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Formulation and evaluation of nutrient-dense gummies with antioxidants and skim milk protein

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Abstract
Consumer demand for healthy, sustainable foods allows agricultural byproducts to be utilized to produce functional foods. Citrus peels, particularly orange and sweet lime peels are among the most plentiful agricultural waste and contain naturally present antioxidant compounds. Therefore, the objective of the study was to formulate a high value green recovery strategy to isolate crude antioxidant compounds from orange and sweet lime peels for a novel nutritionally rich health-promoting food product. The crude antioxidants were isolated from orange and sweet lime peel using the comparison and ultrasound assisted extraction method which enabled increase in yields of bioactive products from peel material. The extracts' antioxidant activities were determined through the 2,2-diphenyl-1-picrylhydrazyl assay based on their capability to scavenge free radicals and thereafter carbohydrates, proteins, lipids, calcium, iron and phosphorus were quantified by the 3,5-dinitrosalicylic acid method, Kjeldahl, Soxhlet, Ethylenediaminetetraacetic acid titration, Wong test and Fiske-Subbarow methods respectively. Three different types of gummies were prepared, one without Citrus peels extract and the other two included sweet lime or orange peels extract respectively. For gummies, agar, pectin and carrageenan were used as gelling agents, with skimmed milk powder as fortifier, stevia and orange flavour were used as a natural sweetener and to improve the taste of the gummy. Sensory evaluation of consumers was carried out to assess the level of acceptance on flavour, texture, appearance and overall acceptability of the gummies and shelf-life studies of the gummies were conducted to know the shelf stability over time at various storage conditions. The finished product exhibits antioxidant potential at 46.65 mg/g for orange and 51.03 mg/g for sweet lime gummies. In the finished products, the carbohydrates were in range 93.9-94.9mg/g, proteins in range 1.7-2.6mg/g, lipids in range 41.7-42.2mg/g, iron was in range 29-31mg/g, calcium 120-152mg/g. The protein, calcium, phosphorus and iron were highest in the orange gummy compared to other formulations. The value of moisture and ash content in the prepared gummies reflects the stability and the mineral content. The flavour and overall acceptability were highest for orange gummy compared to others in the sensory evaluation. The products showed good quality at microbial level up to 30 days, after 45 days only trace amounts of growth were observed in samples. Conclusion has been drawn that citrus waste is a good source of natural antioxidants, suitable for use as a gummy supplement. The products provide an environmentally friendly alternative for agricultural waste and fulfils the needs of wellness-promoting functional food.

Keywords: Antioxidants from citrus peel, gummies, functional foods, ultrasound-assisted extraction

Introduction

Over recent years, due to increased health consciousness, wellness and disease prevention concerns, the demand for foods beyond basic nutrition that possess health-promoting properties have increased substantially (Baker *et al*., 2020) [5]. The health-promoting effects of functional foods have been proven due to their presence of functional ingredients like vitamins, minerals, dietary fibres, antioxidants, among others. The rise of knowledge regarding the connection of food and health has led people to look for foods that could help boost their immunity and prevent diseases.

Post COVID-19 pandemic, health consciousness in consumers further enhanced, resulting in a surge of preference towards foods that comprise of natural and functional components to promote their health (Kaur *et al*., 2023) [15]. Along with the functionality of the food, consumer preference also increased for foods that were easy to consume and modern lifestyles as the food processing industry was spurred to develop and innovate new foods, considering nutrition, functionality, sustainability and convenience. Meanwhile, there is a huge problem for food processing industries to manage large amounts of waste generated from the production of food products. Food processing by-products, including fruit peels, seeds, pomace, and pulp, contain many valuable nutritional and bioactive components but are disposed of as waste materials, resulting in increased environmental pollution and wastage of valuable resources (Gupta and Kumar, 2019) [10].

Therefore, utilization of food processing waste has become a major focus as it represents a cheap and sustainable source of beneficial compounds, thereby forming the basis of a more resource-efficient and environmentally-friendly food industry (Boon *et al*., 2021 [6]. From among the different food-processing wastes, byproducts from citrus fruits constitute one of the most abundant resources. In processing these fruits into juice, jam, concentrates, and other essential oils, up to half of the total weight of these citrus fruits is wasted in the form of peels, seeds, and pomace (Aiello *et al*.,2024) [2].

This discarding of residues causes waste disposal problems as well as increased waste disposal costs. Orange and sweet lime peels, on the other hand, are valuable constituents of the fruit that can be recovered to formulate new food products, thus converting waste into edible and nutrient-rich food. Orange and sweet lime peels comprise of many nutritious constituents including vitamin C, flavonoids, phenolics, dietary fibre, carotenoids, and other phytochemicals which confer many antioxidant properties to these wastes (Aiello *et al*., 2024; Permatananda *et al*., 2024) [2, 19]. The science behind these constituents is gaining increasing attention for their role in promoting health and well-being. Recovery of these bioactive components is not only beneficial in enhancing the nutritional quality of the food product but also sustainable in waste management practices via waste valorisation. In addition to other beneficial compounds, orange and sweet lime peels are very cheap sources of functional ingredients that can be incorporated into foods. Antioxidants are very essential to protect the body against free radicals' damages.

Free radicals damage proteins, lipids and DNA, causing several chronic diseases such as heart failure, cancer, diabetes, neurodegenerative diseases, etc. The body's natural antioxidant defence system helps to counter free radicals, but this system sometimes is overwhelmed by an increased number of free radicals generated within the body or in the external environment (Roh *et al*., 2021) [22]. The inclusion of dietary antioxidants such as citrus peel extracts, helps to provide free radicals that neutralize the free radicals and reduce body damages. The application of ultrasound-assisted extraction in recovering antioxidants from orange and sweet lime peels will contribute towards creating a new functional food product. Ultrasound-assisted extraction has also been identified as one of the techniques which can provide high extraction yield and recovery of bioactive compounds, reduction in extraction time and solvent usage. It is one of the modern green technologies which have become very popular for extraction of bioactive components from various natural resources, including food wastes (Hirondart *et al*., 2020) [12]. Therefore, the study focused on the recovery of antioxidants from orange and sweet lime peels by using ultrasound-assisted extraction.

Protein is one of the important macronutrients for the human body since it is vital in development, tissue repair, hormone production, enzyme production and other biological activities. A proper consumption of protein is capable of enhancing the health status of individuals, both young and old. Milk powder made from skim milk is one of the dried dairy products that have an excellent nutritional content in terms of protein and different micronutrients like calcium, riboflavin, and vitamin B12 (Clarke *et al*., 2021 [7]; Oh *et al*., 2015). Due to its nutrient richness and low-fat content, it is readily incorporated into foods for nutritional fortification. The inclusion of skim milk powder into the developed gummy food would contribute towards its protein richness. In addition to beneficial components, it is crucial to develop food products with favourable sensory qualities to gain wide consumer acceptance. In recent years, functional gummy products have become a trend among people of all age groups. This is mainly due to their taste, appearance, chewiness, convenience and portable. (Baker & Hurst, 2020; Tarahi *et al*., 2023) [5, 23]. This functional food format can be customized for the inclusion of multiple nutrients including, vitamins, minerals, proteins, phytochemicals and antioxidants in a single product. Quality, stability and mouthfeel of gummy products is determined by the gelling agents used in the formulation. Agar-agar, pectin, and carrageenan are used to form gels in the food industry (Yoshida *et al*., 2019; Rawat *et al*., 2024) [21, 24]. Hydrocolloids can influence the mouthfeel and stability of gummies while also being instrumental in the development of plant-based and clean-label food products desired by consumers. While many research efforts Has documented on utilization of citrus peel extract as an antioxidant source, as well as on development of functional food using many gelling agents and ingredients, there were not many literatures available that had focused on the combined formulation of antioxidants derived from orange and sweet lime peel with skim milk powder using sustainable waste valorisation approach to formulate a nutritional gummy product. Hence, the present study focused on extracting crude antioxidants from orange and sweet lime peels by ultrasound assisted extraction technique and used it as an ingredient for the preparation of nutritional gummies with agar-agar, pectin, carrageenan and skim milk powder. Nutritional evaluation, antioxidant capacity analysis, sensory acceptance test and shelf-life stability of the developed product was assessed.

Materials and Methods

1. Procurement of sample

1. Fruits of fresh oranges and sweet limes are bought from local fruit vendors, and peels are harvested after washing them under running water.
2. All the above ingredients such as pectin powder, stevia leaf powder, agar-agar powder, pectin powder, Carrageenan powder, orange flavour and skim milk powder are obtained from reputed suppliers of food ingredients.
3. Citric acid powder as surface treatment.
4. Food-grade moulds for making gummies are from the culinary tools shop.
5. Other equipment’s required for analysis and product development are sourced from accredited suppliers who maintain food-grade standards.

2. Preparation of citrus peel extracts

Orange and sweet lime fruits peels were removed and washed clean, dried and ground into a fine powder. Antioxidant rich extracts of orange and sweet lime were obtained by Ultrasound-Assisted Extraction (UAE). Peel powder was mixed with 70% aqueous ethanol solution and subjected to ultrasonic treatment at 40 kHz for 30 min at ambient temperature. Supernatant was collected after centrifuging at 4℃ for 30 min and stored in the refrigerator.

3. Preparation of antioxidant enriched gummies

Local sourced ingredients such as orange peel and sweet lime peel, agar-agar, pectin, carrageenan, milk powder, stevia, citric acid and flavourings were used in this study.

The citrus peels were washed and dried in an oven at 60°C for 24 hours, after which they were finely grounded into powder and sieved. Antioxidant enriched extract was then obtained by Ultrasound-Assisted extraction method.

For preparing gummies, milk powder and stevia were first dissolved separately in water with constant stirring to avoid lumps. Citric acid and Pectin were then added and mixed properly. Followed by addition of carrageenan and agar-agar, continuous heating and stirring till clear solution was obtained. Citrus peel extract and flavouring agents were then added to the solution to obtain uniformly gummy mixture.

The gummy mixture obtained was poured into moulds and set at room temperature for 2 hrs. Three formulations were prepared-control gummy candies (gummy candies without citrus peel extract), orange peel extract gummy candies, and sweet lime peel Extract gummy candies.

The prepared gummy candies were analysed for nutritional composition, antioxidant activity, moisture content, ash content, sensory acceptability and microbial stability studies were also performed by total plate count method up to visible growth of microbes.

4. Nutritional and Functional Analysis

The amount of protein was estimated by Kjeldahl's method, fat content by Soxhlet's extraction, and carbohydrate by Dinitro salicylic acid method. Iron and calcium content were estimated by Wong's method and ethylenediaminetetraacetic acid titration method respectively, while phosphorus was estimated by Fiske and Subbarao method. Antioxidant activity of extracts was carried out using the 2,2-diphenyl-1-picrylhydrazyl method and readings were taken at 515 nm. Moisture and ash content were estimated by using hot-air oven method and dry ash method respectively.

5. Microbial Analysis

For shelf-life studies the gummies were assessed for Total Plate Count (TPC) at Day 0, 15, 30 and 45 to determine the microbial stability of the product. The extract of the gummies was plated on sterile nutrient agar plates and observed for microbial growth.

6. Sensory evaluation

The developed gummies were assessed for acceptability by 30 panellists (10 semi-trained and 20 untrained), aged 18-40 years. Sensory evaluation parameters like appearance, texture, taste, flavour, odour, chewiness, and overall acceptability were evaluated using a 100-point hedonic scale. Samples were coded as A, B, C and given randomly to panellists.

The panellists recorded their feedback in the evaluation score sheet and results were used to assess the acceptability of developed gummies.

Results and Discussion

In the study of antioxidant activity, nutritional value, sensory acceptance and microbiological stability of both sweet lime and orange peel extract fortified gummies, as well as that control lacking the extracts were used. All analyses were performed in triplicate and reported as a mean.

1. Preparation of gummies

Initial studies demonstrated that composition of the formulation had a marked influence on the textural characteristics and physical stability of the gummies as provided in Figure 1. Addition of excessive milk powder produced failure in gel formation and that a very high level of guar gum resulted in a brittle structure that fractured upon cooling. Pectin produced a gel but the texture was rough and grainy, with evidence of lumpiness. Carrageenan resulted in a formulation which was clear and produced gummies with a tender, firm texture and favourable mouthfeel; this was selected for future studies.

Fig 1: Gummies prepared using various combination of ingredients

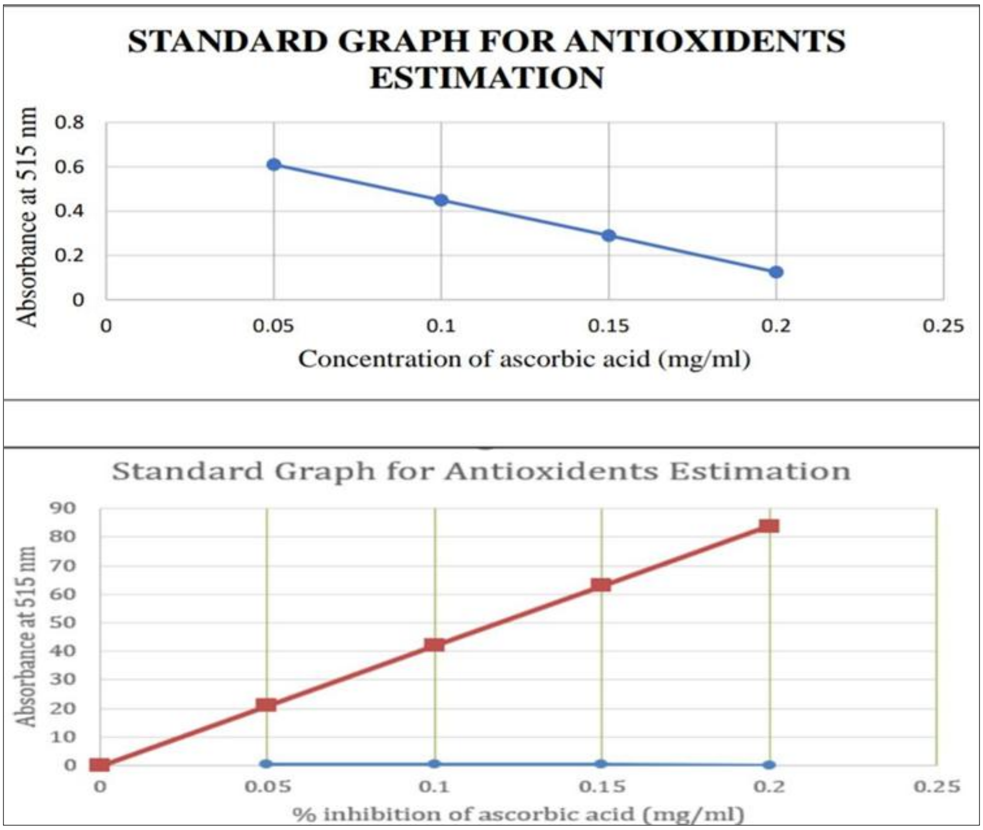
2. Analysis of antioxidant by DPPH method

The analysis of antioxidant content in the extracts and the gummies prepared was carried out by DPPH method. The results are given in Table 1 and graph 1.

Table 1: Results of antioxidant estimation

Test	CG	OG	SLE	OG	SLG
Absorbance at 515 nm	0.69	0.265	0.33	0.41	0.38
% inhibition	10.4	65.83	57.47	46.65	51.03

(Here, CG – Control Gummy, OE - Orange Extract, SLE - Sweet lime Extract, OG - Orange Gummy, SLG - Sweet lime Gummy)



Graph 1: Results of antioxidant analysis

Through the graphical method of 2,2-diphenyl-1-picrylhydrazyl assay, antioxidant activity in 100 g was also observed to be maximum in orange extract with a reading of 65.83 mg/g followed by sweet lime extract with 57.47 mg/g. From the gummies, sweet lime gummies contained more antioxidant activity of 51.03 mg/g than orange gummies, which had the lowest reading of 46.65 mg/g. The control gummies without extract also show lower content of antioxidants than orange and sweet lime gummies are 16.4 mg/100 g. Generally, results show the extracts having stronger antioxidant capacity than the formulated gummies, implying some loss of activity throughout gummy manufacture.

3. Nutritional and Antioxidant Composition

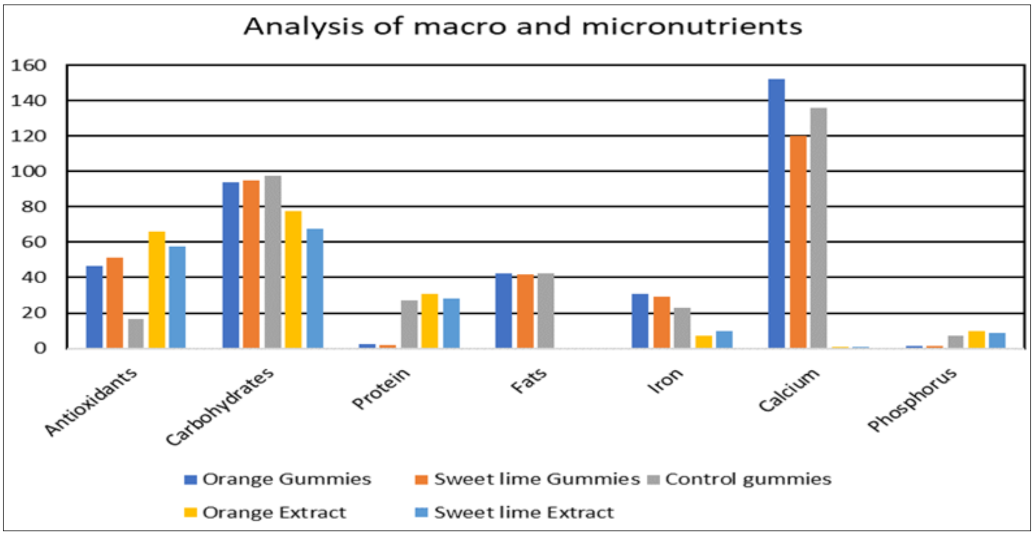
The macronutrients measured, carbohydrate was most abundant in all gummy samples, followed by fats and proteins. Control gummies exhibited the greatest carbohydrate content, 97.36 mg per gram. Sweet lime gummies contained 94.9 mg per gram and orange gummies contained 93.9 mg per gram. Orange gummies contained the highest amount of protein, 30.6% of their weight followed by sweet lime gummies which contained 28% by weight, while the control gummies contained slightly lower, at 27.1% by weight. Fats levels for orange, sweet lime, and control were roughly equivalent, with 42.2 mg/gram, 41.7 mg/gram, and 42.3 mg/gram respectively. Fats levels for the extracts themselves contained trace levels of fats: 0.38 mg/gram for the orange extract and 0.07 mg/gram for the sweet lime extract.

The greatest amount of calcium was present in the orange gummies (1520 mg/gram), followed by the control gummies (1360 mg/gram) and the sweet lime gummies (1200 mg/gram). Phosphorus levels were also higher for orange (9.8 mg/gram) and sweet lime (8.9 mg/gram) gummies compared to the control gummies (7.9 mg/gram). Iron content for the orange gummies was higher at 31 mg/gram than for the sweet lime gummies at 29 mg/gram, control at 27.1 mg/gram, and extracts at 7 mg/gram and 10 mg/gram for orange and sweet lime gummies respectively.

The antioxidant capacity of the gummies was assessed using the DPPH assay; orange extract contained the highest activity, 65.83 mg/gram while sweet lime had 57.47 mg/gram. Sweet lime gummies had a slightly higher antioxidant capacity than orange gummies, with values of 51.03 mg/gram and 46.65 mg/gram respectively. Control gummies contained significantly lower antioxidant levels (16.4 mg/gram). The results obtained for various nutritional parameters and antioxidant content are given in Table 2 and graph 2.

Table 2: Summary of macro, micronutrients and antioxidant content in the gummies

TEST (mg/ml)	Orange gummies	Sweet lime gummies	Control gummies	Orange extract	Sweet lime extract
Antioxidant	46.65	51.03	16.5	65.83	57.47
Carbohydrates	93.9	94.9	97.36	77.6	67.35
Protein	2.6	1.7	27.1	30.6	28
Fats	42.2	41.7	42.3	0.38	0.07
Iron	31	29	23	7	10
Calcium/10	152	120	136	0.8	0.72
Phosphorus	1.6	1.4	7.9	9.8	8.9



Graph 2: Macro and micro nutrient analysis in the gummies

4. Ash and moisture content

The moisture content of both orange and sweet lime gummies was low, indicating good product stability. The ash content of the orange gummies (4.15%) was comparable to that of the sweet lime gummies (4.10%), suggesting similar levels of mineral residue in both samples.

5. Microbial Stability

Total Plate Counts were conducted on the gummies. Microbial growth was not detected in any of the gummy samples up to day 15. Trace growth was evident on day 30 and considerable growth on day 45. This indicates that the gummies are have a shelf life for almost 30 days. The results of microbial analysis are represented in figure 2.

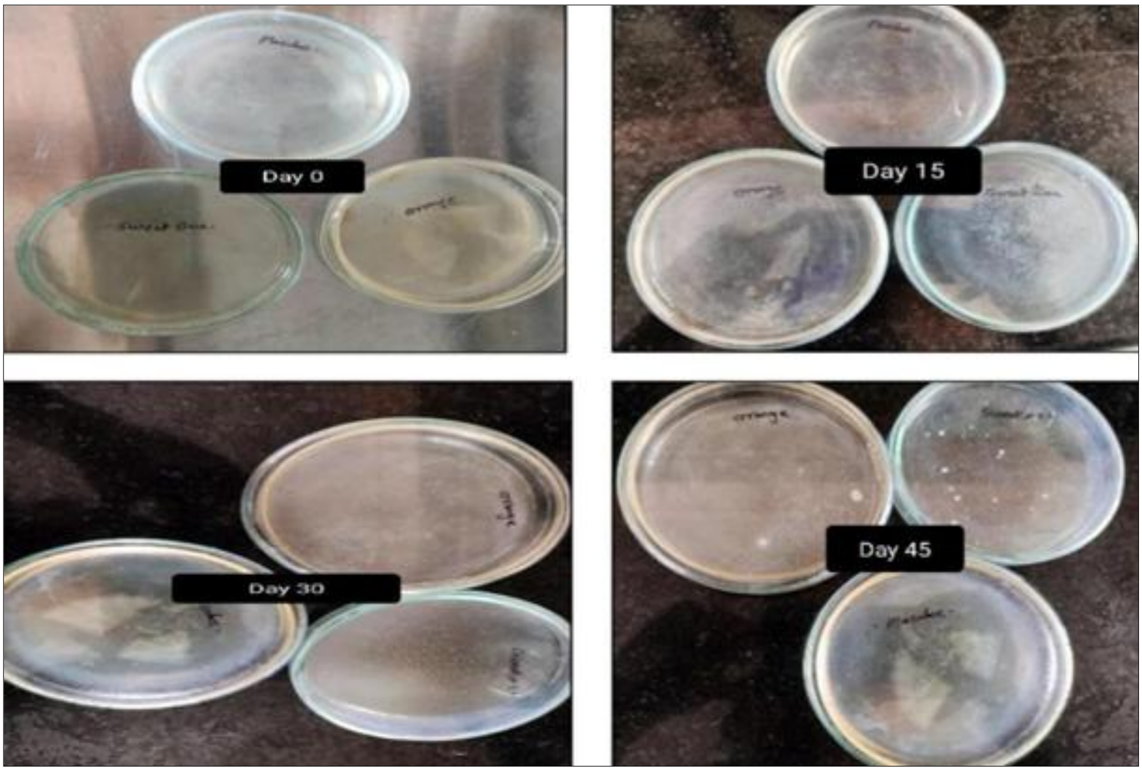
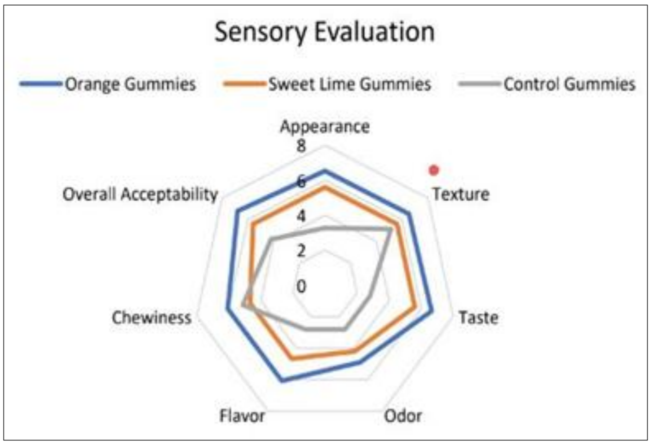


Fig 2: Results of microbial analysis of the gummies prepared

6. **Sensory evaluation**

Sensory acceptability of gummies was rated by 50 panellists using a 7-point Hedonic Scale; the orange gummies were found most acceptable overall (6.8) and the top rating for flavour (6.30). The texture of the orange gummies was rated 5.32. The sweet lime gummies had average ratings and an overall acceptance value of 4.62. The control was the least accepted, scoring 4.20 overall. The summary of the sensory evaluation of the gummies prepared is represented in graph 3.



Graph 3: Sensory evaluation of the prepared gummies

Conclusion

The present study focuses on investigating the nutritional value, antioxidant potential, sensory acceptability and microbial stability of gummies incorporated with the orange and sweet lime peel extracts. Three formulations namely Control, Sweet Lime Peel Extract, and Orange Peel Extract were prepared. Analysis revealed that the orange gummies exhibited higher values for proteins, calcium, phosphorus and iron compared to other samples. Antioxidant analysis showed that, both the peel extract and supplemented gummies were having a significant difference and higher value than the control samples. The values of ash and moisture were in the acceptable limits to predict stability and mineral content of the products. Sensory attributes such as flavour and acceptability of orange peel gummies were reported to be the highest while the control gummies obtained lowest values. Microbial analysis revealed the product as safe and stable for a period of up to 30 days, trace microbial growth was reported after 30 days. Further consumer acceptability needs to be explored. Orange and sweet lime peel extracts could be used as natural functional ingredients to increase nutrition and antioxidants, taste and acceptability of gummies and thus contribute to increased value of by-products.

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