

THE STABILITY OF CHLOROGENIC ACID IN SYRUP OF COFFEE ARABICA (*COFFEA ARABICA* L.) EXTRACT WITH DECAFFEINATION PROCESS

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ABSTRACT

Objective: This research aims to formulate syrup of coffee arabica extract with decaffeination process and stability test of caffeine and chlorogenic acid (CGA) in syrup of coffee arabica extract.

Materials and Methods: An extraction was conducted by using ethanol 70% with soxhletation methods, and decaffeination process (using liquid-liquid extraction with ratio of ethanol and dichloromethane was 1:1). Syrup formulations of coffee arabica extract were made with various concentrations of Na carboxymethylcellulose (CMC) (thickening agent) and sucralose (flavoring agent). The stability of syrup was evaluated through organoleptic, pH, viscosity, hedonic test, microbiological test and determined concentration of caffeine and CGA.

Results: The result showed that the concentration of caffeine before and after decaffeination process was $3.377 \pm 0.091\%$ and $1.028 \pm 0.079\%$. While CGA was $4.159 \pm 0.163\%$ and $3.019 \pm 0.138\%$. Microbiological test showed that no contamination in syrup of coffee arabica extract. The concentration of caffeine in syrup of coffee arabica extract was $1.070 \pm 0.150\%$ and CGA was $4.432 \pm 1.98\%$.

Conclusion: The concentration of caffeine before and after formulation process was $3.019 \pm 0.138\%$ and $4.432 \pm 1.986\%$. The best formula of coffee arabica extract syrup was the formula that contains 5 mg/ml of coffee arabica extract, 1.5 mg/ml of Na CMC, and 0.5 mg/ml of sucralose and no contamination in syrup of coffee arabica extract. Caffeine content would decrease after the decaffeination process.

Keywords: Chlorogenic acid, Coffee arabica extract, Syrup, Decaffeination, Caffeine.

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INTRODUCTION

Indonesia is one of the largest countries producing Arabica coffee in the world. Coffee has been consumed for over 1000 years and making this as the most consumed drink in the world. Coffee contains many active substances such as fenol, flavonoid, mineral, and sugar. Yet, the most widely known compounds in coffee are chlorogenic acid (CGA) and caffeine [1,2].

CGAs are the main phenolic compounds in coffee and can be found in coffee within high concentration [3], for instance: Green coffee contains (5-12 g/100 g) of CGA. CGA is believed to have antioxidant properties which can protect food, cells and any organ from oxidative degenerative. CGA compound is important in preventing various diseases associated with oxidative stress such as cancer, cardiovascular, aging, and neurodegenerative disease. CGA also can be considered as an antiviral, which can inhibit the influenza virus, herpes virus herpes simplex virus Type 1 (HSV-1), HSV-2, and adenovirus [4-6]. In particular, CGA has a function as a brain protector in the treatment of neurodegenerative diseases, such as Alzheimer, Parkinson, and ischemia [7].

Coffee beans contain caffeine that has a pharmacological effect, such as stimulating the central nervous system and relaxing the smooth muscle, especially bronchial and cardiac muscles [8]. Caffeine consumption in the long term can affect the suppression of energy metabolism by causing adrenal fatigue [9]. Previously, descriptive studies indicated that consuming caffeine can cause insomnia, headache, tremor, anxiety, nausea and vomiting [10]. Therefore, it is necessary to reduce the level of caffeine in coffee beans.

Decaffeinated coffee is good for health, especially in gastric disorders. The use of decaffeinated coffee increased and reached 10% of the overall

coffee consumption [11]. Decaffeination process usually uses organic solvents such as dichloromethane and ethyl acetate [4]. According to the previous study, dichloromethane (140 mg/ml) has better ability on dissolving the caffeine compared to water (22 mg/ml) [12].

MATERIALS AND METHODS

Materials

Arabica coffee (Pasir Mulya Village, Pengalengan, Kabupaten Bandung), ethanol 70% (PT Brataco), aquadest, aquabidestilata, toluene (PT Brataco), Mayer reagent (Merck), Dragendorff reagent (Merck), Lieberman-Burchard reagent (Merck), chloroform (PT Brataco), ammonia (PT Brataco), chloride acid 2N (PT Brataco), gelatin 1% (Merck), FeCl₃ (Merck), magnesium powder (Merck), amyl alcohol (Merck), ether (PT Brataco), methanol (PT Brataco), dichloromethane (PT Brataco), acetic acid (PT Brataco), potassium hydroxide solution 5%, n-hexane (PT Brataco), sucralose (Kimia Mart), citric acid (CV. Chemco Prima Mandiri), and sodium benzoate (PT Brataco).

Methods

Extraction

Coffea arabica L. powder was extracted with ethanol 70% using soxhletation methods. Each extract was evaporated by using a rotary evaporator at 50-60°C and followed by water bath at 40°C [13]. Extract was determined including specific standardized and non-specific standardized extracts [14].

Phytochemical screening

Phytochemical screening for secondary metabolites was performed by following standard procedures, including alkaloids, polyphenol, tannin, flavonoid, monoterpen, sesquiterpen, steroid, triterpenoid, kuinon, and saponin test [15].

Decaffeination process

The extract was partitioned with different solvents in a 1:1 (v/v) using water:dichloromethane. The remaining aqueous layer was collected and evaporated by using rotary evaporator at 50-60°C [16].

Formulation of syrup from coffee arabica (*C. arabica* L.) extract

The formula design of syrup from coffee arabica (*C. arabica* L.) extract can be seen in Table 1.

Physical stability of syrup from coffee arabica (*C. arabica* L.) extract was evaluated through organoleptic, pH and viscosity until 28 days.

Bacterial contaminant test

About 1 ml sample was poured into 15-20 ml of medium plate count agar. Petri dishes were incubated at 37°C for 24-48 hrs. Calculated colonies were grown on each Petri dish [17].

Fungal contaminant test

Sample of 0.5 ml pipette was poured on the surface of the potatoes dextrose agar that has been added chloramphenicol. Petri dishes were incubated at a temperature of 20-25°C and observed until 3-5 days. Calculated colonies were grown on each Petri dish [17].

Determination of CGAs in coffee

Concentration of CGAs was detected by high performance liquid chromatography (HPLC). The mobile phase was 40% methanol:60% water containing acetic acid 1%. Mobile phase was filtered under vacuum and degassed before it would be used. The HPLC column was Enduro C-18 (4.6 × 250 mm). The flow rate was 1 ml/min. The ultraviolet (UV) absorbance was detected at 234 nm [18,19].

Determination of caffeine in coffee

Concentration of caffeine was detected by using HPLC. The mobile phase was methanol 37%:water 63%. Mobile phase was filtered under vacuum and degassed before it would be used. The HPLC column was Dionex C-18 (4.6 mm × 250 mm). The flow rate was 1 ml/min. The UV absorbance was detected at 274 nm [20,21].

Statistical analysis

The data were analyzed by one-way analysis variance (ANOVA) and significant differences between the mean of the samples were determined by Newman-Keuls test. The confidence limit was set at $p < 0.05$.

RESULTS

The result from phytochemical screening results can be seen in Table 2.

The result from non-specific parameters non-specific between extract and standard can be seen in Table 3.

The result from organoleptic test and specific parameters extract can be seen in Tables 4 and 5.

The result from decaffeination process can be seen in Table 6.

Physical evaluation of syrup from coffee arabica (*C. arabica* L.) extract can be seen in Table 7, Figs. 1 and 2.

Bacterial and fungi count of the solution (Table 8)

Bacterial and fungi count of the solution can be seen in Table 8.

Concentration of CGA and caffeine (Table 9)

Concentration of CGA and caffeine can be seen in Table 9

DISCUSSION

The parameters of extract including specific and non-specific parameters were approached with the reference value of seed extract arabica coffee

(*C. arabica* L.) that has been established by Rubiyanti et al. [22]. Water was used to keep the quality of extracts by considering the less water on simplicia and extract can avoid contaminants. However, water content in the extract and simplicia can determine acceptability, uniformity, and durability [23]. In particular, loss on drying aims to find out the amount of lost compound during the drying process that can affect the compound number of extract and simplicial [24]. Total ash levels examination describes the internal and external mineral content of extract from the beginning process. Content of acid-insoluble ash aims to find out the amount of internal and external mineral content in the extract and simplicia that cannot dissolve in the solvent acid [24].

Decaffeination was conducted by using an organic solvent, such as dichloromethane or ethyl acetate, associated with the use of water/vapor, before and after extraction. Water has been used to replace organic solvents in the process [4]. At the end of the process,

Table 1: Formulation of syrup from coffee arabica (*C. arabica* L.) extract

Component	F ₁	F ₂	F ₃
Extract	0.5 g	0.5 g	0.5 g
Sucralose	0.05 g	0.05 g	0.05 g
Sodium-CMC	0.15 g	0.45 g	0.75 g
Citric acid	0.2 g	0.2 g	0.2 g
Sodium benzoate	0.1 g	0.1 g	0.1 g
Orange essence	1 ml	1 ml	1 ml
Aquadest	Add 100 ml	Add 100 ml	Add 100 ml

CMC: Carboxymethylcellulose

Table 2: Result of phytochemical screening

Secunder metabolic	Result	
	Dried plant material	Extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	-	-
Polyphenol	+	+
Saponin	+	+
Monoterpen seskuiteperen	+	+
Triterpenoid	+	+
Steroid	+	+
Quinons	+	+

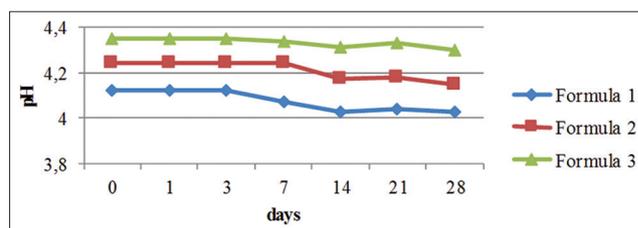


Fig. 1: pH measurements of the solution for 28 days

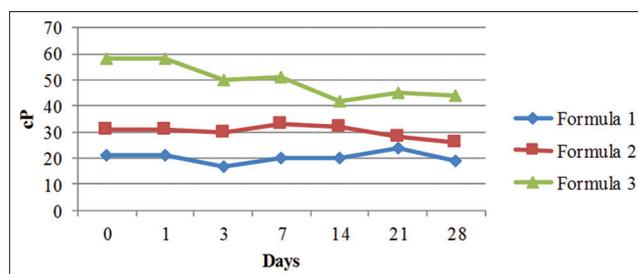


Fig. 2: Viscosity measurements of the solution for 28 days

Table 3: Result of non-specific parameters

Parameters	Extract	Standard
Water content (% v/b)	5.83±1.44	5.83-6.66
Content of ash total (% b/b)	1.75±0.18	1.77-3.28
Content of acid insoluble ash (% b/b)	0.010±0.00	0.01-0.02
Loss on drying (% b/b)	21.83±0.76	18.16-21.83
Density (m/v)	0.91±0.00	0.89-0.92

Table 4: Result of organoleptic

Parameters	Result	
	Dried plant material	Extract
Shape	Oval	Viscous extract
Color	Green	Brown
Odor	Coffee	Coffee

Table 5: Result of specific parameters extract

Parameters	Extract	Standard
Content of water soluble extract (% b/b)	2.50±1.32	29.50-32.50
Content of ethanol soluble extract (% b/b)	35.33±1.44	32.00-38.16

Table 6: Caffeine and chlorogenic acid after decaffeination process

Extract	Concentration (%)	
	Caffeine	CGA
Before decaffeination process	3.377±0.091	4.159±0.163
After decaffeination process	1.028±0.079	3.019±0.138

CGA: Chlorogenic acid

Table 7: Organoleptic evaluation from extract coffee arabica syrup

Parameter	Formula 1	Formula 2	Formula 3
Flavor	++	++	++++
Odor	++++	++++	++++
Color	Orange	Orange	Orange

Table 8: Microbial impurities test of solution

Sample	Result	
	Bacteria	Fungi
Negative control	-	-
Positive control	+	+
Formula 1	-	-
Formula 2	-	-
Formula 3	-	-

-: No growth of bacteria/fungi, +: There is a growth of bacteria/fungi

Table 9: Concentration of the CGA and caffeine

Formula	Levels of (%)	
	Caffeine	CGA
1	1.270±0.150	4.432±1.986
2	0.314±0.006	1.867±0.382
3	0.298±0.015	1.373±0.0016

CGA: Chlorogenic acid

caffeine content is usually reduced. In the decaffeination process, losses of key flavor components of coffee generally might occur, especially when solvents, such as water, are used [25]. Dichloromethane was used because it is relatively nontoxic and has better ability to dissolve caffeine (140 mg/ml compared to water [22 mg/ml]) [12].

Although differences of CGA in decaffeinated coffees may appear to be relatively small, those may be enough to affect the flavor characteristics of the final product. The biopharmacological properties of decaffeinated coffee may differ from those caffeinated coffee due to the pharmacological actions of CGA which were "unmasked" by the absence of caffeine. However, it required further investigations [4].

Sucralose as agent was used in the formulation of syrup. As the sweetening agent, it has 300-1000 times greater of sweetness level compared to sucrose. Next to flavoring agent, citric acid was used as a buffer to stabilize pH of solution. In this formulation, syrup did not use sucrose as sweetening agent because it was incompatible with citric acid, which can be crystallized or hydrolyzed into dextrose and fructose [26].

Carboxymethylcellulose (CMC)-Na was used as a thickening agent since it has good stability in acidic and alkaline (pH 2-10) [6]. Sodium benzoate was used as a preservative agent because of its high water content, which can cause contamination microbes. Plant-derived materials support the growth of microorganisms as a nutrition/food for microorganisms. In addition, preservative agent can prevent from secondary contamination, such as preparation and the environment [27,28].

Regarding the pH observations, data were statistically analyzed by using one-way ANOVA. Previously, homogeneity and normality were analyzed using the Kolmogorov-Smirnov methods test. Since the obtained data have normal and homogeneous values, one-way ANOVA analysis method would be taken into account.

The influence of pH against formula was tested using one-way ANOVA method. Statistic result showed that all formulas have significant differences on the influence against pH value (H_0 was rejected and significant $0.00 < 0.05$). Result of Newman-Keuls test showed that every formula gave different influence against pH.

Influence of storage time against pH value of each formula was analyzed using one-way ANOVA. This statistic results showed that storage time did not affect significantly the pH of solution (H_0 is accepted and significant $0.970 > 0.05$).

Difference of Na-CMC concentration caused different viscosities of each formula since Na-CMC has a role in viscosity value of solution [26].

The influence of formula against viscosity value was tested by using one-way ANOVA method. Statistic result showed that all formula have significant differences on the influence against viscosity value (H_0 was rejected and significant $0.00 < 0.05$). Result of Newman-Keuls test showed that every formula gave different influence against viscosity one another.

Influence of storage time against viscosity value of each formula was analyzed by using one-way ANOVA. This statistic results showed that storage time did not affect significantly the viscosity of solution (H_0 is accepted and significant $0.976 > 0.05$).

Samples in appropriate media have no growth impurities. This might be caused by the presence of sodium benzoate as preservative in solution, which worked effectively as a preservative at pH 3-5 [26]. The results showed that the solutions were eligible, where the bacterial count of solutions was ≤ 104 colonies/ml and the fungi count were ≤ 103 colonies/ml [17].

CONCLUSION

The concentration of caffeine before and after formulation process were $3.019 \pm 0.138\%$ and $4.432 \pm 1.986\%$. Formula containing 5 mg/ml of

coffee arabica extract, 1.5 mg/ml of Na CMC and 0.5 mg/ml of sucralose was considered as the best formula. Microbiological test showed that no contamination in syrup of coffee arabica extract. The concentration of caffeine before and after decaffeination process were $3.377 \pm 0.091\%$ and $1.028 \pm 0.079\%$.

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