmad, "Effect of Differences Methanol Concentration and Extraction Time on the Antioxidant Capacity, Phenolics Content and Bioactive Constituents of Orthosiphon Stamineus Extracts," in *MATEC Web of Conferences*, 2016, vol. 78, pp. 1–7, doi: 10.1051/matecconf/20167801004.

[71] K. J. Wang and J. L. Zhao, "Corn silk (Zea mays L.), a source of natural antioxidants with α-amylase, α-glucosidase, advanced glycation and diabetic nephropathy inhibitory activities," *Biomed. Pharmacother.*, vol. 110, no. 2019, pp. 510–517, 2019, doi: 10.1016/j.biopha.2018.11.126.