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The Influence of Type of Solvent and Extraction Temperature of Corn Silk Extracts

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Abstract— Corn silk has not been well-utilized. Actually it has the capacity to develop into functional food because it contains bioactive compounds and high natural antioxidant such as flavonoids and phenolics and positive effects on human health. There are several factors that influence the quality of corn silks extracts, including ratio (raw:solvents) and extraction temperature. This research was couducted to determine the influence of ratio (raw:solvent) and extration temperature of corn silk extracts. The experimental design used Randomized Block Design (RBD) which consisted of two factors and 3 replications. The first factor was type of solvent. All treatment use (raw:solvents) ratio at (1:4). The first factor consists P1=raw:ethanol (1:4)(w/v), P2=raw:methanol (1:4)(w/v), P3=raw:ethyl acetate (1:4)(w/v), and P4=raw:acetone (1:4)(w/v). and the second factor was extraction temperature that consists S1=60°C, S2=70°C, and S3=80°C. Data were analyzed with analysis of variance, followed by Duncan Multiple Range Test (DMRT) test at 5% significant level. Parameters of chemical analysis were included (water, ash, protein, fat, and carbohyrate), and phytochemical analysis were included (total phenol, total flavonoid, activity antioxidant, beta carotene, and vitamin C). The results showed that the type of solvent and extraction temperature of corn silk extracts have significantly effect on water, ash, protein, fat, and, total phenol, total flavonoid, activity antioxidant, beta carotene, and vitamin C. Methanol solvent and extraction temperature at 60°C is the best treatment which have the highest protein and the lowest fat content at 18.22%±1.13 and 0.17%±0.02 respectively, and the highest total phenol, total flavonoid, activity antioxidant, beta carotene, and vitamin C at 8216.89±132.39 µgGAE/g; 234.29±3.98 µgGAE/g; 215.27ppm±3.87; 80.80%±1.84; and 8.84%±0.45 respectively.

***Keywords*— local corn silk; extract; type of solvents); extraction temperature.**

I. Introduction

As a global staple food, more than half of the world's population considers rice as a staple food. Main rice consumption in Asia, South America, and Sub-Saharan Corn silk is a parts of corn which has not been used effectively, because it has not been well-utilized. It contains antioxidant compounds that are beneficial to the human health. In addition to containing antioxidant compounds, it has potential as traditional medicine. Based on research, it contains sitosterol and stigmasterol, alkaloids, saponins, tannins, flavonoids, steroids, protein, carbohydrates, vitamins, salts, calcium potassium, magnesium, sodium, and essential oils [1]. Different from previous researcher, Bisma local variety of Indonesia have corn silk approximately at 80-90 days (mature), contain water at 11.58%; fat at 0.30%; ash at 3.29%; protein at 17.70%; and carbohydrate at 67.13%; total phenol at 8262.93 µgGAE/g; total flavonoids at 236.03 µgGAE/g; beta sitosterol at 1343.93 ppm; and 75% activity antioxidant [2].

Bioactive compound of corn silk can be obtained by extraction. There are several factors that influence the extraction method namely type solvent, ratio yield weight to volume solvent, temperature, speed stirring, time, tangent to solids, frequency, concentration, particle size, and pH [3;4,5]. One method that is often used to extract bioactive components is maceration method which has advantages such as workmanship and types of tools used are simple, operational cost are relatively low, and can avoid damage to compounds that are thermo-labile [6].

The solvents which is often used for extracting of flavonoid compounds are methanol, ethanol, acetone, and ethyl acetate. Type of solvent influences the extraction process of carotenoid, for example in pumpkin carotenoids extract using a single acetone, ethyl acetate, and n-hexane solvent [7]. The highest activity of inhibitory was found in the active component of corn silk which was purified with 50% ethanol with activity of inhibitory at 69.90%±1.27% [8].

Antioxidant components extracted from corn silk using methanol and water with the amount of polyphenol content in methanol and water extracts each being at 272.81 mgGAE/100g and 256.36 mgGAE/100mg (dry) [9].

Acetone is the most effective solvent for extracting antioxidants from foxtail millet (a type of cereal) compared to water, ethanol, propanol, and methanol [10]. Combination of ethyl acetate (EtO-Ac):water (85:15) solvent produced total flavonoids four times greater than acetone solvents in extracting organic compounds from grape seeds [11]. 50% (v/v) ethanol solvent as the most effective for polyphenols seed grape extraction compared to acetone and water for all concentrations [12], methanol 70% (v/v) chose, with a ratio at 10:1 (v/w) for materials for the same purpose and material [13]. In extracting seeds papaya recruitsusing ethanol, acetone, and ethyl acetate. The best treatment was obtained from the type of solvent which produced the highest total phenol obtained in ethanol solvents [14]. This research was conducted to determine the influence of type of solvent and extraction temperature of corn silk extracts to produce the best characteristics of corn silk extracts.

II. Material and Methods

A. Materials

Fresh corn silk age 70 days were collected from local farmers in Pati, Central Java, Indonesia. Chemicals Folin-Ciocalteu reagent (Merck), sodium carbonate (⩾ 99%, Merck), aluminum chloride (99%, Ferak) were purchased and used without further purification. 2,2-Diphenyl-1-picrylhdrazyl (DPPH, Aldrich), ethanol, methanol, ethyl acetate, and acetone (⩾80%, Merck), gallic acid (⩾99%, Sigma®), rutin (⩾ 94%, Sigma®).

B. Equipments

Some equipment used are becker glass, blender, waterbath, spectrophotometer, pH meter, oven, Whatman filter paper 1, rotary flash evaporator, Folin-Ciocalteu colorimetric, and some glassware for analysis.

C. Preparation

Fresh corn silk weighing 3g, was washed with distilled water, dried in an oven at 60°C for 24h [9] until the final moisture content was 10-11%, ground into a powder using a grinder, and then it dissolved with the proportion of raw and solvent is 1:4 (w/v) at room temperature for 24 hours by maceration. The extraction is done at 70°C with water bath shaker. 1.5 hours later filtered off the dregs. Separation of solvent with rotary flash evaporator at 60°C [15]. Dissolve residue with 5 ml methanol, store temperature at 0-4oC.

D. The Experimental Design

The experimental design used Randomized Block Design (RBD) which consisted of two factors and 3 replications. The first factor was the type of solvent that consists P1=raw:ethanol (1:4)(w/v), P2=raw:methanol (1:4)(w/v), P3=raw:ethyl acetate (1:4)(w/v), and P4=raw:acetone (1:4)(w/v). and the second factor was the temperature of extraction that consists S1=60°C, S2=70°C, and S3=80°C. Data were analyzed with analysis of variance, followed by Duncan Multiple Range Test (DMRT) test at 5% level.

E. Analysis

Chemical analysis was performed on water and ash content using oven method [16], protein content using Micro Kjeldahl method [16], fat content using Soxhlet [16], carbohydrate using carbohydrate by difference [16], and phytochemicals of corn silk (total phenol) [17], flavonoids [18], vitamin C [16], beta carotene [19], and activity antioxidant [18].

III. Result and Discussions

A. Chemical Content of Corn Silk

Different in ratio (raw:solvent) and extraction temperature to chemical content of corn silk extracts as shown in Table 1. Table 1 shows that the methanol solvent and extraction temperature at 60°C showed the highest protein and the lowest fat content at 18.22%±1.13 and at 0.17% 0.02 respectively. Methanol solvent and extraction temperature at 60°C (P2S1) produced the highest protein content that is equal to 18.22% and the lowest ratio (raw:acetone) and extraction temperature at 80°C (P4S3) of 3.19%. The increasing extraction temperature decreases the protein content of corn silk extracts. The protein solubility generally increases when the temperature rises from 0 to 40°C, and the longer of time dissolution, the contact between the solute and solvent is longer, so that many solutes are taken [20].

TABLE I
Chemical Analysis Of Corn Silk Extract

Treat-ment	Component				
	Water (%)	Protein (%)	Fat (%)	Ash (%)	Carbo-hyrate(%)
P_1S_1	1.35 ± 0.04^{b}	14.42 ± 0.56 ^{d}	0.27 ± 0.03^{ab}	2.23± 0.02^{ab}	81.75 ± 0.53^{ab}
P_1S_2	1.46 ± 0.04^{bc}	11.74± 0.52^{cd}	0.40 ± 0.04^{b}	2.36 ± 0.03^{b}	84.05 ± 0.64^{b}
P_1S_3	1.59 ± 0.03^{c}	9.10± 0.75^{bc}	0.72 ± 0.03^{d}	2.36 ± 0.01^{b}	86.10 ± 0.69^{bc}
P_2S_1	1.10 ± 0.02^{a}	18.22± 1.13^{e}	0.17 ± 0.02^{a}	2.08 ± 0.05^{a}	78.45 ± 2.01^{a}
P_2S_2	1.29 ± 0.05^{ab}	14.67 ± 0.59^{d}	0.24 ± 0.03^{ab}	2.31 ± 0.02^{ab}	81.50 ± 0.59^{ab}
P_2S_3	1.41 ± 0.09^{bc}	11.45± 0.6^{c}	0.34 ± 0.01^{b}	2.49 ± 0.09^{bc}	84.32 ± 0.71^{b}
P_3S_1	1.51 ± 0.08^{c}	11.29± 0.91^{c}	0.39 ± 0.04^{b}	2.39 ± 0.03^{b}	84.42 ± 0.89^{b}
P_3S_2	1.67 ± 0.08^{c}	7.90± 0.39^{b}	0.56 ± 0.01^{c}	2.61 ± 0.05^{c}	87.27 ± 0.35^{c}
P_3S_3	1.87 ± 0.05^{cd}	5.04± 1.15^{ab}	0.77 ± 0.03^{de}	2.75 ± 0.03^{c}	89.59 ± 1.11^{d}
P_4S_1	1.78 ± 0.04^{d}	8.27± 0.35^{b}	0.54 ± 0.04^{c}	2.54 ± 0.04^{bc}	86.88 ± 0.40^{b}
P_4S_2	1.98 ± 0.08^{d}	5.60± 0.72^{ab}	0.77 ± 0.06^{de}	2.78 ± 0.08^{c}	88.88 ± 0.50^{cd}
P_4S_3	2.19 ± 0.03^{e}	3.19± 0.77^{a}	0.97 ± 0.02^{e}	2.95 ± 0.01^{cd}	90.71 ± 0.09^{d}

Description: Ratio (raw:solvent):P1=raw:ethanol (1:4) (w/v), P2= raw:methanol (1: 4) (w /v), P3= raw:ethyl acetate (1: 4) (w/v), and P4= raw:acetone (1: 4) (w/v). Extraction temperature: S1=60°C, S2=70°C, and S3=80°C

The level of fat damage varies depending on the temperature used and processing time. The higher the temperature used, the fat damage will increase. Essential fatty acids are isomerized when heated in alkaline solutions and sensitive to light, temperature and oxygen. The process of fat oxidation can cause inactivation of its biological functions and can even be toxic. Apart from fat being damaged by oxidation, fat can also be damaged by hydrolysis [21].

B. Phytochemical Content of Corn Silk Extract

The type of solvent and extraction temperature affect significantly phytochemical content of corn silk extracts as shown in Table 2.

1) Total Phenol

Methanol solvent and extraction temperature at 60°C affect ($p<0.05$) the content of total phenol of corn silk extracts. Table 2 and Figure 1, it can be seen that methanol solvent and extraction temperature at 60°C (P2S1) results in the highest total phenol and acetone solvent and extraction temperature at 90°C (P4S1) resulted in the lowest total phenol. The results of this study are different from those reported by Ref. [5], that to extract soursop leaves using the best ultrasonic treatment at ratio of material and solvent at 1:10 and extraction time at 20 minutes. Empirically, methanol is the most efficient solvent for the extraction of simple phenolic compounds. For phenolic compounds that are more complex (larger molecular weight) acetone-water (aqueous acetone) is used [22]. The solubility of a substance into a solvent is largely determined by the suitability of the properties between the solute and the solvent which is the nature of like dissolve like which is caused by its polarity [23]. The higher ratio of ingredients and solvents, the level higher of phenolic avocado seed extracts will be the higher. [24]. Methanol is used as a solvent, because it is able to dissolve almost all organic compounds both polar, semi-polar and non-polar. Phenol or phenyl alcohol is still one group of compounds with methanol, so its solubility is very suitable, and this compound is not resistant to hot temperatures because it has a low vapor point.

TABLE II
PHYTOCHEMICAL ANALYSIS OF CORN SILK EXTRACT

Treat-ment	Component				
	Total phenols (µg GAE/g)	Total flavonoids (µg GAE/g)	Beta carotene (ppm)	Anti-oxidant (%)	Vita-mine C (%)
P_1S_1	7085.2± 94.72	215.23± 5.76	201.28± 2.65	76.05± 1.63	7.72± 0.28
P_1S_2	6712.6± 129.65	189.25± 5.99	168.8± 4.67	70.35± 1.63	6.61± 0.14
P_1S_3	6056± 67.71	148.92± 10.18	137± 3.08	62.55± 0.78	4.18± 0.04
P_2S_1	8216.89± 132.40	234.29± 3.98	215.27± 3.87	80.80± 1.84	8.84± 0.45
P_2S_2	7395.27± 275.55	213.07± 2.62	190.31± 4.10	73.65± 3.75	7.16± 0.11
P_2S_3	7132.28± 28.17	189.32± 8.05	153.42± 3.27	63.65± 1.77	4.88± 0.30
P_3S_1	6141.20± 159.78	179.71± 4.80	130.10± 3.97	64.25± 1.63	5.39± 0.16
P_3S_2	5715.49± 35.16	136.31± 6.24	114.37± 2.45	57.85± 0.49	3.98± 0.07
P_3S_3	4827.26± 148.85	121.10± 4.35	90.96± 4.82	43.95± 1.20	1.68± 0.19
P_4S_1	4717.52± 146.08	116.31± 5.83	97.33± 1.14	41.99± 3.22	3.29± 0.15
P_4S_2	3368.35± 83.17	100.57± 2.91	73.66± 2.05	32.37± 1.17	2.31± 0.20
P_4S_3	2645.73± 1785.40	69.21± 5.49	49.12± 1.15	20.71± 3.27	1.63± 0.16

Description: Ratio (raw:solvent): of P1=raw:ethanol (1:4) (w/v), P2= raw:methanol (1: 4) (w /v), P3= raw:ethyl acetate (1: 4) (w/v), and P4= raw:acetone (1: 4) (w/v). Extraction temperature: S1=60°C, S2=70°C, and S3=80°C

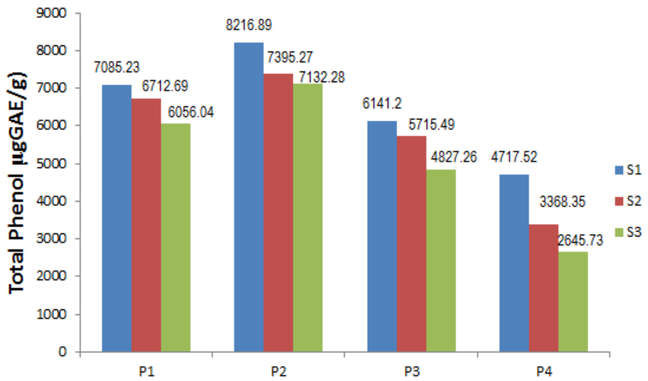

Fig 1. Total fenol

The increase in extraction temperature needs to be considered properly. The higher extraction temperatures and longer extraction time exceed the time limit can cause removal of compounds in the solution due to the evaporation, in addition bioactive components such as flavonoids do not hold high temperatures, thus undergoing structural changes and producing low extracts [25].

2) .Total Flavonoid

Type of solvent and extraction temperature at 60°C affect ($p<0.05$) the content of total flavonoid corn silk extract. The biggest corn silk of flavonoid contents was produced by methanol solvent and extraction temperature at 60°C (P2S1). Flavonoids are included in phenolic compounds, the solvents whose polarity is the same as flavonoids are methanol, resulting in high flavonoids. Flavonoids are heat resistant and easily oxidized at high temperatures. Flavonoids will be degraded at temperatures above 100°C [26]. Flavonoids are sensitive to heat, because groups hydroxyl and ketone, and unsaturated double bonds [27].

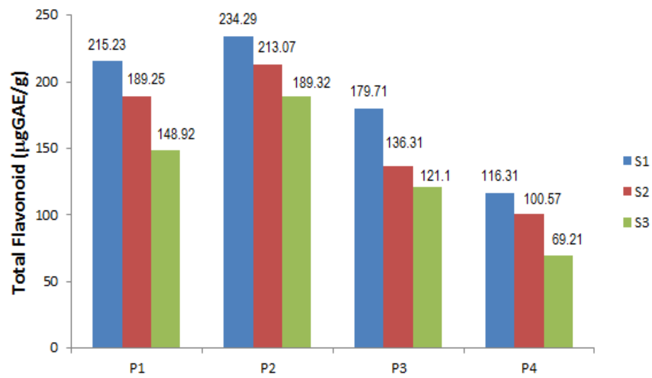

Fig 2. Total flavonoid

3) Activity of antioxidant

Methanol solvent and extraction temperature at 60°C affect ($p<0.05$) the antioxidant activity corn silk extracts. Table 2 and Figure 3 shows that, methanol and extraction temperature at 60°C results in higher antioxidant activity than others. The high total phenol and total flavonoids in corn silk extracts showed high activity of antioxidant. There is a relationship between the ability of phenol compounds as antioxidants and their chemical structure [28]. The configuration and total group hydroxyl is the basis that influences greatly the mechanism of its activity as an antioxidant activity. There is a good correlation between total flavonoids and activity antioxidant, where the higher the total flavonoids and the stronger antioxidant activity [29]. There is a positive correlation between antioxidant activity and the content of polyphenol compounds [30

4) Beta Carotene

Type of solvent and extraction temperature at 60°C affect ($p<0.05$) the content of beta carotene of corn silk extracts. This occurs because the soluble phenol compounds are affected by the polarity of solvent, while the power antioxidant of the solvent is affected by compounds which are soluble in the mixture.

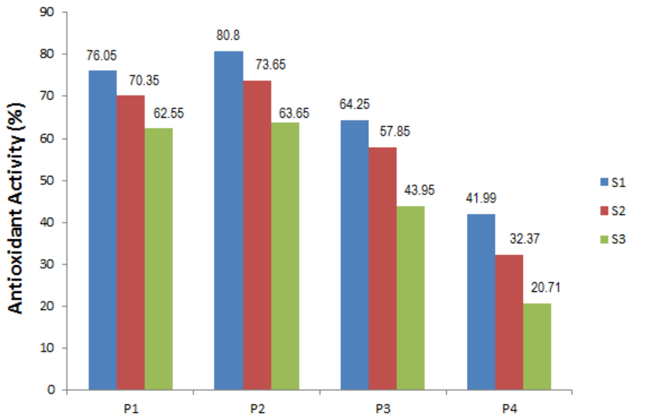

Fig 3. Antioxidant Activity

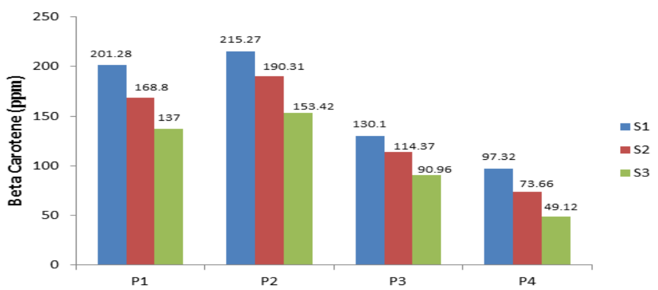

Fig 4. Beta carotene

Antioxidant compounds besides phenolic include alkaloids, vitamin C, vitamin E, and beta carotene etc. [31]. Carotenoids are yellow, red and orange pigments in plants. Carotenoids can function as precursors of vitamin A and antioxidants. Carotene has properties that are very susceptible to oxygen, heat, light, and are not stable under conditions acidic [32]. Table 2 and Figure 4 shows that type of solvent and extraction temperature at 60°C produces the highest levels of beta carotene. This is because beta carotene is a group of antioxidants, it is directly proportional to activity antioxidant. High temperatures are a factor that causes thermal degradation which will damage carotene resulting in carotene decomposition.

5) Vitamin C

Methanol solvent and extraction temperature at 60°C affect ($p<0.05$) the content of vitamin C corn silk extracts. This is directly proportional to the level of antioxidants, because vitamin C is part of antioxidant compounds. Vitamin C is an electron donor and reducing agent. Antioxidants are called, because by donating electrons, this vitamin prevents other compounds from being oxidized. However, vitamin C itself will be oxidized in the antioxidant process, resulting in dehydroascorbic acid [33]. Vitamin C is included in antioxidants so it can not stand at high temperatures. This is because low temperatures can inhibit respiration, enzyme activity, and metabolic reactions.

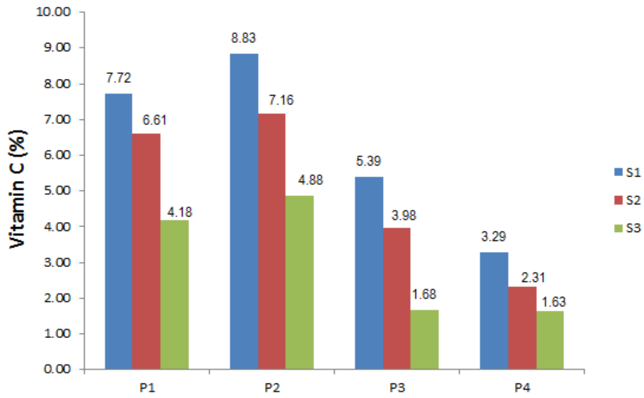

Fig 5. Vitamin C

IV. Conclusions

The type of solvent and extraction temperature will affect the extraction process of corn silk. Methanol solvent and extraction temperature at 60°C is the best treatment which contain the highest protein and the lowest fat content at 18.22%±1.13 and 0.17% 0.02 respectively. It also have the highest total phenol, total flavonoid, activity antioxidant, beta carotene, and vitamin C at 8216.89 ±132.39µgGAE/g; 234.29±3.98 µgGAE/g; 215.27ppm±3.87; 80.80%±1.84, and 8.84%±0.45 respectively.

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