Foods and Raw Materials, 2023, vol. 11, no. 2

E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

https://doi.org/10.21603/2308-4057-2023-2-567
Available online at http://jfrm.ru/en
https://elibrary.ru/NADAEM

Effect of cocoa husk Criollo tea on hypercholesterolemia in animal model

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Received 27.08.2022; Revised 03.12.2022; Accepted 10.01.2023; Published online 04.04.2023

Abstract:
Organic waste is a problem the cocoa industry has to handle. The industry produces a lot of cocoa bean husk, also called *Criollo cocoa husk*. Cocoa bean husk is an underutilized cocoa waste that contains bioactive components in the form of phenols and flavonoids. Processed cocoa bean husk can be brewed as a functional beverage.
The research objective was to test cocoa husk tea for sensory properties, bioactive components, and impact on blood cholesterol. This study used a randomized experimental design with six repetitions. Sensory data were processed using the Friedman and Wilcoxon signed-rank tests ($\alpha = 0.05$) to determine the difference in sensory properties between each formulation of cocoa husk tea.
The sensory evaluation involved 30 untrained panelists who gave the highest score to the formulation with 62.5% cocoa bean husk, 25% lemongrass, and 12.5% aromatic ginger, which could also reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (IC_{50} = 264.8675). The animal test showed that the cocoa husk formulation produced no significant difference ($p > 0.05$) in pre- and post-treatment, but was able to keep cholesterol within normal limits.
Cocoa bean husk showed health benefits by its antioxidant properties and ability to control blood cholesterol.

Keywords: Criollo cocoa husk, sensory characteristic, bioactive value, blood cholesterol, food waste

Funding: This research was supported by the Public Health Faculty of Airlangga University, grant no. 2059/UN3.1.10/PT/2020.

Please cite this article in press as: Adi AC, Tawakal AI, Rasyidi MF, Salisa W, Farapti F, Rachmawati H. Effect of cocoa husk Criollo tea on hypercholesterolemia in animal model. Foods and Raw Materials. 2023;11(2):206–214. https://doi.org/10.21603/2308-4057-2023-2-567

INTRODUCTION

Organic waste produced by the food or beverage industry covers a wide range of substances that cannot be consumed for nutrition purposes [1]. Dried cocoa beans and fermented cocoa beans are the main raw materials for all types of cocoa products, while cocoa bean husk is a by-product of the cocoa industry. The three most popular types of cocoa are *Criollo*, *Forastero*, and *Trinitario*, which together make up 95% of the total cocoa produced in the world. Criollo cocoa husk is a by-product with highly valuable bioactive components. However, it is discarded without recycling [2]. The weight of cocoa husk ranges from 10–17% of the total weight of cocoa beans [3]. A proper utilization of this by-product could prevent many environmental problems that arise from it being dumped as waste.

Cocoa husk has a good biofunctional potential for human health. It contains a lot of polyphenols that possess numerous biofunctional properties and health benefits. Flavonoids are the main polyphenol class in cocoa and their by-products [4]. According to scientific evidence, polyphenols are good for human health due to their antioxidant properties: they act as free radical scavengers and reduce oxidative stress. The bioactive substances contained in cocoa husk have antibacterial, antiviral, anticarcinogenic, antidiabetic,

Table 1 Cocoa bean husk beverage formulations

Ingredients	Formulation									
	Control		1		2		3		4	
	g	%	g	%	g	%	g	%	g	%
Cocoa bean husk	8.0	100.0	5.0	62.5	5.0	62.5	5.0	62.5	5.0	62.5
Lemongrass	–	–	2.0	25.0	1.0	12.5	2.0	25.0	1.0	12.5
Ginger	–	–	1.0	12.5	1.0	12.5	–	–	–	–
Turmeric	–	–	–	–	1.0	12.5	–	–	1.0	12.5
Aromatic ginger	–	–	–	–	–	–	1.0	12.5	1.0	12.5
Mineral water	200.0	96.2	200.0	96.2	200.0	96.2	200.0	96.2	200.0	96.2

and neuroprotective properties, not to mention their beneficial effect on the cardiovascular system [5]. The total polyphenol content in one cocoa pod is slightly higher than in cocoa husk, but the total flavonoid content in cocoa husk is almost twice higher than in cocoa pods [6].

Cocoa polyphenols can affect the lipid profile and enhance the antiatherogenic effect. Saad tested cocoa polyphenols *in vitro* and in cell culture to demonstrate the inhibition of low-density lipoprotein oxidation and reduction of low-density lipoprotein oxidative susceptibility [7]. Experimental rats after a month on a cocoa powder diet improved their lipid profile and demonstrated a low cardiovascular risk. Polyphenols have a protective effect against atherosclerosis: they alter the hepatic cholesterol homeostasis by reducing cholesterol absorption. Polyphenols were also reported to lower blood pressure and the activity of enzymes in the renin-angiotensin-aldosterone system, which are involved in the renin-angiotensin-aldosterone system [8].

A functional cocoa-husk beverage is a solution to the cocoa waste problem, not to mention its potential for human health. Cocoa bean husk can be processed into a functional beverage with a good antiradical and cardioprotective potential because it contains antioxidants in the form of polyphenols (flavonoids). Cocoa bean husk is processed into functional beverage starting with sorting and sterilization, followed by packaging into brewed bags. The present research objective was to find out the sensory profile of cocoa husk functional beverages and their effect on blood cholesterol.

STUDY OBJECTS AND METHODS

Research design. We used a complete randomized experimental design with six repetitions. We developed several tea formulations based on criollo cocoa husk. The research included a qualitative analysis of bioactive and sensory components based on such parameters as color, smell, taste, and concentration, as well as a pre-clinical animal test. The panelists gave an informed consent before the sensory evaluation. The evaluation procedure had no adverse effect on the panelists. The research was approved by the Health Research Ethics Committee, Department of Dental Medicine, Universitas Airlangga (Number: 379/HRECC.FODM/ VIII/2020).

Cocoa husk tea development. We designed four cocoa husk tea formulations (Table 1). Formulations 1 and 2 contained such spices as lemongrass, ginger, and turmeric. Formulations 3 and 4 included lemongrass, turmeric, and aromatic ginger. The spices were intended to improve the taste. Ginger (*Zingiber officinale* L.) was chosen because it contains active compounds with anti-inflammatory and antioxidant properties [9, 10]. Lemongrass (*Cymbopogon citratus* L.) is known to prevent several diseases because it has antibacterial, antifungal, antioxidant, antiseptic, anti-inflammatory, analgesic, and antipyretic properties [11, 12]. Turmeric (*Curcuma longa* L.) is useful as an anti-inflammatory, anti-oxidant, anti-microbial, cancer-prevention, and anti-tumor agent. It can reduce fat and cholesterol in the blood and acts as a blood purifier [13]. It also lowers blood pressure and improves rheumatism [14]. Likewise, aromatic ginger (*Kaempferia galanga* L.) also contains antioxidants, like other spices [15]. The production process required an oven, a baking sheet, a tray, a measuring spoon, a digital scale, and a grinding machine, as well as tea brewing equipment in the form of hollow tea bags.

CHC phytochemical screening. Dry cocoa beans (*Theobroma cacao* L.) served as the main raw material. The list of reagents included Dragendorff's reagent, Mayer's reagent, and Stiasny's reagent. Dragendorff's reagent was prepared by mixing 0.8 g of bismuth nitrate and 20 mL of HNO_3 (p) with 27.2 grams of Kl dissolved in 50 mL of water. The solutions were allowed to stand for 24 h, filtered, and brought up to 100 mL with Aqua Dest. Mayer's reagent was made by mixing 1.36 g of $HgCl_2$ and 60 mL of Aqua Dest with 5 g of Kl dissolved in 10 mL of water. The two solutions were mixed and brought up to 100 mL with Aqua Dest. Stiasny's reagent was prepared by mixing two parts of 30% formaldehyde with one part of concentrated hydrochloric acid. The method used in this research was a laboratory experimental method. The tests covered flavonoids, tannins, quinones, saponins, steroids/triterpenoids, and alkaloids.

The flavonoid examination referred to Farnsworth: the sample was heated with hot water and mixed with magnesium powder, hydrochloric acid, and amyl

alcohol solution [16]. Yellow, orange, and red staining indicated the presence of flavonoid compounds. This test as described by Stefova *et al.* and Nugroho used the standard High-Performance Liquid Chromatography (HPLC) method [17, 18].

The method for examining tannins was approved by WHO and the Association of Official Agricultural Chemists [19]. The tannin testing followed the procedure described by Farnsworth: the heated sample solution was mixed with three different reagents, namely iron (III) chloride, gelatin, and Stiasny's reagent [16]. The positive test for tannins was indicated by a change in color for each reagent: blue-black, white, and pink, respectively.

The quinone test was based on the method developed by Farnsworth [16]. The sample solution was processed through the initial stages of heating. The boiling solution was then mixed with sodium hydroxide. Red staining indicated a positive quinone test. The same initial preparation process was also valid for the saponin tests: after adding a reagent of hydrochloric acid, the vile was shaken vertically for 10 s.

The test for steroids/triterpenoids involved adding 20 mL of ether to 1 g of sample followed by grinding and filtering. The filtrate was put into a vaporizer cup and allowed evaporating. Then few drops of Liebermann-Burchard reagent were added. Green-blue or red-purple staining indicated the presence of steroids/triterpenoids. For the alkaloid test, a solution of 10 mL HCl was mixed with 2 g of sample, crushed in a mortar, and then filtered. After that, 5 mL of 25% ammonia was added to the filtrate and extracted with 20 mL of chloroform. The chloroform layer was removed, and part of it was dropped on filter paper, where it reacted with Dragendorff's reagent. Orange staining indicated a positive alkaloid test.

Cocoa husk antioxidant test. The antioxidant activity test relied on the procedure described by Filbert with several modifications [20]. It involved a sample solution with a concentration of 1000 μg/mL and a 0.4 mM DPPH solution. The sample stock solution was diluted to various concentrations with a total volume of 1.6 mL. The solution was then put into a test tube as a test solution, followed by producing a blank solution. At the next stage, the test solution was mixed with 0.4 mL of DPPH in the test tube and underwent an incubation process for 30 min in the dark. After that, the blank and the test solution were measured for absorbance value using UV-Vis spectrophotometry at a maximal wavelength of 516 nm. Each sample was tested for absorbance value of the inhibition percentage (%) and IC_{50} value [21].

Sensory evaluation. The sensory evaluation revealed the consumer appeal and feasibility [22]. It included such parameters as color, smell, taste, and concentration of cocoa bean husk beverages. Five trained panelists and 30 untrained panelists filled in a questionnaire. The panelists were asked to assess the samples based on their level of preference on a 7-point scale: 1 = disliked very much, 2 = disliked, 3 = disliked a little, 4 = neutral, 5 = liked a little, 6 = liked, and 7 = liked very much. The samples were tea from the cocoa husk with mineral water and spices. The panelists were given mineral water and advised to drink it before moving to the next tea sample.

Blood cholesterol test in experimental animals. The experimental animals used in this study were male Wistar strains rats (*Rattus norvegicus* L.) aged 2–3 months with a weight of 160–240 g. Before entering the treatment stage, all experimental animals were adapted for 7 days. Each rat was placed in a different cage according to the treatment group. The rats were treated and controlled in a fixed environment to make them able to adapt to the new conditions. The conditions presupposed room temperature and sufficient light. Food and drinks were given *ad libitum*. Induction of hypercholesterolemia was performed by testing cholesterol tolerance by giving extra egg yolk.

The experimental animals were grouped into three phases: the adaptation experiment phase, the induction phase, and the intervention phase. During the adaptation stage, all the rats were treated normally using pellet feed for 7 days. During the induction stage, they were divided into five groups: positive control, negative control, Treatment 1, Treatment 2, and Treatment 3. During the induction stage, all the groups were induced using egg yolk, except for the negative control group (–), where the pellet induction lasted two weeks.

The intervention stage involved three types of treatment using simvastatin at a dose of 1 mg/kg BW, as well as 200 and 400 mg cocoa bean husk extract. Figure 1 summarizes the details.

Statistical analysis. The sensory data were first tested for normality and homogeneity by the Saphiro-Wilk test. If the data were distributed normally, they were processed by the analysis of variance (ANOVA) to determine the difference between parameters. The parameters that were found to be different underwent Fisher's test ($p \leq 0.05$). If the assumption of normality was not met, the data were subjected to the Friedman test and the Wilcoxon test ($\alpha = 0.05$).

The statistical data analysis of the cholesterol test was conducted by using the normality test according to the Saphiro-Wilk test. If the data were distributed normally ($p > 0.05$), it was followed by the paired sample t-test analysis. If the data were not distributed normally, then the Wilcoxon test was used. The test was conducted to determine the differences in cholesterol levels in the pre- and post-test through the post hoc test. If the significance value obtained was < 0.05, it meant that there was a difference between cholesterol levels in the pre-test and post-test in all treatments. The statistical analysis was tested using the IBM Statistics SPSS 22 software.

RESULTS AND DISCUSSION

Cocoa husk tea development. The cocoa bean husk tea included several types of spices (Table 1). The spices were intended to improve the taste. Ginger (*Zingiber*

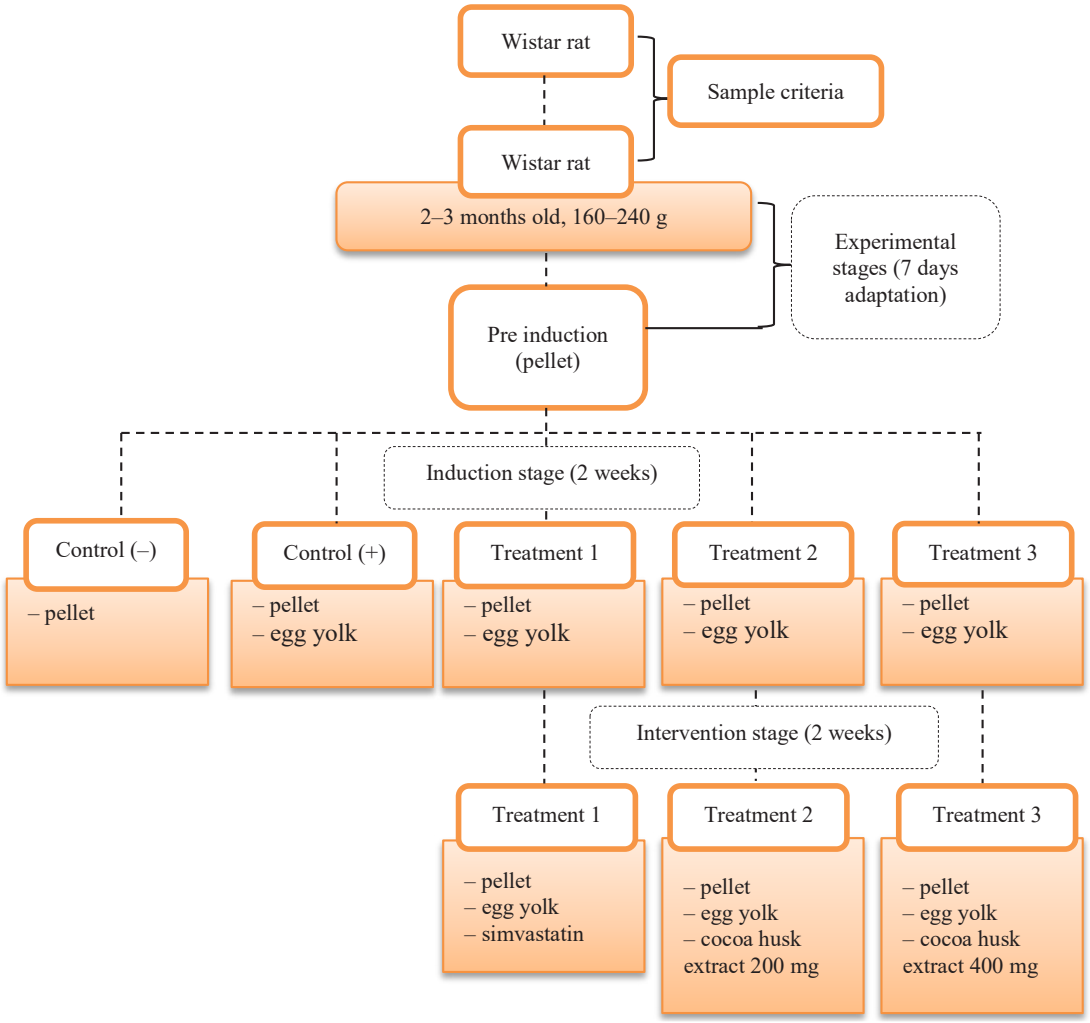

Figure 1 Cholesterol tests in experimental animals

Table 2 Phytochemical screening of cocoa bean husk

Compounds	Result
Alkaloids	–
Flavonoids	+
Saponins	–
Quinones	–
Hydrolyzable tannins	–
Condensed tannins	+
Steroids/triterpenoids	+

+: secondary metabolite detected

–: secondary metabolite undetected

officinale L.) was chosen for its bioactive compounds that have anti-inflammatory and antioxidant properties [9, 10]. Lemongrass (*Cymbopogon citratus* L.) is known to prevent several diseases because it possesses antibacterial, antifungal, antioxidant, antiseptic, anti-inflammatory, analgesic, and antipyretic properties [11, 12]. Turmeric (*Curcuma longa* L.) is an anticoagulant and anti-oxidant. Aromatic ginger (*Kaempferia galanga* L.) also contains antioxidants [15].

Phytochemical screening results. The phytochemical screening employed laboratory experimental methods. It included tests for flavonoids, tannins, quinones, saponins, steroids/triterpenoids, and alkaloids (Table 2).

The results of the phytochemical screening showed that cocoa bean husk contained flavonoids, condensed tannins, and steroids. The fact that it also contained flavonoid compounds, condensed tannins, and steroids/triterpenoids was in line with Yumas, who subjected cocoa bean husk to phytochemical screening and reported flavonoids, tannins, and triterpenoids [23]. The abovementioned substances belong to secondary metabolites. The screening detected no alkaloid compounds, saponins, quinones, or hydrolyzable tannins.

Flavonoids are secondary metabolite compounds that are commonly found in plant tissues. Flavonoids belong to phenolic compounds with a chemical structure of C6-C3-C6 [24]. They are powerful antioxidants as they are able to release hydrogen atoms [25].

Tannins are another type of secondary metabolites found in plants. Tannins are antioxidants: the larger their content, the greater their antioxidant activity. Tannins owe their antioxidant activity to the fact that they contain polyphenolic compounds that are able to capture free radicals [26].

Steroids are natural compounds with a carbon skeleton. They belong to the type of secondary metabolite compounds. Steroids are found in nature and derived from triterpene. Steroids of plant origin are derived from cycloartenol triterpenes. Early in their formation, acetic acid converts into cycloartenol through mevalonic acid and squalene [27]. Steroids are also classified as secondary metabolites and are known to possess antioxidant and antibacterial properties [28].

Antioxidant test results on cocoa bean husk simplicial. Simplicia is a natural material that has not undergone any changes or processing, e.g., it has not been dried [29]. We tested the antioxidant activity of cocoa bean husk simplicia to measure the effect of phytochemical substances in the initial raw material, i.e., before processing.

The simplicia calibration showed that the percentage of inhibition increased together with concentration, which was raised gradually. The resulting value of $R^2 = 0.9956$ demonstrated that these two variables had a strong effect.

The maximal wavelength was 516 nm. The control absorbance values were 0.973, 0.905, and 0.934 with a mean value of 0.937. The values of IC_{50} and IAA for cocoa bean husk simplicia were very weak – 1302.414 and 0.034551, respectively. Thus, the antioxidant activity was weak: IC_{50} < 50 ppm = very strong antioxidant; 50–100 ppm = strong; 101–150 ppm = moderate; and 150–200 ppm = weak [30].

Antioxidant activity of cocoa bean husk extract. The next antioxidant test was carried out on the cocoa bean husk extract to determine the effect of processing on the antioxidant activity of cocoa bean husk. The antioxidant test preceded both the sensory test and the experimental animal test.

The results of the extract calibration showed that the percentage of inhibition increased together with concentration. The obtained value of $R^2 = 0.9949$ demonstrated that these two variables had a strong effect.

The maximal wavelength was 516 nm. The control absorbance values were 0.977, 0.951, and 0.954 with a mean value of 0.961. The IC_{50} and IAA values of cocoa bean husk extract were found weak – 264.8675 and 0.169896, respectively. Thus, the antioxidant activity of cocoa bean extract was still weak. Compared with the results for simplicia, the extract showed even lower IC_{50} values.

The antioxidant activity test was based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Cocoa bean husk simplicia demonstrated antioxidant properties. However, when compared to the antioxidant standards, the antioxidant activity of cocoa bean husk fell into the weak category, as indicated by its IC_{50} value. This antioxidant ability was in line with the phytochemical screening results for flavonoid content (Table 2). The weak antioxidant power might have been due to the low content of flavonoid compounds (not yet quantified) or the type of flavonoid compound that had a chemical structure with a weak electron transfer ability (structural elucidation was not conducted). Therefore, the antioxidant effect could be provided only by regular and continuous consumption of cocoa bean husk infusion.

Table 3 Average sensory test score of cocoa bean husk beverage

	Formulation				
Indicators	Control	1	2	3	4
Color	3.0	5.0	6.5	6.0	6.0
Smell	6.0	4.5	5.0	6.0	5.0
Taste	2.0	5.0	3.8	5.0	4.0
Concentration	1.5	5.5	4.5	5.5	5.5
Total	12.5	20.0	19.8	22.5	20.5
Average	3.1	5.0	4.9	5.6	5.1

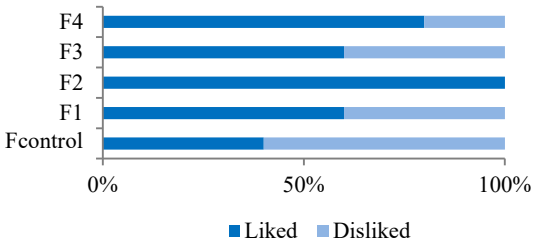

Figure 2 Color assessment of cocoa bean husk beverage, %

Sensory profile. Five formulations of cocoa bean husk beverages (Table 1) where bean husk was substituted with various spices were tested for consumer appeal. Table 3 shows the results of the sensory test for each parameter. Among the five samples, Formulation 3 (62.5% cocoa bean husk with 25% lemongrass and 12.5% aromatic ginger) attained the highest preference value based on the mean value of all parameters, i.e., color, smell, taste, and concentration.

Color is an important aspect that can affect food and beverage preferences [31]. The sensory assessment of color showed that the range of color acceptance scores was 3.0–6.5, which means that it ranged from "disliked a little" to "liked". The highest color score was obtained by Formulation 2 (62.5% cocoa bean husk, 12.5% lemongrass, 12.5% ginger, 12.5% turmeric, 12.5% aromatic ginger), while the lowest preference score was obtained by the control formulation with 100% cocoa bean husk.

The panelists preferred a lighter shade to the dark one: the lighter samples got a higher score than the dark-colored ones. The more cocoa bean husk was added, the darker the color became [22]. When a part of cocoa husk was substituted with spices, it produced a yellowish color or affected the brightness. Extra spices made the drink slightly yellow or bright in color. Turmeric was responsible for the yellow color as it is known to contain three pigments: curcumin, dimethoxy-curcumin, and bis dimethoxy-curcumin [32]. Ginger powder alone produces a yellowish-white color when dissolved in water [33]. Pure lemongrass powder dissolved in water produces a yellowish color [34].

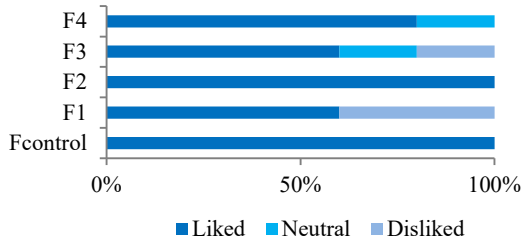

Figure 3 Smell assessment of cocoa bean husk beverage, %

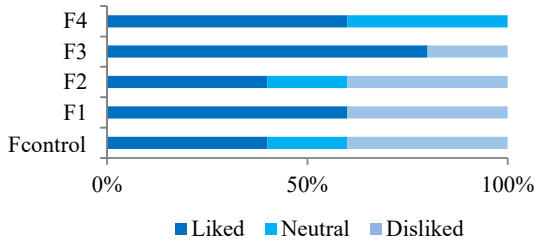

Figure 4 Taste assessment of cocoa bean husk beverage, %

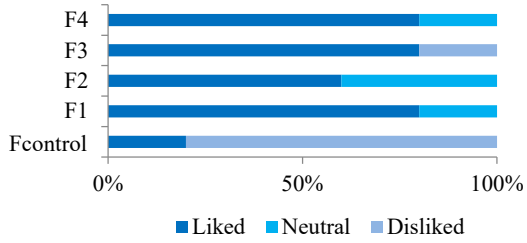

Figure 5 Concentration assessment of cocoa bean husk beverage, %

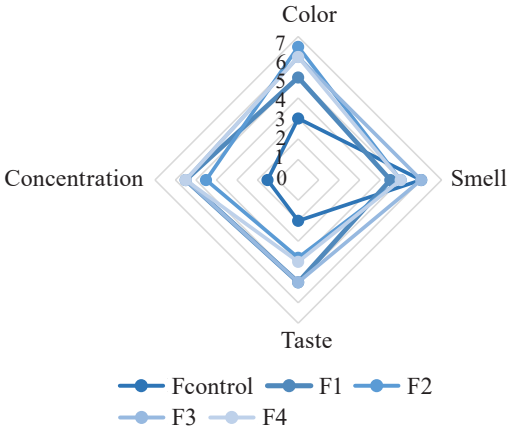

Figure 6 Hedonic test results

The percentage of panelists who claimed to like the color was as follows: Formulation 0 – 40%, Formulation 1 – 60%, Formulation 2 – 100%, Formulation 3 – 60%, and Formulation 4 – 80%. Formulation 2 had the best score for color: all 30 panelists appreciated it (Fig. 2).

Figure 3 demonstrates smell evaluation of the cocoa bean husk beverages. The highest score for smell went to the control sample and Formulation 2 (62.5% cocoa bean husk, 12,5% lemongrass, 12.5% ginger, 12,5% turmeric). The lowest smell score belonged to Formulation 1 (62.5% cocoa bean husk, 25% lemongrass, 12.5% ginger). The panelists preferred the beverage with a distinctive cocoa flavor. The characteristic cocoa smell appears as a result of the reaction between cocoa smell precursors (free amino acids and peptides) and sugar that enter the Maillard reaction to produce such smell components as alcohol, ether, furan, thiazole, pyrone, acid, ester, aldehyde, amine, amine, oxazole, pyrazine, and pyrrole. One of the compounds responsible for the characteristic smell of cocoa is 2,3-butanediol [35].

The highest taste score belonged to Formulation 3 (62.5% cocoa bean husk, 25% lemongrass, 12.5% aromatic ginger), while the lowest score belonged to the control (Fig. 4). The panelists preferred the samples with a lower proportion of cocoa bean husk. Polyphenols that give cocoa bean husk its antioxidant properties produce a slightly bitter and sour taste [23].

The score for the concentration ranged from 1.5 to 5.5, which means that it ranged from “disliked very much” to “liked a little” (Fig. 5). The highest concentration score belonged to Formulation 1 (62.5% cocoa bean husk, 25% lemongrass, 12.5% ginger), Formulation 3 (62.5% cocoa bean husk, 25% lemongrass, 12.5% aromatic ginger), and Formulation 4 (62.5% cocoa bean husk, 12.5% lemongrass, 12.5% turmeric, 12.5% aromatic ginger). The control sample received the lowest concentration score.

The acceptability value of cocoa bean husk beverages was based on the sensory test which included color, smell, taste, and concentration (Fig. 6).

Formulation 3 (62.5% cocoa bean husk, 25% lemongrass, 12.5% aromatic ginger) received the highest preference value based on all the sensory indicators, i.e., color, smell, taste, and concentration.

Table 7 Paired sample t-test: pre- and post-intervention cholesterol levels

Treatment groups	Cholesterol levels, mg/dL		A *p*-value of paired t-test results	Conclusion
	Pre-test	Post-test		
Negative control	29.80 ± 4.38	38.00 ± 8.64	0.902	No difference (normal cholesterol level)
Positive control	33.80 ± 5.54	86.50 ± 8.87	0.002	Difference detected (high cholesterol level)
Treatment 1	34.80 ± 6.18	58.00 ± 4.55	0.606	No difference (high cholesterol level)
Treatment 2	44.00 ± 3.39	42.00 ± 14.16	0.841	No difference (normal cholesterol level)
Treatment 3	33.00 ± 2.86	33.80 ± 8.32	0.942	No difference (normal cholesterol level)

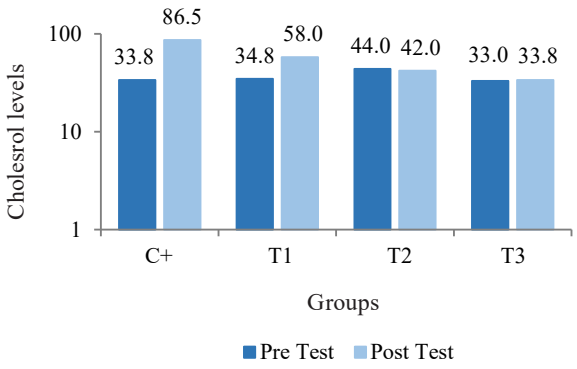

Figure 7 Effect of cocoa bean husk extract on cholesterol in rats

Blood cholesterol value in experimental animals. The data collected for blood cholesterol tests were the initial data before the intervention and the final data collected two weeks after the intervention period. The pre-test and post-test data were compared to reveal differences in the results of the intervention activities (Table 4).

Normal blood cholesterol level in rats is 10–54 mg/dL [36]. The paired sample t-test showed that the negative control treatment with pellets increased cholesterol from 29.8 mg/dL in pre-intervention to 38 mg/dL in post-intervention (Table 7). The negative control demonstrated no significant difference in cholesterol levels (p-value = 0.902 (> 0.05)). Thus, the negative control treatment had no significant effect on cholesterol.

The positive control group received duck egg yolk. As a result, the pre-cholesterol level of 33.8 mg/Dl (normal cholesterol) increased to 86.5 mg/dL (high cholesterol). The difference between pre-test and post-test was significant with a p-value of 0.002 (< 0.05). Hence, the positive control treatment had a significant effect on the changes in cholesterol (Fig. 7).

Treatment 1 included pellets, duck egg yolk, and simvastatin. As a result, the pre-cholesterol level of 34.8 mg/dL reached 58 mg/dL. No significant difference in cholesterol levels was detected (p-value = 0.841 (> 0.05)). Thus, Treatment 1 had no significant effect on cholesterol.

Treatment 2 involved pellets, duck egg yolks, and 200 mg/kg BW of cocoa bean husk extract. From the precholesterol level of 44 mg/dL, it fell down to 42 mg/dL. Therefore, we detected no significant difference in cholesterol levels between pre-test and post-test (p-value = 0.841). Treatment 2 produced no significant effect on cholesterol but was able to maintain it within normal limits.

Treatment 3 involved pellets, duck egg yolks, and 400 mg/kg BW of cocoa bean husk extract. The pre-cholesterol level of 33.3 mg/dL remained almost the same: 33.8 mg/dL. The difference between pre-test and post-test was not significant (p-value = 0.942). Thus, Treatment 3 had no significant effect on cholesterol but was able to maintain it within normal limits.

Treatment 2 and Treatment 3, which involved cocoa bean husk extract, maintained cholesterol levels in the normal range but provided no significant changes. Treatment 1, which involved simvastatin, demonstrated a significant difference in blood cholesterol levels. The positive control also showed a significant difference, which was inversely proportional to the negative control. According to Ahmad & Amy, cocoa injections in rats reduced their low-density lipoprotein (LDL) levels. Darand *et al.* also mentioned the effect of cocoa consumption which could significantly reduce blood cholesterol levels in humans [37, 38]. Sweety also stated that cocoa products had a significant anti-cholesterol impact [39].

The mechanism of reducing LDL cholesterol and total cholesterol in experimental animals could be explained by the high flavonoid content in cocoa bean husk. Flavonoids with their antioxidant and anti-inflammation properties increased the function of high-density lipoprotein (HDL) cholesterol, which decreased the total cholesterol levels. HDL cholesterol removed reverse cholesterol transport in the blood [40]. For instance, Martinez *et al.* also reported that an increase in the consumption of cocoa flavonoids improved the level of HDL cholesterol [41]. Flavonoid-rich foods could improve the endothelial function in cells [42]. Out of the five types of intervention trials, Treatments 2 and 3, which involved cocoa bean extract, managed to keep cholesterol levels in rats within the normal limits, although they provided no significant change.

CONCLUSION

Cocoa bean husk extract, a by-product of the cocoa industry, showed health benefits through its antioxidant activity. It was able to reduce DPPH free radicals (IC_{50} = 264.8675) better than cocoa bean husk simplicia (IC_{50} = 1302.414). The blood cholesterol animal tests registered no significant difference, but the extract was able to maintain cholesterol within normal limits. The lighter-colored samples with little cocoa husk received the best score for color and concentration, while the samples with a higher proportion of cocoa bean husk received a higher score for the characteristic cocoa smell. As for the taste, the panelists preferred the sample with less cocoa bean husk. The highest mean score for sensory properties belonged to the sample with 62.5% cocoa bean husk, 25% lemongrass, and 12.5% aromatic ginger.

CONTRIBUTION

Conceptualization: A.C. Adi and H. Rachmawati; methodology: H. Rachmawati and F. Farapti; software: W. Salisa; validation: A.I. Tawakal; formal analysis: W. Salisa and M.F. Rasyidi; investigation: W. Salisa and A.I. Tawakal; resources: F. Farapti; data curation: W. Salisa; original draft: M. Rasyidi; review and editing: H. Rachmawati, F. Farapti, M.F. Rasyidi; visualization: W. Salisa; supervision: A.C. Adi and H. Rachmawati;

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CONFLICTS OF INTEREST

The authors declared no conflict of interests regarding the publication of this article.

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