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Sensory Characteristics and Antimicrobial Activities of Soft Candy with Essential Basil (*Ocimum Sanctum* L.) Against *Candida Albicans* and *Streptococcus Mutans*

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Abstract

Soft candy is a type of confectionery product that has a chewy texture. Chewing candy can stimulate the activity of salivary secretion, thus it supports the release of active compounds in the mouth. Essential oil contains of active compounds that are able to control bacteria growth that may cause bad breath. The purpose of this study was to observe the antimicrobial activity of soft candy with the addition of basil essential oil (0.2%, 0.4%, 0.6%, and 0.8%) against Streptococcus mutans and Candida albicans and to determine the most acceptable candy by consumers. Parameters of the research observations included antimicrobial activity with solid diffusion and dilution methods, descriptive sensory test with RATA test and acceptability test (aroma, taste, mouthfeel, aftertaste, and overall), and effectiveness test. The results showed that the addition of 0.4% basil essential oil in soft candy performed the highest effectiveness value (0.71). The effectiveness value was obtained from the results of the inhibition zone (disc) test, and the results of the acceptability test. Therefore, from the research, it can be concluded that soft candy with basil essential oil has a great potential to be utilized as antimicrobial agent to overcome mouth problems.

Introduction

Halitosis (bad breath) is common to be found in most people (Kapoor et al., 2016). The causes of the problem of halitosis are the presence of bacteria and food debris left in the mouth (Hawley, 2003; Mubaraki, 2019). These pathogenic bacteria can also cause dental caries and candidiasis (Haryo, 2013) and mouth ulcers (Kumamoto & Vinces, 2005). Several types of *Streptococcus* are known as the cause of caries, one of which is *Streptococcus mutans* (Naderi et al., 2011). Candidiasis is a fungal disease caused by infection of *Candida albicans* (Tjampakasari, 2006).

Nowadays, some efforts to control halitosis problems non-medically can be done by brushing teeth, using dental floss, using mouthwash, and or consuming special food products such as candy (Yaegaki & Coil, 2000). Soft candy is made from boiling sugar, which has a chewy texture, when stretched it is elastic and not sticky. Soft candy is often given additional ingredients such as flavoring or other additives (HP et al., 2013; Sutejo et al., 2015). Chewing candy can stimulate saliva production. As salivary secretion increases, the number of active compounds released in the mouth increases, so it has the potential to increase control or

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inhibition of pathogens that cause dental caries, such as *Streptococcus mutans* (Snow & Wackym, 2008) or halitosis infection.

Essential oils can add aroma, and flavor and act as antibacterial agents while being added to hard candy (Belgis et al., 2021). One of the sources of antimicrobial compounds from the essential oil that can be used is basil leaf extract. Basil essential oil contains a linalool compound of 48.4% (Neveen, Helmy Abou El-Soud Mohamed et al., 2017). It was reported that the addition of a 75% concentration of basil leaf extract in the hard candy dosage form can inhibit the growth of bacteria that cause bad breath (Nirmala, 2011).

The use of hard candy and mouthwash was felt to be less effective. Hard candy takes longer to hydrate since it has a high residence time in the mouth (Saint-Eve et al., 2011), which can be uncomfortable and can make sores worse. Meanwhile, the use of mouthwash sometimes should be limited to a smaller period time depending on the condition of users (Parashar, 2015). Improvement efforts that can be made to overcome the weakness of hard candy and mouthwash preparations are by providing herbal candy in the form of candy with a soft texture. Several studies regarding the making of soft candy in combination with active compounds have been reported (Lukas et al., 2013; Wicaksono et al., 2019). However, the use of basil essential oil in the manufacture of soft candy is not yet known. Therefore, this study was conducted to determine the appropriate concentration of basil essential oil addition in the manufacture of soft candy preparations that have antimicrobial activity against *Streptococcus mutans* and *Candida albicans* and acceptable to consumers.

Methods

Materials

Basil plants (*Ocimum sanctum* L.) (stems, leaves, and flowers) obtained from traditional farmers in Jember, sucrose (Gulaku), maize starch (Maizenaku), corn syrup (Sajo), water, vegetable fat, salt, distilled water, DMSO (Dimethyl Sulfoxide) 10%, NaCl, 75% alcohol, filter paper (Whatman 42 diameter 12,5), aluminium foil, cotton, bacterial cultures of *Streptococcus mutans*, *Candida albicans*, NA media (Nutrient Agar) and Sabouraud Dextrose Agar (SDA) media) obtained from the Laboratory of Food Microbiology and Agricultural Products, Faculty of Agricultural Technology, University of Jember.

Basil Essential Oil Distillation (Nurhadi, 2015)

Fresh basil plants were wilted by air drying. The basil plant was placed on a filter in a distillation kettle and 1:4 water was added. The heater was turned on and the hot steam was held until the water boils. The hot steam was passed to the water cooler so that condensation occurs and will turn into a liquid essential oil. Steam distillation was carried out for 5 hours. The next step was to separate the basil essential oil from the water, then the basil essential oil was stored in a vial, dry, and tightly closed.

The Making of Basil Essential Oil Soft Candy (Dewi, 2014)

Basil soft candy was made by mixing all the ingredients first. The mixture was cooked until it reached a temperature of 120ºC. The ingredients that have been completely mixed were then added with basil essential oil, the amount of basil essential oil added was 0.2%, 0.4%, 0.6%, and 0.8%, respectively. The candy dough was cooled until it was cold and slightly firm, then manual pulling was done. Pulling was done for 5-8 minutes until the dough becomes tough, light in color, not sticky, and can stretch. The dough that has been in clay was then cut into a size of 1x1 cm.

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Test Stages

This research initially underwent the identification of basil plants to determine the plant species used (Suharmiati & Handayani, 2016). The test microbes *S. mutans* and *C. albicans* were prepared by making a suspension in sterile distilled water. Next, there were diluted to 10^{-5} to be used in antimicrobial testing. The 10^{-5} dilution was taken as much as 1 ml and then poured into a petri dish. Furthermore, the liquid NA and SDA media were poured into a petri dish containing the inoculant (Özcan et al., 2005). Then the soft candy sample of basil essential oil was dissolved in 10% DMSO and soaked in paper discs, it was placed on the surface of the agar medium that had been inoculated with microbes and then incubated for 24 hours at 37°C. Antimicrobial activity was observed based on the diameter of the inhibitory area indicated by the clear area formed around the paper disc.

The next antimicrobial test was to determine the MIC (Minimum Inhibitory Concentration) and IC_{50}. First, 1 ml of microbial suspension was prepared. It had been diluted to 10^{-5} and a test solution with various concentrations of 1 ml of volatile oil candy was put into a petri dish and then 10 ml of liquid NA and SDA media were added to each petri dish, levelled, and allowed to solidify. Petri dishes containing inoculants were incubated for 24 hours at 37°C. Observations were made by counting the number of colonies on a petri dish using a colony counter. Furthermore, the percentage inhibition of the growth of *S. mutans* and *C. albicans* colonies were examined to determine the probit value that will be adjusted to the probit table for each known inhibition percentage.

The next tests were descriptive tests using Rate-All-That-Apply (AVERAGE) and acceptance tests (hedonic). The RATA test was chosen to describe the sensory character, carried out in 3 stages, namely the focus group discussion (FGD) stage led by a panel leader who directed the discussion and provides references regarding the attributes of the basil candy according to the literature (Dinar, 2017). The attributes discussed in this stage refer to the literature and the opinion of the panellists (Burdock, 2002; Surburg & Panten, 2006). This stage involved 8 panellists consisting of 4 women and 4 men taken from students of Agricultural Product Technology Department, University of Jember. Sample testing was carried out by panellists tasting the sample presented and giving an assessment of the sample without comparing it with other samples. The assessment was carried out by placing a checkmark on the RATA question table on a five-point scale (1=very weak to 5=very strong) for each attribute that was considered to be able to describe the test sample, and then data collection. The acceptance test was done by the panellists tasting the sample presented and giving an assessment of the sample without comparing it with other samples. The assessment was carried out on a seven-point scale of liking (1 = strongly disliked, 2 = disliked, 3 = somewhat disliked, 4 = neutral, 5 = somewhat liked, 6 = liked, and 7 = very much liked) by placing a checkmark on the questionnaire (Simanungkalit et al., 2018).

The effectiveness test assigns a weighted value to each variable with a range of 0-1. The weighting of the values depends on how important the parameters which the results obtained in the aftermath of the treatment. Normal weight was generated by dividing the weight of the variable by the total weight, after knowing the effectiveness value, it was necessary to calculate the results by multiplying the effectiveness value by the normal weight of several parameters (De Garmo, 1984).

$$\text{Normal weight} = \frac{\text{Parameter Weight Value}}{\text{Total Weight}}$$

$$\text{Value of effectivity} = \frac{\text{Value of Treatment} - \text{The Worst Value}}{\text{The Best Value} - \text{The Worst Value}} \text{ x Normal Weight}$$

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Results and Discussion

Antimicrobial Activity of Basil Essential Oil Soft Candy Against the Growth of *Streptococcus mutans*

The activity of the inhibition zone on the growth of *Streptococcus mutans* increased the diameter of the clear zone on each paper disc that was planted on the surface of the media. The results of the inhibition of the basil essential oil soft candy on the microbial growth of the disc method can be seen in Figure 1 and Figure 2.

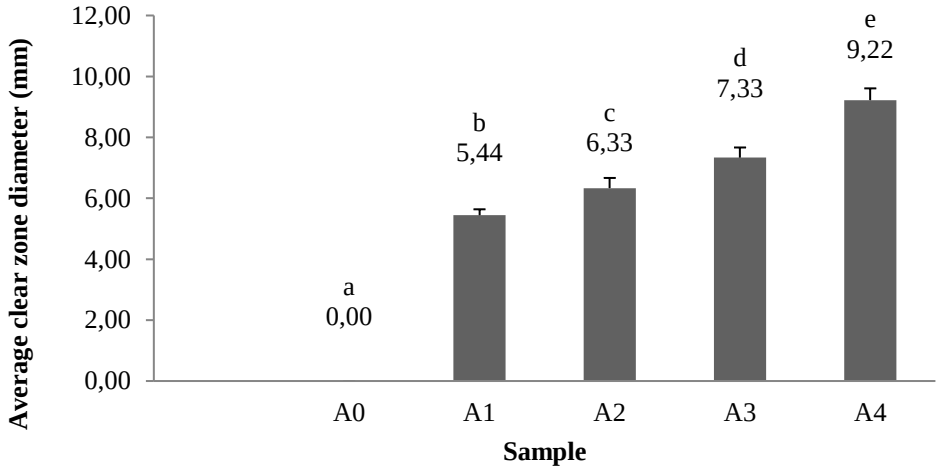

Figure 1. Inhibition zone of basil essential oil soft gum on the growth of Streptococcus mutans at concentrations (A1 = 0.2%; A2 = 0.4%; A3 = 0.6%; A4 = 0.8%)

In Figure 1 it can be seen that the higher the concentration of addition of basil essential oil, the greater the inhibition zone. ANOVA (Analysis of Variance) test with a significant value of 0.05 showed that the difference in the addition of basil essential oil concentration had a significant effect on the diameter of the growth inhibition zone of *Streptococcus mutans*. Figure 1 showed that soft candy A4 (addition of 0.8% basil essential oil equivalent to 1.06 mg/ml) resulted in the largest inhibition zone with a diameter of 9.22 mm (classified as a medium inhibition zone) (Morales et al., 2003).

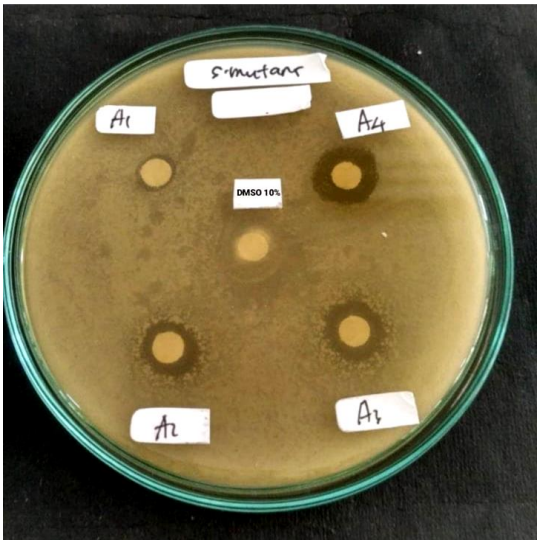

Figure 2. Inhibition zone of basil essential oil soft candy against Streptococcus mutans at concentrations (A1 = 0.2%; A2 = 0.4%; A3 = 0.6%; A4 = 0.8%)

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On average, candy samples A1 to A4 had moderate inhibition against *Streptococcus mutans*. Figure 2 shows the increase in the diameter of the inhibition zone along with the addition of basil essential oil to the candy sample. The greater the amount of basil essential oil added, the greater the amount of active substance contained in the tested material so that the inhibitory power was greater/stronger.

Inhibition Zone Activity of Basil Essential Oil Soft Gum on The Growth of *Candida albicans*

Inhibitory activity on *Candida albicans* growth increased along with the increase in the concentration of basil essential oil added in the manufacture of soft candy. The results of the soft candy inhibition test with variations in the concentration of basil essential oil on the growth of *Candida albicans* with the disc method can be seen in Figure 3 and Figure 4.

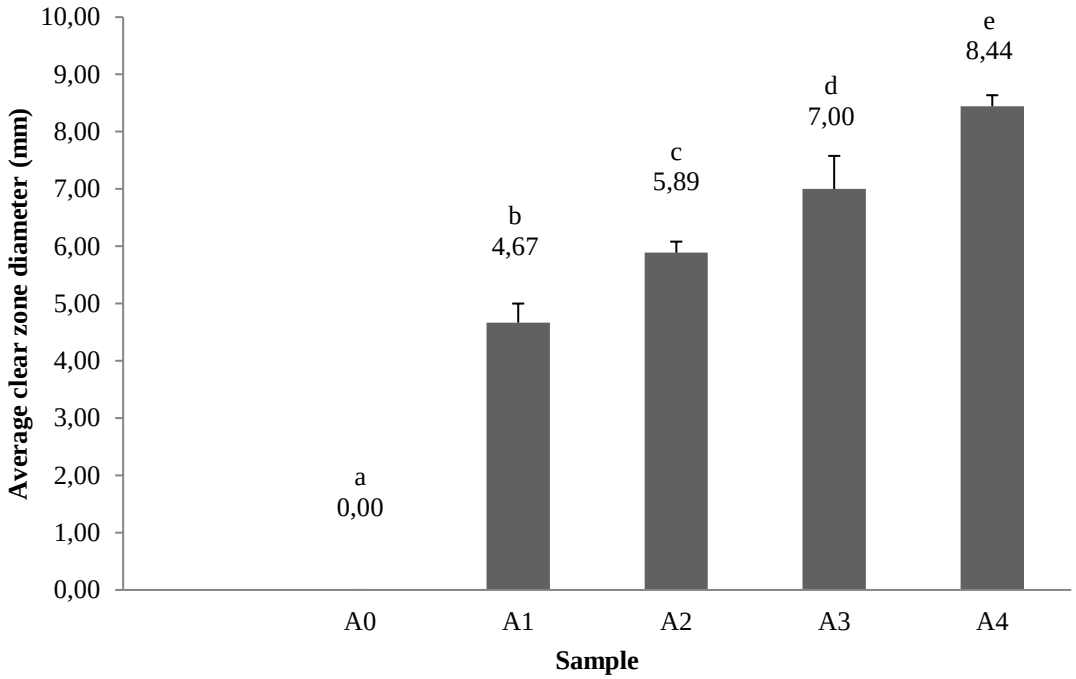

Figure 3. Inhibition zone of basil essential oil soft gum on Candida albicans growth at concentrations (A1 = 0.2%; A2 = 0.4%; A3 = 0.6%; A4 = 0.8%)

Soft candy formulation A4 (concentration of basil essential oil 0.8% (1.06 mg/ml)) resulted in the largest inhibition zone value of 8.44 mm (classified as moderate inhibition zone). On average, candy samples A0 and A1 had weak inhibition, and candy samples A2, A3 and A4 had moderate inhibition against *Candida albicans* growth. It was suspected that the concentration of addition of basil essential oil in soft candy was not sufficient to inhibit the growth of *Candida albicans*. The results of ANOVA (Analysis of Variance) with a significant value of 0.05 showed that the difference in the addition of basil essential oil concentration had a significant effect on the diameter of the growth inhibition zone of *Candida albicans*.

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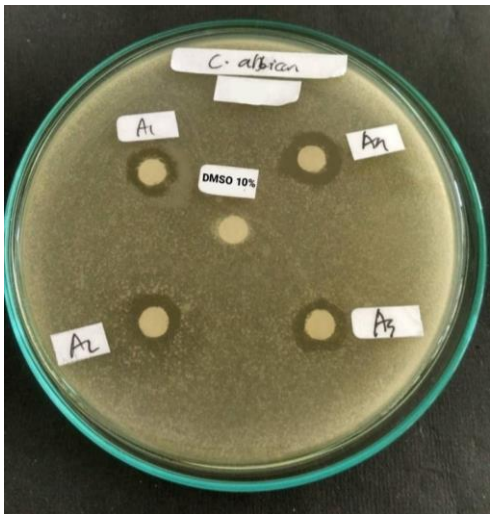

Figure 4. Inhibition zone of basil essential oil soft candy against Candida albicans at concentrations (A1 = 0.2%; A2 = 0.4%; A3 = 0.6%; A4 = 0.8%)

Figure 4 shows an increase in the diameter of the inhibition zone along with the addition of basil essential oil in the soft candy sample. The greater the amount of basil essential oil added, the greater the power, and the greater the inhibition. This condition was following the opinion of Harmely, Deviarny and Yenni (2014), the growth of pathogens commonly found in the oral cavity, including *Candida albicans*, can be inhibited by bioactive compounds of basil essential oil.

Antimicrobial Activity of Basil Essential Oil Soft Candy Against the Growth of *Streptococcus mutans*

Based on the results of the probit curve of soft candy with various concentrations of basil essential oil, the equation y = 1.5266x + 5.565 was obtained. From the calculation according to the equation, the IC_{50} and MIC values were 0.4 (mg/ml) and 2.94 (mg/ml), respectively. At the concentration of 0.4 (mg/ml) soft candy was able to inhibit 50% growth of *Streptococcus mutans*. At the concentration of 2.94 (mg/ml) basil essential oil soft candy was able to provide 90% inhibition of the growth of *Streptococcus mutans*.

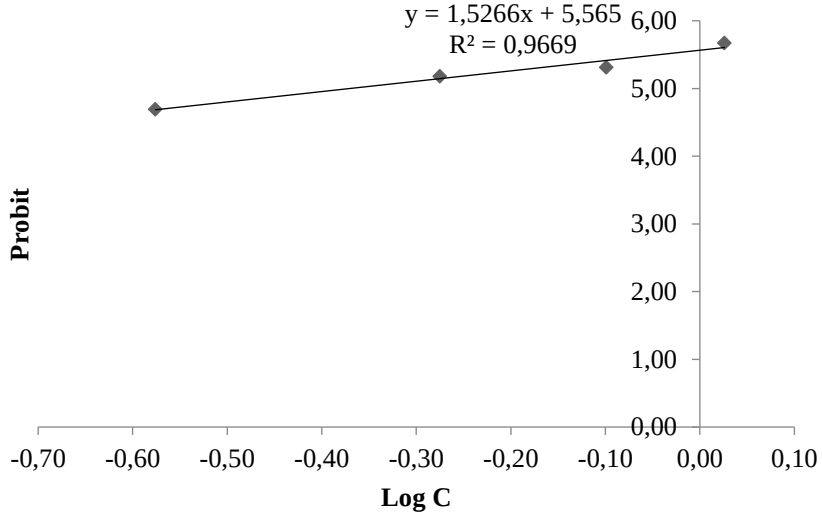

Figure 4. The curve of the inhibition relationship of the basil essential oil soft candy on the growth of Streptococcus mutans using the probit curve

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The results of the probit curve calculation showed that the relationship between the addition of basil essential oil concentration shows a linear line, where the greater the amount of basil essential oil added to the soft candy, the higher the inhibitory linear line was produced.

Antimicrobial Activity of Basil Essential Oil Soft Candy Against the Growth of *Candida albicans*

The results of the calculation of the probit curve equation of the basil essential oil soft candy against *Candida albicans* can be seen in Figure 6, then the equation y= 1.629x + 5.376 was obtained. According to the calculation using this equation, the IC_{50} and MIC values for *Candida albicans* were 0.53 mg/ml and 3.58 mg/ml, respectively. At both concentrations (0.53 mg/ml and 3.58 mg/ml) the basil essential oil soft candy was able to inhibit 50% and 90% of *Candida albicans* growth, respectively.

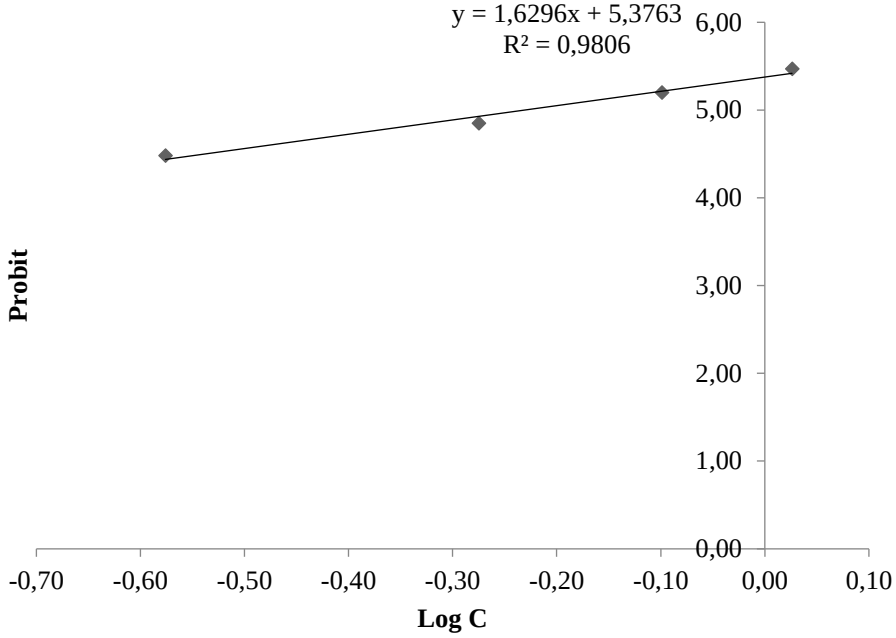

Figure 6. Inhibition curve of basil essential oil soft candy on Candida albicans growth using probit curve

The results of the probit curve calculation showed that the relationship between the addition of basil essential oil and the inhibition of *Candida albicans* resulted in a higher linear line. Calculation of IC_{50} and MIC values of basil essential oil soft candy on the growth of *Streptococcus mutans* and *Candida albicans* based on the probit curve.

PCA (Principal Component Analysis) Sensory Characteristics of Basil Essential Oil Soft Candy

The use of PCA to display the relative location of the sample by taking into account the characteristics of each attribute in the sample. The PCA was used to find the relationship between variables by generating a correlation matrix so that new groups of variables will be known through the highest percentage of variance in the data set (Setyaningsih et al., 2010)

Rate All That Apply (RATA)

The PCA method was used to determine the relationship between the different treatments of adding basil essential oil to the intensity of its sensory attributes. Based on Figure 7, the resulting 99% of total data can be explained by both PCs, namely PC1 at 22% and PC2 at 77%.

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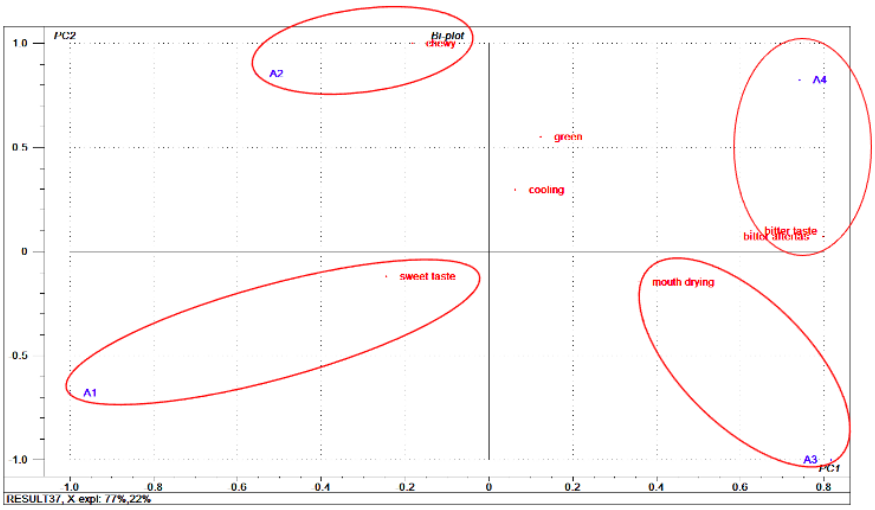

Figure 7. PCA biplot graph of the intensity level of sensory attributes on soft candy basil essential oil treatment A1 (essential oil 0.2%), A2 (essential oil 0.4%), A3 (essential oil 0.6%), and A4 (essential oil 0.8%)

The 0.2% basil essential oil soft candy (A1) was PC2 negative, this can be characterized by the high intensity of the sensory attribute of sweetness. Soft candy A2 was on PC2 positive with high intensity of the sensory attribute chewy texture. Soft candy A3 was on PC1 positive and PC2 negative with high sensory attribute intensity of mouth-drying. Soft candy A4 was on PC1 and PC2 positive with high intensity of sensory attributes and bitter aftertaste. The more essential oils were added, the soft candy will have a high intensity of sensory attribute of bitter aftertaste (bitter taste left in the mouth after being swallowed), green aroma (fresh aroma like leaves), and mouthfeel cooling (cool sensation in the mouth).

Acceptance Rate

The PCA method was used to determine the relationship between the different treatments of adding basil essential oil to the level of acceptance of the panelists. Based on Figure 8, the resulting total data of 98% can be explained by both PCs, namely PC1 at 13% and PC2 at 85%.

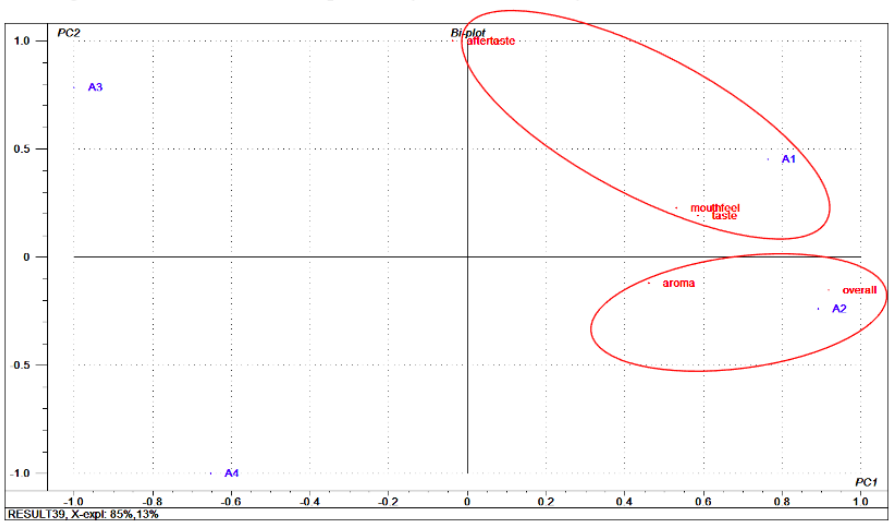

Figure 8. PCA biplot graph of panelists' acceptance rate of soft candy with basil essential oil treatment A1 (essential oil 0.2%), A2 (essential oil 0.4%), A3 (essential oil 0.6%), and A4 (essential oil 0.8%)

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The basil essential oil soft candy 0.2% (A1) was located on PC1 and PC2 positive, this could be characterized by the high level of acceptance of the mouthfeel and aftertaste sensory attributes. Soft candy A2 was PC1 positive and PC2 negative, which can be characterized by a high level of acceptance of aroma and overall sensory attributes. For soft candy A3 and A4 were not present in the graph because the value of the acceptance rate of soft candy A1 and A2 were greater.

The Effectiveness Value of Basil Essential Soft Candy

The result of the highest effectiveness value showed the best sample of this research. The best treatment of soft candy with various concentrations of addition of basil essential oil, was selected based on the results of the inhibition zone (disc) test, and the results of the acceptability test (aroma, taste, mouthfeel, aftertaste, and overall). The higher the level of importance, the higher the value of the weight of the given variable. The results of the effectiveness of the basil essential oil soft candy with various concentrations of basil essential oil can be seen in Table 1.

Based on Table 1, it was known that the best treatment for soft candy with basil essential oil was obtained in treatment A2, namely the concentration of addition of basil essential oil of 0.4% (0.53 mg/ml) with an effectiveness value of 0.71. The panelists' acceptance rate of aroma was 48.2%, taste was 48.2%, mouthfeel was 38.8%, aftertaste was 36.5%, and overall was 64.7%. The activity of the inhibitory zone on the growth of *Streptococcus mutans* and *Candida albicans* had a clear zone diameter of 6.33 mm and 5.89 mm, respectively and was classified as moderate inhibition. This inhibitory zone is comparable with gummy candies produced using bovine colostrum (BC) and essential oils (Eos) and probiotics (LAB) as reported by Bartkiene et al. (2017). The candies contained C. *reticulate* L. X LUHS244 and C. *paradise* L. X LUHS244 showed inhibition of Pseudomonas aeruginosa strain. These inhibition zones 9.0 ± 0.5 and 7.0 ± 0.5 mm, respectively. The BC, EOs and LAB combinations increased the antimicrobial activity and inhibited a broader spectrum of the analysed pathogenic bacteria, compared to the separate ingredients. Therefore, the antimicrobial activity of the soft candy which only contain basil essential oil is slightly lower then that of the gummy candy.

Table 1. The value of the effectiveness of the best treatment soft candy basil essential oil

Samples	Value of Effectivity
A1	0,66
A2	0,71
A3	0,39
A4	0,33

Source: Primary Data (2022).

Conclusion

The highest effectiveness value of the basil essential oil soft candy was 0.71, which was obtained from the soft candy with the addition of 0.4% essential oil. It has antimicrobial activity against Streptococcus mutans and Candida albicans which was classified as moderate inhibition and was favored and well received by panellists in terms of aroma 48.2%, taste 48.2%, mouthfeel 38.8%, aftertaste 36.5%, and overall by 64.7%. Based on these results thus it can be concluded that soft candy with basil essential oil has a great potential to be utilized as antimicrobial agent to overcome mouth problems. In the future, the shelf life of this product can also be analysed to obtain information on the length of time for which this product remains usable and fit for consumption.

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