

Effect of green okra and strawberry ratio on antioxidant activity, total phenolic content, and organoleptic properties of jelly drink

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Abstract. Okra (*Abelmoschus esculentus* L.) is a flowering plant which contain vitamin A and antioxidant in its pods. Vitamin A and antioxidant are good for the eyes and cancer prevention. The objective of this study was to study the effect of green okra and strawberry ratio on antioxidant activity, total phenolic content, and organoleptic properties of jelly drink. This study was conducted on April until June 2017 in Community Nutrition Department Laboratories. Jelly drink was made with five formulas based on the ratio of green okra and strawberry concentrates, such as F1 (100:0), F2 (80:20), F3 (70:30), F4 (60:40), and F5 (50:50). The gelling agent used was carrageenan and konjac combination. Chemical analysis such as antioxidant activity and total phenolic content was analyzed using DPPH method and folin-ciocalteau method. The organoleptic test consisted of hedonic test and hedonic quality test. This jelly drink has antioxidant activity values range from 0.557-1.056 mg AEAC/100g. Total phenolic content of jelly drink was about 20.84-31.17 mg GAE/100g. Green okra and strawberry ratios has a significant effect on the value of antioxidant activity but not on the total phenolic content. Organoleptic showed that the difference of green okra and strawberry ratios significantly affect the panellist acceptance on color and aroma attributes on hedonic test, and color, taste, and aftertaste attributes on hedonic quality test.

1. Introduction

Trend of disease suffered by community has changed significantly. Based on data, in 1900, the death caused by infectious disease such as pneumonia and influenza reached almost 210 per hundred thousand people and decrease to 16 per hundred thousand people in 2011. Opposite with that, death caused by noninfectious disease such as malignant neoplasma increase from 81 per hundred thousand people in 1990 to 174 per hundred thousand people in 2011 [1]. In line with the data from [2] that there was a significant change in the proportion of the cause of death between infectious and noninfectious which can be caused by free radical.

Antioxidant itself can stop free radical scavenging and its chain reaction that can cause the damage or even the death of the cell. Antioxidant can be produced by the body orexogenously from vegetables and fruit consumption [3]. Responding to this, people become more aware of the importance of healthy living and begin to change their eating habits. Food nowadays has expanded its function, not only to fulfill nutritional needs but also expected to have an impact on health. This generated a concept of functional food [4].



In its development, food in Indonesia has passed through several generations. First generation is food as supplement, second is as whole foods, and third is as functional food [4]. Functional food is food product that not only to fulfill nutrition needs by the body but also decrease disease risk [5]. Functional food can be implemented as a food and beverages, such as rice bran drink, kombucha drink, tomat juice, and variative tea made by many kinds of leaves. Beside those, okra has been known as well for its health benefit. Okra (*Abelmoschus esculentus* L.) is a plant with its height can reach 2 meters. Okra pods contain vitamin A and antioxidant such as flavonoid that good for sight and cancer prevention. Vitamin contained in okra can work as antioxidant to maintain immunity. Okra has been proven to potentially be antidiabetic and antihyperlipidemia [6]. Okra pods have viscosius liquid inside that contains fiber [7].

Fiber in okra is suitable to be made as chewy textured food such as jelly. Jelly is a kind of semi solid beverage that generally has clear appearance and chewy texture. Jelly is made by fruits extract and sugar using boiling technique with the addition of gelling agent to form its chewy texture. Processing fruit and vegetables as jelly or other kind of food is one of the way to utilize them so that it can decrease amount of fruit and veggies that can be wasted and as the attempt to increase income of farmer. Fruits that usually be processed as jelly are mango, apple, strawberry, and grape [8] so that's why in this research, green okra was used as the main material to make jelly drink with the addition of strawberry. Based on those considerations, the food development such as jelly drink made from green okra (*Abelmoschus esculentus* L.) and strawberry to give another option of beverage that has health beneficial.

2. Objective

This research is objected to make jelly drink from green okra and strawberry in several ratio and the effect on its antioxidant activity, total phenolic content, and organoleptic characteristics.

3. Method

3.1. Time and place

This research held on April until June 2017. It took place in Food Development Laboratory, Food Chemistry and Nutrient Analysis Laboratory, Nutrition Biochemistry Laboratory, and Organoleptic Laboratory. Those laboratories are located in Community Nutrition Department, Faculty of Human Ecology, Bogor Agricultural University.

3.2. Material and equipment

Materials used in jelly drink making were green okra from Agribusiness Development Station IPB, Bogor, strawberry, lemon, and food coloring Red Rose from Supermarket in Bogor, sucralose, potassium citrate, gelling agent, water bottled, and cup. Chemical reagents used were methanol, ascorbic acid, DPPH, gallic acid, folinciocalteau reagent, buffer acetic pH 5.5, sodium carbonate, aquades, and free ion water.

Equipments used were divided into three groups. Pot, gas stove, electric scale, knife, bowl, measurer glass, blender, food filter, cup, and stirrer were used in the making of jelly drink. Equipments used in the analysis were spectrophotometer, vortex, evaporator, *magnetic stirrer*, centrifuge, pH meter, refrigerator and chemical glasses. Tools used for organoleptic test were questionnaire, cup, and pen.

3.3. Procedure

This research was divided to three phases. First phase was formulating and making of jelly drink. Second phase was chemical analysis such as antioxidant activity and total phenolic content analysis and organoleptic analysis. Third phase was determining the best formulation of jelly drink. Diagram of the research procedure can be seen in the figure 1 below.

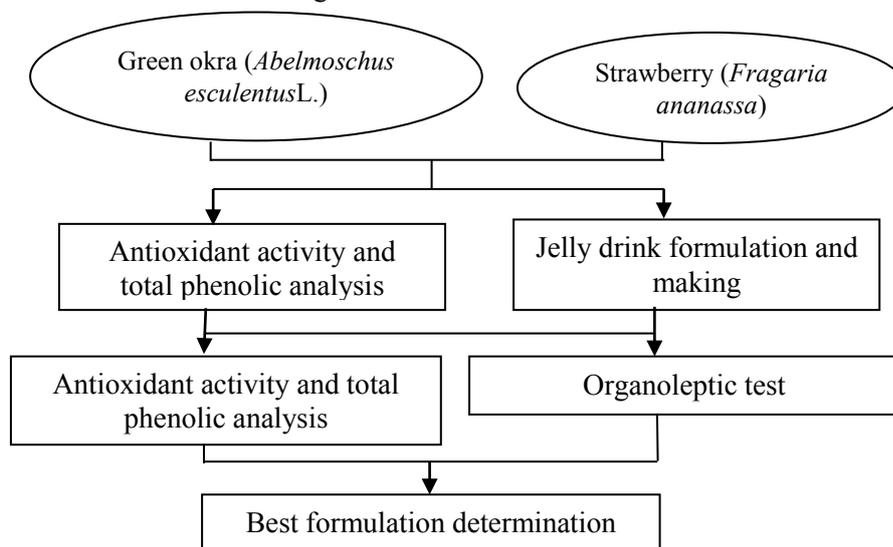


Figure 1. Procedure of the research.

3.3.1. The making of jelly drink product. The making of jelly drink product started with preparing green okra and strawberry. Okra was selected which had green color, in a good condition, undamaged, and easy to cut. It then blanched for about 3 minutes to remove unwanted odor and taste. Fresh strawberry was chosen and firstly cleaned by water and cut. Green okra and strawberry were blended using blender and filtered to get the extract. Procedure in the making of strawberry and okra extract was based on [9] with the ratio 1:5 compared with the water. Extract was filtered then using food filter. Strawberry extract was added to green okra extract as formulation made as well as other materials needed. After that, all materials were mixed and boiled in the pot. The unformed jelly was put then in the container and kept in cold temperature. Jelly drink was stored in the refrigerator.

3.3.2. The formulation of jelly drink. Gelling agent used was carrageenan and conjac combination with sucralose as sweetener. Two of them produced the desire texture. Here is the formulation of jelly drink.

Table 1. Jelly drink product formulation.

Material	Unit	F1	F2	F3	F4	F5
Green okra extract ^a	%	100	80	70	60	50
Strawberry extract ^a	%	0	20	30	40	50
Gelling agent ^b	g	0.4	0.4	0.4	0.4	0.4
Potassium citrate	g	0.15	0.15	0.15	0.15	0.15
Sucralose	g	0.01	0.01	0.01	0.01	0.01
Lemon	ml	3	3	3	3	3

^acalculated by total of green okra and strawberry extract per 100 ml. Extract was made with water bottled with the ratio 1:5

^bGelling agent used was carrageenan and conjac combination 0.4% [13]

3.3.3. Chemical and organoleptic analysis. Chemical analysis done was analysis of antioxidant activity based on [10] and total phenolic content using folin-ciocalteau method based on [11]. Organoleptic characteristics were assessed using hedonic and hedonic quality test with the attribute such as color, clarity, taste, after taste, in mouth texture, biting texture, aroma, and mouthfeel. The result of analysis was used to determine best formula based on statistical test and total sensory acceptance from each component [12].

3.3.4. Best formula determination. The best formula was chosen by considered organoleptic characteristic, antioxidant activity, and total phenolic content. Hedonic test is one of the tools to assess the most preferable formula. Preferable level was seen from the total amount of hedonic test with percentages given by researcher as its importance level, such as 20% for the taste, 15% for the texture, and 10% for other attributes. Consideration of 3 aspects was done to make sure that the best formula not only have the best health beneficial but also liked by the consumers.

3.4. Design experiment

Design experiment of this jelly drink product was complete randomize design with two repetitions. Experiment unit assessed was green okra and strawberry jelly drink in the second phase of the research. Treatment done in this experiment was green okra and strawberry extract ratio. Response variable observed was organoleptic characteristic, antioxidant activity, and total phenolic content of jelly drink. Mathematic model used is written below.

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

Information:

- Y_{ij} = Response variable caused by the ratio between green okra and strawberry extract in level-i repetition-j
- μ = mean
- τ_i = Effect of the formula in level-i to response variable
- ϵ_{ij} = error ratio of green okra and strawberry in level-i repetition-j
- i = proportion level of green okra and strawberry
- j = repetition ($j=1,2$)

3.5. Data Processing and Analysis

The data was processed using *Microsoft Excel* 2013 and SPSS 18 software. Organoleptic data was analyzed statistically using nonparametric differentiate Kruksal Wallis. If the treatment effect significantly, further test would be conducted, such as Mann Whitney test. Total sensory acceptance was calculated to assess chosen product by organoleptic test. Chemicals analysis was conducted using ANOVA test and if the treatment effect significantly, further test conducted by Duncan test. Correlation in chemical analysis was tested using Spearman test.

4. Result and Discussion

4.1. Formulation and making of green okra and strawberry jelly drink

Jelly drink making process was started by choosing the materials, such as green okra and strawberry. Before making the extract, okra was firstly cleaned. Blanching itself was done to decrease unwanted odor and flavor [14]. Okra and strawberry blended using blender with water bottled ratio 1:5 to make the extract [9]. The percentage in the formula was based on [8]. The addition of strawberry was purposed to cover the bitterness of okra.

Other materials added were gelling agent, potassium citrate, sucralose, lemon, and water bottled. Gelling agent is polymer component with much molecular weight and such as a group of molecules and coils of polymers that can add chewy and gel characteristic to the product [15]. Potassium citrate or potassium chloride can be used as basic component in food product to form gel strength [16]. Sugar concentration used was firstly 13% and potassium citrate as much as 0.15% from its total weight

between green okra and strawberry [17] but in this research, sweetener used was sucralose that can increase consumers, and using sucralose was objected to form the shape that researcher wanted.

4.2. Antioxidant activity

Antioxidant is chemical compound that plays important role to defense or retard the damage caused by oxidation [18]. Antioxidant activity can be analyzed by DPPH method that based on the color changing of DPPH compound by reacting with antioxidant. Its reaction is measured by spectrophotometer. Result of antioxidant analysis is expressed by AEAC (*Ascorbic acid Equivalent Antioxidant Capacity*). Value of antioxidant activity can be seen on the table 2 below.

Table 2. Antioxidant activity of green okra and strawberry jelly drink.

		Antioxidant (%)	Antioxidant activity (mg AEAC/100g)		
Raw extract	Green okra	90.612	10.10 ± 0.56		
	Strawberry	89.473	9.90 ± 0.75		
Ratio of Okra : Strawberry Extract ^a					
Jelly drink extract	100	:	0	33.215	0.56 ^a ± 0.12
	80	:	20	44.737	0.76 ^b ± 0.02
	70	:	30	56.615	0.97 ^c ± 0.09
	60	:	40	60.384	1.03 ^d ± 0.00
	50	:	50	61.878	1.06 ^d ± 0.10

^ain percent per 100 ml mixed extract

Result of the analysis showed that green okra jelly drink with the addition of strawberry extract as much as 50% has the highest antioxidant activity. This means that every 100 grams jelly drink with the ratio of green okra and strawberry has radical scavenging ability 1.056 times as vitamin C. Compared with other beverage such as rice bran drink and tomato juice which have antioxidant activity 28.74 and 1.87 mg AEAC/100 g [19] this green okra and strawberry jelly drink has lower antioxidant activity but higher if it's compared with black jelly with antioxidant activity as much as 0.131-0.301 mg AEAC/100 g [20].

Statistical analysis of the data shows that the treatment has significant effect to its antioxidant activity. According to further test, antioxidant activity of jelly drink without the addition of strawberry is different with antioxidant activity of jelly drink with the addition of strawberry as much as 30% to 50%. The data shows the opposite if it's compared with raw material's data since it shows that green okra has higher antioxidant activity than strawberry. Spearman correlation test shows that there is a strong significant correlation and in line between treatment and antioxidant activity with coefficient correlation 0.862 and p=0.001. Table shows that antioxidant activity of green okra is 10.099 mg AEAC/100 g meanwhile strawberry is 9.901 mg AEAC/100g.

This could be caused by the process in the making of jelly drink. Blanching process, that was done to reduce unwanted odor and taste, is proven can decrease antioxidant activity [21]. Factors in cooking such as the method and temperature can effect antioxidant activity in food [22]. This can be happening cause its ability to release antioxidant compound. Aquathermal process in vegetables can reduce antioxidant activity caused by the decreasing of vitamin C and polyphenol in water [22].

4.3. Total phenolic content analysis

Green okra and strawberry have about 8000 kinds of phenolic compound, such as flavonoids and phenolic acid [23]. Phenolic compound can be analyzed using many kinds of methods, such as Folin-Ciocalteu method. Total phenolic content is calculated principally on the electron transfer on alkaline media from phenolic compound as phosphomolybdic acid complex or phosphotungstate. This complex forms blue color that can be read with spectrophotometer on 760 nm [24]. The average of total phenolic content in green okra and strawberry jelly drink can be seen below.

Table 3. Total phenolic content of green okra and strawberry jelly drink.

Ratio of green okra : strawberry ^a			Total phenolic content (mg GAE/100g)
100	:	0	25.07 ± 1.16
80	:	20	20.84 ± 0.92
70	:	30	29.81 ± 5.85
60	:	40	31.17 ± 4.00
50	:	50	25.42 ± 1.97

^ain percentage per 100 ml mixed extract

The highest total phenolic content (31.17 mg GAE/100g) is in the formula with the ratio of green okra and strawberry about 60:40. The lowest total phenolic content is in the formula with ratio between green okra and strawberry about 80:20. Its total phenolic content is about 20.84 mg GAE/100g. This result is not in line with the result of antioxidant activity and it can be caused by phenol instability. Anthocyanin stability can be decreased during storage because of light and temperature [25]. Even though the result is not quite good, statistical analysis showed that there is no significant effect of treatment to total phenolic content of jelly drink.

According to [26], total phenolic content from okra extract in ethanol solution is about 12.99 mg±1.75 mg/g/GAE meanwhile strawberry is about 228.9 mg/100 g [27]. The big difference between total phenolic content in okra extract and jelly drink can be caused by jelly making process which include much water (about 1:5) so that it became more diluted. This made the component in jelly drink is less than the raw material. Even though, based on [28], if it's compared with hantap leaf jelly drink that has total phenolic about 10.6 mg/100 grams, green okra and strawberry jelly drink contains more total phenol.

4.4. Organoleptic analysis

Organoleptic analysis was done to assess product quality by panelist's senses. By this test, response and impression obtained from the senses are expected to be known. In this research, organoleptic characteristic was assessed using hedonic and hedonic quality tests with 35 semi-trained panelists.

4.4.1. *Hedonic quality test.* Hedonic Quality Test shows the impression of food product. It can show characteristics of product specifically, such as the attributes. Here is the average of hedonic quality scores of jelly drink.

Table 4. Hedonic quality average score of green okra and strawberry jelly drink.

Attribute	Average score on every formula				
	F1 (100:0)	F2 (80:20)	F3 (70:30)	F4 (60:40)	F5 (50:50)
Clarity	2.79	2.81	2.84	2.80	2.87
Color	2.53 ^a	3.12 ^b	3.39 ^b	3.64 ^c	3.71 ^d
Aroma	2.71	3.01	3.00	3.09	3.06
Taste	2.81 ^b	2.73 ^b	2.67 ^b	2.48 ^b	2.33 ^a
<i>Aftertaste</i>	3.11 ^c	3.34 ^c	3.13 ^c	3.03 ^b	2.77 ^a
In mouth texture	3.50	3.56	3.49	3.50	3.59
Biting texture	2.91	2.74	2.67	2.64	2.63
<i>Mouthfeel</i>	3.20	3.36	3.31	3.14	3.13

Description: Classification of clarity attribute, 1=very turbid, 2=turbid, 3=normal, 4=clear, 5=very clear. Classification of color attribute, 1=*peach*, 2=*salmon*, 3=*coral*, 4=*pink*, 5=*soft pink*. Classification of aroma attribute, 1=very strong, 2=strong, 3=so so, 4=light, 5=very light. Classification of taste attribute, 1=sweet sour, 2=sweet slightly sour, 3=little sweet, 4=sweet, 5=very sweet. Classification of *aftertaste* attribute, 1=very strong, 2=strong, 3=normal, 4=light, 5=very light. Classification attribute in mouth texture, 1=very rough, 2=rough, 3=normal, 4=soft, 5=very soft. Classification of biting texture attribute, 1=very light, 2=light, 3=normal, 4=strong, 5=very strong. Classification of *mouthfeel* attribute, 1=very strong, 2=strong, 3=normal, 4=light, 5=very light. Different letter on the same line means significant difference on formula.

Result of hedonic quality test on clarity, aroma, in mouth texture, biting texture, and *mouthfeel* attributes show variative average scores. Statistical test shows that there is no significant difference on the ratio of green okra and strawberry added in jelly drink to clarity, aroma, in mouth texture, biting texture, and *mouthfeel* attributes of jelly drink.

Result of hedonic quality test on color attribute shows the average score is between 2.53 to 3.71 in the scale of *coralto* pink. Statistic test shows that there is significant difference on color attribute between all formulas, except formula F2 with F3, F3 with F4, and F4 with F5.

Result of hedonic quality test on taste attribute shows the average score is between 2.33 to 2.81 in the scale of sweet slightly sour to slightly sweet. Statistic test shows that there is significant difference on taste attribute between all formulas, except formula F5 with F4. Sourness of jelly drink can be caused by strawberry and lemon that added in the making.

Result of hedonic quality test on aftertaste attribute shows the average score is between 2.77 to 3.34 in the scale of strong to light. Statistic test shows that there is significant difference on taste attribute between formula F5 with almost of all formulas, except with F4 and F2 with F4. Aftertaste felt by the panelists is expected to cause by tannin that taste bitter in mouth [28].

4.4.2. Hedonic Test. Hedonic test or preference test is done to find out the most preferable formula chosen by the panelists. Hedonic test of green okra and strawberry jelly drink is done by [29]. Average score of panelist acceptance on five formulas of jelly drink is between “do not like” and “so so” scale. Result of the test can be seen in the table below.

Table 5. Average score of green okra and strawberry jelly drink hedonic.

Attribute	Average score on every formula				
	F1 (100:0)	F2 (80:20)	F3 (100:0)	F4 (60:40)	F5 (100:0)
Clarity	2.84	2.84	2.91	2.90	3.04
Color	2.32 ^a	2.98 ^b	3.44 ^c	3.70 ^c	3.90 ^d
Aroma	2.75 ^a	3.00 ^b	3.21 ^b	3.24 ^b	3.18 ^b
Taste	2.84	2.84	2.60	2.71	2.60
<i>Aftertaste</i>	2.88	3.05	3.20	3.12	2.92
In mouth texture	3.61	3.67	3.58	3.64	3.84
Biting texture	2.91	2.97	3.11	3.12	2.91
<i>Mouthfeel</i>	3.24	3.20	3.25	3.27	3.14

Description: 1=don't like very much, 2=don't like, 3=so so, 4=like, 5=like very much. Different letter on the same line means significant difference on formula.

Acceptance level of clarity, taste, *aftertaste*, in mouth texture, biting texture, and *mouthfeel* attribute was tested statistically and it shows that there is no significant difference on clarity, taste, *aftertaste*, in mouth texture, biting texture, and *mouthfeel* characteristics in every formula of jelly drink. This means that although there is difference on the ratio between green okra and strawberry extract in jelly drink, but the characteristics such as clarity, taste, *aftertaste*, in mouth texture, biting texture, and *mouthfeel* are same.

Result of hedonic test of color attribute shows the average score is between 2.32 to 3.90 in the scale of “don't like” to “like”. The most preferable formula based on the color is formula F5. Statistic test shows that there is significant difference on all formulas, except formula F3 with F4.

Result of hedonic test of aroma attribute shows the average score is between 2.75 to 3.24 in the scale of “don't like” to “so so”. The most preferable formula based on the aroma is formula F4. Statistic test shows that there is significant difference on formula F1 with F3, F1 with F4, and F1 with F5.

4.5. Best Formula Determination

Best formula is determined by considering 3 aspects, such as organoleptic test, antioxidant activity, and total phenolic content. Total sensory acceptance is calculated to assess the most preferable formula by the score of hedonic test. Different percentage is given to every attributes based on its importance to jelly drink, such as 20% for taste attribute, 15% for in mouth texture and biting texture attributes, and 10% for color, aroma, clarity, and *mouthfeel attributes*. Statistic test is done to find out the differences on the formulas after scoring. Result of the analysis shows that there is no effect on every formula so that the preferable formula is chosen by its score at the highest. Here's the table of best formula determination.

Table 6. Best formula determination.

Formula	Ratio	Total sensory acceptance	Antioxidant activity (mg AEAC/100g)	Total phenolic content(mgGAE/100g)
F1	100 : 0	2.95	0.557 ^a ± 0.12	25.07 ± 1.16
F2	80 : 20	3.07	0.757 ^b ± 0.02	20.84 ± 0.92
F3	70 : 30	3.13	0.966 ^c ± 0.09	29.81 ± 5.85
F4	60 : 40	3.18	1.032 ^d ± 0.00	31.17 ± 4.00
F5	50 : 50	3.15	1.056 ^d ± 0.10	25.42 ± 1.97

Different letter on the same line means significant difference on formula ($p < 0.05$).

Based on the data in table 11, it can be known that the most preferable formula is formula F4. Considering the antioxidant activity, formula with ratio of green okra and strawberry 60:40 and 50:50 are jelly drink with higher antioxidant activity compared to other formulas and both are not different statistically. Result of total phenolic content analysis showed that formula F4 has the highest content of all. Beside that, determining formula F4 as the best formula is because it contains more green okra than formula F5. Okra is vegetable that has many healthy beneficial, such as antidiabetic and antihyperlipidemia [6]. As the conclusion, best formula determined is formula F4 with the ratio of green okra and strawberry as much as 60:40.

5. Conclusion and recommendation

5.1. Conclusion

Green okra (*Abelmoschus esculentus*) and strawberry can be made as jelly drink with the addition of gelling agent such as carrageenan-conjac combination. Result of hedonic quality test showed that ratio between green okra and strawberry has significantly effect on color, taste, and aftertaste attributes. Treatment has affected significantly as well to panelist's acceptance on color and aroma attributes.

Antioxidant activity of green okra and strawberry jelly drink is in the range of 0.557-1.056 mg AEAC/100g. Result of analysis showed that jelly drink with the addition of strawberry as much as 50% has the highest antioxidant activity. Total phenolic content of green okra and strawberry jelly drink is in the range of 20.84-31.17 mg GAE/100g. Jelly drink with the ratio of green okra and strawberry 60:40 has the highest content of total phenolic content. Based on statistic test, treatment has significant effect on antioxidant activity but not its total phenolic content.

5.2. Recommendation

Blanching and boiling process in the making of jelly drink beside to reduce unwanted odor and aroma, also to form jelly texture. However, this process reduced the antioxidant activity. Another method is still need to be developed to reduce unwanted aroma and taste but not the antioxidant activity so that the quality of similar product can be enhance.

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