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Acceptability Analysis of (Carob, Tempeh, Honey Truffle) as an Alternative Snack Rich in Antioxidants (Cartemuffle Isoflavones and Polyphenols) to Prevent Breast Cancer

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ABSTRACT
The most common cancer that affects women is breast cancer. Carob, honey, and tempeh are potential food modifications in the form of antioxidant-rich chocolate snacks that effectively ward off free radicals that cause breast cancer. The study was conducted to identify the acceptability (color, aroma, taste, and texture) and isoflavone and polyphenol levels of the control formula and CARTEMUFFLE formulas as alternative breast cancer prevention snacks. The study method used a true experimental design with a completely randomized design (CRD). There was a control formula and 3 modified formulas, namely LF1, LF2, and LF3. Further-more, an acceptability test was conducted on 25 moderately trained panelists to determine the most preferred. Due to cost constraints, isoflavone and polyphenol antioxidant content tests were conducted on the control and the most favored formulas. The Results showed that the most preferred formula was formula LF3 4.01 in the preferred category. Isoflavone and polyphenol antioxidant content were higher in LF3 than the control formula/LF0. This study shows the most pre-ferred formula is LF3, containing 0.12 mcg isoflavones and 1.73 mg polyphenols higher than the control. Further research can analyze all formulas' total antioxi-dant, macronutrient, and fiber content and change the cooking technique.

Keywords: Breast Cancer, Carob, Tempeh, Honey, Antioxidant

INTRODUCTION
Breast cancer is a type of cancer that emerges in the breast and then spreads throughout other breast cell organs. It is crucial to recognize and comprehend the many cases of benign and non-cancerous (malignant) breast lumps[1]. Breast tumors that aren't considered cancerous are caused by abnormal growths that don't spread to other breast tissues. They are generally not life-threatening. However, these benign lumps called tumors can increase the risk of developing breast cancer [2].

GLOBOCAN (Global Burden of Cancer), a report by the International Agency for Research on Cancer (IARC) in 2020, reported 19,292,789 cases of cancer and 9,958,133 mortalities caused by cancer worldwide [3]. Breast cancer cases in Indo-nesia, according to GLOBOCAN, in 2020 reached 16% or 68,858 confirmed cases out of a total of 396,914 new cancer cases, followed by 22 thousand mortalities [4].

The risk factors that increase the incidence of breast cancer are age, family histo-ry, gender (women mostly suffer the incidence of breast cancer), physical activ-ity, smoking habits and passive smokers exposed to cigarette smoke, alcohol con-sumption, obesity, poor diet such as consumption of foods high in fat and low in fiber, high in preservatives/colors and other factors [5–7]. Consumption of fatty foods is a risk factor for breast cancer, especially consuming saturated fats such as fried chicken, fast food, meat, whole cream milk, butter, cheese, and fried foods. With frequent frequency and excessive amounts, fat will accumulate in the body exces-sively, which can cause the body to

produce more estrogen, triggering an abnormal cell division process.

The prehistoric civilizations of China, India, Egypt, and Sumeria stated that food prevents and treats disease effectively. The Ayurveda system of traditional medicine describes the health and therapeutic benefits associated with various bioac-tive foods [8]. Bioactive Food has various effects on the human body, and the com-pounds contain the highest antioxidant activity. These antioxidant compounds are important to decrease or reduce inflammation and carcinogenesis caused by oxida-tive stress. ROS (Reactive Oxygen Species) are directly destroyed during protein phosphoryla-tion due to inhibition of cell proliferation [9–11]. Tempeh, a food known for its nutri-tional value, is a significant source of the antioxidant isoflavones [12]. Honey, which also contains antioxidants in the form of polyphenols, and some foods that are still rarely known and used for their properties, such as carob, can be used as an alterna-tive to chocolate, which is high in fat and fiber [13].

The main physiological activity of isoflavones is as an antioxidant that inhibits angiogenesis so that the proliferation of cancer cells will die. It also includes the case of breast cancer [14]. The isoflavone content of tempeh is better than soybean. This is because soybean seeds are transformed during the fermentation process so that the free-form isoflavone compounds of tempeh (aglycones) change into new trans-formant compounds that have higher biological activity, this is indicated by factor II (6,7,4 'tri-hydroxy isoflavones) which are present in tempeh but not in soy-beans [15]. In in-silico research related to "stimulation of isoflavone tethering to es-trogen recep-tor β as a breast cancer treatment," isoflavones that are low in affinity energy have a percentage of interactions above 90% with hydrogen bonds, so that amino acid residues Glu 305, Arg 346 and Leu 476 have an active role in binding to estrogen receptor β [16].

Carob, with its low-fat and high fiber content, has an antioxidant polyphe-nol and flavonoid count of 1.76 mg, equivalent to 0.30 mg of gallic acid per gram dry weight and equivalent to quercetin per gram dry weight [17]. The nutritional composition of carob varies greatly depending on the part of the plant (such as the fruit, flesh, or seeds), cultivar, genetic factors, and the growing environment [18]. Although carob is high in sugar, most studies agree that it is rich in insoluble fiber and micro-constituents, not to mention vitamins and phenolic compounds that are anti-hyperglycemic, antioxidant, and known for their anti-inflammatory properties [17–19].

Antioxidant diversity in the form of polyphenols is also found in honey, which has pro-inflammatory and anti-inflammatory properties. In vitro experiments showed honey's antiproliferative and proapoptotic effects on breast cancer cells and in-creased apoptosis; this animal study showed that honey reduced tumors' number, growth rate, volume, and weight [20]. The polyphenols replace the entire tumor micro-environment and reduce the process of angiogenesis so that the body is resistant to continued cancer growth and metastasis of cancer cells [21]. Another related study supporting polyphenol intake, in which 134 newly diagnosed breast cancer patients and 267 healthy individuals were studied with a food frequency questionnaire (FFQ), showed that adopting a diet rich in polyphenols can reduce the potential risk of breast cancer [22].

The diversity of foodstuffs available gives rise to a wide variety of foods, with many popular food-related trends appearing tempting and delicious but lacking in nutritional aspects [23]. Chocolate truffles are one of the most popular snacks that have a sweet taste, with a variety of block and round shapes originating from France. The main

ingredients of chocolate truffles are chocolate, heavy cream, and cocoa powder, which are high in saturated fat, which can lead to obesity and various dis-eases, including breast cancer.

The results of the above explanation are the reason why researchers are inter-ested in making chocolate truffle snacks that are substituted with carob powder, tempeh, and honey as a binder instead of heavy cream. The modification will consist of sev-eral formulas and then will be tested on several panelists through the Organo-leptic/Acceptability Test, Hedonic test, and statistical test. In addition, the most pre-ferred formula will be tested for isoflavone and polyphenol antioxidant content, and the control formula will be used as a comparison. Therefore, this snack can be con-sumed as an alternative snack that is high in fat and rich in antioxidants to pre-vent breast cancer, which is suffered by many women.

RESEARCH METHOD

The research that will be carried out is a true experimental design with a type of research design called CRD (Completely Randomized Design), which involves testing the antioxidant content [24]. The research experiments occurred from November 2023 - March 2024. Meanwhile, preparation and implementation of the CARTEMUFFLE chocolate organoleptic test was carried out in the "Taste Test" Laboratory and the Cooking Laboratory of the Surabaya Ministry of Health Polytechnic, Jalan Pucang Jajar Selatan No. 24 B, Gubeng District, Surabaya City. Then, the antioxidant content test was carried out at the Nutrition Laboratory of the Faculty of Public Health, Airlangga University Campus C, Jl. Dr. Ir. H. Soekarno No.123, Mulyorejo, Mulyorejo District, Surabaya, East Java. The samples used were 4 formulas, 1 control formula, and 3 modified CARTEMUFFLE formulas, which are presented in the following table:

Table 1. Control Formulation and CARTEMUFFLLE (Carob, Tempeh, Honey Truffle) Chocolate Formulation

Ingredients	Control Formula (Code LF0)	Formula 1 (Code LF1)	Formula 2 (Code LF2)	Formula (Code LF3)
Carob Powder	0g	50g	45g	35g
Tempeh	0g	30g	40g	50g
Honey	0g	50g	45g	45g
Chocolate Bar	125g	30g	30g	30g
Whipping Cream	50g	0g	0g	0g
Cocoa Powder	10g	10g	10g	10g

The best samples will be tested for isoflavone and polyphenol antioxidant levels. For the acceptability/organoleptic test sample, there were 25 panelists with a type of moderately trained panelist. Physical assessment data collection techniques consist of 4 indicators, including color, aroma, taste, and texture, using the Hedonic Scale Test method, which consists of 5 levels of preference (Very dislike, Dislike, Neutral, Like, Very Like). Furthermore, data analysis will use statistical tests, namely the Kruskall-Wallis test, followed by the Mann-Whitney test if there are differences related to the hypothesis, whether accepted or not, with an error rate of 0.05 (α = 0.05). After testing, the data will be presented in the form of a table.

RESULT AND DISCUSSION

Table 2. Characteristics of CARTEMUFFLE (Carob, Tempeh, Honey Truffle) Chocolate Formulation

Indicator	CARTEMUFFLE (Carob, Tempeh, Honey Truffle) Chocolate Formulation			
	LF0	LF1	LF2	LF3
Color	Dark Brown	Dark Brown	Dark Brown	Dark Brown

Aroma	Typical Chocolate	Chocolate mixed With Carob	Chocolate mixed with carob	Chocolate mixed with carob
Texture	Soft	Soft and slightly crunchy flavor from the roasted tempeh crumbs	Soft, and more crunchy texture from roasted tempeh crumbs	Soft and slightly crunchy texture from roasted tempeh crumbs
Flavor	Sweet chocolate Flavor	Sweet, carob dominates and a hint of sourness	Sweet, slightly carob flavor and slightly sour.	Sweet, honey is more flavored, crumbs from tempeh are felt more than the other 3 formulas

Table 3. Average Distribution of CARTEMUFFLE Formulation Ratings

Indicator	CARTEMUFFLE (Carob, Tempeh, Honey Truffle) Chocolate Formulation			
	LF0	LF1	LF2	LF3
Color	3.8	3.92	4	4.04
Aroma	3.96	3.69	4.08	4.08
Texture	4.08	3.23	3.44	3.92
Flavor	4	3.19	3.4	4
Average	3.96	3.50	3.73	4.01

Score Categories: 1 (Strongly Disliked), 2 (Disliked), 3 (Neutral), 4 (Liked), 5 (Strongly Liked)

According to the results of the organoleptic test or acceptance test of the CARTEMUFFLE (Carob, Tempeh, Honey Truffle) product using a hedonic scale, it is known that the formula most preferred by panelists in general based on the color, aroma, texture and taste of the CARTEMUFFLE product is the most preferred LF3 formula with the addition of 35 g carob powder, 50 g tempeh, and 45 g honey.

Table 4. Kruskall-Wallis Test Results CARTEMUFFLE (Carob, Tempeh, Honey Truffle) Chocolate

No.	Indicator	Kruskall Wallis Test Values
1	Color	0.600
2	Aroma	0.240
3	Texture	0.008
4	Falvor	0.01

In the Kruskall Wallis test results table, the texture and taste indicators have a P-value <0.05, where the texture value is 0.008, and the taste value is 0.01, which means there is a difference. As for the color and aroma indicators of the control formula and 3 CARTEMUFFLE formulas, the results of the Kruskall Wallis test value for color are 0.600 and aroma is 0.240, the P-value> 0.05, which means that there are no significant differences related to indicators between the control formula and the 3 CARTEMUFFLE formulas tested.

Table 5. Mann Whitney Test Results on CARTEMUFFLE

Indicator	Texture		Falvor	
	Asymp. Sign	Description	Asymp.Sign	Description
LF0 with LF1	0.002	LF0 ≠ LF1	0.002	LF0 ≠ LF1
LF0 with LF2	0.009	LF0 ≠ LF2	0.021	LF0 ≠ LF2
LF0 with LF3	0.136	LF0 = LF3	0.115	LF0 = LF3
LF1 with LF2	0.852	LF1 = LF2	0.639	LF1 = LF2
LF1 with LF3	0.097	LF1 = LF3	0.068	LF1 = LF3
LF2 with LF3	0.140	LF2 = LF3	0.274	LF2 = LF3

Asymp. Sign < 0.05 (there is a difference the formulas)

The Mann-Whitney follow-up test results showed significant differences in texture indicators. Of the 4 texture formulas, the significant differences were

formula LF0 with LF1 and LF0 with LF2. Likewise, the same significant difference was seen related to the taste and texture indicators, where formula LF0 with LF1 with asymp was used. Sign obtained 0.002 and the formula between LF0 and LF2 with asymp. Sign 0.021, where the meaning of the two value scores shows the difference between formulas.

Table 6. Test Results of Antioxidant Content of Isoflavones and Polyphenols

Sample Codes	Isoflavones (μ g/g)	Polyphenols (mg as. galate/100 g)
Formula LF0	0.06	1.64
Formula LF3	0.12	1.73

The test results of the antioxidant content of isoflavones and polyphenols in 100 grams of CARTEMUFFLE most favored by panelists, namely formula LF3, obtained for isoflavones of 0.12 micrograms while for polyphenols, 1.73 milligrams of gallic acid were obtained per 100 grams of CARTEMUFFLE LF3. While the isoflavone content in formula LF0 was 0.06 micrograms and for polyphenols, it was 1.64 milligrams.

Color

Color is a sensory that is the main concern of panelists, color can be a determinant of food quality that can be assessed in general. Food that has high nutritional value and has an attractive color appearance will increase appetite [25].

Based on the results of the color indicator organoleptic test on CARTEMUFFLE, the highest score is 4.04 which is obtained by formula LF3 and the lowest score is obtained by LF0 with a score of 3.8. This assessment shows the difference in liking between LF3 and LF0, but based on the Kruskal Wallis test, it is known that from the 4 formulas, there is no significant difference because the 4 formulas have a uniform color. The color seen in the 4 products is a typical brown color obtained from a combination of the color of the chocolate bar ingredients and the dominating carob color.

Aroma

The aroma or fragrant and delicious odors of food can increase a person's appetite. The smell is determined by volatile substances that are smelled in the nasal passages and responded to by the olfactory system or sense of smell, namely the nose [25].

The highest value obtained from the control formula and 3 CARTEMUFFLE formulas is the value of formulas LF2 and LF3 with a score of 4.08 which means that the aroma of these 2 formulas is in the category favored by panelists. While the lowest value is the value obtained by formula LF1 with a score of 3.69, which is included in the category favored by panelists.

Observations made through organoleptic tests related to sensory aroma to panelists in the control formula and 3 CARTEMUFFLE formulas were tested through data processing, namely the Kruskall Wallis test with a significance value of 0.240 which proves that there is no significant difference in aroma between formulas. From this assessment, the aroma of chocolate combined with carob powder is the most preferred aroma.

Texture

Texture plays an important role in the sensory experience of food, and it is often the basis for determining overall flavor value through hearing and taste [25]. The texture of the various CARTEMUFFLE formulas is influenced by the comparison of the use of ingredients, where carob itself has a rather tough texture and looks a little fibrous, honey makes some formulas such as formula LF3 which feels a little melted when it is at room temperature which almost matches the texture of LF0. And tempeh as an enhancer of the crunchy texture of the 3 CARTEMUFFLE formulas compared to the control formula or LF0.

The highest average score based on the texture indicator was obtained by formula LF0 with a score of 4.08. While the

lowest average value was obtained by formula LF1 with a score of 3.23 which means neutral, where the comparison material from carob is more so that the texture feels a little bit astringent.

Based on the texture of LF0, there is no addition of carob, while formulas LF1, LF2 and LF3 have a texture that feels astringent, which is influenced by carob powder, besides that LF1, LF2 and LF3 have a crunchy texture from roasted tempeh. Based on this assessment, this soft texture is one of the most preferred by panelists.

Flavor

Taste assessment is done using the sense of taste, namely the tongue, where the taste itself arises due to the taste of food when it enters the mouth [25]. All 4 formulas are made using chocolate bars; the control formula uses more chocolate bars, while the 3 CARTEMUFFLE formulas use the same ratio, for some of the added ingredients themselves have different comparisons. This is what affects the taste of each formula.

Based on the results of the organoleptic test, the liking for the taste of the CARTEMUFLLE formula with the highest average score is formula LF0 and LF3 with a score of 4 which means it is in the preferred category. The lowest average value was obtained by formula LF1 with a score of 3.19, which means that the panelists were neutral towards the taste of formula LF1.

The assessment results, prove that panelists dislike the taste that has a composition of carob powder ingredients that are too high, besides that the taste of LF0 and LF3 is the formula that tastes the most preferred, where carob in formula LF3 is used less at 27% compared to formula LF1 as much as 38% carob powder and LF2 as much as 35%. Honey and tempeh, which are made in a higher ratio than carob, show that they can obscure the less delicious taste of the carob itself.

Antioxidant Content of CARTEMUFFLE (Carob, Tempeh, Honey Truffle)

CARTEMUFLLE (Carob, Tempeh, Honey Truffle) is a modified food product to prevent breast cancer with its antioxidant content of isoflavones and polyphenols. Antioxidants that protect the body from free radical attacks can stop the process of cell damage by giving free radicals electrons so that antioxidants neutralize free radicals so as not to steal electrons from DNA cells [26].

The test of antioxidant content of isoflavones and polyphenols was only carried out on the control formula and the most preferred CARTEMUFFLE formula, namely formula LF3, this is because the limited costs in the study were insufficient to test the antioxidant content of all formulations that had been made.

The antioxidant test results of the control formula and LF3 formula have different values, where LF3 contains isoflavones as much as 0.12 mcg and 1.73 mg per 100 grams of CARTEMUFFLE chocolate. The cause of the increase in both contents is the carob, tempeh, and honey, which are the ingredients of the chocolate truffle modification in formula LF3. Where tempeh has isoflavone antioxidant content is not found in the control formula, as well as carob and honey increase the polyphenol content in LF3.

The required daily intake of isoflavones is 5-20 mg per day to reduce the risk of death from breast cancer, and studies have shown that consuming isoflavone-containing foods as early as possible when cells are in good condition can prevent breast cancer [27],[28]. The recommended intake of isoflavones if distributed in a day for snack intake is required as much as 10% or equivalent to 0.5-2 mg of isoflavones. CARTEMUFFLE snack formula LF3 has an isoflavone content of 0.12 mcg which is not enough to fulfill the intake for 2 snacks.

The content of isoflavones in tempeh is variable, depending on the variety and the way it is processed. In the LF3 CARTEMUFFLE formula, 50 grams of tempeh should contain 21.76 mg or 43.52

mg of isoflavones per 100 grams [29, 30]. The decrease in isoflavone levels may be influenced by many factors such as the degradation of levels at long room temperature, the cooking process, especially frying and roasting dishes where tempeh in the composition of the CARTEMUFFLE formula is roasted for 30 minutes, in contrast to the steaming process where cooking tempeh by steaming will minimize the cause of the decrease in isoflavone levels [31].

The recommended daily intake for polyphenol antioxidants is 125 - 250 mg which if distributed for 2x snacks in a day is 10% or 12.5 - 25 mg per snack. However, the polyphenol content in the LF3 CARTEMUFFLE formula is low at 1.73mg/100gram with the recommended consumption of 2 meals of morning and afternoon snacks with a total of 3.46 mg.

In CARTEMUFFLE chocolate formula LF3, the content of isoflavone antioxidants and polyphenols that have been tested by the spectrophotometric method is not adequate for the dietary intake requirements for morning and afternoon snacks, but CARTEMUFFLE chocolate still has other opportunities that need to be researched regarding the content of different nutrients contained in it such as macro content, such as total energy, protein, fat, carbohydrates and fiber content. Fiber and low-fat content are supporting factors to reduce the risk of breast cancer. The carob used in the 3 CARTEMUFFLE chocolate formulas has a high fiber content and low fat compared to chocolate or cocoa powder, which has a high-fat content and low fiber [32].

CONCLUSION AND RECOMMENDATION

This research was conducted by making 4 different formulations, 1 control formula-tion and 3 formulations that were substituted with carob powder and modified with the addition of tempeh and honey with the ratio of Formulation LF0 (Without substi-tution and modification of food ingredients), Formulation LF1 (Carob: Tempeh: Honey) (38% : 24%: 38%), Formulation LF2 (Carob: Tempeh: Honey) (35%: 30%: 35%), Formulation LF3 (Carob: Tempe: Honey) (27%: 38%: 35%), the most pre-ferred CARTEMUFFLE chocolate formulation is formula LF3 with a mean score of 4.04 in the preferred category. The results of the Kruskall Wallis Test and the Mann Whitney test with an error rate ($\alpha = 0.05$), showed that there was a significant differ-ence in the texture indicator with a value of $0.008 < 0.05$ and taste of $0.01 < 0.05$. As for color and aroma, no significant difference was found, the color indicator obtained a value of $0.60 > 0.05$ and aroma $0.240 > 0.05$. These results were proven through further tests where between LF0 and LF1, LF0 and LF2 there were significant differ-ences in texture and flavor indicators. CARTEMUFFLE chocolate formula LF3 con-tained 0.12 mcg isoflavone antioxidants and 1.73 mg polyphenols, higher than LF0 with 0.06 mcg isoflavone antioxidants and 1.64 mg polyphenols. Further research can analyze all formulas' total antioxidant, macronutrient, and fiber content and change the cooking technique.

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