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**SENSORIAL AND MICROBIAL ASSESSMENTS OF CANDY
DEVELOPED FROM HOLY BASIL LEAF**

BY

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Submitted to the Department of Nutrition and Food Engineering in the partial fulfillment
of B.Sc. in Nutrition and Food Engineering

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APPROVAL

This Project titled “**sensorial, and microbial assessments of candy developed from holy basil leaf**”, submitted by **Prashad Kanti Roy** to the Department of Nutrition and Food Engineering, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of B.Sc. in Nutrition and Food Engineering and approved as to its style and contents. The presentation has been held on **September, 2023**.

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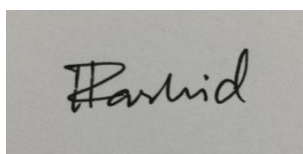
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DECLARATION

I hereby declare that, this project has been done by me under the supervision of **Md. Harun-Ar Rashid, Assistant Professor, Department of NFE**, Daffodil International University. I am also declaring that neither this project nor any part of this project has been submitted elsewhere for award of any degree or diploma.

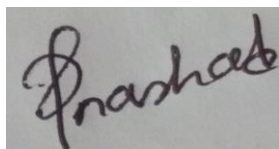
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ABSTRACT

Holy basil leaf, is a highly valued medicinal plant in traditional medicine systems. It contains a variety of bioactive chemicals, which contribute to its anti-inflammatory, antioxidant, and antibacterial activities. Holy basil leaf, it is a nutrient-dense herb and that is rich in a variety of vitamins, minerals, and other beneficial compounds. Corn syrup and sucrose are the basic ingredients used to make hard candies. Other ingredients are also used to improve taste and attractiveness, like color flavor etc. Therefore, the purpose of this study was to use holy basil leaves to develop a type of candy that would be an appealing and enjoyable way for consumers to consume the healthful components that these leaves contain. Holy basil leaf candy has made in the NFE lab and its Sensorial qualities, and microbial were analyzed. Holy basil leaf candy contained 2.63% and 0.95% Moisture and Ash. Brix and pH contained 69% and 4.16 respectively. It's a quick, easy and inexpensive recipe. It also had an enjoyable sensorial quality. Only 1% of participants disliked slightly the holy basil leaf Candy, while 48% liked it very much.

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CHAPTER 1

1. Introduction

1.1 Introduction of holy basil leaf candy

Recently, there has been a rise in interest in the creation of novel and practical herbal medicine formulations. One such formulation is candy made from holy basil leaf extract, which is a tasty and simple way to consume holy basil leaf. The holy basil leaf extract combined with sugars and others ingredients to create the candy. Due to its possible beneficial effects and ease of use, the holy basil leaf extract candy has grown its popularity. The candy can be eaten anywhere, making it a great option for people in their busy schedules. Additionally, the candy making from holy basil leaf is a fantastic substitute for raw holy basil leaf intake, because many people can't take disagreeable odor and bitter flavor in holy basil leaf (Patwardhan 2005).

The holy basil leaf (*Ocimum sanctum* L.) is a well-known Ayurvedic herb. It includes bioactive compounds including as eugenol, rosmarinic acid, and apigenin, which contribute to its anti-inflammatory, antioxidant, immunomodulatory, and antibacterial properties. This leaf is used to treat the signs and symptoms cough and cold. The leaf can be consumed in various kinds of forms, including herbal tea, dried powder, candy produced from the extract of the leaves, fresh leaf extract mixed with honey, and so on. The leaves of this herb have traditionally been used to treat a wide range of diseases, including respiratory problems, digestive issues, and even as a natural stress and anxiety reliever (Cohen, 2014).

Holy basil leaf is high in vitamins and minerals like calcium, magnesium, potassium, and iron. Holy basil is one of the most important medicinal plants. This secondary metabolite and essential oil constituents of the leaf have a wide range of medicinal applications, including the treatment of chronic fever, diarrhea, ulcers, dysentery, bronchitis, and other skin and respiratory disorders. Holy basil leaf also contains diaphoretic, anti-cancer, anti-diabetic, anti-microbial, and anti-fungal properties (Mandal, 2022).

Due to its medicinal and spiritual properties, holy basil leaf is considered the most essential herb in Ayurveda. It is also known as "the Mighty Queen of Herbs" or "the Elixir of Life". Different parts of holy basil, specifically leaves, stems, flowers, roots, seeds, and even whole plants, are used to treat a variety of ailments, including bronchitis, bronchial asthma, malaria, diarrhea, skin diseases, arthritis, eye diseases, chronic fever, anxiety, cough, indigestion, hiccups, and insect bites, in traditional medical practices such as Ayurveda (Singh, 2002).

Regular consumption of (holy basil leaf) has been shown to enhance overall health, well-being, and lifespan, aid in illness prevention, improve the body and mind in dealing with a variety of chemical, physical, infectious, and emotional pressures, and restore physiological and psychological function. Also, these leaves can improve beauty, intelligence, stamina, and emotional stability, while also adding shine to the face and honey to the voice (Prakash, 2005). These beneficial effects have been proven for a range of bioactive compounds present in holy basil leaf. Antioxidants are essential in protecting the body against oxidative

stress, which can lead to the beginning of a variety of diseases. Anti-inflammatory substance may decrease the body's inflammation, which is related to a variety of chronic health issues (Kumar, 2010).

1.2 Medicinal importance of holy basil leaf

- **Anti- Inflammatory properties:** According to the presence of compounds like eugenol, rosmarinic acid, and ursolic acid, holy basil leaf has been proven to have powerful anti-inflammatory properties. By preventing the production of inflammatory mediators including prostaglandins and cytokines, these substances reduce inflammation (Sampath, 2015).
- **Antioxidant Activity:** This leaf is also known to have significant antioxidant activity due to the presence of flavonoids, phenols, and terpenoids. These compounds destroy free radicals and protect cells from oxidative damage, reducing the risk of chronic diseases like cancer, diabetes, and heart disease (Jahanger, 2022).
- **Immunomodulator Effect:** Holy basil leaf has been proved to have immunomodulatory qualities, which means it may impact the immune system's response to infections and other foreign chemicals. This property is due to the presence of chemicals such as osmium sides, eugenol, and ursolic acid, which aid the growth of immune cells and antibodies (Jagetia, 2004).
- **Anti-microbial Properties:** This leaf is known for its antibacterial properties due to the presence of essential oils such as eugenol, eucalyptol, and camphor. These oils have antibacterial, antiviral, and antifungal properties and have long been used to treat diseases (Thosar, 2013).
- **Anti-Cancer Activity:** The presence of compounds such as ursolic acid, rosmarinic acid, and apigenin in holy basil leaf has been evidence of anti-cancer activity. These substances work to prevent cancer cell growth and spread by causing apoptosis (programmed cell death) and decrease inflammation (Tong, 2018).
- This leaf is a versatile herb with a variety of medicinal properties, making it an essential component in a wide range of health supplements and herbal medicine.

Glucose syrup and sucrose are the main ingredients used to make hard candies, while a broad variety of additional ingredients may be added to generate significantly various qualities (flavor, texture, look, etc.). The glucose syrup content may range on a dry basis from a low of 10-15% to a high of over 60%. The type of glucose syrup used, how much of it is used, how much inversion occurs during cooking, and of course the candy's final water content, all have a big impact on how it looks and feels. The doctoring action of glucose syrup allows the production of hard candies in large-scale continuous procedures where sucrose would otherwise be prone to crystallize. To add to the value of a product or to make it more appealing to consumers, additional ingredients may include organic acids, colors, flavors, fruit juices, fats, and cream. Although hard candies exist in a variety of shapes and sizes, they are typically all made in the same way. In order to make hard candies, sugar syrup is heated to a temperature known as "hard crack" (146-154 °C; 295-310 °F) (Hartel, 2018).

1.2 Objective of the study

The present work has been undertaken with the following objectives:

- ✓ To develop candy with holy basil leaf extract.
- ✓ To transfer the healthiness of holy basil leaf into candy
- ✓ To evaluate the physical properties of holy basil leaf extract candy
- ✓ To assess the sensory appeal of the holy basil leaf candy

CHAPTER 2

2.1 Literature Review

Hard candy is sometimes known as rock candy. It is totally made of sugar. It is made only water and sugar. The hard candy manufacturing technique is based on the crystallization principle. The process of crystallization is characterized by Le Chatelet's principle as "a system that is shifted away from equilibrium acts to restore the equilibrium by reacting in opposition to the shift." Maillard browning produces hard candies. Maillard browning is a non-enzymatic browning process in which the carbonyl group of the free sugar reacts with the amino group of the amino acid to create browning. Hard candies stand out over all other sorts due to their lowest final moisture content. The hard candy is available in a range of flavors, sizes, shapes and color (Namdev, 2022).

Hard candy is a popular type of candy all around the world. It is produced by dissolving sugar, corn syrup and flavorings in water and then heating the liquid to a high temperature till it becomes hard and brittle. The candy can be formed into a number of shapes, sizes, and colors, making it a versatile delicacy that can be enjoyed by people of all ages (Wang, 2017). Recently, there has been a rise in interest in using natural ingredients, such as plant extracts and essential oils, to create hard candies that are not only delicious but may also have health benefits (Jamshidi, 2017).

Holy basil is renowned as the "Queen of Herbs" for good cause. It contains a number of bioactive compounds. The leaf of this herb has traditionally been used to treat a range of ailments, including respiratory disorders, digestive issues, and even as a natural stress and anxiety reducer. Evidence from science indicates holy basil may protect organs and tissues from industrial toxins and heavy metals. Considering the various toxins, we are exposed to in modern life, holy basil may be an ancient respond to certain modern concerns (Cohen, Tulsi-*Ocimum sanctum*: A herb for all reasons., 2014).

Several studies on the production of holy basil leaf candy and its possible health benefits have been done. Researchers discovered that the candy holds antioxidant and anti-inflammatory properties in one study (Upadhyay, 2017).

Holy basil leaf has incredible medicinal benefits. This leaf was found to helped diabetics decrease their blood glucose levels. The same study found that holy basil significantly reduced overall cholesterol levels. Another study revealed that its antioxidant properties improved blood sugar levels. Holy basil can effectively treat severe acute airborne sickness. Coughs, colds, flu, and bronchitis are all relieved by her leaf juice. Holy basil leaf oil can also be used as an ear drop. This helps in the treatment of malaria. It is especially effective for cholera, hysteria, indigestion, fever, and other health problems (Kumar K. P., 2010).

Holy basil fresh leaf is consumed daily by millions of people. The legendary healing powers of this leaf often known as the "Medicinal Queen," have been well-known for many years. A high level of antioxidants and other nutrients, as well as the reduction of inflammation, fevers, and stress, have all been scientifically proven to be beneficial. Holy basil leaf protects the heart, blood vessels, liver, and lungs in particular and regulates blood pressure and blood sugar. Due to its high quantity of eugenol (1-hydroxy-2-methoxy-4-

allylbenzene), recent research suggests that holy basil leaf, like many contemporary medications, may be a COX-2 inhibitor. Holy basil also claims to protect against cataracts and toxicity. Holy basil leaf anti-flu properties have recently been discovered by medical researchers around the world. Holy basil leaf improves the effectiveness of the world defense system, notably its ability to control infectious disease (Goswami, 2020).

Holy basil is an annual herbaceous plant in the Liliaceae family. Its pale green, slightly hairy leaves are commonly used as a flavoring component in Southeast Asian cuisine, particularly in Thai stir-fries. Many Indians eat immature holy basil leaves as a meal addition or as an offering after visiting temples to say prayers to the gods. A spicy, lemony flavor can be found in holy basil leaf. (Archana, 2002). Through traditional use, several qualities of holy basil have been attributed to it. These include cancer-fighting, antiseptic, and antiallergic properties, as well as revitalizing, tonic, and vitalizing properties that promote longevity and health (Reen, 1996).

Holy basil was traditionally used to improve health in a variety of ways. Anti-arthritis, anti-coagulant, anti-diabetic, anti-inflammatory, antimicrobial (including antibacterial, antifungal, and antiviral), antioxidant, cardioprotective agent, and neuroprotective (Cohen, Tulsi-*Ocimum sanctum*: A herb for all reasons., 2014).

Holy basil is a plant rich in phytochemicals, minerals, and vitamins, earning it the title of "Mother Medicine of Nature." Holy basil is a modern-day amazing plant. Although holy basil is an adaptogen, it may help to balance cortisol (the stress hormone) levels in the body, improve brain function, boost the immune system, decrease inflammation, and increase energy levels. (Mohan, 2011). Holy basil leaf candy is made from holy basil leaf. Because of its many medicinal properties, holy basil has been used in traditional Ayurvedic medicine for millennia. Eugenol, rosmarinic acid, and ursolic acid are three active compounds found in the leaves of the holy basil plant that have been found to have anti-inflammatory, antioxidant, and antibacterial properties (Tarannum, 2019).

The addition of holy basil leaf extract to candy can improve its flavor while also providing health benefits. Candy is a popular confectionery deliciously consumed all around the world. Because of a growing demand for functional foods—foods that give health benefits beyond their basic nutritional value—plant extracts are now being employed more frequently in the production of candies (Kumar K. P., Traditional Indian herbal plants tulsi and its medicinal importance., 2010).

The holy basil plant is not native to Bangladesh, but it is said to have come from trade and cultural exchange with neighboring India. The holy basil plant is grown in Bangladesh and used in traditional medicine, cuisine, and religious activities. It is particularly popular among the Hindu population, who see it as a sacred plant and use it in their devotion and ceremonies. Holy basil is often grown in household gardens and small-scale farms throughout Bangladesh, particularly in the districts of Rajshahi, Rangpur, and Chittagong. It is also grown commercially in certain regions, mostly for its medical benefits (Rahman, 2022).

CHAPTER-3

3. Materials and Methods

3.1 Materials

- Holy basil leaf
- Water
- Sugar
- Salt
- Corn syrup

Table 1: Ingredients and Amount

Ingredients	Amount
Holy basil leaf	20 gm
Water	120 ml
Sugar	200 gm
Salt	1tsp
Corn syrup	60 ml

3.2 Methods

Flow chart of holy basil leaf candy

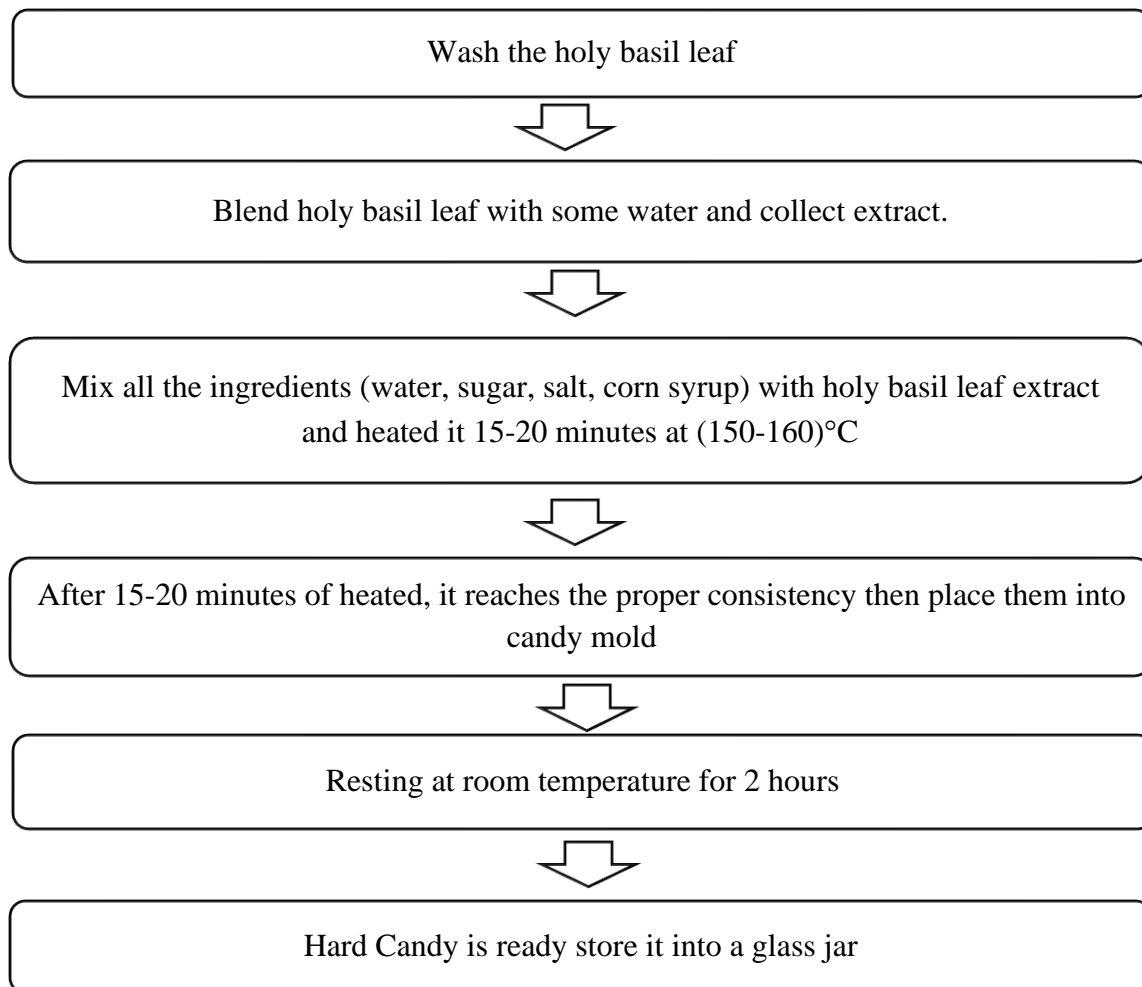


Figure 3.1: Holy basil leaf candy

3.2.1 Determination of moisture

The easiest technique to determine moisture from solid or semi-solid samples is to use a moisture analyzer.

Procedure:

- First, took 2 grams of sample.
- After that, the sample was put in a digital moisture analyzer, and left there until the moisture analyzer showed that it was finished.
- After the analyzer indicated that it was finished, I checked the moisture reading.

Materials for analyzing moisture:

- Moisture analyzer
- Aluminum foil
- Sample
- Weight balance



Figure 3.2: Moisture Analyzer (newstareenvironmental.com, 2023)

The formula was as follows:

Moisture content (%) = [Final weight of the sample (in gram)/ Raw weight of the sample (in gram)] x 100.

Final weight of the sample = (Crucible + Raw sample)- (Crucible + dried sample)

3.2.2 Determination of Ash

Procedure:

- First of all, take a crucible and heat this crucible in the oven at 105°C for 30 minutes.
- Then cool the crucible into the desiccator, and after cool the crucible take weight the crucible without sample. And then take a sample of 2 grams of hard candy. And transfer it to the crucible and weigh the crucible with the sample (note them both).
- Then transfer the crucible with the sample to the muffle furnace at 600–620°C Celsius temperature for 6-8 hours. Until turns to the ashes
- Finally, the percentage of the ash content was calculated.

Calculation Formula:

$$\% \text{Ash} = (\text{weight of white dry ash} / \text{weight of sample}) \times 100$$



Figure 3.3: Muffle Furnace (lithmachine.com, 2023)

3.2.3 Determination of Brix

Procedure:

- First, take the brix analyzer (refractometer) and clean the refractometer prism with distilled water.
- Then take a dropper and place the sample inside it.
- Put a drop of the sample on the prism of the refractometer.
- Take the readings.
- At last Rinse the prism with distilled water between samples and dry with a soft lint-free tissue.



Figure 3.4: Refractometer (Microscopes.com.au, 2023)

3.2.4 Determination of pH

It is now simple to determine pH with a pH meter. it also shows temperature.

Procedure:

- Before taking measurements, the pH meter should be calibrated using a buffer solution with a known pH value. This typically involves adjusting the meter to read the correct pH value for the buffer solution.
- Then crush or grind the hard candy to a fine powder. Weigh 10 grams of the powdered candy and add it to 50 mL of distilled water. Heat the mixture on a hot plate, stirring constantly, until the candy is dissolved.
- Then immerse the electrode in the candy solution and wait for the pH reading to stabilize. The pH value will be displayed on the digital screen of the pH meter.
- To ensure the accuracy of the results, repeat the measurements several times and calculate the average pH value.
- After each measurement, rinse the electrode with distilled water and blot it dry with a lint-free cloth.
- Compare the average pH value of the candy solution to the acceptable pH range for hard candy products.



Figure 3.5: Portable pH meter (lidinco.com 2023)

3.2.5 Microbiological analysis of holy basil leaf candy

Requirements (Instruments and chemical)

Nutrient agar, sodium chloride, Lab thermometer, Distilled water, Glass stir rod, Heat resistant hand protection, water bath, laminar flow, Boiling mixture (autoclave), Sterile Petri dish, Beaker/flask, test tube, pipette, Petri dish, Incubator.

Preparation of Sodium Chloride Solution:

Take 2.5 gm of sodium chloride in 200 ml of distilled water and mix properly.

Preparation of Agar Media:

Take 5gm of nutrient agar powder in 200 ml distilled water and mix well.

Procedure:

- First, gather all the equipment required for the test, including the test tube, beaker, measuring cylinder, pipette, micropipette, and petri dishes, and place it all in the autoclave to be sterilized at 121°C for 30 minutes.
- All are removed from the autoclave after 30 minutes and kept in the laminar air flow.
- After that, add 1 ml of the sample for serial dilution along with 9 ml of sodium chloride to the test tube.
- Take 1 ml of sample using a micropipette from the first test tube and place it in the second test tube. Then, take 1 ml of sample from the second test tube and place it in the third test tube. And each test tube shows numbers such as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} .
- Then, using a pipette, add 9 ml of agar media to each petri dish and 1 ml of sample from each test tube, dropping each petri dish in turn. Distribute each medium evenly and watch for the medium to bind.
- After that, place all of the petri dishes in the incubator for 24 hours at 37°C.
- After 24 hours, remove all petri dishes from the incubator and count them, then make a note of it.

The CFU/ml can be calculated using the formula:

Colony-Forming Unit (CFU) = (No. of Colony x Dilution Factor) / Volume of Sample

3.2.6 Sensory Evaluation**Sensory evaluation of the holy basil leaf candy**

The hedonic rating test is used in this sensory assessment. Color, flavor, texture, and taste are all significant sensory aspects. In the hedonic scale approach, stimuli are presented separately and scored on a 9-point scale ranging from "like extremely" to "dislike extremely". Suitable for a diverse range of demographics. Hedonic tests are used to assess the amount of liking for one or more products. For instance, determining how well consumers appreciate a novel product concept or comparing the favorability of standard items to the favorability of market leaders.

Procedure:

- A hedonic scale is a series of language expressions that represent the degree of liking and dislike.
- More kid-friendly words, or images of facial expressions or cheerful faces, with a 9-point scale to indicate your level of likes and dislikes.
- Tasters can also provide feedback on the appearance, taste, smell, and texture of the product.
- Finally, we must evaluate the outcomes.

Table 2: Sensory Evaluation form of holy basil leaf Candy.

Name:

ID:

- Taste this sample and checking how much you like or dislike.
- Use the appropriate scale to show your attitude by checking at point that best describe your feelings about the sample.

Preference	Color	Texture	Flavor	Taste
Like Extremely				
Like very much				
Like moderately				
Like slightly				
Like or Dislike				
Dislike slightly				
Dislike very much				
Dislike moderately				
Dislike extremely				

Signature

CHAPTER – 4

4. Results and Discussions

4.1 Proximate Composition of holy basil leaf Candy

The proximate composition of holy basil leaf candy has shown in table 2.

Table 3: Nutrient Content

Product	Moisture	Brix	Ash	pH
Holy basil leaf Candy	2.63%	69%	0.95%	4.16

Moisture Content: The holy basil leaf candy around 2.63% moisture, and other hard candy moisture content around 2-5%.

Brix Content: Holy basil leaf candy has present about 69% sugar. Because there is a lot of sugar in this, the bitterness is effectively removed, making the flavor pleasant.

Ash Content: Holy basil leaf candy has present almost 0.95% ash.

pH: The holy basil leaf candy pH almost 4.16

4.2 Sensory quality

4.2.1 Taste Preference

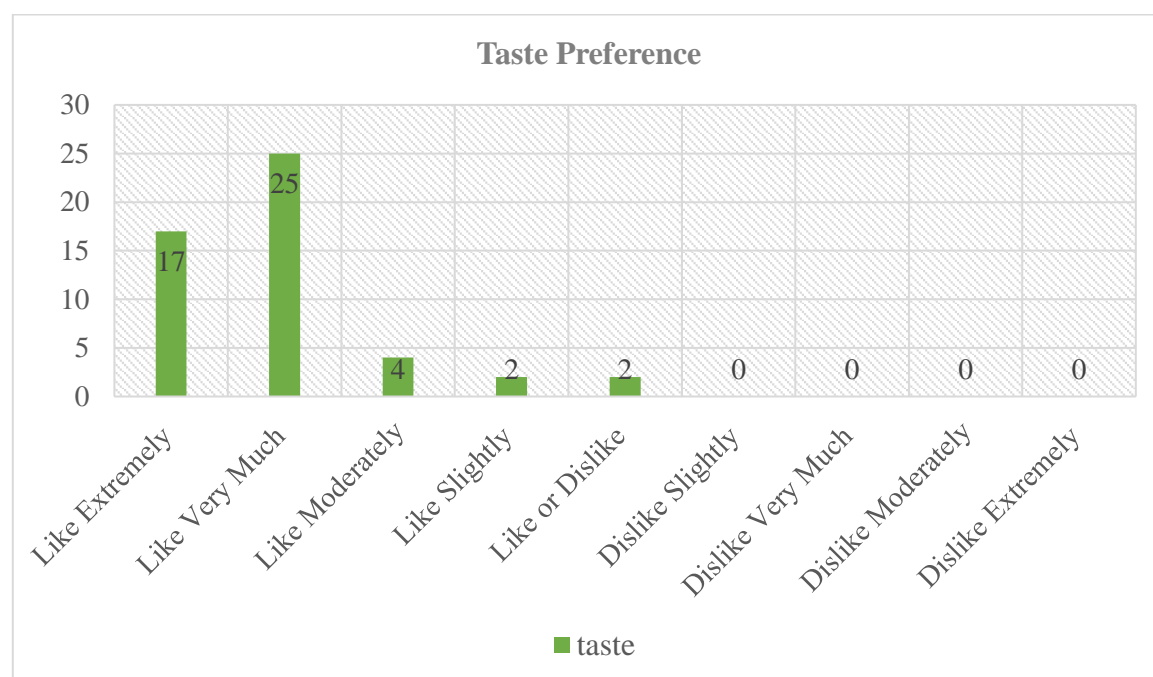


Figure 4.1: Taste preference

The holy basil leaf candy's Taste ratings on a 9-point hedonic scale is shown in bar charts, In Figure 4.1. the purpose of the data analysis was to evaluate the taste preference of holy basil leaf candy, based on the responses of 50 participants. Of the 50 participants, 34% people liked the taste of holy basil leaf candy extremely. The largest group of 50% people chooses Like Very Much. Also 8% people indicated that they liked it moderately. On the

other hand, 4% individuals have selected Like slightly of candy taste preference. 4% participants were in mixed thoughts between preferring Like or Dislike response.

4.2.2 Flavor Preference

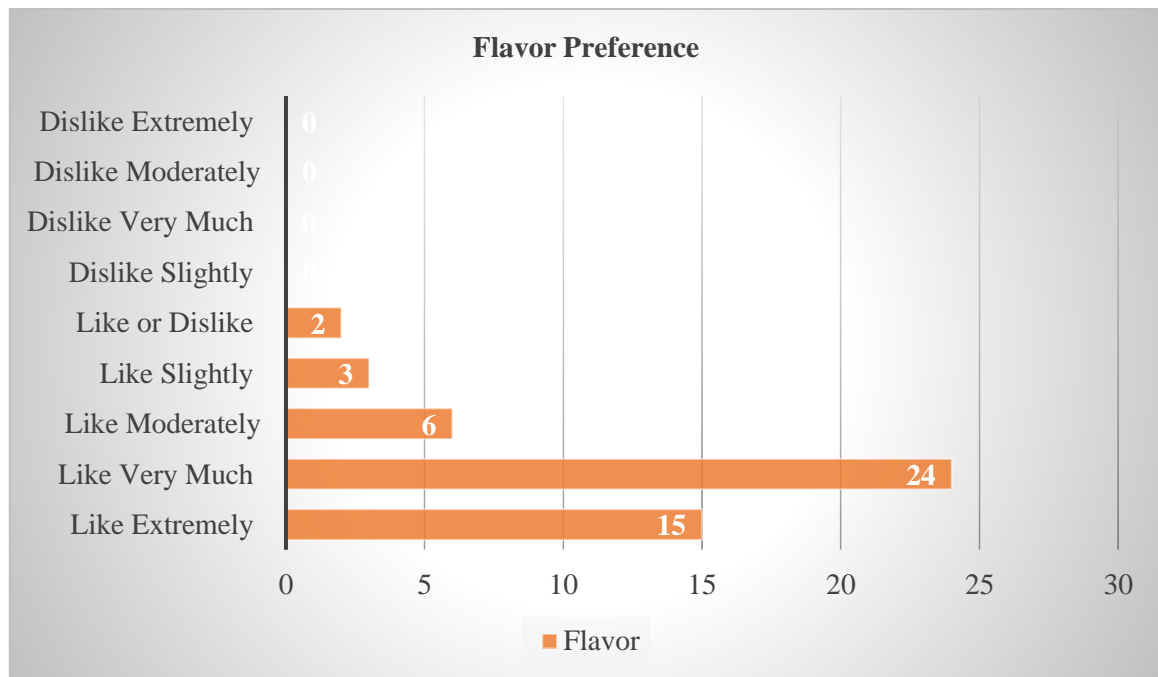


Figure 4.2: Flavor preference

Figure 4.2 indicates the 9-point hedonic scales Flavor Ratings for the holy basil leaf candy in bar charts. According to the flavor preference data analysis report, most participants liked the flavor of the holy basil candy. The candy's flavor was "liked extremely." by 15 participants' out of 50. As well as 24 people have responded on "Like Very Much". On the other hand, 6 respondents have chosen "like moderately". 3 participants have indicated "like slightly" flavor preference of candy. And 2 people were in mixed reaction and chooses "like or dislike". However, no one has chosen the remaining options of the given criteria.

4.2.3 Texture Preference

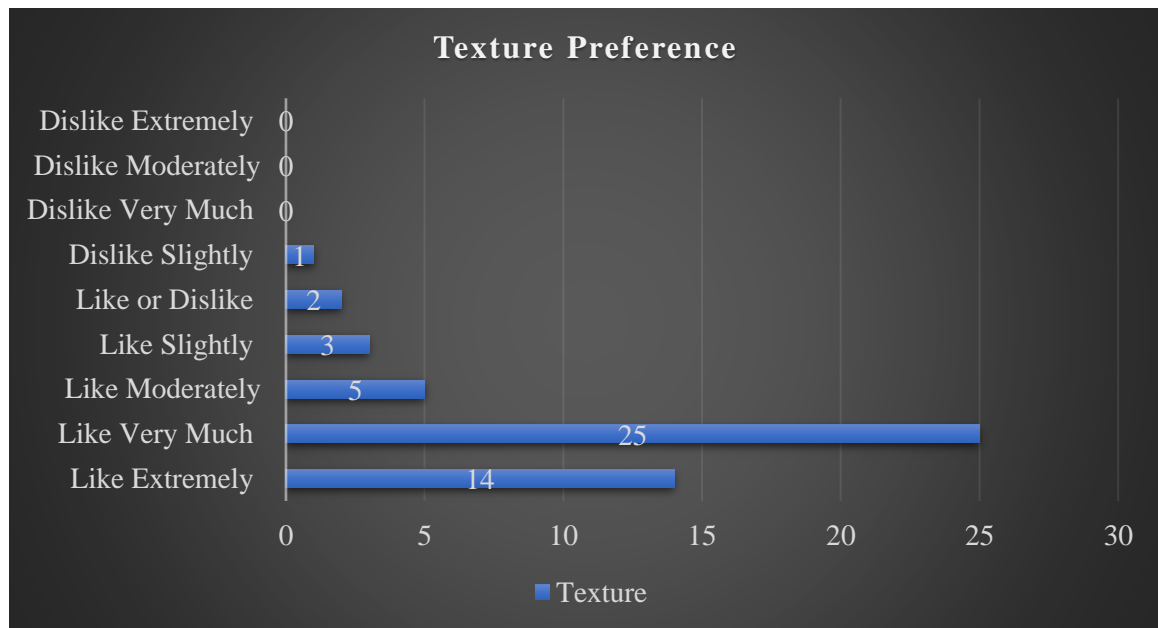


Figure 4.3: Texture preference

Figure 4.3 indicates most of the sensory evaluator liked the texture of the holy basil candy. 14 people gave holy basil candy's texture a score of "like extremely." Also, this bar graph indicates that Sample holy basil leaf Candy received the highest rating of 25 participants "like very much." On the other hand, 5 respondents have indicated "like moderately" texture preference of holy basil leaf candy. Also 3 participants have selected "like slightly" from the candy texture criteria. Besides that, 2 members were neutral (like or dislike). Notably, only 1 participant has indicated "dislike slightly". But nobody has picked any of the other options remaining in texture preference test criteria.

4.2.4 Color Preference

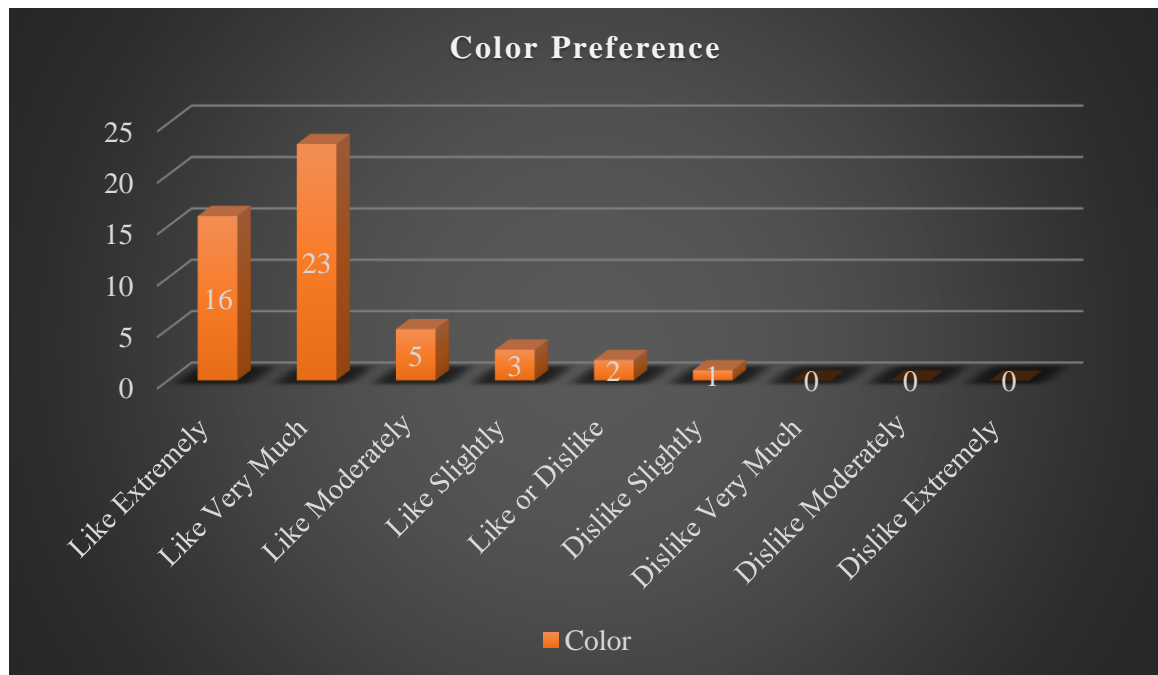


Figure 4.4: Color preference

In bar charts, Figure 4.4 shows the 9-point hedonic scales Color Ratings for the holy basil leaf candy. The color had the rating "like extremely" by 16 of the 50 participants. As well as the bar graph shows that 23 participants liked it very much. The graph also shows that the five participants chose the candy color "like moderately". Then again three respondents have selected "like slightly" of color preference of candy. On the other hand, two persons have indicated like or dislike of its color. This bar graph also shows only one people have indicates "dislike slightly" of candy color. One thing is that the participators group did not receive any other option from the color preference graph.

4.3 Overall Sensory Perception

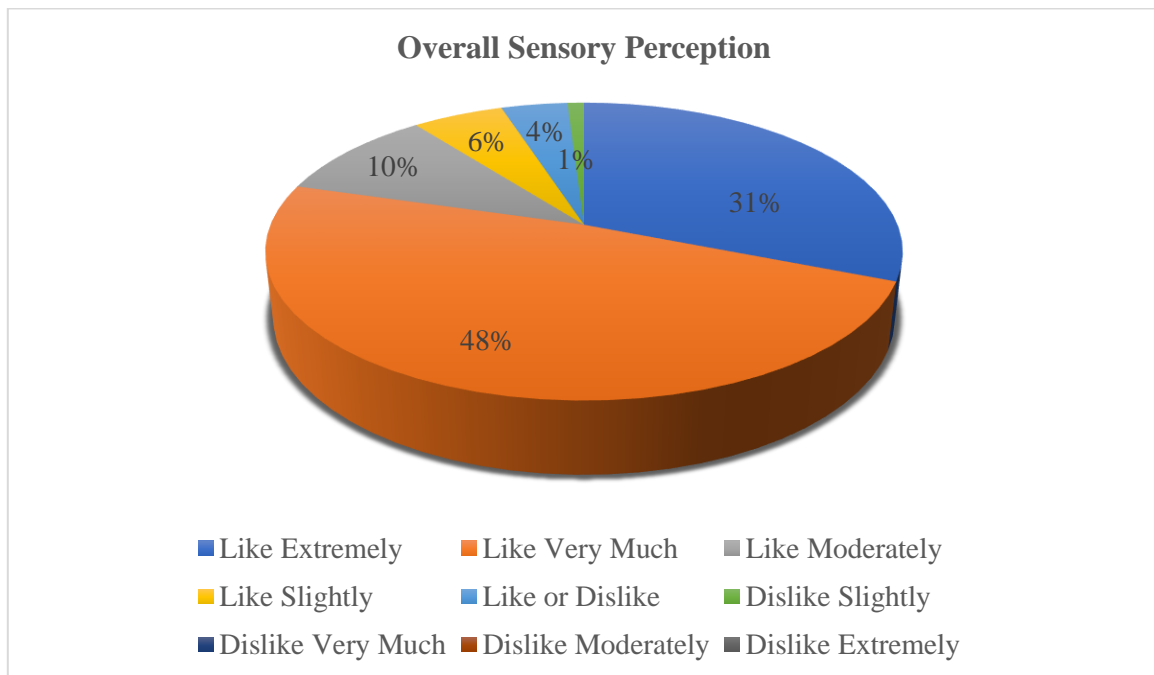


Figure 4.5: Overall Acceptance of holy basil leaf Candy

The overall sensory acceptances of holy basil leaf Candy are shown in the figure 4.5. Among all participants 31% liked the candy extremely. Likewise, 48% participants liked it very much. And 10% participants liked it moderately. And other 6% and 4% participants liked it slightly and like or Dislike. On the other hand, 1% participants are slightly Dislike the holy basil leaf Candy.

CHAPTER -5

5.1 Conclusion

Holy basil leaf Candy was developed in the NFE lab and its nutritional, sensorial and microbial qualities were analyzed. The holy basil leaf Candy reveals a low amount of moisture, which will allow it to last longer. The overall organoleptic acceptability of holy basil leaf candies in terms of organoleptic properties, 31% participants liked extremely holy basil leaf candy and 48% participants liked it very much. Likewise, 1% participants are slightly Dislike the holy basil leaf Candy. From the literature it is already known that holy basil leaf is full of beneficial bioactive compounds. So, "Holly Basil Leaf Candy" is a unique product that combines the special flavor and probable health advantages of basil leaves into an appealing candy form.

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