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Utilization of Essential Oil from *Citrus sinensis* (Orange) Peel in the Development of Functional Gummy Candy

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Abstract: Today consumers have become health conscious and are inclined towards functional foods that provide added health benefits. Gummy candy in the market contain variety of harmful synthetic flavours and colours. The aim of this study was to evaluate *Citrus sinensis* peel essential oil as healthy source of natural flavouring and colouring substance and erythritol as a non-caloric sweetener in the manufacture of functional gummy candy. Two formulations F1 and F2 were developed with varying ratio of orange essential oil and eryhtritol. Agar agar was used as gelling agent and its concentration was kept constant in all the formulations. The ratio of orange essential oil and erythritol in F1 and F2 formulations were (0.1:35) and (0.15:40), respectively. These gummy candies were examined for physicochemical parameters such as moisture, ash, protein, fat, carbohydrate content, and antioxidant activity. Also, microbial count and sensory attributes like appearance, aroma, texture, taste and overall acceptability of the gummy candies were evaluated. The F1 gummy candy formulation was chosen as the best based on sensory assessment. Selected gummy candy included 50.31% moisture, 0.57% ash, 1.35% protein, 38.59% carbohydrate, 1.75% fiber, and 42.27% antioxidant activity. Microbiological analysis revealed no coliform bacteria. Results indicate that orange essential oil positively contributed to the sensory characteristics of the candies. Additionally, erythritol has proven to be satisfactory sweetener suitable for candy. Thus, in conclusion orange peel essential oil can be effectively used as a primary flavoring agent in the development of functional gummy candies.

Keywords: Orange peel essential oil, functional foods, gummy candy, eryhtritol, agar agar

I. INTRODUCTION

Today consumers have become health conscious and there is an increased demand for functional food. The recent COVID-19 pandemic situation is accelerating the rise in demand for functional foods. Functional foods are foods that have a good influence on one or more bodily functions beyond basic nourishment in a way that is important to either a better state of health and well-being and/or a lower risk of a specific disease. According to the functional food survey (2021), 29% of people consume more functional foods, while 31% take more supplements. These factors have an impact on the confectionary market, and producers are improving their goods by incorporating technology and ingredients that suit to consumer demands (Yadav N et.al, 2021 and Kruger CL, Mann SW 2003).

Gummy candies are a form of confectionary that is popular among all age groups, and not just children, because of their soft texture, glossy appearance, and sweet taste. Use of gummy candies as health supplements is popular that act as a vehicle to deliver vitamins C, D, and E. (Lele, V et.al, 2018). The principal components used in the production of gummy sweets are sucrose, glucose syrup, gelling agent (gelatin, starch, and pectin), and food acids, flavourings, and colouring. (Kurt, A., et.al, 2021) The majority of artificial flavouring components come from petroleum and contain a variety of toxic compounds. Consumption of artificial flavouring has been linked to a wide range of negative side effects, including headaches, exhaustion, nausea, chest discomfort, disorientation are some of the short term effects. Long term effects include cancer, kidney damage, brain damage, damage to central nervous system (Ramesh, M., and Muthuraman, A. 2018). Additionally, due to the high glycemic index or high calorie content that comes with sugar gummy candy can be the cause of health issues like obesity, tooth decay, and hyperglycemia to their daily consumptions. As a result, health-conscious customers and the food industry are constantly

looking for healthy yet low caloric alternatives in candy manufacture while keeping taste, shape, texture, and qualities parameters constant (Kurt, A., et.al, 2021) (Bartkiene, E. et.al, 2021) provided one solution to the problem by developing antimicrobial gummy candies containing bovine colostrum, essential oil and probiotics. Also, antioxidant gummy candy was developed by (Charoen R. et.al, 2015) those were enriched *Psidium guajava* leaf extract.

Citrus sinensis is commonly known as sweet orange or orange. Worldwide increasing production of orange results in huge amount orange byproducts particularly its peel. Orange peel accounts for half of an orange's weight. Thus orange processing industry produces 50% waste of its input amount. One of the best way to manage orange peels peel waste is by extracting essential oil from it. (Manjarres-Pinzon, K. et.al, 2013). Essential oils are volatile compounds that plants naturally produce as a result of secondary metabolism which serve as protective and signaling molecules in plants. (Yeshi k., Wangchuk P., 2022) These essential oils are also beneficial to humans and have potential application in food, pharmaceutical and cosmetic fields.

Citrus sinensis peel oil include a high concentration of monoterpene hydrocarbons (70-95%), with d-limonene dominating all of the sweet orange oils tested. Sweet orange peel oils have been shown to include sesquiterpene hydrocarbons, which are responsible for its the distinctive citrus flavour. (Geraci, A. et.al 2016). Additionally orange essential oil contain bioactive compounds like carotenoids, limonene, terpenoids, polyphenols and flavonoids compounds. The glycosides hesperidin and naringin are primarily responsible for the antioxidant action of citrus peel extracts. Coniferin and phlorin are two other phenols found in orange peels that have been shown to help in radical scavenging isolated from orange peel extracts (Carey A. William 2013). The oil has wide application in food industry in food preservation, food packaging, and food flavouring sector. Orange essential oil, which is both refreshing and stimulating, is a boost to digestion and immune system. Very versatile, orange essential oil is be blended as flavoring substance with both sweet and savory food dishes (Wardee Harmon 2018). Orange oil is established as potential flavouring substance due typical citrus aroma and flavor.

Erythritol ((2R,3S)-Butan-1,2,3,4-tetrol) is a smallest sugar alcohol in the polyol family that is formed when the aldehyde or ketone group in certain carbohydrates is hydrolyzed. Polyols are abundant in fruits and vegetables such as grapes and mushrooms, as well as fermented foods such as soy sauce (Regnat et.al, 2017). It has a nearly no calorie value. It has an extraordinarily high digestive tolerance and will not create laxative effects when used as intended (De Cock, P. 1999). Erythritol has a 70% sweetness relative to sugar ratio and provides a cooling sensation. (Rzechonek D. A. et.al, 2017). Thus, erythritol was found to be safe natural sweetener suitable to be used in gummy candy formulation.

Agar agar is water soluble polysaccharide extracted from red seaweed is widely used as gelling, thickening agent in candy, jellies, soups, yoghurt due to its strong gelling properties (Selvalakshmi et.al, 2017). Agar agar is a vegan substitute to gelatin which on the other hand is made from animal hides, skin, bone and cartilages. Scientists (Patel K. K. et.al and Grétarsdóttir K. G. 2016) have investigated the use of agar agar in the production of gummy candy.

The aim of this research study was to utilize orange essential oil obtained from the peels of *Citrus sinensis* as the primary flavouring agent in the formulation of gummy candy. Additionally to use Erythritol as a sweetener in order to reduce its caloric content. Further to assess the formulated gummy candies for their physicochemical, antioxidant, microbial and sensory properties.

II. MATERIAL AND METHODOLOGY

2.1. Materials

Oranges used in preparation of gummy candy were procured from local market of Vadodara. Food grade agar agar of By nature and erythritol sweetener of So sweet were purchased online from Amazon India. All the chemicals and glassware of analytical grade required for the study were available in the department of Food Analysis and Food Processing lab, Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara.

2.2. Preparation of oranges

Oranges were sorted, and only fresh, disease-free, and undamaged oranges were selected. The selected oranges were washed with tap water to remove any dust, dirt or contamination present on surface and were allowed to air dry. Washed oranges were manually peeled in such a way that peels and segments were collected separately. The orange peels were processed for essential oil extraction and segment were processed into orange juice.

2.3. Extraction of orange peel essential oil

Obtained orange peels were washed with potable water, allow to air dry and subsequently dried in tray drier at 60^0C for 24 hours. The dried peels were then grounded into powder using mixer grinder. Orange peel powder was then sieved using sieve.

10 g of orange peep powder was weighed and transferred into porous filter paper to create a thimble. 150 ml of methanol was taken in round bottom flask which was used as solvent in extraction process. Soxhelt apparatus was setup with solvent in round bottom flask, thimble in the extractor, water inlet and outlet attached to the condenser. Condenser was checked to ensure that the cooling supply was right to prevent and heating damage to the apparatus. Heating mantle was set at 80ºC. The methanol in the round bottom flask evaporated and got condensed

into the sample extracting the oil it along the way. Reflux was conducted for 4 hours. The mixture of oil and solvent was then placed in the water bath to completely evaporate the solvent. (Fakayode et.al, 2018 and Fekadu et.al, 2019) The weight of oil obtained was recorded and the yield of oil was calculated by the below formula:

$$\text{Essential oil Yield \%} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

2.4. Extraction of orange juice

Orange segments which were previously separated from peels are then utilized for extracting of orange juice. Orange segments were cut into small pieces using clean knife and seeds and pits were carefully. Orange pieces were then transferred into juicer. The obtained orange pulp was then strained to provide final orange juice.

2.5. Formulation of gummy candy

Table 1 – Formulation of gummy candy

Ingredients	F0 (g)	F1 (g)	F2 (g)
Water	50	-	-
Orange juice	-	50	50
Orange essential oil	-	0.1	0.15
Erythritol	35	35	40
Agar agar	6	6	6

2.6. Preparation of gummy candy:

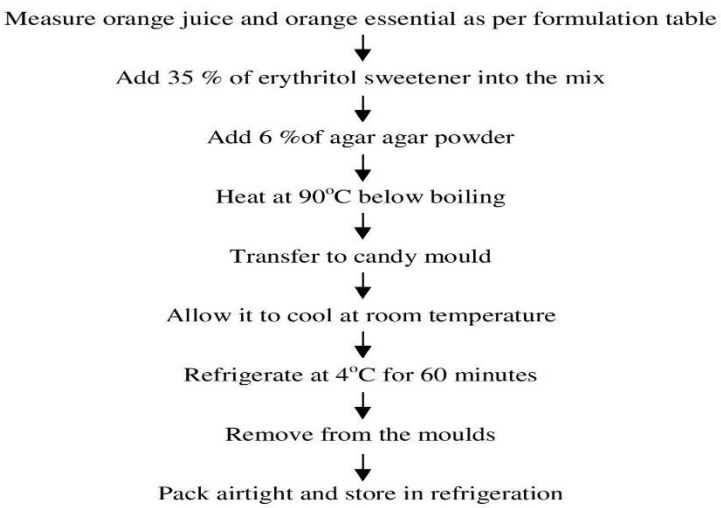

Figure1 – Flowchart for preparation of gummy candy

2.7. Physio-chemical Analysis

2.7.1. Moisture and ash

The moisture content were determined gravimetrically in an oven set to 105^0 C. The ash content was determined by using muffle furnace at 550^0c. The samples were heated before being placed in the oven for ash and moisture content. Both ash and moisture can be calculated by following formula (Moghaddas Kia, E., 2020).

$$\text{Moisture / Ash \%} = W_1 - W_2 \times 100$$

W_1

Where, W_1 is initial weight of sample and W_2 is weight of sample after treatment.

2.7.2. Protein

Protein content of gummy candies were analyzed using Kjeldhal's method. (AOAC method)

- **Digestion**

In a digestion flask, 1 g of sample was taken and Kjeldhal catalyst (K2SO4: C2So4 in 9:1 ration) was added. Digestion of the solution was carried for 2-3 hours at 420°C (till it turns clears or colorless). Sample was allowed to cool followed by slowly diluting it 50 mL of distilled water.

- **Distillation**

10 ml of resulting sample was taken and % 40 NaOH solution was added. Distillation was done till all NH_3 was liberated in 10 ml of Boric acid.

- **Titration**

0.4 ml of methyl red indicator was added and titrated with 0.1 n HCL till faint pink colour appeared. Similarly a blank sample with water was analyzed.

$$\text{Crude protein \%} = \frac{(\text{Sample titre} - \text{Blank titre}) \times 0.0014 \times 6.25}{\text{Weight of sample (g)}} \text{ X } 100$$

2.7.3. Fat content

Soxhlet apparatus was used to analyses the fat content of the gummy candy. A sample of 5g was measured and transferred thimble. The round bottom flask were wash and oven dried at 130°C for 15 minutes. 70 ml of petroleum ether was measured and added into extraction flask. Thimble containing sample was placed in the extractor and Soxhlet was assembled. Fat extraction was carried at 65°C for 4 hours. The recovered ether was separated and recovered, and the amount of fat in the round bottom flask was calculated.

$$\text{Crude fat \%} = \frac{W_3 - W_2 \text{ X } 100}{W_1}$$

Where, W_1 is weight of sample,

W2 is weight of empty beaker and

W3 is weight of beaker + extracted fat.

2.7.4. Carbohydrate content

The amount of carbohydrates was determined by subtracting the total amount of moisture, fat, protein, total ash from 100. The following formula was used to estimate the carbohydrate.

Carbohydrate % = 100 - % Moisture - % Protein - % Fat - %Ash

2.8 Microbial analysis

Microbial analysis is the ideal quality evaluation procedure used in food product quality analysis. The prepared gummy candy's microbiological quality was identified on different microbiological characteristics, including the total plate count, yeast and mold count, and the E. coli count. The samples were also analyzed while they were being stored at room temperature. Microbial tests were performed using the APHA's recommended procedures (1992).

Sample preparation: 9 Sterilized tubes were taken and in each tube 9 ml of sterile distilled water was added aseptically and labelled. 1g of sample was first diluted in 9 ml of sterile distilled water tube labelled 1 and then 1 ml was transfer in tube 2. In this way serial dilution was carried up to 10 fold.

A. Total plate count

Nutrient agar medium: Standard nutrient agar was prepared by dissolving 28 g of nutrient agar in 1 litre of distilled water. Nutrient media was then sterilized at 121^0C for 15-20 mins in an autoclave.

Pour plate method: Petri plates and pipettes were also sterilized in autoclave. The work station was disinfected with 70% alcohol. 1 ml of diluted sample was poured into petri plate followed by that molten media was poured into it. All the transfer were made under aseptic conditions to prevent contamination. The plates were steadily swirled and then allowed to solidify. Then plate were incubated at 37^0Cfor 48 hours and checked for growth of colonies.

B. Yeast and mold count

Potato dextrose agar medium preparation: Potato dextrose media was prepared by dissolving in 1 litre of water 39 g of potato dextrose agar. Media was then sterilized at 121^0C for 15-20 mins in an autoclave.

Pour plate method: Petri plates and pipettes were also sterilized in Autoclave. The work station was disinfected with 70% alcohol. 1 ml of diluted sample was poured into petri plate followed by that molten media was poured into it. All the transfer were made under aseptic conditions to prevent contamination. The plates were steadily swirled and then allowed to solidify. Then plate were incubated at 37^0Cfor 48 hours and checked for growth of colonies.

C. Coliform test:

Procedure: 28 grams of Bromocresol purple broth with lactose media was dissolved in 1000 ml of distilled water. Heat was supplied to dissolve the media contents.10 ml media was then dispense into sterilized test tubes containing inverted Durham's tube. Sterilization of media was carried at 1210C for 15 mins. 1 ml of selected dilution was added in the test tubes and incubated at 24 and then 48 hours. Checked for the presence of coliform indicated by colour change from purple to yellow and gas formation in the inverted tube.

2.9. Antioxidant activity:

To determine the antioxidant activity of gummy candy procedure given by (Moghaddas Kia et.al, 2020 and Hooda R. 2015) were followed but with slight modifications. A mass of 5 g was roughly dissolved in 20 mL of 50% ethanol for each sample. The combined solution was then homogenized for three minutes at a speed of 1000 rpm. The homogenate was further solubilized by heating at 40 C for about 20 minutes with constant magnetic stirring, followed by a 20-minute centrifugation at 10,000g. The supernatant was then collected and the procedure outlined was used to determine the DPPH radical-scavenging activity. Fresh DPPH solution was prepared at the time of experiment by dissolving 1 mg DPPH (1,1 -diphenyl-2-picryl-hydrazyl) in 50 ml methanol and kept in dark place. 1 mL of extracted sample was then mixed with 3 mL of DPPH solution and incubated in the dark for 30 minutes. At 517 nm, absorbance was measured using a blank of methanol without DPPH. A control sample was also run that contained only DPPH solution and no sample extract. Percent DPPH inhibition was calculated by the following formula:

$$\text{DPPH Inhibition \%} = \frac{A_{CONTROL} - A_{SAMPLE}}{A_{CONTROL}} \times 100$$

Where, $A_{CONTROL}$ = absorbance of control and A_{SAMPLE} = absorbance of sample.

(Moghaddas Kia et.al)

2.10. Sensory evaluation

The sensory evaluation of the gummy sweets was undertaken using a 9-point hedonic scale acceptance test (1 = dislike extremely; 9 = like extremely). Ten semi-trained panelists between the ages of 25 and 30 were chosen. Panelists were given water to rinse their mouths between evaluating samples. The candy was judged on five criteria: appearance, colour, texture, flavour, and overall acceptability. Panelists were given questionnaires of 9-point hedonic scale in order to evaluate the samples.

2.11. Statistical analysis

The data were presented as the mean value and standard deviation of three measurements (n=3). Data were analyzed using the ANOVA to determine the influence of the various formula components on the quality parameters for gummy candy. When $p < 0.05$, the differences were deemed significant.

3. RESULT AND DISCUSSION

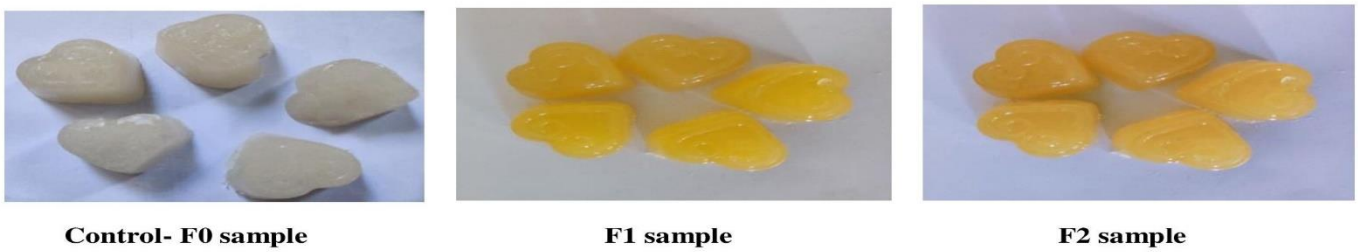

Figure 2- Picture of formulated gummy candy

3.1. Orange essential oil yield

The yield of orange essential oil for 10 gram sample of peel powder was found to be 1.9±0.02 %. The result was in accordance to the result obtained by (Giwa O.S. et.al, 2018) 2.54% of sweet orange essential oil by Soxhlet extraction method. Also (Fakayode et.al, 2018) found yield of sweet orange essential oil of 3.24% by Soxhlet extraction method.

3.2. Physicochemical characteristics of Gummy candy

Moisture content

The moisture content in the gummy candies was found to be in the range of 50.31±0.20 to 58.46± 0.19 (figure 3). Highest moisture percentage was found to be in control F0 formulation which was around 58.46 % followed by F1 and F2 as 50.31±0.20% and 51.88±0.19% respectively. The $p < 0.05$ revealed a significant difference between three gummy candies.

Ash

The ash content of the gummy candy was found in the range of 0.54±0.89 to 0.59±0.73 (figure 3). Highest ash percentage was reported in F2 formulation of 0.59±0.73 %, followed by F0 and F1 as 0.54±0.89 and 0.57±62 respectively. The ($p > 0.05$) revealed no significant difference between in ash content of three gummy candies.

Protein

The protein content in the candies was in the range of 0.55±0.02 to 1.42±0.07. Highest percentage of protein was observed in the formulation F2 which was 1.42±0.07. F0 and F1 was recorded with 0.55±0.02% and 1.35±0.11% protein content. The p value was (<0.05) indicated significant difference in three candies protein.

Fat

The F2 formulation had the highest fat content of 0.84±0.03 (figure). F0 and F1 was recorded with fat content of 0.56±0.02 and 0.73±0.06 respectively. There ($p < 0.05$) was significant difference in three gummy candies.

Carbohydrate

Highest carbohydrate content 39.76±0.23 was recorded in the F2 formulation of gummy candy. F0 and F1 formulation of gummy candy had carbohydrate percentage of 20.50±0.35% and 38.59±0.23% respectively (figure). The p <0.05, indicated the carbohydrate percentage of different formulations increased notably.

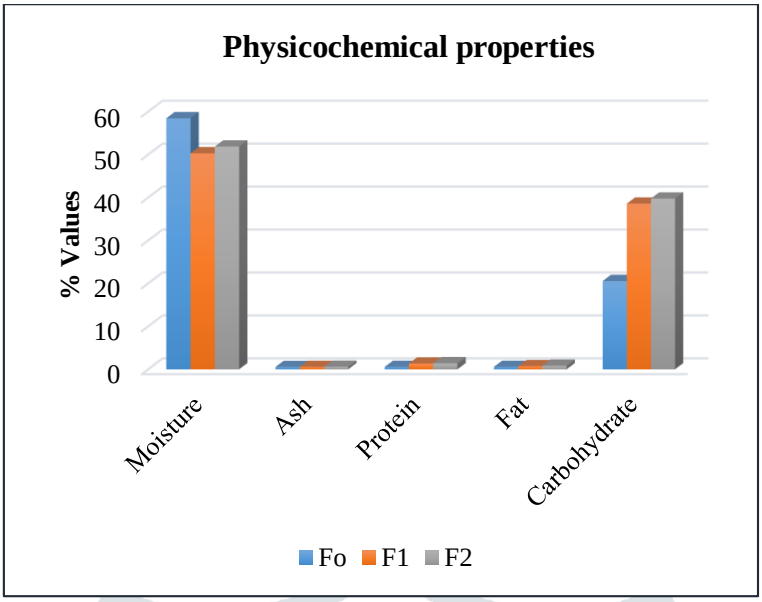

Figure 3- Physicochemical properties of gummy candy

3.3. Antioxidant activity of candies

Figure 4 shows a plot of the DPPH inhibition percentage for each of the three gummy candies. There was a significant difference (p 0.05) between the gummy candies. The amount of DPPH radical scavenging activity was given as a percentage, with T2 having the highest percentage (50.13%), followed by T1 (42.27%), and control (16.94%), respectively.

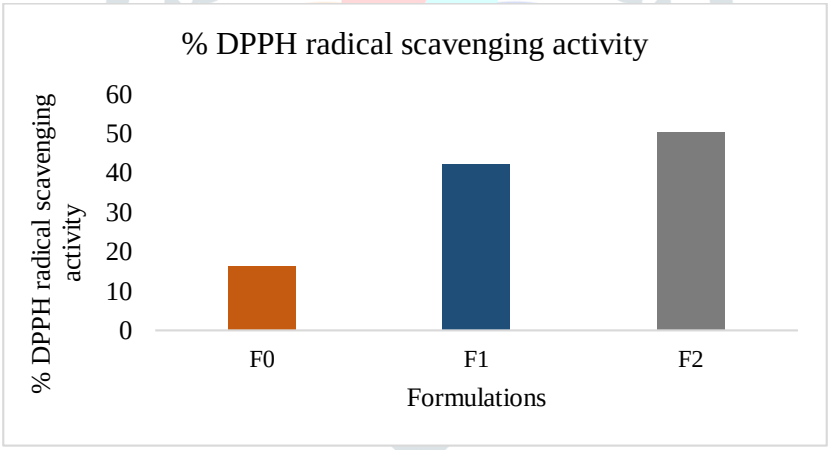

Figure 4- DPPH antioxidant activity of gummy candy

3.4. Microbial analysis of gummy candy

Total plate count (TPC) of gummy candy: The total plate count of gummy candies were found 5.7, 5.3 and 4.9 log10 cfu/g for F0, F1 and F2 formulations of gummy candies respectively. The highest count was found in control sample. With the change in orange essential oil concentration the colony count of TPC also varied. In accordance with the analysis, F0, F1, and F2 had statistically significant differences ($p < 0.05$).

Yeast and mould count (YMC) of gummy candy: The total yeast and mould count of gummy candies were found 8.8, 8.3 and 7.5 log10 cfu/g for F0, F1 and F2 formulations of gummy candies respectively. The highest count was found in control sample. With the increase in orange essential oil concentration the colony count of gummy candy decreased. In accordance with the analysis, F0, F1, and F2 had statistically significant differences ($p < 0.05$).

Coliform count: The coli form count was absent in control and test samples F1 and F2.

3.5. Sensory Analysis

The organoleptic properties of gummy candy all had a significant impact on their quality. Due to the variable orange essential oil concentration, there were noticeable alterations in the aroma and taste of gummy candies. The control gummy candy F0 formulation received a 6.0 hedonic score on aroma, 7.6.6 hedonic score on appearance, a 5.8 hedonic score on texture and 6.2 hedonic score on taste. The control F0 formulation had a 6.15 hedonic score for overall acceptability, indicating that the gummy candy ranged between like slightly and like moderately. The F1 formulation received a 8.4 hedonic score on aroma, 8.0 hedonic score on appearance, a 8.8 hedonic score on texture and 8.6 hedonic score on taste. Overall acceptability of the F1 candy was 8.2 score, indicating that the gummy candy ranged between like very much and like extremely. The F2 formulation received a 7.2 hedonic score on aroma, 8.4 hedonic score on appearance, a 8.2 hedonic score on texture and 6.6 hedonic score on taste. Overall acceptability of the F2 candy was 7.6 score, indicating that the gummy candy ranged between like moderately and like very much. It might be because the gummy candy had a strong aroma and a taste that wasn't as well-liked as in earlier versions. Thus, F1 formulation was selected as the best gummy candy. In accordance with the analysis, F0, F1, and F2 had statistically significant differences ($p < 0.05$).

Table 2 – Sensory evaluation

	Aroma	Appearance	Texture	Taste	Overall acceptability
F0	6.0± 0.63	76.6± 1.01	5.8± 0.74	6.2± 0.74	6.15± 0.71
F1	8.4± 0.48	8.0± 0.19	8.8± 0.64	8.6± 0.48	8.2± 0.18
F2	7.2± 0.74	8.4± 0.48	8.2± 0.4	6.6± 0.47	7.6± 0.25

CONCLUSION

From above research it can be concluded that the objectives of this study were met by extracting essential oil from orange peels, using it in the development of gummy candy, examining and comparing the physicochemical and antioxidant characteristics of gummy candy, and determining sensory acceptability of the gummy candies. According to the results of all the formulations containing varied quantities of orange juice, orange essential oil, erythritol, and agar agar, F1 was found to be the best among all the different treatments. F1 formulation gummy candy contains 50.31% moisture, 0.57% ash, 1.42% protein, 0.84% lipids, and 39.76% carbohydrates. In addition, F1 gummy candy also depicted good antioxidant activity of 42.27% having added health benefits. Utilisisation of erythritol a non-caloric sweetener in gummy candy was found to be a suitable option for health conscious and diabetic people. Orange essential oil acted as a best natural orange flavouring and colouring ingredients with nutritional benefits. Thus, orange essential oil is found to be the safe and natural flavouring agent to be used in the formulation of healthy orange gummy candy.

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