**Short Research Article**

**An Analysis of Microbiological Quality and Bioactive Compounds for Preparing Immunoboost Confectionery**

**Abstract.** Functional foods derived from plants are gaining popularity due to their potential for treatment and safety. The confectionery market is expanding due to increased demand for functional foods and health advantages. This study investigates the development and optimisation of immune boosting and vitamins-infused gummies with natural extracts known to support. With growing consumer interest in functional foods and nutraceuticals, the research aims to create a palatable, convenient, and health-promoting alternative to conventional supplements. Natural ingredients, particularly those rich in antioxidants and essential micronutrients, were selected for formulation based on their known efficacy in immune modulation. The research process involved ingredient selection, formulation trials, sensory evaluation, and nutritional analysis. The gummy matrix was developed using suitable gelling agents, natural colourants, and flavour enhancers to ensure consumer acceptability. Key parameters such as texture, stability, shelf life, and vitamin retention were optimised through iterative testing. Sensory evaluation was conducted with trained panellists to assess taste, texture, appearance, and overall acceptability. Statistical tools were employed to identify the optimal formulation with the highest acceptability and nutritional benefit. The final product demonstrated a balanced profile of taste and health benefits, with active compounds retained at effective levels. The study concludes that natural-extract-enriched gummy vitamins offer a viable and appealing method for delivering essential nutrients, especially for populations that prefer non-pill-based supplements. This innovative approach has potential applications in public health, nutrition and the functional food industry. The recommended intake for immune-boosting gummies is two gummies per day for adults.

**Keywords:** Vitamins enriched gummies, natural extracts, immune function, functional foods, micronutrients..

1. **Introduction**

The confectionery market is expanding due to increased demand for functional foods and health advantages. Nowadays, consumers are aware of the necessity of following a healthy diet, and there is a demand for natural and nutritious food products. Consequently, new trends in the food industry are focused on the development of foods with low levels of sucrose and artificial additives (e.g., flavours and colourants), as well as high antioxidant, protein, and fibre content (Tarahi et al.,2023). Gummy candies, a chewable gelatin-based candy, are popular for their distinct texture, look, and flavour. Traditionally, gummy candies are made using a combination of sugar, water, and gelatin. However, to improve candy formulations, there are several common techniques employed such as substituting the gelling agent for gelatin, utilizing natural colorants, incorporating plant extracts, vitamins, or fruit derivatives, and substituting sugar with other sweeteners (Roudbari et al., 2024; Vojvodić Cebin et al., 2024). They originated in Germany in 1864 and are available in a variety of flavours, including Jelly Babies, Gummi bars, and Gummibärchen. Gummies are also available in ring-shaped versions with popular flavours such as peach ring. Multivitamin candy, such as Flintstones, appeals better to youngsters. Grapes, a major agricultural crop, are also used to treat a variety of ailments in nations such as Pakistan, Italy, and Turkey. Grapes have a variety of health benefits, including antiviral, anticancer, antibacterial, antifungal, anti-inflammatory, anti-acne, anti-ageing, antihypertensive, protective, anti-asthma, antiplatelet, anticataract, anti-obesity, anticholinergic, anti-sunburn, anti-hyperpigmentation, wound-healing, and antiviral activity. Butterfly pea blossoms contain flavonoids, which have antioxidant properties and can help prevent upper respiratory tract infections. Sunflowers are a major crop worldwide, generating seeds and vegetable oil. Gelatin is used in gummy jelly compositions to increase texture and flavour. Fruits are being added to the formulation of gummy jelly for various purposes, including acting as a flavouring and colouring agent, and to provide phytonutrients beneficial for health (Renaldi et al., 2022; Tarahi et al., 2023). Citrus byproducts, such as polyphenols, flavonoids, essential oils, and polysaccharides, are rich sources of biomolecules. Gummy sweets, including grapes, butterfly pea flowers, and sunflower seeds, are popular fortification formulas due to their high protein content. Fruits and vegetables contain antioxidants, which help maintain nutrition, texture, flavour, and freshness. They can be added to the diet to inhibit lipid decomposition, or they can exist naturally. Healthy dietary habits, a stress-free mentality, and adequate sleep are essential for the immune system's healthy operation. Fruits and vegetables high in vitamins, carotenoids, and phytochemicals can boost the immune system and have anti-inflammatory, antibacterial, antiviral, antioxidant, immunomodulatory, and anticancer activities. Vitamin C, a reducing agent, neutralises reactive oxygen species and promotes radical scavenging. Vitamin E, a potent antioxidant, regulates host immune responses and improves T cell function. Phytochemicals, non-nutritive secondary metabolites with disease-protective properties, improve the immune system's ability to combat malignancies. Functional foods derived from plants are gaining popularity due to their potential for treatment and safety.

1. **Objectives:**

* To develop and prepare a immune-boosting and vitamins-enriched gummies
* To test the microbial load and shelf life of the developed product
* To analyse the phytochemical properties and physicochemical properties of the formulated product.

**Materials and methods**

**Standardisation of immune-boosting gummies**

**Table 1:** Variations of immune-boosting gummies

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.no** | **Ingredients** | **Control** | **Sample 1** | **Sample 2** | **Sample 3** |
| 1 | Black grape juice | 80ml | 60 ml | 50ml | 45ml |
| 2 | Butterfly pea flower extract | 60ml | 40 ml | 35ml | 30ml |
| 3 | Sunflower seed powder | 25 g | 20 g | 15g | 10g |
| 4 | Gelatin | 1 tbsp | 1 tbsp | 1 tbsp | 1 tbsp |
| 5 | Sugar | 2 tbsp | 2 tbsp | 2 tbsp | 2 tbsp |
| 6 | Honey (optional) | 1 tsp | 1 tsp | 1 tsp | 1 tsp |
| 7 | Lemon juice | 15ml | 10ml | 8ml | 5ml |

Sample 1 was the most desired formulation out of the four that were assessed since it scored the highest on the sensory evaluation. This sample included constant amounts of gelatin (1 tbsp), sugar (2 tbsp), honey (1 tsp, optional), lemon juice (10 ml), 60 ml of black grape juice, 40 ml of butterfly pea flower extract, and 20 g of sunflower seed powder. Sample 1 outperformed the control and other samples due to its well-balanced component combination, which produced an enticing colour, nice aroma, smooth texture, and well-rounded taste.

**Microbial analysis of the developed immune-boosting gummies**

Microbial analysis is crucial to ensure product safety and quality standards. The tests for plate count and harmful pathogens. Low counts indicate good manufacturing hygiene and preservation. Factors like moisture content, pH, and sugar concentration influence microbial stability. Preparing the sample, a certain amount, typically measured in grams, of the immune-boosting candies is weighed. A sterile diluent, such as peptone water, is then used to homogenise the sample and produce a homogenous solution. In order to lower the concentration of microorganisms and facilitate counting, the homogenised sample is put through a series of dilutions. Usually, several 1:10 dilutions are made. A measured volume from the chosen dilution—typically 1 mL—is inoculated into sterile petri dishes. Plate Count Agar (PCA) that has been melted and cooled is added to the sample, gently stirred, and left to set. Incubation for a predetermined amount of time (24–48 hours), the infected plates are incubated at a particular temperature (usually 30-37°C). Depending on the microbiological profile being targeted, different incubation conditions may apply.   
Colonies that are visible on the plates are counted following incubation. To guarantee accuracy, only plates with colony numbers between 30 and 300 are taken into account for computation. Total Plate Count (TPC) computation: The following formula is used to calculate TPC:

TPC = Colony count x Dilution factor

Sample volume plated

Colony-forming units per gram (CFU/g) of the gummy sample are how the results are interpreted. The Food and Drug Administration (FDA), World Health Organisation (WHO) , or local health authorities or other regulatory guidelines determine the permissible level for TPC in immune-boosting candies. The total plate count of the immune-boosting gummies is tabulated below:

**Table 2:** Microbial analysis of the developed immune boosting gummies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.no | Parameter | Test method | Unit | Results |
| 1 | Total plate count | IS 5402 Part-  1:2021 | CFU/g | 1100 |

Fig 1- Pie chart showing microbial analysis of the developed immune boosting gummies

IS 5402 is a method for determining total plate count (TPC) in food and animal feeding stuffs. It specifies a horizontal method for enumerating microorganisms by counting colonies that grow on a solid medium after incubation at 30°C. IS 5402 includes both pour plate and spread plate techniques.

1. **Keeping the quality of immune-boosting gummies**

To determine the sample's storage behaviour, the keeping quality is assessed. For 15 days, they are stored in the refrigerator and at room temperature. The developed gummies are stored in the refrigerator as well as at room temperature. The samples are sent to Idhayam Parikshan Labs Limited in Virudhunagar for microbiological investigation. A 15-day shelf-liferesearch is conducted on the items.

**Table 3:** Keeping the quality of immune-boosting gummies

|  |  |  |  |
| --- | --- | --- | --- |
| Days | Appearance | Odour | Texture |
| First day | Bright, no spots | Fruity, fresh | Slightly softer |
| Second day | No change | Mild fruity | Chewy |
| Third day | No change | Slight fruity | Slightly sticky |
| Fourth  day | No visible change | Mild odour | Slightly soft |
| Fifth day | Slight stickiness | Slight off-odour | Soft |
| Sixth day | Slight dullness | Noticeable off odour | Very soft |
| Seventh day | Sticky, slight discolouration | Off-odour | Mushy |

**Results and discussion**

**Phytochemical properties of the developed product**

Black grapes and butterfly pea flowers are rich in phytochemicals with numerous health benefits. Black grapes contain resveratrol, a powerful antioxidant, and flavonoids, while butterfly pea flowers have anthocyanins, flavonoids, and antioxidant properties. Both botanicals support cellular defence mechanisms and may reduce chronic disease risk. Their synergistic use in health products can enhance antioxidant capacity and promote wellness. Immune-boosting gummies often contain a blend of vitamins, minerals, and plant-derived compounds, many of which have known phytochemical properties that contribute to their health benefits. In immune boosting gummies, the total amount of polyphenols on a dry basis is

* 8.29 mg in 10 g = 83 mg per 100 g (dry basis)
* That’s a rich amount for a supplement
* Given that daily consumption is likely around 5–10 g of gummies, the full 8.3 mg per day, which is a good contribution, especially if paired with other nutrients (e.g., vitamin C, zinc, etc.).

A known quantity of the immune-boosting candies is weighed during sample preparation. Until a consistent weight is reached, dry the sample in an oven at a regulated temperature (often between 50 and 60°C). Pulverise the dehydrated candies until they are a fine powder. Weigh a quantity of the dried gummy powder precisely to extract the polyphenols. Use the proper solvent to extract the polyphenols (usually 70–80% methanol, ethanol, or acetone). Permit the sample to be extracted using techniques such as: Sonication: Submerging the sample for a predetermined amount of time in an ultrasonic bath. Shaking is the process of using a shaker at a regulated temperature and speed. Maceration: a predetermined amount of time spent with the sample in the solvent. Use Whatman filter paper or a comparable grade to filter the extract.

Gather the transparent filtrate for examination. Reagent preparation: Make the Folin-Ciocalteu reagent, which is a common reagent for polyphenol analysis. Make a 7.5% sodium carbonate solution. Pipette an aliquot of the extract into a test tube using the colourimetric reaction method. A measured amount of Folin-Ciocalteu reagent should be added. Give it a set amount of time to react, usually three to five minutes. After adding the sodium carbonate solution, let the mixture rest at room temperature in the dark for 30 to 60 minutes. A UV-Vis spectrophotometer is used to measure the reaction mixture's absorbance at 760 nm. To compute find the total amount of polyphenols in the sample extract, utilise a calibration curve of a standard polyphenol component, such as gallic acid. Utilising the following formula, determine the total polyphenol content on a dry basis: Total Polyphenols = C x V x D (mg GAE/g dry weight)   
 M   
C is the polyphenol concentration (mg/mL) based on the standard curve.   
V is the extract's volume (mL). D stands for dilution factor, if any. M is the dried gummy sample's mass (g).

**Table 4:** Phytochemical properties of the developed product

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.no | Parameter | Test method | Unit | Results |
| 1 | Total polyphenols on the dry basis | IPL.CH.INS.STP.35 | 10g | 8.29 |

The IPL method, or High-Performance Liquid Chromatography (HPLC), is a widely used analytical technique for identifying and quantifying polyphenols in various samples. It separates and measures different polyphenol compounds based on their chemical properties, providing detailed information about the polyphenol composition of a sample.

Fig 2- Pie chart showing Phytochemical properties of the developed product

1. **Conclusion**

The best antioxidant sources are vitamin C and vitamin E. Vitamin C is a water-soluble antioxidant that helps regenerate other antioxidants and supports immune defence and collagen synthesis, neutralises free radicals, supports the immune system Food sources are Black grapes. Vitamin E is a fat-soluble vitamin known for its strong antioxidant properties, particularly in protecting cell membranes from oxidative damage. food sources are Sunflower seeds. The microbial analysis is done to ensure product safety and quality. The total plate count is analysed in immune boosting gummies The result of TPC is 1100CFU/g is a moderate and reasonable level of microbial activity. Polyphenols broad group of plant compounds with strong antioxidant activity Food sources include Butterfly pea flower. The phytochemical analysis is performed on the total polyphenols content of immune boosting gummies in a 10 g sample, that 8.29 mg of polyphenols are present. The recommended intake for immune-boosting gummies is two gummies per day for adults.

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