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Enhancement and Preservation of Fresh Orange Juice Using Citrus Essential Oils

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Abstract: Nowadays, consumers are demanding for minimally processed, additive-free, high quality, fresh-like and microbiologically safe foods. However, as food deterioration is the constant threat along the entire food chain, food preservation remains as necessary today as in the past. In this paper, several citrus essential oils, including sweet orange, lemon and lime essential oil applied directly in fresh orange juice as a natural preservative, were analyzed for their antimicrobial activity. The antimicrobial activity of each citrus essential oil was assessed against *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Aspergillus niger* using broth macrodilution. Among all citrus essential oils tested, sweet orange essential oil with 0.10% concentration showed significant antimicrobial activity against microorganisms tested. Hence, it was chosen to be applied in the fresh orange juice. Then, shelf life analysis was conducted during 7 d of storage at 5 °C and during 2 d of storage at 25 °C using selected concentration of the selected citrus essential oil in the fresh orange juice. Based on the results obtained, the addition of sweet orange essential oil with 0.10% concentration was not able to prolong the shelf life of the fresh orange juice according to the Indonesian National Standard (SNI). However, the formulation of fresh orange juice itself was able to improve its shelf life when compared to the shelf life of the commercial orange juice.

Key words: Fresh orange juice, spoilage, citrus essential oils, preservative, antimicrobial.

1. Introduction

Fruit juice, particularly citrus juice, is one of the most consumed beverages on the market [1, 2]. In statistics terms, citrus juices are the most popular fruit juices with more than 50% of the international commercialization volume of juices [2]. Within citrus juices, orange juice represents an approximate 60% of all Western Europe consumption of juices and juice-based drinks and a similar amount (60%) of all fruit juice sales in the United States [2]. Consumption of fruit juice itself has been increasing over the last few years, due to the high demand for low caloric-food products with fresh characteristics [3]. Consumers perceive fruit juice as a nutritive, healthy and functional product [2].

However, most of the fruit juices sold on the market are processed fruit juices with worse nutritional and organoleptic properties than fresh juices. Pasteurization and sterilization are commonly used to extend the shelf life of fruit juices by inactivating microorganisms and enzymes. Nevertheless, the process can destroy nutritional components, such as vitamins [4-6]. Although fruit juices on the market have a long shelf life, consumers find it unattractive when the nutrients they contain are depleted [7]. For that reason, the global juice industry faces new challenges, as today's consumers are demanding minimally processed, additive-free, high quality, fresh-like and microbiologically safe foods [5, 6, 8, 9]. However, fresh juices that are not processed and contain no preservatives have a short shelf life. It can only last for several days, as they can be easily spoiled by microorganisms [10, 11]. Many organisms, particularly fungi and lactic acid bacteria, can use fruit as substrate and cause spoilage, producing off flavors,

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odors and discoloration of the product [10].

In the past few years, many researchers have studied the characteristics of essential oils. They have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications, especially in pharmaceutical, sanitary, cosmetic, agricultural and food industries since the middle ages [12]. Therefore, instead of applying heat treatment which may decrease the nutritional value of the fruit juice, addition of essential oil is more preferable. The purpose of this study was to evaluate fresh orange juice with fresh-like flavor preserved with citrus essential oil. However, several challenges may be encountered with the addition of citrus essential oil, as the oil itself is highly concentrated. Furthermore, it has a strong aroma and flavor. Therefore, the addition of citrus essential oil should be of an appropriate amount to prevent the alteration of the final taste of the fresh orange juice, but the amount itself still has the ability to act as antifungal and antibacterial agents.

2. Materials and Methods

2.1 Selection of Orange Juice Base

Several variants of orange with two categories: (1) sensory contributing orange base, such as Jeruk Mandarin, Jeruk Medan, Jeruk Pontianak, Jeruk Sunkist (Navel USA) and Jeruk Sunkist (Valencia), and (2) pH reduction contributing orange base, such as Jeruk Limau and Jeruk Nipis, were purchased from AEON supermarket (BSD city, Tangerang) and evaluated. Two variants of orange with high yield of extraction and low pH were selected and combined to produce orange juice with pH of 2.60. The pH itself was measured using a digital pH meter, Lutron PE-03 Electrode pH (Taiwan).

2.2 Orange Juice Produced with Selected Variants

2.2.1 Orange Juice Production

The materials required for orange juice production were Jeruk Pontianak and Jeruk Nipis, cane sugar and table salt. The stages of the production process were

Fig. 1 Orange juice production.

simplified according to Falguera and Ibarz [13], as shown in Fig. 1.

After combining both variants of orange, the pH of final orange juice was measured using Lutron PE-03 Electrode pH (Taiwan) in order to ensure that pH of 2.60 was achieved. Moreover, sugar content of final orange juice was also measured using Atago Pocket Refractometer PAL-1 (Japan) after the addition of 5% w/w sugar and 0.2% w/w salt to ensure that the sugar content was above 10 °Brix, according to requirement from Indonesian National Standard (SNI) [14].

2.2.2 Microbial Analysis

Freshly squeezed orange juice (Jeruk Pontianak, Jeruk Nipis and its combination, respectively) was produced in a small scale without the addition of sugar and salt and analyzed using standard plate count analysis (pour plate technique) according to the description by Da Silva et al. [15]. The materials required were 1 mL of each sample for serial dilutions, sterile 0.85% saline solution (8.5 g of sodium chloride in 1 mL of distilled water, Himedia, India) as diluting agent, sterile Petri dish, potato dextrose agar (PDA; Merck, Germany), de Man, Rogosa and Sharpe agar (MRS; Merck, Germany) and plate count agar (PCA; Merck, Germany).

Samples and serial dilutions ranging from 1 (without dilution) to 1:1,000 were prepared by taking 1 mL of each sample and putting it into sterile Petri dish. Another 1 mL of the sample was also taken and put into a test tube which contained 9 mL of sterile distilled water. The solution was mixed by using vortex for 15 s and resulted in 1:10 dilution. Afterward, 1 mL of 1:10 dilution was taken and put into another sterile Petri dish and test tube which also contained 9 mL of sterile distilled water, resulted in 1:100 dilution. The process was repeated until 1:1,000 dilution was obtained. Sterile PDA, MRS agar and PCA were poured into each sterile Petri dish containing the sample. By using pour plate method, it was mixed immediately by rotation motion to ensure the uniformity. After all the plates poured with sterile PDA dried, they were stacked for three plates and incubated at 25 °C in the dark condition for 5 d. Resulted yeast and mould colonies were counted and the calculation was made for the plates with 15 to 150 colonies. While, Petri dishes poured with sterile MRS agar and PCA were incubated at 37 °C for 2 d. Resulted bacteria colonies were counted and the calculation was made for the plates with 30 to 300 colonies.

2.2.3 Determination of Vitamin C Content

Vitamin C determination was conducted by using iodine titration according to the procedure of University of Canterbury [16]. The materials required for the titration were 0.005 mol/L iodine solution (2 g of potassium iodide; BDH Laboratory Supplies, United States), 1.3 g of iodine (BDH Laboratory Supplies, United States) and distilled water until the solution up to 1 L, 0.5% starch indicator solution (clear solution of 0.25 g of soluble starch in 50 mL of boiling water, BDH Laboratory Supplies, United States) and 20 mL of sample.

First, iodine solution (0.005 mol/L) was prepared by weighing 2 g of potassium iodide and 1.3 g of iodine into a 100 mL beaker glass. A few milliliters of distilled water were added and the solution was swirled for a few minutes until iodine was completely dissolved. Next, the solution was transferred into a 1 L volumetric flask. All traces of the solution in the beaker glass were rinsed by adding distilled water and transferring it to the volumetric flask continuously. Distilled water was added until the solution up to 1 L mark. Second, starch indicator solution (0.5%) was prepared by diluting 0.25 g of soluble starch into 50 mL of boiling water until the solution was clear. Stirring was needed during the process. Moreover, the solution was cooled first before using.

Sample of 20 mL (Jeruk Pontianak, Jeruk Nipis and its combination, respectively) was taken into 250 mL Erlenmeyer flask by using graduated pipette. Next, 150 mL of distilled water and 1 mL of starch indicator solution (0.5%) were added. All of the samples were

titrated with iodine solution (0.005 mol/L) until the endpoint of the titration was reached, which was the first permanent trace of a dark blue-black color or permanent color changes due to the starch iodine complex.

2.3 Determination of Type and Concentration of Citrus Essential Oil

Three types of citrus essential oils, including sweet orange (*Citrus sinensis* L.), lemon (*Citrus lemon* L.) and lime essential oil (*Citrus latifolia* L.) were purchased from Lansida (Indonesia) and tested against *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Aspergillus niger* purchased from the Bogor Agricultural University in form of agar slant. During the research, all of the microorganisms were stored under refrigerator temperature in order to maintain their longer lifespan. Moreover, the inoculum used for broth macrodilution was prepared from pure cultures of *S. cerevisiae* grown on potato dextrose broth at 37 °C for 24 h, *L. plantarum* grown on nutrient broth at 37 °C for 24 h and *A. niger* grown on potato dextrose broth at 37 °C for 7 d.

The turbidity of each inoculum was adjusted by diluting the culture in 0.85% sterile saline solution to the density of a 0.5 McFarland standard at 530 nm wavelength for *S. cerevisiae* and *A. niger*, and a 0.5 McFarland standard at 625 nm wavelength for *L. plantarum*. Distilled water was used as the blank. Furthermore, accepted standardized inoculum should have absorbance A = 0.08-0.13, which yielded a stock suspension of 1-5 × 10^6 cells/mL for *S. cerevisiae*, 1.5 × 10^8 CFU/mL for *L. plantarum* and 0.4-5 × 10^6 viable conidia/mL for *A. niger*, respectively. Furthermore, the final concentration required for broth macrodilution was 5 × 10^2 to 2.5 × 10^3 CFU/mL for *S. cerevisiae*, 5 × 10^5 CFU/mL for *L. plantarum* and 0.4-5 × 10^4 CFU/mL for *A. niger*, respectively.

The broth macrodilution procedure was conducted according to the method designed by CLSI (M27-A2) for *S. cerevisiae* [17], CLSI (M07-A9) for *L. plantarum* [18] and CLSI (M38-A) for *A. niger* [19]. Potato dextrose broth (Himedia, India) and nutrient broth (Merck, Germany) were used for the analysis. In the beginning, preliminary research was conducted first by applying five different concentrations ranged from 0.10%, 0.20%, 0.30%, 0.40% until 0.50% [12, 20, 21]. From the result obtained, 0.10% concentration already showed significant antimicrobial activity against microorganisms tested. Therefore, the other three different concentrations of each essential oil: 0.06%, 0.08% and 0.10% were tested by diluting each essential oil with 3% of Tween 80 (3.18 g of Tween 80 in 100 mL of distilled water, Brataco, Indonesia), as lower concentration of the added essential oil was more preferable due to sensory characteristics consideration. The results of the analysis were measured using 10S UV-vis spectrophotometer (Thermo Electron Corporation Genesys, United States).

2.4 Sensory Analysis

Scoring and hedonic tests were used to evaluate the final orange juice added with selected citrus essential oil and concentration with the most significant inhibitory activity against all of the microorganisms tested. The tests were conducted according to the protocol design by Meilgaard et al. [22]. The total number of 50 untrained panelists was required to evaluate several perceived attributes, including color, citrus aroma, fresh flavor, sourness, sweetness, viscosity and aftertaste (bitterness) in terms of their intensities (Table 1) in scoring test. They were required to choose the suitable scale used as a description for each sample presented according to the intensities they perceived. In hedonic test, the panelists were required to evaluate the overall acceptance of the samples given using score from 1 to 9 (Table 1).

2.5 Comparative Analysis of Commercial Fruit Juices

Several characteristics of 17 commercial orange juices sold on the market, including their types of

Table 1 Verbal scale used in scoring and hedonic tests.

Attributes	Intensities
Color	1 = very light, 2 = light, 3 = slightly light, 4 = slightly dark, 5 = dark, 6 = very dark
Citrus aroma, fresh flavor, sourness, sweetness, aftertaste (bitterness)	1 = very weak, 2 = weak, 3 = slightly weak, 4 = slightly strong, 5 = strong, 6 = very strong
Viscosity	1 = very watery, 2 = watery, 3 = slightly watery, 4 = slightly viscous, 5 = viscous, 6 = very viscous
Overall acceptance	1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely

processing (evaporated, pasteurized, sterile processed, or cold pressed), ingredients (with or without artificial preservative), storing condition (room temperature storage or refrigerator temperature storage) and age were observed (days, weeks, months or years). Commercial fruit juice that had similar characteristics as fresh orange juice was going to be studied as a comparison for the shelf life analysis.

2.6 Shelf Life Analysis

In this analysis, total microbial count was conducted to enumerate the number of viable yeast, mould and bacteria responsible for the fresh orange juice spoilage, followed by iodine titration to determine the vitamin C degradation in the fresh orange juice. The materials required for the total microbial count were 1 mL of each sample for serial dilutions, sterile distilled water as diluting agent, sterile Petri dish, sterile PDA (Merck, Germany) and PCA (Merck, Germany). Total microbial count analysis was conducted for every 24 h during 7 d of storage at 5 °C and for every 6 h during 2 d of storage at 25 °C. The procedure conducted was similar with the procedure written in the standard plate count analysis. In addition, the materials required for the determination of vitamin C content were similar with the determination of vitamin C content conducted before. Iodine titration was also conducted for every 24 h during 7 d of storage at 5 °C and for every 6 h during 2 d of storage at 25 °C. The procedure conducted was similar with the procedure written in the standard plate count analysis. However, the serial dilutions prepared may differ for each analysis, as the resulted colonies for yeast and mould should be within 15 to 150 colonies, while the resulted colonies for bacteria should be within 30 to 300 colonies. Thus, the results can be calculated and converted into CFU/mL.

The shelf life of the fresh orange juice preserved with selected citrus essential oil was determined, after the total number of responsible spoilage microorganisms exceeded their maximum numbers according to the requirements in SNI Sari Buah Jeruk [14], after the total number of the yeast and mould colonies were exceeding 50 CFU/mL and the total number of the bacteria colonies exceeding 2×10^2 CFU/mL, specifically. Hence, the effectiveness of selected citrus essential oil applied in the fresh orange juice as natural preservative to prolong the shelf life was obtained.

2.7 Statistical Analysis

Each analysis was conducted in duplicate. Conventional statistical methods were used to calculate means and standard deviations. Statistical analyses (ANOVA, t-test, Wilcoxon) were applied to the data to ascertain significant differences between the levels of the main factor with $P < 0.05$ using the Design Expert 6.0.8 and Microsoft Excel.

3. Results and Discussion

3.1 Selection of Orange Juice Base

According to the results of all evaluated orange variants (Table 2), Jeruk Pontianak was selected due to its higher yield of extraction and lower pH. One way ANOVA statistical analysis showed that Jeruk Pontianak with the highest mean score of yield of extraction and the lowest mean score of pH was

Table 2 Evaluated characteristics of several orange variants sold in Indonesian market.

Sensory contributing orange base	Yield of extraction (%)	pH
Jeruk Mandarin	33.97 ± 0.00^{cd}	3.96 ± 0.17^{a}
Jeruk Medan	34.27 ± 0.01^{c}	4.18 ± 0.02^{a}
Jeruk Pontianak	50.76 ± 0.05^{a}	3.31 ± 0.03^{c}
Jeruk Sunkist (Navel USA)	39.93 ± 0.03^{b}	4.10 ± 0.45^{a}
Jeruk Sunkist (Valencia)	37.75 ± 0.03^{bc}	3.42 ± 0.14^{b}
pH reduction contributing orange base		
Jeruk Limau	31.17 ± 0.01^{a}	2.28 ± 0.02^{a}
Jeruk Nipis	25.54 ± 0.01^{b}	2.25 ± 0.00^{b}

[a-d] Means with different superscript letters are significantly different with each other ($P < 0.05$).

Table 3 Standard plate count analysis of Jeruk Pontianak, Jeruk Nipis and its combination.

Orange variants	pH	Sugar content (°Brix)	Microorganisms	Microbial growth (total colonies/mL)
Jeruk Pontianak	3.31 ± 0.03	8.23 ± 0.04	Yeast and mould	5.00 ± 0.00
			Lactic acid bacteria	2.75 ± 0.35
			General bacteria	3.25 ± 1.06
Jeruk Nipis	2.48 ± 0.03	7.55 ± 0.00	Yeast and mould	3.50 ± 0.71
			Lactic acid bacteria	0.25 ± 0.35
			General bacteria	3.25 ± 2.47
Combination of Jeruk Pontianak and Jeruk Nipis	2.60 ± 0.00	8.55 ± 0.07	Yeast and mould	9.00 ± 2.12
			Lactic acid bacteria	2.50 ± 1.41
			General bacteria	5.25 ± 0.35

significantly different with the other variants of orange. Jeruk Nipis was also selected, as it has slightly lower pH and is easier to be extracted compared to Jeruk Limau, although its yield of extraction in percentage is lower. Based on *t*-test statistical analysis, Jeruk Limau and Jeruk Nipis were significantly different in terms of yield of extraction and pH. Both selected Jeruk Pontianak and Jeruk Nipis were combined to produce orange juice with pH of 2.60. The combination ratios of Jeruk Pontianak and Jeruk Nipis varied from 1:1 until 2:1, as it depended on the initial pH of each variant. Therefore, controlling the pH during the production process of orange juice was essential.

3.2 Orange Juice Produced with Selected Variants

3.2.1 Microbial Analysis Results

Due to the fresh condition of the orange juices, the deterioration of the product had not occurred rigorously. The low number of total microbial was not able to be calculated in CFU/mL, as the results from the lowest dilution were not fall between the calculated ranges. Hence, it was presented in direct number of the total colonies/mL. According to the results shown in Table 3, combination of Jeruk Pontianak and Jeruk Nipis had higher growth of yeast, mould and general bacteria, followed by Jeruk Pontianak and Jeruk Nipis. This may be due to a rise in nutrients content of both variants when combined and longer preparation time. Longer preparation time may lead to higher microbial growth due to the temperature abuse. Hence, both factors promote higher growth of yeast, mould and bacteria. On the other side, Jeruk Nipis had lower growth of lactic acid bacteria, followed by combination of both variants, and Jeruk Pontianak itself. This may be due to that the pH of Jeruk Nipis was very acidic (2.48 ± 0.03). Both the growth and the growth rate of microorganisms are greatly affected by pH. The very acidic pH may cause growth inhibition of the lactic acid bacteria [23].

3.2.2 Vitamin C Analysis Results

Based on the titration results in Table 4, Jeruk Nipis

had higher vitamin C content compared to its combination with Jeruk Pontianak and Jeruk Pontianak itself. Despite of large difference in pH, the vitamin C content from both variants were between 115.36 mg/100 mL and 117.12 mg/100 mL.

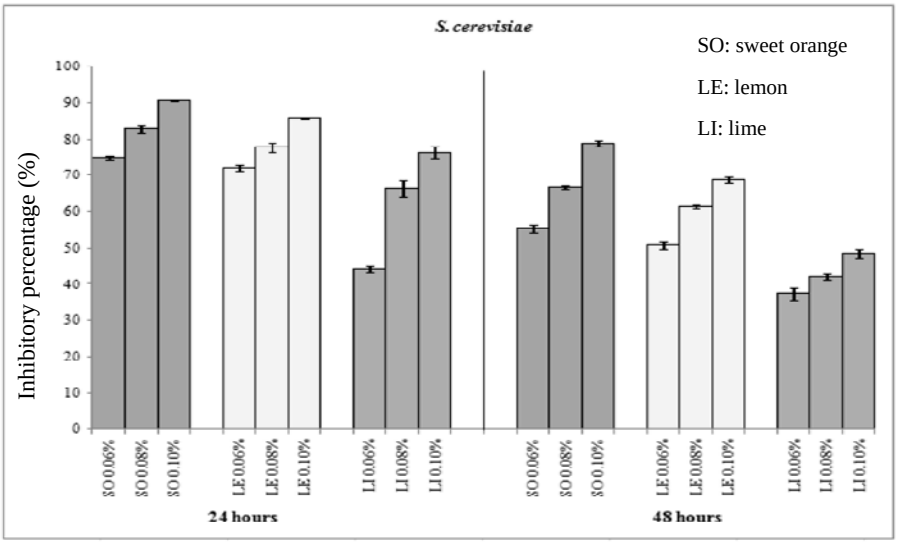
3.3 Determination of Type and Concentration of Citrus Essential Oil

Based on the results showed in Fig. 2, the inhibitory percentage of each citrus essential oil tested was increasing proportionally to the increment of the concentration and decreasing over the incubation time. It may be due to the composition of the essential oil, which consists of volatile compounds.

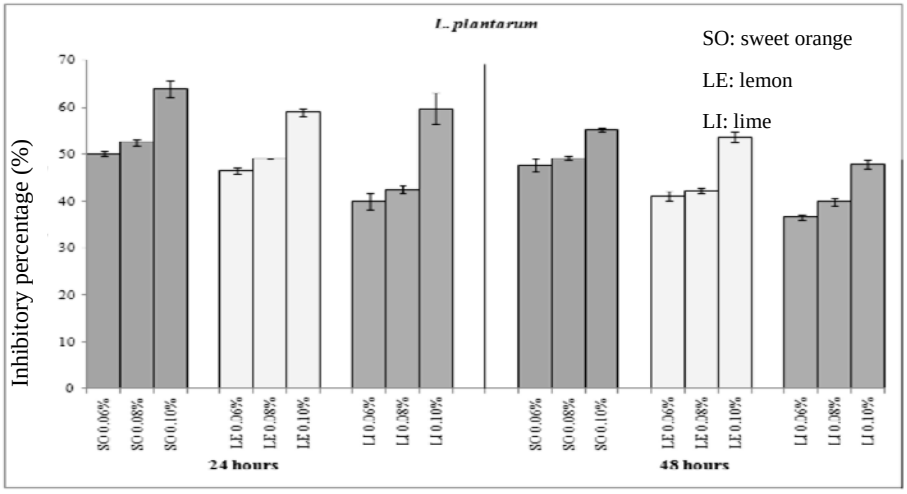
The inoculums dose used during the analysis was 5 × 10^2 to 2.5 × 10^3 CFU/mL for *S. cerevisiae*, 5 × 10^5 CFU/mL for *L. plantarum* and 0.4-5 × 10^4 CFU/mL for *A. niger*. Moreover, positive (growth control) and negative (sterility) controls were also included. Positive control contained 0.9 mL of the standardized

Table 4 Vitamin C content in freshly squeezed juice.

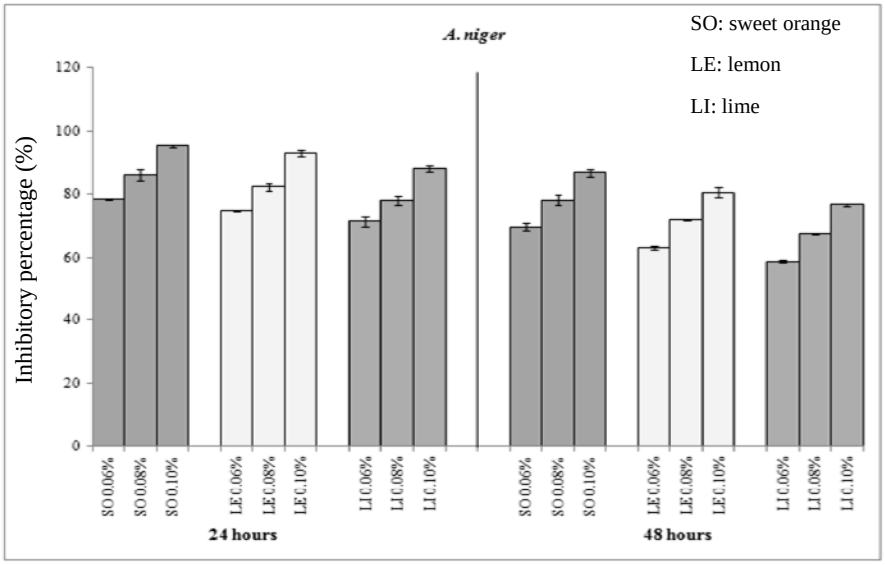
Orange variants	Vitamin C content (mg/100 mL)
Jeruk Pontianak	115.36 ± 0.00
Jeruk Nipis	117.12 ± 0.62
Combination of Jeruk Pontianak and Jeruk Nipis	116.46 ± 0.31



(a)



(b)



(c)

Fig. 2 Inhibitory percentage of citrus essential oil against *S. cerevisiae* (a), *L. plantarum* (b) and *A. niger* (c) after 24 h and 48 h of incubation.

inoculum with the addition of 0.1 mL of potato dextrose broth for *S. cerevisiae* and *A. niger* or 0.1 mL of nutrient broth for *L. plantarum*. Positive control was used as a growth comparison with other tubes containing the antimicrobials. Negative control contained only 1 mL of sterile potato dextrose broth for *S. cerevisiae* and *A. niger* or sterile nutrient broth for *L. plantarum*. Negative control used to ensure the sterility of the working procedure.

According to two ways ANOVA statistical analysis, there was no interaction between the types of citrus essential oil and concentrations toward antimicrobial activity. It also showed that lime essential oil was significantly different with the other citrus essential oils tested, while sweet orange essential oil and lemon essential oil were not significantly different (Table 5). However, sweet orange essential oil has higher mean score of inhibitory percentage compared to lemon essential oil. Thus, sweet orange essential oil has higher inhibitory activity against all microorganisms tested. This may be due to its higher composition of 68%-98% of limonene [20]. Limonene is the most popular phenolic compound found in citrus essential oils and has been recognized for its antimicrobial activity [24, 25]. Therefore, high composition of limonene leads to high antimicrobial activity.

The results shown in Tables 5 and 6 were obtained by calculating all of the results of the inhibitory percentage of each citrus essential oil tested for all microorganisms, all concentrations and all observation periods in order to acknowledge whether all citrus essential oils tested were significant different or not.

Moreover, 0.10% concentration of citrus essential oil has the highest inhibitory percentage among all concentrations tested (Table 6). Two ways ANOVA also showed that all of the concentrations tested were significantly different. However, 0.10% concentration of citrus essential oil has the highest mean score, followed by 0.08% and 0.06% concentrations. Thus, 0.10% sweet orange essential oil was selected to be applied in the fresh orange juice.

3.4 Sensory Evaluation of the Final Orange Juice Added with or without Sweet Orange Essential Oil

The results of the sensory tests were analyzed using Wilcoxon statistical analysis. Among all of the evaluated attributes, color, citrus aroma, fresh flavor and viscosity were significantly different (Table 7). The

Table 5 Inhibitory percentage of each type of citrus essential oil tested.

Types of citrus essential oil	Inhibitory percentage (%)
Sweet orange	70.14 ± 15.58[a]
Lemon	65.23 ± 15.76[a]
Lime	56.79 ± 16.67[b]

[a, b] Means with different superscript letters are significantly different with each other ($P < 0.05$).

Table 6 Inhibitory percentage of each concentration of citrus essential oil tested.

Concentration	Inhibitory percentage (%)
0.06%	56.29 ± 14.41[c]
0.08%	63.15 ± 16.13[b]
0.10%	72.72 ± 15.39[a]

[a-c] Means with different superscript letters are significantly different with each other ($P < 0.05$).

Table 7 Mean score of the evaluated attributes and overall acceptance obtained from scoring and hedonic tests.

Scoring test attributes	Without sweet orange essential oil 0.10%	With sweet orange essential oil 0.10%
Color	3.46 ± 0.89[a]	2.78 ± 0.93[b]
Citrus aroma	3.78 ± 1.17[a]	4.92 ± 0.97[b]
Fresh flavor	4.12 ± 1.33[a]	4.44 ± 1.03[b]
Sourness	5.18 ± 0.77[a]	5.34 ± 0.69[a]
Sweetness	2.22 ± 1.07[a]	2.02 ± 0.94[a]
Viscosity	2.82 ± 0.98[a]	3.08 ± 0.94[b]
Aftertaste (bitterness)	4.66 ± 1.19[a]	4.54 ± 1.11[a]
Hedonic test		
Overall acceptance	4.70 ± 1.91[a]	4.78 ± 2.03[a]

[a-c] Means with different superscript letters are significantly different with each other ($P < 0.05$).

The addition of the essential oil resulted in the lighter color of the fresh orange juice, due to the bright yellow color of the sweet orange essential oil; enhanced the citrus aroma and fresh flavor of the orange juice, due to the large percentages of monoterpenes (C_{10}/H_{16}) and smaller amounts of sesquiterpenes (C_{15}/H_{24}) that carry the oxygenated compounds comprising alcohols, aldehydes, ketones, acids and esters which are responsible for the characteristic odor and flavor profiles [26, 27]; and affected the viscosity from slightly watery to watery due to the high viscosity of the essential oil itself.

In terms of their overall acceptances, the mean score of the fresh orange juice with and without the addition of essential oil was 4.78 and 4.70, respectively, which were between slightly dislike to neither like nor dislike. Low overall acceptances may be caused by the low the pH of the fresh orange juice (2.60). Statistical analysis also showed no significant difference. Thus, sweet orange essential oil with 0.10% concentration was used and applied directly to the fresh orange juice for the shelf life analysis, as the addition itself did not affect the overall acceptance of the juice.

3.5 Comparative Analysis of Commercial Fruit Juices

Based on the comparative analysis, the majority of the commercial orange juices sold on the market are produced by evaporation which covers 59% of the orange juices, followed by pasteurization with 29%, and both sterile processing and cold pressing with 6%. Among all commercial products, orange juice produced using sterile processing (commercial product A) has similar characteristic as fresh orange juice used for the whole study. Both are natural orange juices and minimally processed. The shelf life of the selected commercial orange juice, which is 3 d, was used as the comparison for the shelf life analysis of the fresh orange

juice added with sweet orange essential oil 0.10%.

The total yeast and mould count and the total bacteria count of the commercial product A during 3 d of storage at 5 °C are shown in Fig. 3. According to the graphs, the total yeast and mould count and the total bacteria count were already exceeded the requirement of SNI at day 2.

3.6 Shelf Life Analysis of Fresh Orange Juice with Addition of Sweet Orange Essential Oil

The total microbial count of the samples stored at 5 °C and 25 °C are shown in Figs. 4 and 5, respectively. Among all of the samples observed, the negative control stored at both storage temperatures had the maximum number of microbial colonies. The results also showed that the samples stored at 5 °C had lower total microbial count compared to the samples stored at 25 °C, as lower storage temperature can slow down the rate of microbial growth [28].

There are also other differences in terms of their sensory characteristics. At 25 °C, all samples deteriorated more rapidly as the separation of the juice, the production of off-flavors and gas occurred after 24 h of storage. At 5 °C of storage temperature, the separation of the juice, the production of gas and off-flavors did not occurred during 7 d of storage for all samples.

Moreover, the total microbial count of the sample added with essential oil was already exceeded the requirement of SNI at day 3 when stored at 5 °C and even before 6 h when stored at 25 °C (Figs. 4 and 5). ANOVA single factor showed that the sample added with essential oil and the negative control stored at 5 °C and 25 °C were not significant different. Thus, the

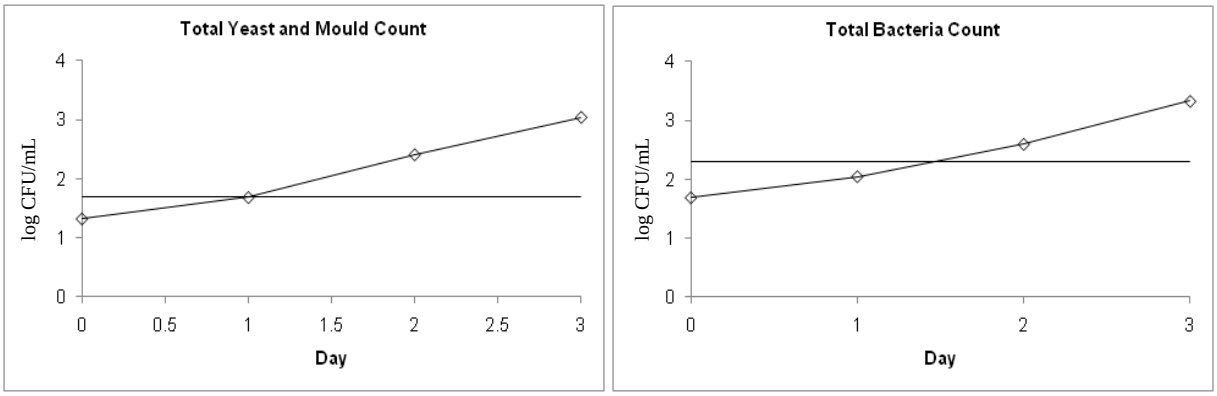


Fig. 3 Total microbial count of commercial product A during 3 d of storage at 5 °C.

◊: commercial product A; –: SNI.

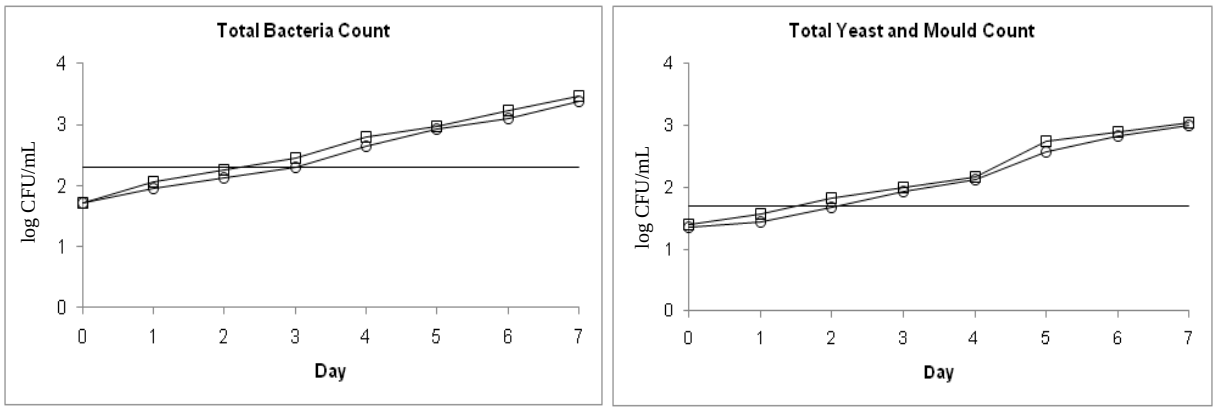


Fig. 4 Total microbial count of fresh orange juice with addition of 0.10% sweet orange essential oil during 7 d of storage at 5 °C.

□: negative control; ○: with essential oil addition; –: SNI.

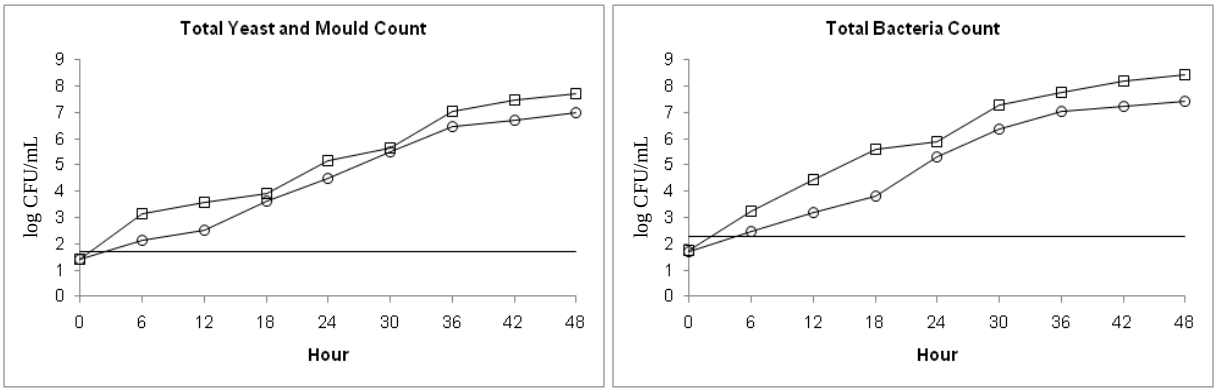


Fig. 5 Total microbial count of fresh orange juice with addition of 0.10% sweet orange essential oil during 2 d of storage at 25 °C.

□: negative control; ○: with essential oil addition; –: SNI.

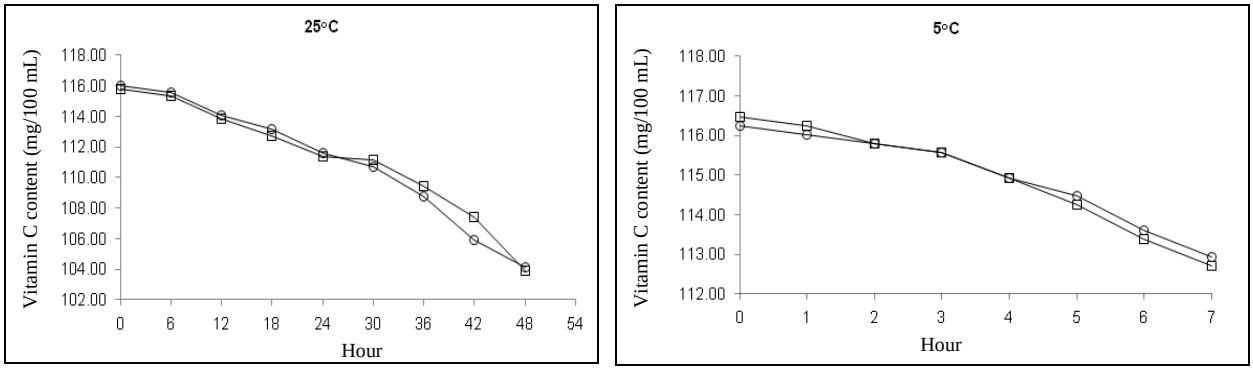


Fig. 6 Vitamin C content of fresh orange juice with addition of 0.10% sweet orange essential oil at 5 °C and 25 °C storage temperatures.

□: negative control; ○: with essential oil addition.

addition of the essential oil was not effective to inhibit the growth of spoilage microorganisms in the fresh orange juice and not able to prolong the shelf life of the product.

According to Fisher and Phillips [20], it has been found that higher minimum inhibitory concentration (MIC) of the essential oils is often required when applied to food. Some researchers also found that antimicrobial agent tend to lose its effectiveness inside food model, although it has strong antimicrobial activity when tested alone [29]. Therefore, the concentration of the sweet orange essential oil added (0.01%) may not be high enough to be effective in inhibiting the growth of the spoilage microorganisms.

On the other side, the essential oil itself is a hydrophobic compound. There is a possibility that the essential oil added was not dispersed completely in the fresh orange juice. In a study conducted by Boroski et al. [30], a homogenizer is used to disperse oregano essential oil in dairy beverage and maintain the oil stability in the dairy beverage. Therefore, the usage of homogenizer is more preferable to produce more homogenize dispersion of oil in liquid medium.

However, the total microbial counts of the negative control and the sample with the essential oil (Fig. 4) were lower compared to the commercial product A (Fig. 3). The total microbial count of the commercial product A exceeded the requirement of SNI at day 1, while the negative control and the sample with the essential oil addition stored at 5 °C exceeded the requirement of SNI at day 3. Thus, the shelf life of the fresh orange juice was improved when compared to the commercial product A due to the fresh orange juice formulation with pH of 2.60.

The vitamin C contents of the samples stored at 5 °C and 25 °C are shown in Fig. 6. Both graphs showed the vitamin C content of all samples decreased gradually over the storage time. However, the samples stored at 25 °C were decreased more dramatically compared to the samples stored at 5 °C.

According to Burdurlu et al. [31], degradation of vitamin C depends upon factors, such as oxygen, heat, light, storage temperature and storage time. Moreover, very low storage temperature also maintains unaltered the chemical and physical characteristics of the juice [32]. Based on the results, storage temperature was the main factor affecting the vitamin C content degradation of the samples. Moreover, ANOVA single factor showed that there was not significant difference between the sample added with 0.10% sweet orange essential oil and the negative control stored at 5 °C and 25 °C. Thus, the addition of 0.10% sweet orange essential oil did not affect the vitamin C content degradation.

4. Conclusions

Combination of Jeruk Pontianak and Jeruk Nipis creates orange juice with pH of 2.60. Based on broth macrodilution analysis, sweet orange essential oil with 0.10% concentration has the highest inhibitory activity. The addition of the essential oil did not affect the overall acceptance of the fresh orange juice, however enhanced the citrus aroma and fresh flavor. The addition of the essential oil was not able to prolong the shelf life of the fresh orange juice stored at 5 °C and 25 °C according to the standard of SNI. However, the addition itself was able to improve the shelf life of the fresh orange juice, when compared to that of the commercial orange juice.

As for recommendations, further formulation of orange juice base should be conducted in order to improve the taste, particularly to reduce the sourness and aftertaste (bitterness) and increase the overall acceptance. In addition, higher concentrations above the three concentrations of citrus essential oil tested during the study should be analyzed to maximize the antimicrobial activity of the essential oil in the fresh orange juice. Moreover, the dispersion of the essential oil added should be observed and analyzed. Further processing with homogenizer should also be applied. In general, the spoilage in fresh orange juice is caused due to the growth of *S. cerevisiae*, *L. plantarum* and *A. niger*. All of the microorganisms are facultative anaerob, facultative anaerob and aerobic microorganisms, respectively. Hence, the application of citrus essential oil in the fresh orange juice as natural preservative could also be combined with vacuum packaging by removing the oxygen to reduce the growth of the microorganisms responsible for the spoilage in the fresh orange juice.

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