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Utilization of rose flower extract as antioxidant rich-drink

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Utilization of rose flower extract as antioxidant rich-drink

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Abstract. Rose flower extract containing high levels of anthocyanin pigment has the potential to be further processed into healthy functional drinks. The addition of natural flavor to the production process is expected to improve the sensory and functional aspects of this rose extract-based drink. The research was aimed to study the effect of natural flavor addition at various concentration on pH, total dissolved solids, color intensity, sensory quality, total anthocyanin levels, and antioxidant activity of rose extract-based drink. Rose filtrate was obtained by maceration method using water solvent. The added natural flavors were mint leaves, ginger and lemon juice, with concentration levels of 1.5% (b/v), 3% and 4% (v/v). Antioxidant activity and anthocyanin levels were determined using the DPPH and pH difference methods, respectively. Sensory quality was determined using hedonic rating test with test parameters of taste and flavour. The results showed that different types and concentrations of natural flavors significantly affected the pH, redness and yellowish level of rose extract-based drink. The addition of natural flavors significantly enhanced its antioxidant activity. While the concentration of natural flavor only affected its total dissolved solids. The increase in total anthocyanin content of rose juice extract due to the addition of mint leaves is 46.48%, ginger is 34.50% and lemon is 16.70%. Based on the results, the best treatment was shown by rose extract-based drink with the addition of 3% of mint leaves given the antioxidant activity of 84.44%, brightness (L) of 35.30, redness (a +) of 8.00, yellowness (b +) of 4.60, pH value of 3.45, total dissolved solids of 21.17 ° Brix, total anthocyanin level of 12.14 mg/ L, taste score of 3.20 (quite tasty) and flavor score of 3.27 (quite like).

1. Introduction

Nowadays, benefits of bioactive compounds within foods and beverages as non-communicable diseases prevention have been taking public attention [1]. In globalization era, ethnical barriers have been gradually phased out and these products, although from various regions, are now universally available as international pro-health products. This has placed functional food products as the trend of current food products and encourage various industries, both the pharmaceutical and food industry, leading to the concept of "Healthy, Functional and Satisfied Foods". One of the functional foods that has been improved is antioxidant drinks.

Antioxidant drinks can improve body immunity and decrease disease risk. It is different from any supplement or carbonated drinks [2]. The development of antioxidant drinks also suppress the growth of drink that still use harmful dyes, excessive synthetic sweeteners and antioxidants. Concern about

the side effects of synthetic antioxidant as a source of free radicals causes natural antioxidants to be a much-needed alternative. Natural antioxidant usually obtained from fruit and vegetable as vitamins and phytonutrients like vitamin C, vitamin E, and carotenoid (lutein, beta carotene, and lycopene) and also natural bioactive compounds like phenolic acid, flavonoid, coumarin, alkaloid, polyacetylene, saponin, and terpenoid. There are a lot of research that has been prove that natural bioactive compounds have biological effect like antioxidant, antibacterial, antivirus, anti-inflammation, anti-allergic, anti-thrombotic, vasodilation action, anti-mutagenesis, anti-carcinogenic, and antiaging.

Some countries are famous for their local antioxidant-rich drinks. Kombucha, a light, bubbly and sweet fermented black or green tea from China [3], Hibiscus extract-drink from Malaysia [4], Clitoria, an extract drink from Thailand, Rhododendron, a traditional herb drink from India, and white turnip extract from Turkey. Wine from grape contains anthocyanin pigment that can prevent obesity. Anthocyanin gives health effect not just from its compound but also from its degraded products or metabolites, and it can be transport using passive diffusion or active transporter within intestine epithelium [5]. Anthocyanin consumer in Europe are 19.8% in Netherland, 64.9 mg/day (male) and 44.1 mg/day (female) in Italy, and 18.4% in Spain. The reports said that it can prevent cardiovascular disease and functions as anti-proliferative [6].

Indonesia is one of the rose-producing countries in the world. Rose productivity in Indonesia ranges from 120-280 flowers/m2/year. East Java is one of the rose-producing regions such as in Batu Malang and Bangil Pasuruan. Rose is one of agricultural product that usually used as decoration. It produces flower continuously and its production time is repeated.

In Iran, *Rosa canina* L., has been used as vitamin source, drug supplement, and food for ages. Its hip has red-orange color and contains vitamins (mostly vitamin C) and another functional compound like polyphenol, carotenoid and carbohydrate [7]. Batu and Pasuruan local rose from Indonesia have a lot of functions which can be utilized, especially its petals contains anthocyanin pigment, vitamin C, and high in antioxidant [8]. Rose anthocyanin pigment identification has been proved using HPLC analysis by Saati [9]. The research was done to analyse anthocyanin using molecule weight observation with LCMS method. Anthocyanin pigments showed as red in acid pH and bluish in base condition [10]. Anthocyanin has beautiful color that can captivated consumer.

As cut flowers, roses are generally displayed 1 to 3 days, which the petals will slowly fall out and the fragrance will disappear as the days passed. The nature of its antioxidant compound which are water soluble, make rose petals has high potential as functional drink and it is rarely been utilized in Indonesia. Processing of healthy drink from rose extract is a way to increase the added value of roses. This study aimed to determine the effect of natural flavor addition at various concentration on rose extract drink pH, total dissolved solids, color intensity, sensory quality, total anthocyanin level, and antioxidant activity.

2. Materials and Methods
This research was conducted in February 2018 until June 2018 at the Laboratory of Food Science and Technology, University of Muhammadiyah Malang. The materials used in this study were the local variety red rose (one day storage period) obtained from Bangil-Pasuruan farmers, mint leaves, imported lemons, emprit gingers, refined sugar (Gulaku, Sugar Group Companies) and citric acids purchased from a local supermarket. The study was conducted using Factorial Randomized Design consisting of two factors. The first factor was a type of natural flavor which includes mint, lemon and ginger leaves while the second factor was natural flavor concentration of 1.5%, 3% and 4.5%. Thus there were 9 treatment combinations and each combination was repeated 3 times.

2.1. Rose Extract Production and Natural Flavor Preparation
Rose filtrate production referred to modified Saati [9] method. About 45 grams of crushed rose petals were soaked in 300 mL of distilled water and 1% citric acid at room temperature for 24 hours. Filtering was done using filter paper to separate the pulp and filtrate. The filtrate was then evaporated using rotary evaporator at a temperature of 50-60 ºC until the volume shrinks 3-4 times to obtain rose

extract. Washed mint leaves were dried at room temperature. Cleaned ginger was cut into 0.2 cm pieces, boiled with the ratio of ginger to water (1:3) for ± 10 minutes with a temperature of 85-90ºC and then strained with filter cloth [11]. Lemon was squeezed using orange squeezer [12].

2.2. Rose Extract Drink Production

The making procedure of rose extract drink referred to modified Saati [9] method. About 65% of water (v/v) was heated and then sugar was dissolved (b/v) into it. The temperature was conditioned to around 80-85ºC. Furthermore, rose extract was added as much as 35% (v/v), natural flavor (1.5%, 3% and 4.5%) and citric acid while continuing to stir for 10 minutes. The temperature of the mixture was lowered to around 60-65ºC. Rose extract drinks were filtered and put in the packaging. Rose extract drinks were analyzed for pH, antioxidant activity, color intensity and organoleptic (taste and aroma) and total anthocyanin content.

2.3. pH, Total Dissolved Solids, and Color Intensity Analysis

pH analysis was done according to National Standarization Agency of Indonesia [13] method using pH-meter. Total dissolved solids and color intensity were measured based on Yuwono and Susanto [14] method. Total dissolved solids were measured using a refractometer while the color intensity using color reader.

2.4. Antioxidant Activity Analysis

Measurement of antioxidant activity was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [15]. 1 mL of rose extract was diluted in 9 mL of 70% methanol and centrifuged at 4000 rpm for 10 minutes. 1 mL of the centrifugation supernatant was diluted in 7 mL of methanol 70% and then 2 mL of DPPH 0.25 mM solution was added into it. After the mixture was homogenized and stored in a dark place for 30 minutes, the absorbance of the mixture was read with a UV Vis spectrophotometer at $\lambda = 517$ nm. % inhibition calculated by the formula:

$$\% \text{ Inhibition} = \frac{\text{Blank Abs - Sample Abs}}{\text{Blank Abs}} \times 100\%$$

2.5. Total Anthocyanin Content

Determination of total anthocyanin content was carried out using the pH difference method [16]. Making a pH 1.0 solution: 1.49 grams of KCI were dissolved in distilled water in a 100 ml volumetric tube. Then, 25 mL of the KCL solution was added with 67 ml of 0.2 N HCL solution until pH reached 1.0 ± 0.1. Making a pH 4.5 solution: 1.64 grams of potassium acetate were dissolved in distilled water in a 100 ml volumetric tube. 0.2 N HCL solution was added until the pH reached 4.5 ± 0.1. Two sample solutions were prepared from each filtrate, the first sample used a pH 1.0 solution and the second sample used a pH 4.5 solution. then the absorbance of each solution was measured at wavelengths of 510 and 700 nm. The absorbance of the dissolved sample (A) was determined by the formula:

$$A = (A_{510 \, nm} - A_{700nm})_{\text{pH } 1.0} - (A_{510 \, nm} - A_{700nm})_{\text{pH } 4.5}$$

$$\text{Anthocyanin concentration (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

A	= Absorbance
MW	= Molecular Weight (cyanidin glucoside: 449.2)
DF	= Dilution Factor (10 mL/0.1 mL)
ε	= Molar absorbance/molar extinction coefficient (29600 L cm^{-1})
l	= cuvette size (1 cm)

2.6. Sensory Analysis
Sensory tests were carried out based on the Yustiyani and Setiawan [17] method using hedonic rating tests on 30 untrained panelists. The parameters measured were preference for the taste and aroma of rose extract drink.

2.7. Statistical Analysis
Data analysis was done using ANOVA method ($p < 0.05$) with $\alpha = 5\%$. If there were a significant differences, the analysis was continued with the DMRT (Duncan's Multiple Range Test). De Garmo analysis [18] used to determine the best treatment combination in this study.

3. Results and Discussion

3.1. Rose Extract Drink pH
The pH of rose extract drink increased in line with the increase in amount of mint leaves and and ginger added, while the addition of lemon will reduce its pH (Table 1). This showed that the pH value of rose extract drink was affected by the type and concentration of natural flavor added.

Table 1. pH differences between natural flavor ingredients variance and concentration.

Treatment	pH
P1K1 (Mint : 1.5%)	3.36 [c]
P1K2 (Mint : 3%)	3.45 [d]
P1K3 (Mint : 4.5%)	3.54 [e]
P2K1 (Ginger : 1.5%)	3.38 [cd]
P2K2 (Ginger : 3%)	3.39 [cd]
P2K3 (Ginger : 4.5%)	3.44 [cd]
P3K1 (Lemon: 1.5%)	3.15 [b]
P3K2 (Lemon: 3%)	3.00 [a]
P3K3(Lemon : 4.5%)	2.96 [a]
Control (Without Natural Flavor)	3.48 [de]

[a]The same letter on the same column indicated insignificant result according to Duncan 5%

Yazakka and Susanto [19] stated that the addition of ginger concentration will reduce the pH value, because ginger contains phenolic compounds which are capable of releasing protons (H+) in the solution. However, in Table 1 showed that the pH value of ginger treatnent (1.5%, 3%, and 4.5%) was not significantly different. This was caused by small difference between concentration levels.

3.2. Rose Extract Drink Total Dissolved Solids
Overall, the addition of natural flavors increased the total dissolved solids of rose extract drink (Table 2). Natural flavor concentration did not give a significant difference in total dissolved solids in the treatment group. This was caused by small difference between concentration levels. Some compounds which are measured in the total dissolved solids value are sugar, protein, vitamins and organic acids [20]. The total dissolved solids given were in a slightly higher range than the total dissolved solids value in Indonesian National Standard (SNI 3719-2014) for fruit juice drinks (min, 7.5-16 °Brix) [13].

Table 2. The effect of natural flavor concentration on rose extract drink total dissolved solids.

Treatment	Total Dissolved Solids (^{0}Brix)
K1 (1.5%)	20.0 [b]
K2 (3%)	20.2 [b]
K3 (4.5%)	20.0 [b]
Control (Without Natural Flavor)	18.3 [a]

[a]The same letter on the same column indicated insignificant result according to Duncan 5%

3.3. Rose Extract Drink Antioxidant Activity Analysis

Natural flavor addition increased rose extract antioxidant activity (Table 3). This showed that antioxidant compounds in natural Flavor (mint, ginger and lemon leaves) synergize with antioxidant compounds in the basic formula (control). According to Rajalakshmi and Narasimhan [21], a synergistic phenomenon will occur if two types of antioxidants meet in a food system, one as a free radical scavenger and the other as a hydroperoxide decomposer. If both were used in combination, the overall effect will exceed their respective use separately.

The antioxidant activity in the natural flavor group of mint leaves and ginger were lower than lemon. This is because mint leaves and ginger contain a lot of volatile compounds, which are unstable especially in the presense of heat. According to Muawanah *et al.* [22], heating process can accelerate the oxidation of antioxidant compounds contained in food, resulting in lowering of antioxidant activity of these foods.

Table 3. The effect of natural flavor variance on rose extract drink antioxidant activity

Treatment	Antioxidant Activity (%)
P1 (Mint Leaves)	84.05 [b]
P2 (Ginger)	84.28 [bc]
P3 (Lemon)	84.42 [c]
Control (Without Natural Flavor)	83.02 [a]

[a]The same letter on the same column indicated insignificant result according to Duncan 5%.

Mint leaf extract contained essential oils 1-2%, menthol 80-90%, menthon, d-pipirition, hexanolfenilasetat, ethyl amylcarbinol, and neomentol [23]. In addition, mint leaves also contained resin and tannins [24]. Tanin is one type of polyphenol which is antioxidant.

Consuming anthocyanin pigment concentrates can replace two antioxidant-vitamin supplements (vitamins A and C) [18]. Al-Juhaimi and Ghafoor [25] stated that the total phenolic content of mint leaf extract (*Mentha arvensis*) was 1.24 mg GAE / 100mL, while the value of its antioxidant activity (free radical scavenging activity) was 34.21%. Mayani *et al.* [11] showed that total phenol in ginger was 420.27 ppm and Azizah [26] stated that antioxidant activity in ginger was 77.65%. Measurement of lemon antioxidant activity carried out by Sun *et al.* [27] showed results of 42.8 ± 1.0 μmol of vitamin C equivalent/ g edible weight, using the Total Oxyradical Scavenging Capacity (TOSC) method.

3.4. Rose Extract Drink Color Intensity

3.4.1. Brightness (L). Figure 1 showed that the lowest brightness was lemon treatment, while the highest one was mint leaves treatment. If the brightness value gets lower than the color of rose extract

drink will get darker. Anthocyanin pigment will be more stable in acidic or in low pH condition. This causes its concentration maintained, resulting in brightness reduction [28]. Ariviani [29] stated that the acidic condition made up most of the anthocyanin pigment in the form of flavylium or oxonium cation which have darker color.
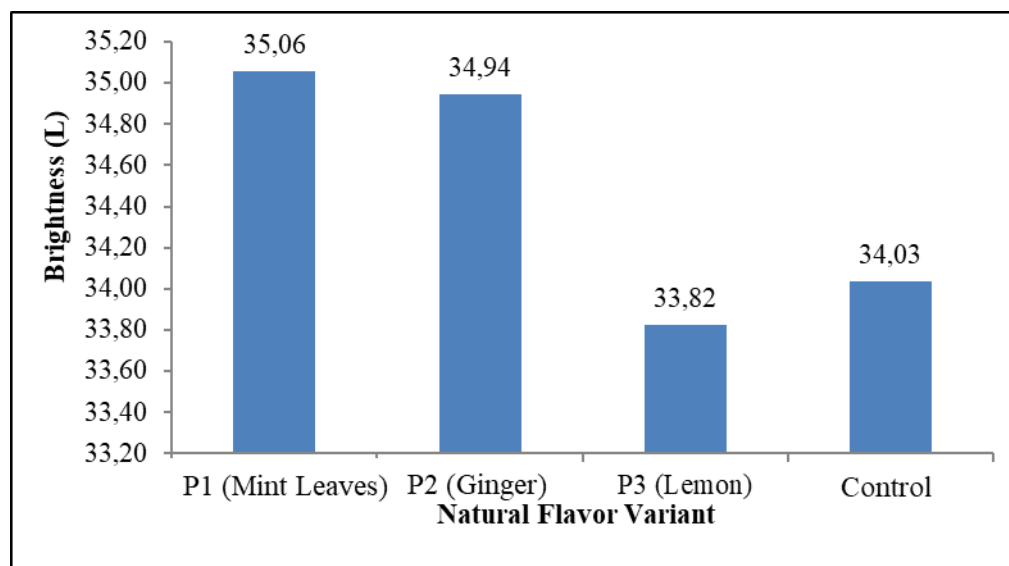
Figure 1. Effect of natural flavor variance on rose extract drink brightness level

3.4.2. Redness (a) and Yellowish (b) Level. The highest redness value was from lemon treatment (Table 4). Lemon made the drink pH lower than mint leaves and ginger did. Acid condition make rose extract anthocyanin pigment more stable during processing, causing its red color become deeper. The lowest redness level got from ginger treatment. That caused by ginger essential oil which has a cloudy yellow color which will affect the redness value, while the mint leaves have green color [29].

Table 4. Effect of natural flavor variance and concentration on rose extract drink redness (a+) and yellowish (b+) level.

Treatment	Redness (a+)	Yellowish (b+)
P1 (Mint Leaves)	8.40 [b]	3.50 [b]
P2 (Ginger)	5.77 [a]	1.25 [a]
P3 (Lemon)	11.46 [c]	2.46 [b]
Control (Without Natural Flavor)	8.95 [b]	4.70 [c]
K1 (concentration 1.5%)	2.94 [b]	2.94 [b]
K2 (concentration 3%)	2.72 [b]	2.72 [b]
K3 (concentration 4.5%)	1.55 [a]	1.55 [a]
Control (Without Natural Flavor)	4.70 [c]	4.70 [c]

Note: The same letter on the same column indicated insignificant result according to Duncan 5%

The higher the concentration of natural flavor caused the redness and yellowish of rose extract drink got lower. Higher concentration of natural flavor added will reduce the redness of the rose extract drink, but it depends on the flavor properties [30].

Mint leaves   Ginger

Lemon   Control
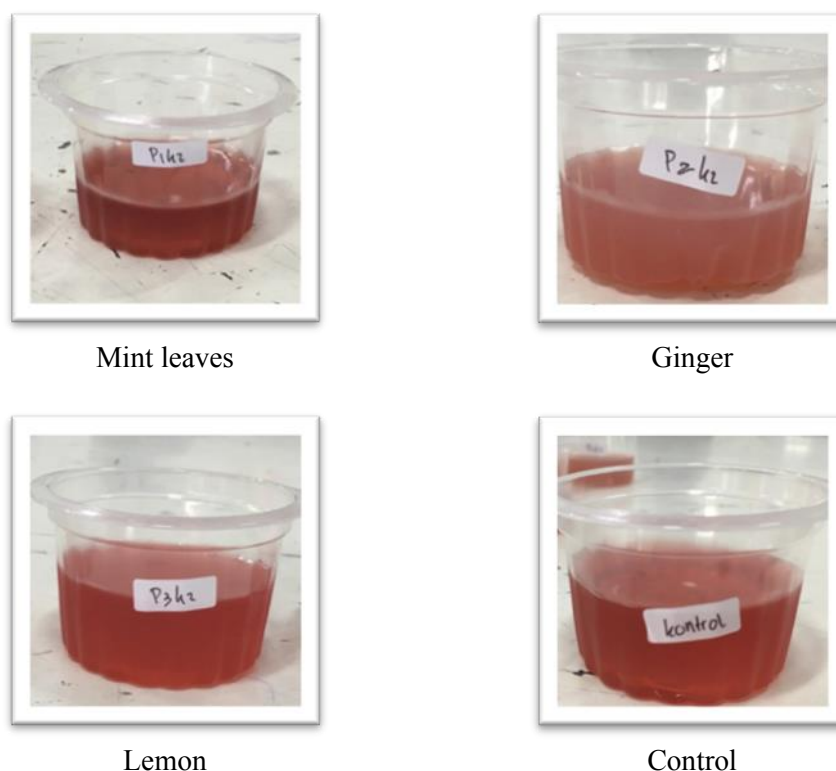
Figure 2. Rose extract drink with added natural flavor.

3.5. Rose Extract Drink Sensory Quality

3.5.1. Rose Extract Drink Taste Score. Panelists quiet dislike K3 (4.5%) treatment while for K1, K2, and control treatment quiet likeable (Table 5). This showed that natural flavor addition will affect panelist acceptance of rose extract drink taste. Flavor addition with certain concertation will increase panelist acceptance of rose extract drink taste. But, if the concentration added is too high it will lower rose extract taste acceptance [30].

Table 5. Effect of natural flavor concentration on rose extract drink taste score.

Treatment	Taste
K1 (concentration 1.5%)	3.21 [b]
K2 (concentration 3%)	3.19 [b]
K3 (concentration 4.5%)	2.98 [a]
Control (Without Natural Flavor)	3.53 [b]

[a]The same letter on the same column indicated insignificant result according to Duncan 5%. Taste Score : 1= really dislike, 2 = dislike. 3 = like enough, 4 = like, and 5 = really like.

Figure 3 showed that the least likeable rose extract drink was P2 (ginger) treatment with 2.93 score, while P1 (mint leaves), P3 (lemon), and control treatment score are quite similar to each other. This was because panelist favor rose extract drink with fresh natural flavor rather than natural flavor that taste like strong herb. Essential oil and oleoresin in ginger is the reason that cause gingery characteristic. Ginger odor caused by its essential oil, while oleoresin cause ginger hot taste [31].
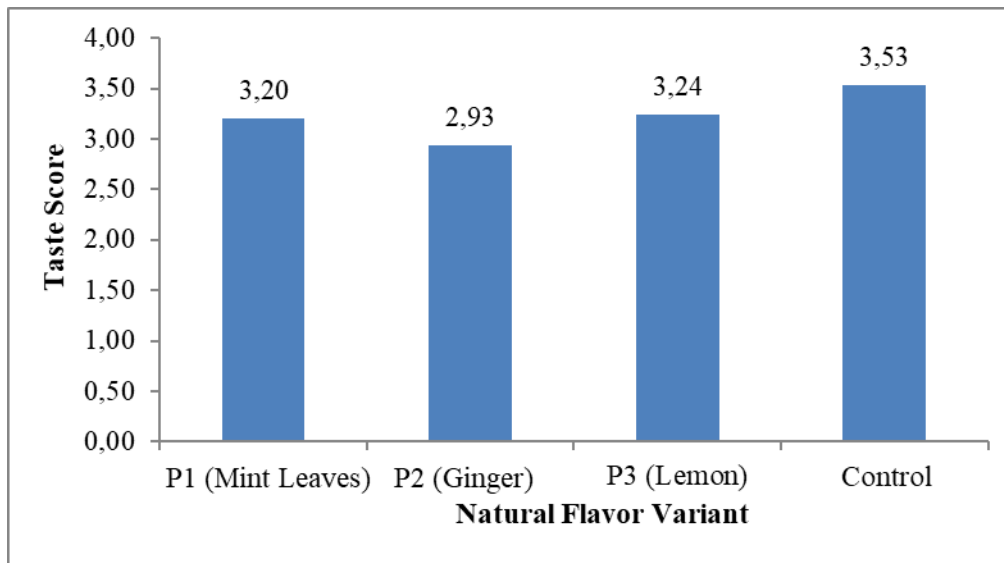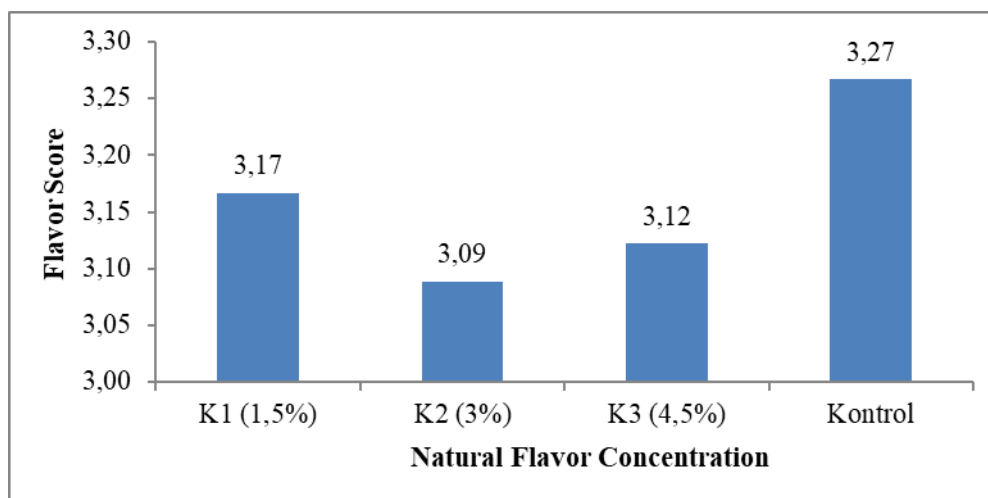
Figure 3. Effect of natural flavor variance on rose extract taste score. Taste Score: 1= really dislike, 2 = dislike. 3 = like enough, 4 = like, and 5 = really like.

Control got the highest taste score with 3.53 point because rose extract without natural flavor addition had fresh taste and rosy flavour liked by panelist. According to Adi [23], mint leaves has cool sensation and fresh flavor and usually used in food with fresh flavor. Nagy and Shaw [32] stated that lemon has soft, watery, and sour characteristic thus rarely consumed directly and usually used as additional flavor and natural acidulant.

3.5.2. Rose Extract Drink Flavor Score. The least favorable treatment is P2 (Ginger) (Fig. 4). This was because panelist like fresh flavor over herb and spice flavor. Wijayakusuma [33] stated that according to individual sensory analysis, the addition of ginger extract in drink will produce spicy and pungent flavor so it will cover the other ingredients flavor.
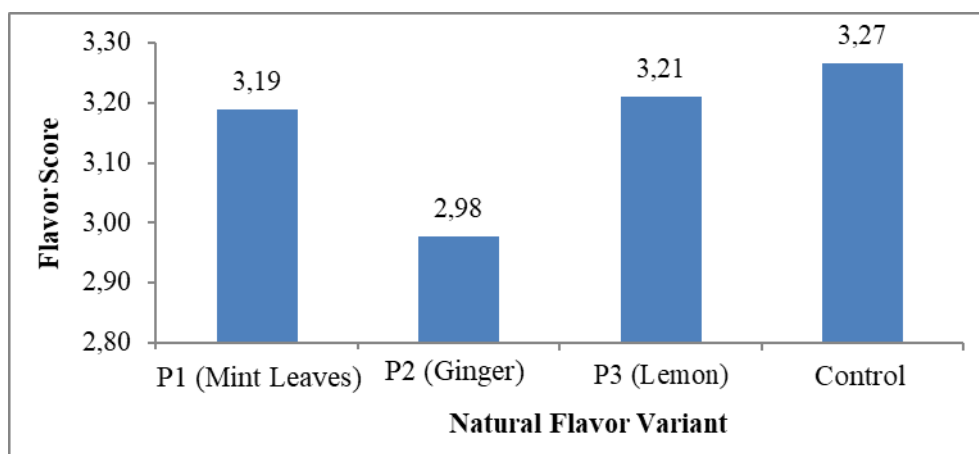
Figure 4. Effect of natural flavor variance on rose extract drink flavor score. Taste Score : 1= really dislike, 2 = dislike. 3 = like enough, 4 = like, and 5 = really like.

Figure 5. Effect of natural flavor concentration on rose extract drink flavor score. Taste Score : 1= really dislike, 2 = dislike. 3 = like enough, 4 = like, and 5 = really like.

Figure 5 showed that natural flavor concentration did not have specific effect on rose extract drink flavor score because the range of concentration differences are not high so panelist did not really taste the differences between each treatment. Snyder *et al.* [34] stated that food volatile compound have important role to produce taste and flavor. The higher volatile compound contained in food will produce more strong and pungent flavor. Mint leaves contain menthol which is aromatic compound that has strong flavor and volatile [35].

3.6. Total Anthocyanin of The Best Combination Treatment
Total anthocyanin analysis was performed on the combination of natural flavor types with the best concentration based on de Garmo test results. The best combination treatments are P1K2 (Mint : 3%), P2K1 (Ginger : 1.5%), P3K1 (Lemon : 1.5%) and control as comparison.

Tabel 6. Rose extract drink best treatment total anthocyanin results at λ 520 nm.

Treatment	Total Anthocyanin (mg/L)	Enhancement (%)
P1K2 (Mint : 3%)	12.10	46.48
P2K1 (Ginger : 1.5%)	11.11	34.50
P3K1 (Lemon : 1.5%)	9.64	16.70
Control (Without Natural Flavor)	8.26	

The highest total anthocyanin treatment is P1K2 (mint Leaves: 3%), while the lowest total anthocyanin is control. Table 6. showed that natural flavor addition and concentration is directly proportional with total anthocyanin value. This is because the compounds in natural flavor synergized with rose extract drink anthocyanin pigment and make it more stable. According to Mishra [36], anthocyanin degradation could be happen during extraction, production, and storage process. Factors that affect anthocyanin stability are anthocyanin specific structure modification (glycosylation, acylation with aliphatic or aromatic acid), pH, temperature, light, metal ion existence, oxygen, sugar content, enzyme and sulphur oxide.

The other factor that affect anthocyanin stability are anthocyanin structure and the other component in food ingredients. Anthocyanin can form complex compound with other polyphenolic compounds. Flavonol and flavon compound that usually conjugated with anthocyanin have contribution to keep

anthocyanin in stable condition. Heating process is one of crucial factor which can damage anthocyanin. Anthocyanin color will change as it pH change. The amount of hydroxyl or methoxy group in anthocyanidin structure will affect anthocyanin color [37].

4. Conclusion

Rose extract drink best treatment is P1K2 (mint leaves : 3%) with antioxidant activity 84.44%, brightness (L) 35.30 points, redness (a+) 8.00 points, yellowish (b+) 4.60, Ph 3.45, total dissolved solids 21.17 °Brix, total anthocyanin 12.14 mg/L, taste 3.20 points (tasty enough), and flavor 3.27 points (like enough). Natural flavour addition increases total anthocyanin of rose extract drink, which mint leaves with 46.48%, ginger 34.5% and lemon 16.70%. All rose extract treatment pH and total dissolved solids consistent with fruit sari quality requirements (SNI 3719-2014) which maximal pH of 4 and minimal total dissolved solids of 7.5 – 16.0 °Brix.

Acknowledgements

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