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Application of Lemongrass Essential Oil as a Natural Preservative Agent for Pineapple Juice

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Abstract: Pineapple juice is frequently consumed for its extensive health benefits, but is prone to microbiological degradation. Pasteurization – as a current preservation technique – have been degrading the nutritional qualities of fruit juices, and the use of synthetic preservatives have been perceived negatively by consumers. In this study, lemongrass essential oil was applied for the preservation of pineapple juice. Agar Well Diffusion Method was used for antimicrobial activity screening of lemongrass essential oil against *E. coli*, *S. cerevisiae*, and *A. niger*, while Broth Macrodilution Method was used in determining Minimum Inhibitory Concentration (MIC) of lemongrass essential oil, which was evaluated to be 0.1%. Furthermore, the addition of 0.1% lemongrass essential oil into pineapple juice had a negative impact regarding the sensorial acceptability, but was able to significantly suppress the microbial count for bacteria, yeast, and mould during 5 days of storage time in chilling temperature (4^{o}C). Vitamin C content degradation and pH changes during storage were not significantly affected by the addition of lemongrass essential oil. Furthermore, the addition of 0.1% lemongrass essential oil was observed to be not synergistic with the enzyme activity of pineapple juice, and has negatively impacted the enzyme stability of the pineapple juice.

Keywords: antimicrobial, *Cymbopogon citratus*, essential oil, *Ananas comosus*, preservative, storage stability

1. Introduction

Pineapple (*Ananas comosus*) is a commercially significant fruit that serves as a major export commodity for Indonesia. Pineapple has vibrant tropical flavor and promote extensive health benefits (Farid Hossain, 2015). Because the growing condition of pineapple is directly above the ground, it is quite impossible to completely prevent contamination when the fruit is made into juice. Furthermore, inappropriate storage conditions might lead to microbial contamination during storage by both spoilage and pathogenic microorganisms (de Sousa Guedes *et al*., 2016).

Methods that are currently and commonly used to prevent microbial deterioration of fruit juices during storage include thermal processing (pasteurization) and the utilization of allowed food preservatives (Eissa, *et al*., 2008). However, thermal treatment causes a significant loss regarding the nutritional quality and the freshness of fruit juices (Elez-Martinéz *et al*., 2006). Furthermore, the use of synthetic preservatives has been perceived negatively by consumers.

Many researchers have reported that lemongrass (*Cymbopogon citratus)* and its essential oil was bactericidal and fungicidal against a broad range of microorganisms, both pathogenic and spoilage (Naik *et al*., 2010). Among eight essential oils that were evaluated, lemongrass essential oil showed the strongest fungicidal effect against the target microorganisms in fruit juices (Helal *et al*., 2006). Furthermore, it was found that the addition of 0.3% lemongrass essential oil to unpasteurized apple juice significantly decreased the microbial load during storage, but created a negative impact on the sensorial acceptance (Eissa, *et al*., 2008). As essential oil is highly concentrated and consists of mainly volatile compounds, the incorporation of essential oils into a food system has its limitations such as strong organoleptic properties regarding its flavor and aroma (Prakash *et al*., 2012).

2. Materials and Methods

Materials

The materials that were used for this research are pineapple fruit (Nanas Honi from SUNPRIDE, Indonesia), sugar, salt, commercial lemongrass essential oil from Nusaroma Indonesia, Tween 80, test organisms (Saccharomyces cerevisiae, Aspergillus niger, and Escherichia coli), Mueller Hinton agar (Oxoid, United Kingdom), D-glucose (Merck, Germany), methylene blue, sodium chloride (Merck, Germany), potato dextrose agar (Merck, Germany), nutrient broth (Merck, Germany), agar bacteriological no. 1 (Oxoid, United Kingdom), potato dextrose broth *(Merck, Germany), plate count agar (Merck, Germany), chloramphenicol, potassium iodide (Merck, Germany), iodine (Merck, Germany), soluble starch, potassium phosphate monobasic, sodium hydroxide (Merck, Germany), casein from bovine milk (Sigma-Aldrich, United States), trichloroacetic acid, Folin's Phenol Reagent, anhydrous sodium carbonate, sodium acetate, calcium chloride, L-Tyrosine (Merck, Germany), and distilled water.*

Methods

2.1. Preliminary Analysis of Lemongrass Essential Oil and Pineapple Juice

Antimicrobial activity screening of lemongrass essential oil was performed by agar well diffusion method against bacteria (E. coli), yeast (S. cerevisiae), and mould (A. niger). Concurrently, the initial conditions of pineapple juice such as pH, microbial count, vitamin C content, and enzyme activity were analyzed.

2.1.1. Agar Well Diffusion Method

Prior to the assay, microbial strains (E. coli, S. cerevisiae, A. niger) were sub-cultured into slant agar and incubated at 37°C for 24 hours. After 24 hours of incubation, the turbidity was adjusted using 0.85% saline solution such that they are identical to 0.5 McFarland standard turbidity. One mL of each microbial suspension was pipetted into petri dish and by pour plate method, 25-30 mL of agar (Mueller Hinton Agar for bacteria, MHA + 2% glucose + 0.5 µg/mL methylene blue for yeast, Potato Dextrose Agar for mould) was poured and mixed thoroughly in a circular motion and let dried. Wells of 5 mm were cut using glass pipettes and 50 µL of diluted lemongrass essential oil (2%, 4%, 6%, and 8% v/v in 0.05% Tween 80) were pipetted into each wells. Fifty µL of Tween 80 was pipetted into another well as blank (negative control). Fifty µL of positive controls were also used (gentamycin 1 mg/mL for bacteria, nystatin 0.5 mg/mL for yeast, nystatin 1 mg/mL for mould). Petri dishes were incubated at 37°C for 24 hours for bacteria and yeast, and 72 hours for mould. Zones of inhibition were measured.

2.1.2. pH Analysis

pH meter was calibrated using buffer solution of pH 4, 7, and 10 prior to measurement. pH of pineapple juice was measured.

2.1.3. Total Plate Count (TPC)

Stock samples were prepared by pipetting 1 mL of sample into 9 mL of 0.85% saline solution. Then, 1 mL of stock sample was pipetted into another 9 mL of saline solution, resulting in *1:10 dilution. The steps were repeated until 1:1000 dilution was obtained. By using pour plate method, 15-20 mL of Plate Count Agar was poured into petri dishes containing the sample, mixed thoroughly, and let dried. Petri dishes were incubated at 37°C for 24 hours. Resulted bacteria colonies were enumerated (30-300 colonies).*

2.1.4. Total Yeast and Mould Count (TYMC)

Stock samples were prepared by pipetting 1 mL of sample into 9 mL of 0.85% saline solution. Then, 1 mL of stock sample was pipetted into another 9 mL of saline solution, resulting in 1:10 dilution. The steps were repeated until 1:1000 dilution was obtained. By using pour plate method, 15-20 mL of Potato Dextrose Agar supplemented with 100 mg/L chloramphenicol was poured into petri dishes containing the sample, mixed thoroughly, and let dried. Petri dishes were incubated at 37°C for 72 hours. Resulted fungal colonies were enumerated (15-150 colonies).

2.1.5. Iodine Titration

Twenty mL of sample and 5 mL of 0.5% starch indicator solution was added. Then, the sample was titrated with 0.005 mol/L iodine solution while stirring until the end point was reached (blue-black color). Volume of titrant was recorded.

2.1.6. Protease Activity Assay (Sigma-Aldrich)

Into each tube, 5 mL of 0.65% w/v casein solution (diluted with 50 mM potassium phosphate buffer) was added. The tubes were put into a 37°C water bath for 5 minutes. Then, 1 mL of diluted enzyme sample (1 part sample : 3 parts enzyme diluent solution) was pipetted into the sample tube. One mL of enzyme diluent solution was pipetted into sample blank tube. The tubes were incubated at 37°C for exactly 10 minutes. After incubation, 5 mL of 110 mM TCA solution was added into each tubes to stop the reaction. The mixtures were further incubated at 37°C for 30 minutes. During 30 minutes of incubation, tyrosine standard solutions were set up by diluting 1.1 mM L-Tyrosine standard into the following volumes in mL: 0.05, 0.1, 0.2, 0.4, and 0.5. Then, varying volumes of distilled water was added until the volume of each tubes were 2 mL. Standard blank was set up by adding only 2 mL of distilled water into a tube. After 30 minutes of samples incubation, the mixtures were filtered using filter paper. Two mL of filtrate containing sample, sample blank, standard, standard blank were transferred into a new set of test tubes. Afterwards, 5 mL of 500 mM sodium carbonate was added into all tubes containing sample, sample blank, standard, and standard blank. Immediately after, 1 mL of 0.5 M Folin's Phenol Reagent was added into each tube. All mixtures were incubated at 37°C *for 30 minutes. Finally, 1 mL of each tube was pipetted into cuvettes and the absorbance values were read at 660 nm.*

2.2. Determination of Essential Oil Concentration

Determination of lemongrass essential oil concentration for product application was done by performing Minimum Inhibitory Concentration (MIC) determination and sensory evaluation.

2.2.1. Broth Macrodilution Method

Broth macrodilution was performed to find the MIC of lemongrass essential oil. Broth macrodilution was conducted according to CLSI M07-A9 for bacteria, M27-A2 for yeast, and M38-A for mould. Prior to the assay, microbial strains (E. coli, S. cerevisiae, A. niger) were sub-cultured into agar slant and incubated at 37°C for 24 hours. After 24 hours of incubation, the turbidity was adjusted using 0.85% saline solution such that they are identical to 0.5 McFarland standard turbidity. For standardizing the inoculum, further 1:150 dilution by nutrient broth was done for bacteria. For yeast, further 1:1000 dilution followed by 1:20 dilution by potato dextrose broth was done. For mould, further 1:100 dilution by potato dextrose broth was done. For bacteria, 2 mL of standardized suspension was pipetted into tubes with 2 mL of lemongrass essential oil of various concentrations (0.1%, 0.2%, 0.3% v/v in 0.05% Tween 80). For yeast and mould, 0.9 of standardized suspension was pipetted into tubes with 3.6 mL of lemongrass essential oil of various concentrations (0.1%, 0.2%, 0.3% v/v in 0.05% Tween 80). Both negative and positive controls were used. Initial absorbance values were recorded (600 nm for bacteria, 530 nm for yeast and mould), and change of absorbance values were recorded for the next 24 and 48 hours.

2.2.2. Hedonic Scale Sensory Analysis

Thirty panelists were given 4 samples (control sample, with 0.1%, 0.2%, 0.3% v/v lemongrass oil). Panelists were asked to determine the liking with the values ranging from 1 (dislike extremely) to 9 (like extremely). The acceptance of color, aroma, taste, viscosity, aftertaste, and overall acceptance were evaluated.

2.3. Shelf Life Analysis

Shelf life analysis was done in terms of microbial count, vitamin C content, pH, and enzyme activity during 5 days of storage at a chilling temperature (4°C). Two samples (control sample and sample with 0.1% lemongrass oil) were evaluated. Total microbial count was evaluated in terms of TPC and TYMC for every 24 hours during storage period. pH of both samples were recorded every 24 hours during

storage period. Iodine titration for vitamin C determination was also done every 24 hours during storage period. Enzyme activity analysis of both samples was conducted at the beginning and at the end of storage period. The container that was used throughout shelf life analysis were 20 mL autoclave-sterilized glass bottles with rubber stoppers.

3. Results and Discussions

3.1. Pineapple Juice Initial Analysis

Table 1. demonstrated the initial conditions of the pineapple juice sample. It was observed that both of the initial total plate count (TPC) and total yeast and mould count (TYMC) of pineapple juice was already numerous. The initial TPC was close to exceeding the TPC allowed by the national standard (SNI) requirements for fruit juice (1 x 10^4 CFU/mL). Furthermore, the initial TYMC of the pineapple juice had already exceeded the TYMC allowed by the SNI (1 x 10^2 CFU/mL).

High microbial contamination was presumably because of unsterile working procedures and environment. Furthermore, since the pineapple juice was not pasteurized, microbial contamination was very likely to occur.

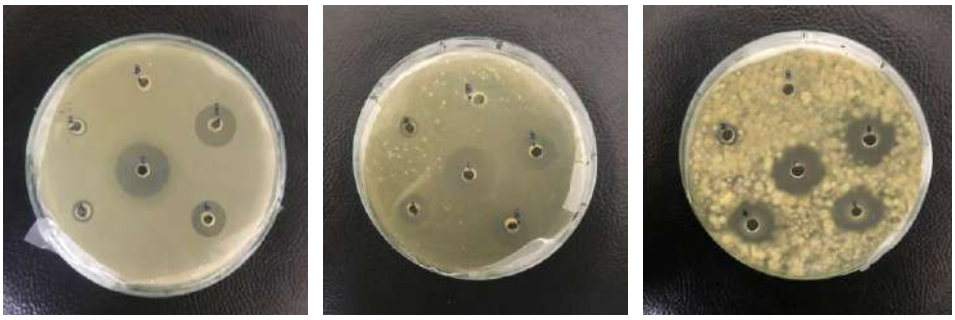
Table 1. Initial conditions of pineapple juice sample

Parameter	Unit
pH	3.73 ± 0.01
Vitamin C	35.93 mg/100 mL
Total plate count	9.65 x 10^3 CFU/mL
Total yeast and mould count	1.77 x 10^3 CFU/mL
Enzyme activity	1.21 ± 0.02 Units/mL

3.2. Well Diffusion Method Results for Lemongrass Essential Oil

Screening of antimicrobial activity of lemongrass essential oil was performed using well diffusion method at 2%, 4%, 6%, and 8% concentrations against *E. coli, S. cerevisiae,* and *A. niger*. Figure 1 (a), (b), (c) demonstrated the zone of inhibition against the target microorganisms. Figure 2. represented, in millimeters, the respective zones of inhibition.

Based on the well diffusion method results, lemongrass essential oil was proven to have antimicrobial activities against the tested microorganisms. Furthermore, as the concentration of lemongrass essential oil was increased, the zones of inhibition for the target microorganisms were increasing as well. This was related to the inhibition mechanism by the well diffusion method itself. With more concentrated load of lemongrass essential oil inside the well, the more it has the ability to diffuse outwards to the surroundings, making the zone of inhibition larger as the concentration was increased (Tendencia, 2004)



(a) (b) (c)

Figure 1. Well diffusion method results against (a) *E. coli,* (b) *S. cerevisiae*, and (c) *A. niger*

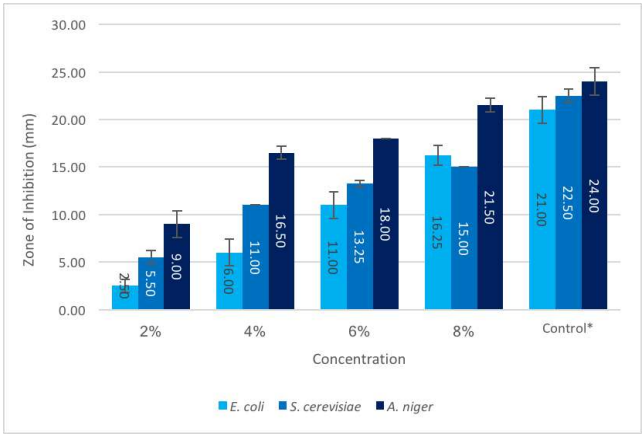


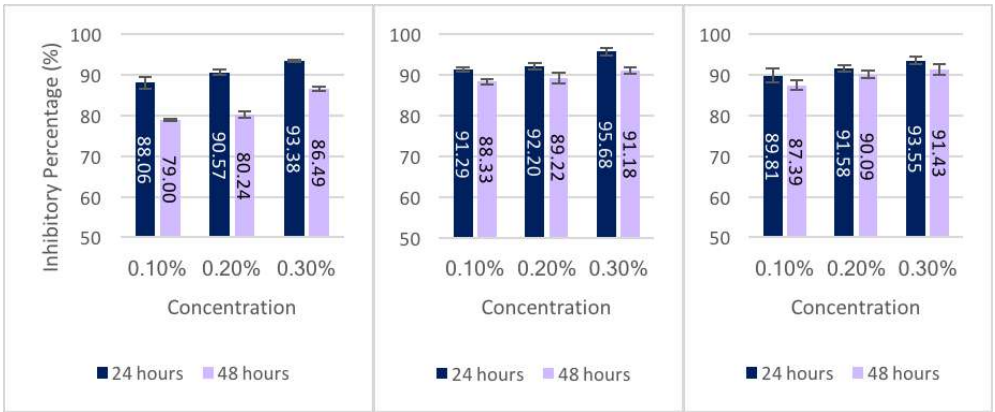
Figure 2. Zone of inhibition (mm) of lemongrass essential oil against *E. coli*, *S. cerevisiae*, and *A. niger*

For each of the microbial strain, positive controls were differed. Gentamycin (1 mg/mL) (Kaur Monga *et al*., 2017), nystatin (0.5 mg/mL) (Dar *et al*., 2016), and nystatin (1 mg/mL) (Akinpelu *et al*., 2015) was used as the positive controls for *E. coli*, *S. cerevisiae*, and *A. niger* inhibition zone respectively.

3.3. MIC Determination of Lemongrass Essential Oil

Minimum Inhibitory Concentration (MIC) determination following well diffusion method assay to precisely obtain the antimicrobial characteristics of the essential oils in regards to the application in food preservation (Mith *et al*., 2014). Minimum Inhibitory Concentration (MIC) of lemongrass essential oil was evaluated at 0.1%, 0.2%, and 0.3% concentrations.

Figure 3 (a), (b), and (c) represented the results of broth macrodilution method of lemongrass essential oil against *E. coli*, *S. cerevisiae* and *A. niger* as inhibitory percentage of microbial growth after 24 and 48 hours. Lemongrass essential oil demonstrated a rather strong antimicrobial activity against the tested microorganisms, and was able to inhibit at least 80% of all target microorganisms over 24 hours of incubation. For this reason, all of the microorganisms were considered as sensitive to lemongrass essential oil. Furthermore, inhibitory percentages for each concentration decreased after 48 hours of incubation. The occurrence is presumably due to the release of volatile compounds that was the main constituent that plays role in inhibiting the microorganisms.



(a) (b) (c)

Figure 3. Inhibitory percentage (%) of lemongrass essential oil against (a) *E. coli*, (b) *S. cerevisiae*, and (c) *A. niger* after 24 and 48 hours of incubation

The compounds responsible for the antimicrobial mechanism of lemongrass essential oil was a group of terpenoids. Geranial (α-citral) and neral (β-citral) were the derivatives of the terpenoids found in the lemongrass essential oil fraction (Arswendiyunma in Antara *et al*., 2013).

Table 2. represented the means of inhibitory percentage of lemongrass essential oil for each concentration after 24 and 48 hours of incubation time. At 0.1% concentration, lemongrass essential oil had already shown a strong inhibitory activity, and was able to inhibit 87.31% of the target microorganisms. Statistical analysis has shown that at 0.1%, 0.2%, and 0.3%, the means of inhibitory percentages were not significantly different. Therefore, 0.1% concentration was determined as the MIC for this study.

Table 2. Mean of inhibitory percentage of lemongrass essential oil after 24 and 48 hours of incubation

Concentration	Inhibitory Percentage (%)
0.1%	87.31 ± 4.31^{a}
0.2%	88.98 ± 4.41^{a}
0.3%	91.95 ± 3.14^{a}

*Different in superscripts denotes significant difference (P-value < 0.05)

3.4. Sensory Evaluation Results

Thirty untrained panelists were chosen for the hedonic scale sensory evaluation (Watts *et al*., 1989), and were presented with 4 pineapple juice samples. Table 3. demonstrated that the addition of lemongrass essential oil had affected all of the sensory attributes that were evaluated.

Table 3. Hedonic scale sensory evaluation results

	Control	0.1%	0.2%	0.3%
Color	6.30 ± 1.66^{b}	6.63 ± 1.47^{ab}	6.83 ± 1.18^{a}	6.50 ± 1.46^{ab}
Aroma	6.97 ± 1.47^{a}	5.97 ± 1.45^{b}	6.00 ± 1.62^{b}	5.63 ± 2.11^{b}
Taste	7.17 ± 1.39^{a}	6.10 ± 1.34^{b}	4.87 ± 1.30^{c}	4.03 ± 1.97^{d}
Viscosity	6.97 ± 1.00^{a}	6.77 ± 1.17^{a}	6.63 ± 1.30^{a}	6.07 ± 1.41^{b}
Aftertaste	6.57 ± 1.55^{a}	5.83 ± 1.58^{b}	5.27 ± 1.80^{c}	4.43 ± 1.87^{d}
Overall	7.13 ± 1.01^{a}	6.30 ± 1.18^{b}	5.83 ± 1.37^{c}	5.00 ± 1.31^{d}

*Different in superscripts denotes significant difference (P-value < 0.05)

*Hedonic Scale:

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

The most observable change for the attributes were the taste, aftertaste, and the overall acceptance, since it had a decreasing value as the lemongrass essential oil concentration was increased. Some panelists described the addition of lemongrass essential oil contributed to the pungent and bitter taste. This might account for the lower acceptance values of the samples with added lemongrass essential oil compared with the control. Furthermore, it was observed that the decrease of acceptance values was significant between each concentration.

Aroma of the pineapple juice was also significantly affected by the addition of lemongrass essential oil. The compound that might be responsible for the aroma is citral, as it is both a major

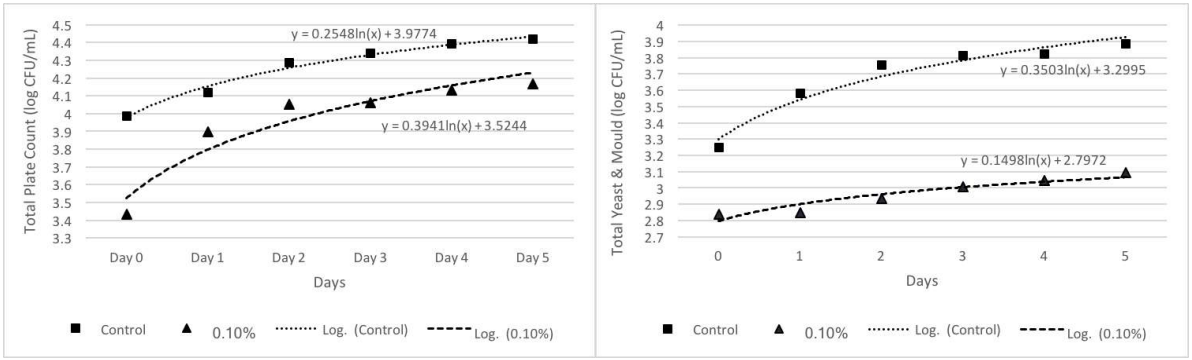
constituent of lemongrass essential oil and a volatile compound. Monoterpenes are volatile substances that are responsible for the aroma and flavor profile of essential oil (Ganjewala, 2009).

3.5. Shelf Life Analysis of Pineapple Juice

Shelf life analysis was done to acknowledge whether or not the addition of lemongrass essential oil significantly affected the microbial and chemical degradations. Pineapple juice with 0.1% (v/v) lemongrass essential oil concentration was chosen for the shelf life analysis because at that concentration, the sensorial acceptability was closest compared to the control sample, while having strong in-vitro antimicrobial activity assessed by broth macrodilution method. Two samples were evaluated during shelf life analysis; which were pineapple juice without the addition of lemongrass essential oil as control sample, and pineapple juice with the addition of 0.1% lemongrass essential oil.

3.5.1. Total Microbial Count

Total microbial count was evaluated in terms of Total Plate Count (TPC) and Total Yeast and Mould Count (TYMC). Figure 4. (a) and (b) presented the total microbial count for pineapple juice samples during storage. In TPC, control sample was observed to have a smaller slope (0.2548) compared to 0.1% sample (0.3491). The values imply that faster rate of bacterial growth was observed in the sample with 0.1% lemongrass essential oil compared to the control sample. However, in TYMC, the control sample had a larger slope (0.3503) compared to the 0.1% sample (0.1498). This indicated a higher rate of yeast and mould growth in the control sample when compared to the sample with the addition of 0.1% lemongrass essential oil.



(a) **(b)**

Figure 4. (a) Total plate count and (b) total yeast and mould count of pineapple juice with and without 0.1% lemongrass essential oil

The difference for both sets of data were significant (P-value < 0.05), implying that the addition of 0.1% lemongrass essential oil significantly suppressed the microbial load of pineapple juice, but was better at inhibiting yeast and mould than bacteria (if the slopes were to be compared).

3.5.2. pH Analysis

Figure 5. presented the increasing pH values of both pineapple juice samples over 5 days of observation. At day 0, it was observed that the addition of 0.1% lemongrass essential oil has increased the pH of pineapple juice (from 3.73 to 3.83). This was in accordance to the sensory evaluation results performed earlier in this thesis work, which indicated that bitter taste was sensed along with the addition of lemongrass essential oil.

pH of both samples was increasing during 5 days of storage time. Similar findings were observed (Dhaliwal and Hira, 2001; Cortés et al., 2008; and Del Caro et al., 2004), reported that the pH of fruit juices was increased significantly during storage at a refrigerated temperature. pH is one of significant quality parameters of fruit juices that indicates the bioactive compound stability. The increase in pH value was caused by growth of spoilage microorganisms in fruit juices (Sanchez-Moreno et al., 2006)

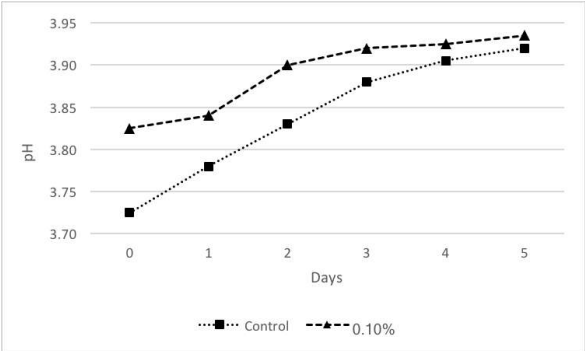


Figure 5. pH of pineapple juice with and without the addition of 0.1% lemongrass essential oil

The values of both sets of data were not significantly different (P-value < 0.05), which implies that the addition of 0.1% lemongrass essential oil was not responsible for the increase of pH *in pineapple juice.*

3.5.3. Vitamin C Degradation Analysis

Figure 6. presented the vitamin C content of both samples during 5 days of storage period, which showed that the vitamin C content of both samples were degraded during storage. When statistically analyzed, the addition of 0.1% lemongrass essential oil to the pineapple juice did not significantly affect the vitamin C degradation (P-value > 0.05).

However, the vitamin C content of the pineapple juice were deteriorated greatly over the storage period. The vitamin C content of pineapple juice control sample was degraded by 24.90% during storage and had a rate loss of 1.79 mg/100 mL per day. The vitamin C content of the sample with 0.1% lemongrass essential oil was degraded by 28.69% during storage had a rate loss of 2.11 mg/100 mL per day. In 2009, Uckiah et al. observed a rate loss of 0.6 mg/100 mL of vitamin C even at a low temperature storage.

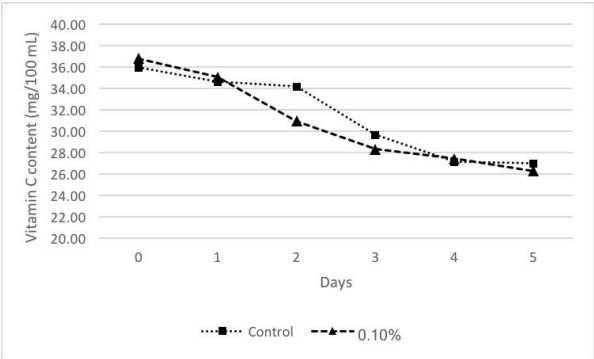


Figure 6. Vitamin C content (mg/100 mL) of pineapple juice with and without the addition of 0.1% lemongrass essential oil

In this study, oxidation was accounted for the great loss of vitamin C during storage period. Even though the samples were kept in a glass bottle with rubber stopper, the headspace of the bottle was presented with little oxygen (Uckiah et al., 2009), which eventually caused the oxidation of vitamin C during storage period.

3.5.4. Enzyme Activity Analysis

Figure 7. showed the enzyme activity, expressed as Units/mL of both samples at the beginning and at the end of the storage period. At day 0, addition of 0.1% lemongrass essential oil was not synergistic with the enzyme activity of pineapple juice. Addition of 0.1% lemongrass essential oil reduced 12.06% of enzyme activity of the pineapple juice at day 0, from 1.21 ± 0.02 Units/mL to 1.06 ± 0.08 Units/mL.

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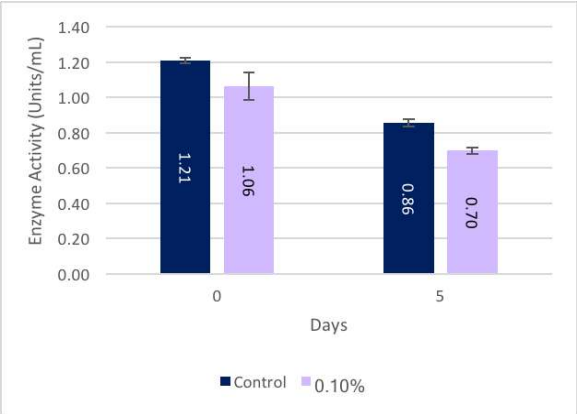

Figure 7. Enzyme activity (Units/mL) of pineapple juice samples at day 0 and day 5

According to the findings of Mohan et al. in 2016, the addition of synthetic preservative (sodium meta bisulfate) reduced the enzyme activity of pineapple extract from 4.28 ± 0.06 Units/mL to 1.68 ± 0.06 Units/mL (60.75% reduction) at day 0. The decrease in enzyme activity observed in this study is in accordance to the findings of Mohan et al., but the percentage reduction was much smaller.

The stability of enzyme was evaluated at the end of the storage period. The enzyme activity of the control sample was reduced by 29.24% (from 1.21 ± 0.02 Units/mL at day 0 to 0.86 ± 0.02 Units/mL at day 5). Furthermore, the enzyme activity of the 0.1% sample was reduced by 34.36% (from 1.06 ± 0.08 Units/mL at day 0 to 0.70 ± 0.02 Units/mL at day 5). The difference in the percentage reduction was 5.13%, indicating that the addition of 0.1% lemongrass essential oil has negatively impacted the enzyme stability of pineapple juice.

4. Conclusions and Recommendations

4.1. Conclusions

Based on the antimicrobial activity screening by well diffusion method, the best response for inhibitory activity of lemongrass essential oil against *E. coli*, *S. cerevisiae*, and *A. niger* was shown at the highest tested concentration (8%). The MIC of lemongrass essential oil in this study assessed by broth macrodilution was 0.1%. The addition of 0.1% (v/v) lemongrass essential oil into the pineapple juice product has negative impact on the sensory attributes of pineapple juice evaluated in this thesis work.

The effect of 0.1% addition of lemongrass essential oil into pineapple juice during 5 days of storage period was evaluated and the results have shown that lemongrass essential oil significantly suppressed the microbial count for both TPC and TYMC during storage period (P-value < 0.5), though the initial microbial load was already high. The addition of 0.1% lemongrass essential oil does not significantly affect vitamin C degradation and the pH changes during storage period (P-value > 0.5). It was found out that the addition of 0.1% lemongrass essential oil was not synergistic with the enzyme activity of pineapple juice, by reducing 12.06% of enzyme activity right after the addition, and had a negative impact on the enzyme stability of pineapple juice during storage.

4.2. Recommendations

Since lemongrass essential oil had a negative impact on the taste, aftertaste, and overall acceptance of pineapple juice, further studies are needed to improve the sensorial acceptance. Finally, since lemongrass essential oil has shown an outstanding performance as antibiotic and antifungal, lemongrass essential oil might be useful for the development of drugs.

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