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The Development of Functional Beverage Combinations of Green Tea, Green Coffee, and Cinnamon for Hypercholesterolemia

Pengembangan Produk Minuman Fungsional Kombinasi Teh Hijau, Kopi Hijau, dan Kayu Manis bagi Hiperkolesterolemia

Aviani Harfika[1]*, Hardinsyah Hardinsyah[2], Listhia Hardiati Rahman[1]

[1]Nutrition Science Program, College of Holistic Health Sciences, Purwakarta, Indonesia
[2]Department of Community Nutrition, Faculty of Human Ecology, IPB University, Bogor, Indonesia

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Correspondent:
Aviani Harfika
aviani_harfika@stikesholistic.ac.id

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ABSTRACT

Background: Green tea, green coffee, and cinnamon are high in antioxidants and can improve blood lipid profiles.
Objectives: The purpose of this study was to develop functional beverages consisting of green tea, green coffee, and cinnamon for people with hypercholesterolemia.
Methods: This study was purely experimental with a completely randomized design. Three formulas of the functional beverages were: F1 (50% : 25% : 25%), F2 (40% : 35% : 25%), and F3 (35% : 40% : 25%). This study used organoleptic tests with 30 semi-trained panelists. The research data were analyzed using the Kruskal-Wallis H test.
Results: The results of the hedonic test showed that there were no significant differences in the average color, flavor, aroma, viscosity, or after-taste characteristics ($p>0.05$). The panelist's most preferred formula was F2, with the highest acceptance for all test characteristics. The results showed that one serving of F2 beverage (100 mL) contained 1.1 g carbohydrates, 3.7 g protein, 0.7 g fat, and 304 mg AEAC/100 g antioxidants.
Conclusions: The development of functional beverages consisting of green tea, green coffee, and cinnamon has the potential to be an alternative high-antioxidant beverage for hypercholesterolemia.

INTRODUCTION

Hypercholesterolemia is a lipid metabolic disorder characterized by an increase in total blood cholesterol (cholesterol level >200 mg/dL)[1]. The prevalence of hypercholesterolemic disease in Indonesia is currently quite high at 28%, and its increase is estimated to cause 2.6 million deaths[2]. Hypercholesterolemia is one of the risk factors for metabolic syndrome that can increase the incidence of non-communicable diseases. High cholesterol levels are associated with the risk of hypertension, stroke, coronary heart disease, and obesity[3]. Risk factors for hypercholesterolemia are categorized into factors that can be modified and not modified. Those that can be modified include consumption patterns, smoking, drinking habits, physical activity, and obesity[4].

Consumption habits are one of the most commonly used factors for the prevention and management of hypercholesterolemia. Numerous studies linking bioactive components and the prevention of non-communicable diseases have been done. These studies have found that antioxidants have a significant effect on reducing oxidative damage and inflammation and can improve antioxidant status in patients with dyslipidemia[5]. Previous literature studies have also shown that herbal and plant foods have affect on decreasing total blood cholesterol[6].

According to many studies, green tea contains minerals, vitamins, and antioxidants such as polyphenols that can lower the risk of cardiovascular disease. The bioactive content of green tea that is beneficial to health is Epigallocatechin Gallate (EGCG). Total cholesterol and triglycerides in overweight or obese subjects can be significantly reduced by consuming two glasses of green tea per day[7]. Meta-analysis studies also showed that green tea can significantly lower total cholesterol levels (−4.66 mg/dL, $p<0.01$) and Low-Density Lipoprotein (LDL) cholesterol (−4.5mg/dl, $p<0.01$) compared to control treatments[8]. Another type of food that is also high in

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antioxidants is green coffee. Nowadays, research into the effects of green coffee on health has begun. The compound of green coffee that affects on the blood lipid profile is chlorogenic acid. Research found that consumption of green coffee has significant effects on weight loss, the prevention of cardiovascular disease, and type 2 diabetes[9]. Consumption green coffee extract for eight weeks significantly decreased LDL-oxidation (31.18 ng/ml, $p<0.01$) and could improve the total antioxidant concentration capacity of the subject (71.73 µmol/l, $p=0.029$)[10]. Studies on the effects of supplementation with green coffee extracts also showed the promising effect of the bioactive substance chlorogenic acid on the decrease of total and LDL serum cholesterol and also an increase in high-density lipoprotein (HDL) cholesterol. Similar to green tea and green coffee, cinnamon contains cinnamaldehyde-type polyphenols and has high antioxidant activity. Studies found that supplementation with cinnamon extract for two months improved the levels of fasting insulin, blood sugar, total cholesterol, and LDL cholesterol in the subjects[12].

Functional foods contain active components that can be beneficial for health and disease prevention. Previous studies showed the positive effects of bioactive substances in some functional food combinations. Some studies report that a combination of several functional foods has a synergistic effect that is beneficial to health[13]. For instance, a combination of decaffeinated tea and green coffee products given for 90 days to patients with metabolic syndrome can significantly lower total cholesterol levels compared to controls[14]. Meanwhile, the combination of other products such as green coffee and beta-glucose can improve the levels of total cholesterol, LDL, VLDL, and triglycerides[15].

Unfortunately, functional food development associated with the prevention and treatment of hypercholesterolemia has not been much developed, mainly in association with active substances from a combination of herbal or local foods. Functional beverages are promising product developments because they are easy to consume and practical for the community. However, currently, no research has been conducted on the development of a functional food combination of green tea, green coffee, and cinnamon, especially for hypercholesterolemia. For this reason, this research aimed to develop functional beverage products from a combination of green tea, green coffee, and cinnamon, in particular for hypercholesterolemia.

METHODS

This research was purely experimental with completely randomized designs. The study used three formulas with differences in the ratio of green tea, green coffee, and cinnamon: F1 (50% : 25% : 25%), F2 (40% : 35% : 25%), and F3 (35% : 40% : 25%). The ratios of these formulas were based on the findings of literature studies and previous research that formed the basis for the development formula[16]. The proportion of the difference between green tea, green coffee, and cinnamon is shown in Table 1. The formula development was carried out in the Food Experimental Laboratory of the Department of Community Nutrition, IPB University.

Table 1. Characteristics of ingredients in each formula

Material	Amount (g)		
	F1	F2	F3
Green tea	5	4	3
Green coffee	2	3	4
Cinnamon	2	2	2
Whey protein	10	10	10
Stevia	1	1	1
Emulsifier (arabic gum)	2	2	2
Water	100	100	100

The main ingredients were selected based on their quality and they were obtained from an institution that guaranteed quality. For the ingredients, this study used powdered forms of green tea, green coffee, and cinnamon. The green tea powder (*Camellia sinensis*) was obtained from the *Balai Penelitian Teh dan Kina Bandung*, the green coffee powder of the type of robusta Lampung (*Coffea robusta*) was obtained from the Southeast Asian Food and Agricultural Science and Technology Laboratory (SEAFAST) of the IPB University, and the cinnamon powder (*Cinnamomum burmanni*) was obtained from *Balai Penelitian Tanaman dan Rempah Bogor*. In addition, to consider the receptivity, flavor, and color of the product, supporting ingredients were added, such as water, whey protein isolates (90%), stevia, and emulsifiers. The addition of whey protein and stevia to this product was to improve the flavor and reception of the product. Previous research has shown that whey protein affects blood lipid profiles[17], while stevia contributes as a calorie-free sweetener. Furthermore, Arabic gum-type emulsifiers were added to make the beverage homogeneous with a Hydrophilic-Lipophilic Balance (HLB) of 8-16. Some pieces of equipment used included glasses, a spoon, a bowl, food scales, shakers, spatulas, and measuring glasses. The process of making a beverage started with weighing ingredients in each formula. The main ingredients used were already in powder form and had been combined with other supporting ingredients. After each ingredient was weighed, the ingredients were combined into powder and ready for use. The process of making the beverages involved mixing the powder of the main ingredients (green tea, green coffee, and cinnamon) and the supporting ingredient (whey protein isolate 90%, stevia, and emulsifiers) with 100 mL of room temperature (25°C) mineral water using a shaker for ±1 minute. After that, they were served in glasses to be ready to drink.

After developing the formula, an organoleptic test was performed to determine the level of panelists' preference for the formula. Organoleptic hedonic test

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questionnaires were used based on Wirawanti's (2017)[18] research questionnaires for functional beverage *tempe* given to adult population panelists of IPB University students. The hedonic test questionnaire consisted of 6 questions including color, flavor, aroma, viscosity, after-taste, and overall product, with 9 scales used on each question (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely). The organoleptic panel consisted of 30 semi-trained panelists who met the criteria of bachelor and graduate students in the Department of Community Nutrition at IPB University. The inclusion criteria include willingness to take an organoleptic test, age ≥18 years, having taken training or examination related to organoleptic tests, not having an allergy or sensitivity to beverage ingredients (green tea, green coffee, cinnamon, and whey protein), and willingness to sign an informed consent, while the exclusion criteria include resigning or not completing the organoleptic test until completed. The questionnaires were given by the researchers in paper-based form in Bahasa Indonesia. Panelists were asked to fill in each question without comparing the assessment of each formula. The data obtained from organoleptic tests were analyzed using SPSS version 16.0 for Windows. The results of the reliability test showed an alpha-Cronbach coefficient of 0.78, indicating the good reliability of the questionnaire instrument. In this study, a Kruskal-Wallis H test analysis was performed to obtain the best formula with significance ($p<0.05$). The research has obtained ethical approval from the IPB Research Ethics Commission with number 132/IT3.KEPMSM-IPB/SK/2018.

Following the organoleptic test, proximate analysis was performed, which included water and fiber testing using the Gravimetry method, total fat analysis using the Soxlet-Hydrolysis test, protein content analysis using the Kjeltec method, and carbohydrate content testing using the By Difference test. Spectroscopic photometry was used to analyze the total antioxidant content. Proximate and total antioxidant analysis tests were carried out twice to ensure standardization of the results. Proximate and total antioxidant analysis tests were conducted at the MBRIO Food Laboratory, Bogor, West Java.

RESULTS AND DISCUSSION

Functional beverage product which is mainly composed of green tea, green coffee, and cinnamon, can serve as beverage alternatives that have the potential to decrease cholesterol levels in hypercholesterolemia. In general, previous studies showed that the main ingredient in this study has high levels of antioxidants[7,11,19]. Green tea is a variety of tea where the leaves are not fermented or dried. It is generally processed directly without a prolonged process of withering to prevent excess oxidation of tea leaves[20]. The most common bioactive component in green tea is Epigallocatechin Gallate (EGCG). The EGCG content in green tea is higher than in black and oolong tea[21]. Meanwhile, green coffee is made without roasting. The homogeneity of green coffee beans ranges from 94.3% to 97%, and the water content of coffee seeds ranges from 9.056%-9.243%[22]. Green coffee has health benefits because it contains more polyphenols[23]. Lastly, cinnamon is commonly used as an aromatic food, containing phenolic components such as cinnamaldehyde and eugenol[24]. Antioxidants and other bioactive compounds found in functional foods may have positive health effects[24]. The consumption of functional foods as beverages from a variety of foods can be accomplished to improve health benefits and effectiveness. Previous research has shown that a combination of several types of herbal plants can significantly increase the antioxidant content[25]. Table 2 shows the results of the organoleptic test formula.

Table 2. Organoleptic test results of the average preference formula's

Formula	Color	Flavor	Aroma	Viscosity	After-taste	Overall
F1	5.5^{a}	4.8^{b}	5.9^{c}	5.6^{d}	4.8^{e}	5.1^{f}
F2	5.2^{a}	5.9^{b}	6.0^{c}	5.5^{d}	5.2^{e}	5.4^{f}
F3	5.1^{a}	5.3^{b}	6.2^{c}	5.5^{d}	5.2^{e}	5.3^{f}

The same-letter notation shows an insignificant difference ($p>0.05$).

Color

Color plays an important role in a product. Unappealing color will make the panelists less receptive, even if the product contains more nutrients[26]. The average test results of preference on color characteristics showed no significant difference between formulas F1, F2, and F3 ($p>0.05$). Based on the results of the panelists, all formulas were rated neutral, although F1 had the highest average preference. The F1 color was preferred because it had a greater proportion of green tea than other formulas. The concentration of the green color increases with the addition of more green tea powder. The resulting green color is due to the high chlorophyll content of green tea, which does not undergo prolonged drying and makes the color concentrated green[27]. The beverage's produced green color is similar to that of matcha, therefore green tea dominates over green coffee and cinnamon in appearance. This is consistent with previous research that showed panelists preferred concentrated green-colored beverages made from green tea powder[27]. Previous research also showed that panelists favor vivid colors over muted ones in powdered herbal drinks[28].

Flavor

The characteristics of flavor indicate there were no significant differences in each formula. However, F2 had the highest average preference among panelists. This indicated that the more green tea added, the more unfavorable the panelists. Based on prior research, green tea has a bitter after-taste in functional beverages, regardless of whether honey is included. The bitter taste

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of green tea comes from the caffeine and L-theanine content of the beverage [27]. In the development of the beverage, stevia or calorie-free sugar was added to enhance flavor sensitivity. Previous research has shown that the addition of stevia leaf powder to Arabica coffee drinks has a significant influence on the level of flavor, aroma, and after-taste parameters[29]. Studies on *temulawak* and *serai* powdered herbal beverages have also shown that panelists' receptivity tends toward a sweet flavor[28]. This functional beverage contained whey protein in addition to sweeteners to improve flavor sensitivity. This is a result of considering that the three main ingredients-green tea, green coffee, and cinnamon-have bitter flavors by nature. The addition of whey protein made the flavor characteristics creamier, and the after-taste bitterness was reduced. The results of the meta-analysis study showed that whey protein affects improving the blood lipid profile[17]. Thus, the addition of whey protein not only improves the receptivity of flavor parameters but is expected to improve the blood lipid profile in hypercholesterolemia.

Aroma

The results of the aroma parameters showed there were no significant differences between F1, F2, and F3. Still, the most preferred aroma parameter was F3, which had the largest proportion of green coffee compared to F1 and F2. Although green coffee does not undergo roasting like coffee seeds in general, it still has volatile and nonvolatile compounds that affect the characteristic aroma. Volatile compounds are evaporative substances that contribute to the aroma of the senses, such as aldehyde, ketone, and ester. Non-volatile substances contribute to the flavor of coffee, such as caffeine, protein, and sugar. The content of carbohydrate compounds in coffee seeds is determined by the formation of aroma components. Aromatic components are formed through the low-molecular-weight caramelization of sugar as well as through Maillard's reaction. Aromatic compounds are produced when amino acids and reduced sugars interact[30]. In addition to the aroma of green coffee, cinnamon also contributes to functional beverage products. Cinnamon's characteristic aroma from cinnamaldehyde and eugenol improves the product's receptivity to aroma parameters.

Viscosity

The results of the preference on the viscosity parameter showed no significant differences in each formula. The panelists assessed the viscosity parameter as generally neutral. Similar amounts of water added to the formula might not significantly change the viscosity. Furthermore, the Arabic gum-type emulsifiers were added to each formula to create a homogenous beverage product. As the main ingredients were in powder, residue might be visible, which was caused by the separation between powder and water when prepared. Arabic gum was used for its Hydrophilic-Lipophilic Balance (HLB) of 8-16, which facilitated the quick homogenization of the main ingredients in water. In addition, shakers were used in developing the beverages to make the homogenization process faster. This finding was in line with a smoothie study that showed panelists' preferred viscosity was smooth and residue-free[31].

After-taste

The result of the preference of the after-taste parameter indicated that the panelists were neutral, and there was no significant difference between the formulas ($p>0.05$). The bitter after-taste of the main ingredients in green tea, green coffee, and cinnamon could be minimized by including additional supporting ingredients such as whey protein and stevia. The bitterness of green tea and green coffee comes from caffeine and chlorogenic acid. Compared to grained coffee seeds, green coffee has a slightly acidic taste due to its high chlorogenic acid content[32]. Chlorogenic acid is one of the antioxidants found in green coffee[33]. In addition to green tea and green coffee, cinnamon also has a specific after-taste that is spicy and sweet. However, the after-taste disappeared in each formula because of the lower proportion of cinnamon.

Overall

The overall parameter result is the preference level of the panelists assessing the overall functional beverage products, covering color, flavor, aroma, viscosity, and after-taste. There was no significant difference in the overall parameters of each formula. However, the panelist's highest preference was F2 over F1 and F3. Therefore, F2 was selected as a functional beverage consisting of green tea, green coffee, and cinnamon. Overall, F2 had a composition of less green tea than F1 and less green coffee than F3. This implied that F2 was the most preferred formula because it had less bitterness and after-taste of the main ingredients.

Proximate Analysis Test

After determining the formula from the organoleptic test, a proximate analysis test was conducted covering the carbohydrate content, protein, fat, water content, and raw fiber content. Proximate test results are presented in Table 3. The carbohydrate content was 1.1 grams and was derived from whey protein and a small portion of green coffee[34]. The fat content of the selected formula was determined to be 0.7 grams. The low-fat content per serving of the drink showed that the drink was low in fat and might be beneficial for hypercholesterolemia. The 3.7 grams of protein could be derived from the whey protein ingredient. These results show that functional beverages such as green tea, green coffee, and cinnamon have a higher protein content than green tea or green coffee alone[35]. The water content of 94.2 g/100 g indicated a high-water content due to the ready-to-drink samples used in the testing. Raw fiber selected with a formula of 0.2% already meets SNI 4320 1992 criteria. Traditional beverage powder has a maximum raw fiber content of 1.5%[6,36]. Green tea contains many minerals, including manganese, iron, selenium, copper, and zinc[35]. In addition, previous studies also showed that the content of the proximate analysis differs significantly in the type of coffee depending on the region of origin and the difference in altitude[37].

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Table 3. Proximate and total antioxidant analysis results of the selected formula

Analysis Test	Result
Carbohydrate (g)	1.1
Fat (g)	0.7
Protein (g)	3.7
Raw fiber (%)	0.2
Water content (g/100 g)	94.2
Antioxidant total (mg AEAC/100 g)	304

Antioxidant Total Analysis

The analysis results showed that functional beverages such as green tea, green coffee, and cinnamon have a total antioxidant content of 304 mg AEAC/100 g. These results indicated that the functional beverage contained high levels of antioxidants[38]. Previous research showed that a combination of several types of herbal plants has a total antioxidant content of 52.92% DPPH[38]. These findings also suggest that the combination of functional beverages contains higher levels of antioxidants than green tea, green coffee, and cinnamon individually. Previous studies showed that green coffee contains 57-85% DPPH-TEAC (mmolT/100 g) and green tea 60-70% DPPH. This study's findings are in line with previous meta-analysis studies that showed that the catechins in green tea, 576-714 mg or equivalent (3-4 g of green tea powder), can significantly reduce weight and body fat for 8-12 weeks. Green coffee with 30-100 mg of caffeine or the equivalent (1-3.5 g) of green coffee powder can reduce body fat percentage and improve blood lipid profile levels for 3-24 weeks[16].

Based on the results of organoleptic tests and analytical tests, F2 was the most preferred by the panelists and had an antioxidant content of 304 mg AEAC/100 g. When compared to the total antioxidant content of one particular ingredient, such as green tea, green coffee, and cinnamon, the F2 combination of green tea, green coffee, and cinnamon had a higher total antioxidant content[38]. This study has found a combination of functional beverage products from green tea, green coffee, and cinnamon, where the combination of these ingredients can be synergistic to improve the functionality of each ingredient. The analysis of the total antioxidant levels obtained demonstrates this conclusion. This research however did not do an analysis test on the antioxidant levels of each main ingredient before they were combined into a functional beverage.

CONCLUSIONS

The organoleptic test results showed that the selected formula was F2, which has the highest level of preference for the overall characteristics. There were no significant differences in the characteristics of color, aroma, flavor, viscosity, and after-taste among the formulas. The results of the selected formula showed that one portion of the beverage contained 1.1 g carbohydrates, 3.7 g protein, 0.7 g fat, water content of 94.2 g/100 g, 0.2% raw fiber, and 304 mg AEAC/100 g of antioxidants. This study recommended analyzing the total antioxidant content of each component separately before combining and further investigating the effects of the intervention on blood lipid profile levels in hypercholesterolemia.

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