RESEARCH PAPER

Assessment of different hydrocolloids as stabilisers in the development of kiwi gummies fortified with vitamin D2-enriched *Cordyceps militaris* powder

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Abstract: The present study examined the effects of hydrocolloids (pectin, xanthan gum, and agar-agar) on the physicochemical stability, sensory characteristics, microbiological quality, and vitamin D_2 retention of kiwi-based gummies enriched with *Cordyceps militaris* during 90 days of ambient storage. A control formulation lacking gelling agents was employed for comparison. Water activity rose steadily across all samples; however, pectin-based gummies showed much lower values (0.512–0.589), indicating greater moisture immobilisation. A steady decrease in pH (3.58–3.25) was seen across treatments, with pectin formulations consistently displaying lower pH, thereby boosting microbial stability. Texture profile analysis found that agar-agar had the highest hardness, while pectin showed superior cohesiveness, springiness, and chewiness, resulting in a more desirable gel structure. Sensory evaluation indicated significantly higher consumer approval for pectin-based gummies. The preservation of Vitamin D_2 declined after storage, yet it was best in pectin samples (86.67%), followed by agar-agar and xanthan gum, whereas the control displayed the greatest deterioration. Microbiological testing found a progressive rise in total plate count; nevertheless, pectin formulations demonstrated the lowest microbial load, with negligible yeast, mould, and coliform present. Pectin demonstrated the highest efficacy among hydrocolloids in increasing structural integrity, nutrient stability, and shelf life of vitamin D_2-fortified kiwi gummies.

Keywords: Hydrocolloids, kiwi gummies, vitamin D_2-enriched *Cordyceps militaris*, microbial growth, storage stability

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INTRODUCTION

The world's maximum population is vitamin D-deficient. Numerous studies have reported this deficiency with musculoskeletal, cardiovascular, and neurological disorders [1]. Vitamin D plays a crucial role in enhancing calcium and phosphate absorption, which is essential for bone mineralization and overall skeletal health. Adequate vitamin D intake helps prevent rickets, osteomalacia, osteoporosis, and fractures, and it is often used therapeutically in combination with Magnesium and Vit K to manage calcium and phosphate deficiencies [2]. Vitamin D is either synthesized endogenously in the skin through sun radiation(ultraviolet) UVb–induced conversion of 7-dehydrocholesterol to vitamin D_3 or it can be sometimes obtained from dietary products such as dairy products, oily fish, liver, egg yolk etc. Since natural foods contain insufficient amounts of vitamin D, that why fortified foods increased as per the necessity and requirement. Dietary health has gained increasing importance among consumers, leading to a growing demand for fortified foods and health-supporting supplements. In the human body, 25-hydroxyvitamin D is the major circulating form, which is subsequently converted to the biologically active 1,25-dihydroxyvitamin D. Present conducted study a result, to introduced in dosage forms as a gummies product as reported that chewable dosages form is gradually replacing conventional tablets and capsules, as they offer improved palatability and ease of consumption [3]. Being more food-like than traditional dosage forms, gummies provide a suitable matrix for the incorporation of naturally derived functional ingredients, while their inherent sweetness can help mask the undesirable flavors of certain bioactive compounds [4].

Gummies are widely consumed confectionery products, making them attractive platforms for product innovation. Their characteristic chewy texture, diverse shapes, appealing colours, and flavours contribute to high consumer acceptance. Typical gummy formulations contain sweeteners, acidulates, flavouring agents, colorants, and hydrocolloids, which are responsible for developing the desired structural and sensory attributes [5]. Hydrocolloids are hydrophilic, high-molecular-weight polymers that interact with water to form viscous solutions or gel structures. In food systems, they are used to regulate texture, enhance stability, and control moisture migration by binding free water. Owing to their effectiveness at low concentrations and wide range of natural sources, hydrocolloids are extensively employed in confectionery products to improve structural integrity, shelf-life, and sensory quality [6]. Among commonly used hydrocolloids, pectin, xanthan gum, agar-agar, and gelatine, pectin plays a major role in defining the texture of gummy jellies. Pectin is particularly valued for its ability to form clear, elastic gels with desirable chewability. It can be used alone or in combination with other hydrocolloids like starch, guar gum, gum arabic, gelatin, and carrageenan to achieve market-preferred textures [7]. Its gelling, thickening, and stabilising properties, along with thermos-reversible behaviour and melt-in-mouth characteristics, have made pectin one of the most widely applied hydrocolloids in the food industry [8]. To prevent adverse effects associated with excessive vitamin D intake, the Institute of Medicine (IOM) established the tolerable upper intake level (UL), which represents the maximum daily intake unlikely to pose health risks for most individuals. The UL for vitamin D from food and supplemental sources is defined as 1,000 IU/day for newborns (up to 6 months), 1,500 IU/day for infants aged 7–12 months, 2,500 IU/day for children aged 1–8 years, 4,000 IU/day for adolescents aged 9–18 years, and 4,000 IU/day for adults, including pregnant and lactating women.

In regions with limited sunlight exposure, vitamin D fortification of foods is commonly practised to improve the population's vitamin D status. Vitamin D contributes to calcium and phosphorus absorption, bone and dental health, and immune function. In this context, the present study aims to improve the stability and bioavailability of vitamin D in kiwi-based gummies through the incorporation of vitamin D-enriched *Cordyceps militaris* powder. Kiwi (*Actinidia deliciosa*), a commercially important fruit from the Actinidiaceae family, is rich in vitamin C, dietary fiber, potassium, vitamin E, folate, polyphenols, carotenoids, flavonoids, anthocyanins, lutein, and various antioxidant and enzymatic compounds with established health benefits [9]. These bioactive constituents have been associated with digestive, renal, ocular, skeletal, cardioprotective, antidiabetic, anticancer, immunomodulatory, and antibacterial effects [10]. However, despite its high nutritional value, kiwi naturally lacks vitamin D.

To address vitamin D deficiency, kiwi-based gummies fortified with UV-treated *Cordyceps militaris* powder enriched in vitamin D_2 represent a promising functional food approach. Although previous studies have examined vitamin stability, sensory properties, and microbial behaviour in gelatin-based confections, limited research is available on gummies fortified with mushroom-derived vitamin D_2. Moreover, no studies have reported on kiwi-flavored gummies incorporating UV-enhanced *Cordyceps militaris* powder. Therefore, the present work investigates the sensory attributes, microbiological safety, physical stability, and shelf-life of vitamin D-fortified kiwi gummies stored at ambient conditions for 90 days, to support product formulation, shelf-life validation, and commercial scalability.

1. MATERIALS AND METHODS

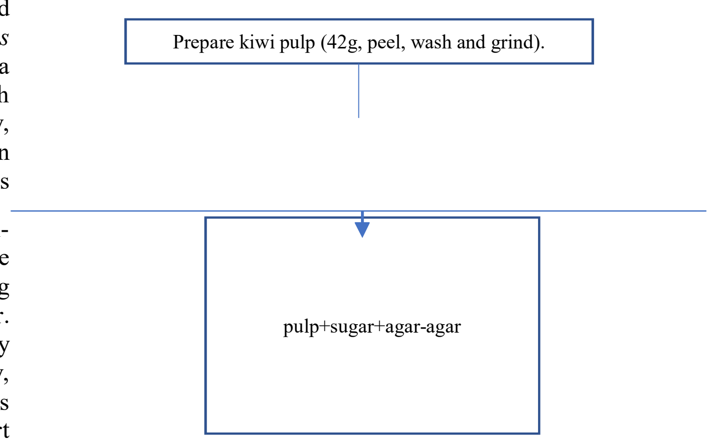
The kiwi fruits (250 g) were obtained from the 24*7 store in Greater Noida, Uttar Pradesh, India, and stored at 0-5 °C. *Cordyceps militaris* was produced in the Sharda University laboratory, and the dried powder was UV-treated in the lab only. Pectin, Xanthan gum and agar-agar were obtained from Store Direct Trading Pvt. Ltd. in Mumbai, India. This experiment also included corn syrup and sugar.

Gummies preparation (As in formulation table 1)

Formulation table

Ingredient	Control	Pectin	Xanthan Gum	Agar-Agar
Kiwi puree (g)	42	42	42	42
Cordyceps militaris (Vitamin D_2 enriched) powder (g)	2.5	2.5	2.5	2.5
Powdered sugar (g)	24.5	24.5	24.5	24.5
Corn syrup (g)	18	18	18	18
Stablising agent (g)	0	4.2	1.2	2.8
Citric acid(g)	1.2	1.2	1.2	1.2
Potassium sorbate(g)	0.1	0.1	0.1	0.1
Added water(g)	11.7	7.5	9.5	9
Total (g)	100	100	100	100

Table 1: formulation table for kiwi gummies



pulp+sugar+pectin

↓

stirring till desired consistenc(85-90°C)

↓

Cool mixture to <40°C

↓

Add Vitamin D2 enriched Cordyceps mushroom powder

↓

Pour into silicone molds, tap to remove bubbles

↓

Cool it for 45-60 min at room temperature and after that refridgerate @4°C

↓

demold and cure at 40-50% RH for 6-24hours

↓

Packaging and storage: airtight amber container, cold/dry storage

Adjust pH, Add citric acid (target 3.2-3.5 pH value) and potassium sorbate

Figure 1: Vitamin D2-fortified Kiwi gummies.

Analytical Methods

Water activity (a_w)

The water activity was measured with a Hygro-Palm GP23 device from Rotronic, Switzerland. About 10 g of gummy candy was carefully cut into small pieces with sterilized scissors and placed in sealed measuring vials. They were then left to settle at room temperature for at least 30 minutes. After equilibration, the samples were promptly transported for measurement utilizing the a_w mode [11]. The findings are displayed as mean values accompanied by standard deviation, with measurements conducted in triplicate.

Determination of pH Value

The pH value of a gummy solution was measured using a pH meter (Five Easy FE20, Mettler Toledo, Zürich, Switzerland) in accordance with the methodology outlined by [12]. To achieve this, approximately 4 g of candies (equivalent to 2 gummies) was combined with 2 mL of demineralized water and cooked at 50°C for 30 minutes until fully dissolved. The dissolved candies were quantitatively transferred to a 10 mL flask and allowed to cool at room temperature for a brief period (15 minutes) [11]. The measurements were conducted in triplicate, and the results are reported as the mean accompanied by the appropriate standard deviation.

Texture profile analysis (TPA)

Texture profile analysis (TPA) was conducted utilising the TA.HD.Plus Texture Analyser (Stable Micro Systems; Godalming, UK), fitted with a 5 kg load cell. Measurements were performed at ambient temperature. The experiment employed a cylindrical plate with a diameter of 50 mm. The experimental configuration comprised two consecutive compression cycles to a distance of 1 mm, commenced with a trigger force of 509.9 g, with a 5-second pause between cycles. The testing velocity was documented at 5 mm/s. The parameters were measured from the force–time curve: hardness, cohesiveness, springiness, and chewiness [13]. Data were gathered using Texture Exponent (v6.2) from Stable Micro Systems. Measurements were performed in triplicate, and the results are expressed as the mean with standard deviation.

Sensory Evaluation

A panel of 20 people conducted sensory analysis internally. Ten participants were staff from the Faculty of Food Science and Technology with expertise in sensory evaluation, while the remaining ten were students of food technology. A comprehensive evaluation measured the intensity of appearance, texture, flavour, chewability, aftertaste, and overall acceptability using a hedonic scale from 1 to 9, where 1-4 signified "no intensity," 5-8 indicated "moderate intensity," and 9 represented "extreme intensity." “General acceptance” was evaluated on a hedonic scale ranging from 1 to 9, where 1 signifies “not acceptable,” 5 denotes “moderately acceptable,” and 9 indicates “extremely acceptable” [14]. The samples were categorised into three groups: pectin, xanthan gum, and agar-agar. All samples were labelled and assessed in daylight and at ambient temperature. The panelists were provided with plain rice crackers and mineral water in separate cubicles to refresh their palates between tests.

Vitamin D2 retention analysis

Vitamin D_2 retention in kiwi gummies was evaluated by measuring vitamin D_2 content at different storage intervals (0, 30, 60, and 90 days) and expressing the results as a percentage of the initial concentration. Vitamin D_2 quantification was performed using high-performance liquid chromatography (HPLC) equipped with a C18 reverse-phase column and UV detection at 265 nm. The mobile phase consisted of methanol and water (95:5, v/v) at a flow rate of 1.0 mL/min. Calibration was carried out using standard vitamin D_2 solutions, and retention was calculated as:

$$\text{Vitamin } D_2 \text{ Retention (\%)} = (\text{Vitamin } D_2 \text{ at time t} / \text{Vitamin } D_2 \text{ at day 0}) \times 100$$

The results were expressed as percentage retention relative to the initial vitamin D_2 content.

Microbial Analysis of the Kiwi Gummies

- **Total Plate Count (TPC):** Total Plate Count was determined using the pour plate method. One millilitre of diluted sample was inoculated into sterile Petri plates, followed by the addition of Plate Count Agar and incubation at 35–37°C for 24–48 hours. Colonies were counted and expressed as CFU/g.
- **Yeast and Mould Count:** Yeast and mould counts were determined using Potato Dextrose Agar (PDA) by the spread plate method and incubated at 25–28°C for 3–5 days. Colonies were counted as CFU/g.
- **Coliform Count:** Coliforms were estimated using Violet Red Bile Agar (VRBA) through the pour plate technique and incubated at 35–37°C for 24 hours. Typical colonies were counted and expressed as CFU/g.

  Microbial load was calculated using the standard formula:

$$\text{CFU/g} = (\text{Number of colonies} \times \text{Dilution factor}) / \text{Volume plated}$$

Microbial counts were expressed as colony-forming units per gram (CFU/g). All analyses were conducted in triplicate to ensure accuracy and reproducibility [15].

Statistical analysis

All experiments were conducted in triplicate to ascertain the statistical significance of the data. The mean values and standard deviations were computed and later displayed. Statistical analysis was conducted utilising one-way ANOVA, followed by Tukey’s test, and performed with SPSS software. A p-value under 0.05 was deemed significant for all experimental data.

RESULTS AND DISCUSSION

During a 90-day storage period, the formulated products were tested for key quality characteristics, including water activity, texture profile, sensory acceptability, microbiological stability, and vitamin D_2 retention.

Water activity (a_w) during 0-90 days of storage

Water activity (a_w) is a critical parameter influencing microbial stability and shelf life of confectionery products. The control sample exhibited the highest water activity during storage, increasing from 0.617 at day 0 to 0.721 at day 90, which may increase the susceptibility of the product to microbial spoilage.

In contrast, the incorporation of hydrocolloids significantly reduced the water activity of the gummies. Among the tested formulations, pectin gummies consistently exhibited the lowest a_w values (0.512–0.589) throughout storage compared with xanthan gum and agar-agar formulations. The reduced water activity may be attributed to the high water-binding capacity and gel-forming properties of pectin, which effectively immobilize free water within the gel network. Similar observations have been reported in a study, where hydrocolloid-based matrices significantly influence moisture mobility and water binding in confectionery gels [16, 17]. Lower water activity is desirable because it reduces microbial growth and enhances the storage stability of food products (Table 2).

Storage Day	Control	Agar-agar	Xanthan Gum	Pectin
Day 0	0.617 ± 0.005[a]	0.602 ± 0.004[b]	0.575 ± 0.004[c]	0.512 ± 0.004[d]
Day 15	0.628 ± 0.003[a]	0.614 ± 0.003[b]	0.589 ± 0.004[c]	0.526 ± 0.003[d]
Day 30	0.644 ± 0.005[a]	0.630 ± 0.004[b]	0.603 ± 0.004[c]	0.542 ± 0.006[d]
Day 60	0.680 ± 0.008[a]	0.665 ± 0.006[b]	0.631 ± 0.008[c]	0.566 ± 0.008[d]
Day 90	0.721 ± 0.008[a]	0.697 ± 0.008[b]	0.668 ± 0.007[c]	0.589 ± 0.007[d]

*Table 2: Effect of hydrocolloids on water activity (a_w) during storage

Effect of pH during 0-90 days of storage

The pH of all gummy formulations dropped over the 90-day storage duration (Table 3). The initial pH values varied from 3.42 (pectin) to 3.58 (control). By day 90, they diminished to 3.25 (pectin) and 3.40 (control). One-way ANOVA indicated a significant influence of hydrocolloid type on pH across all storage intervals ($p < 0.05$). Pectin-based gummies consistently showed lower pH values (3.42–3.25), followed by xanthan gum (3.50–3.34) and agar-agar (3.53–3.36), whereas control samples revealed higher values (3.58–3.40) (Tukey's HSD). The reduction in pH may be ascribed to moisture redistribution and the production of organic acids during storage [18]. The comparatively reduced pH in pectin formulations may result from its robust gel network and enhanced acid retention ability [19]. The acidic pH range of 3.25 to 3.58 enhances microbiological stability and prolongs shelf life [20]. These results align with other research indicating the impact of hydrocolloids on the physicochemical stability of food gels [21].

Storage Day	Control	Pectin	Xanthan Gum	Agar-Agar
Day 0	3.58 ± 0.02[a]	3.42 ± 0.02[c]	3.50 ± 0.02[b]	3.53 ± 0.02[b]
Day 15	3.55 ± 0.01[a]	3.39 ± 0.02[c]	3.47 ± 0.02[b]	3.50 ± 0.02[b]
Day 30	3.50 ± 0.02[a]	3.35 ± 0.02[c]	3.43 ± 0.02[b]	3.46 ± 0.02[b]
Day 60	3.45 ± 0.02[a]	3.30 ± 0.02[c]	3.38 ± 0.02[b]	3.41 ± 0.02[b]
Day 90	3.40 ± 0.02[a]	3.25 ± 0.02[c]	3.34 ± 0.02[b]	3.36 ± 0.02[b]

*Table 3: Effect of pH during storage

Texture profile analysis during 0-90 days of storage

Texture is one of the most important quality attributes influencing consumer acceptance of gummy products. The results of texture profile analysis revealed that the type of hydrocolloid significantly affected the mechanical properties of the developed gummies (Table 4). Agar-agar produced the highest hardness (15.24 N), indicating the formation of a rigid gel structure. However, excessively high hardness may negatively affect chewability and overall product acceptability.

In comparison, pectin gummies exhibited a more balanced and desirable texture profile, characterized by lower hardness (2.63 N) but significantly higher cohesiveness (0.818), springiness (0.902), and chewiness (9.27 N×mm). These parameters indicate improved structural integrity, elasticity, and resistance during mastication. Similar findings were reported by Zhang et al. (2021) and Wang et al. (2023) [22, 23], who observed that pectin-based gel matrices provide superior elasticity and structural stability in gummy confectionery compared with other hydrocolloids.

Additionally, pectin gummies exhibited the lowest stickiness (0.098 N), which is advantageous for product handling and mouthfeel. The improved textural properties of pectin gummies may be attributed to the formation of a stable three-dimensional polysaccharide gel network capable of retaining moisture while maintaining elasticity.

Parameter	Control	Agar-agar	Xanthan gum	Pectin
Hardness (N)	5.26 ± 0.04[c]	15.24 ± 0.07[a]	9.51 ± 0.04[b]	2.63 ± 0.02[d]
Cohesiveness	0.375 ± 0.020[d]	0.493 ± 0.002[c]	0.615 ± 0.005[b]	0.818 ± 0.002[a]
Springiness	0.419 ± 0.002[d]	0.506 ± 0.002[c]	0.736 ± 0.003[b]	0.902 ± 0.002[a]
Chewiness (N×mm)	0.842 ± 0.003[d]	2.70 ± 0.02[c]	4.31 ± 0.02[b]	9.27 ± 0.09[a]
Stickiness (N)	0.110 ± 0.010[c]	0.251 ± 0.001[a]	0.182 ± 0.002[b]	0.098 ± 0.002[d]

*Table 4: Effect of different hydrocolloids on texture profile parameters

Sensory evaluation during 0-90 days of storage

Sensory characteristics are crucial factors determining consumer acceptance of confectionery products. The results indicated significant differences among treatments for all sensory attributes (Table 5). Pectin gummies obtained the highest scores for appearance (8.33), texture (8.67), flavour (8.00), chewability (8.00), aftertaste (8.33), and overall acceptability (8.00).

The superior sensory performance of pectin gummies may be attributed to their balanced texture, improved elasticity, and pleasant mouthfeel. Previous studies on hydrocolloid-based gummy formulations have also reported higher consumer preference for pectin-based gels due to their desirable chewiness and smooth texture [24, 25]. In contrast, xanthan gum and agar-agar formulations showed moderate sensory scores, while the control sample received the lowest ratings.

Attribute	Control	Pectin	Xanthan	Agar-Agar
Appearance	6.33 ± 0.58^{c}	8.33 ± 0.58^{a}	6.67 ± 0.58^{bc}	7.00 ± 0.00^{b}
Texture	5.33 ± 0.58^{c}	8.67 ± 0.58^{a}	7.00 ± 1.00^{b}	6.33 ± 0.58^{bc}
Flavor	6.00 ± 1.00^{c}	8.00 ± 1.00^{a}	6.67 ± 0.58^{c}	7.67 ± 0.58^{b}
Chewability	6.00 ± 1.00^{c}	8.00 ± 1.00^{a}	7.00 ± 1.00^{b}	6.33 ± 0.58^{bc}
Aftertaste	7.33 ± 0.58^{b}	8.33 ± 0.58^{a}	6.00 ± 1.00^{c}	6.67 ± 0.58^{b}
Overall acceptability	5.67 ± 0.58^{c}	8.00 ± 1.00^{a}	7.33 ± 0.58^{b}	7.00 ± 1.00^{b}

*Table 5: Sensory evaluation of hydrocolloid-treated samples (Mean ± SD)

Vitamin D_2 retention during 0-90 days of storage

The stability of vitamin D_2 during storage is an important consideration in the development of fortified functional foods. In the present study, vitamin D_2 retention gradually decreased during storage for all formulations. However, the degree of degradation varied significantly among treatments (Table 6).

Among the tested hydrocolloids, pectin gummies exhibited the highest vitamin D_2 retention, maintaining 86.67% of the initial vitamin content after 90 days, whereas xanthan gum and agar-agar retained 76.67% and 82.33%, respectively, and the control retained only 53.33%. The improved vitamin stability observed in pectin gummies may be attributed to the protective gel network that limits oxygen diffusion and protects vitamin molecules from oxidative degradation.

Storage Day	Control	Pectin	Xanthan gum	Agar-agar
0	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
30	83.67 ± 1.53^{c}	95.00 ± 1.00^{a}	86.33 ± 2.52^{b}	89.33 ± 3.06^{b}
60	69.00 ± 1.00^{c}	90.33 ± 0.58^{a}	81.67 ± 1.53^{b}	84.33 ± 4.04^{b}
90	53.33 ± 4.16^{c}	86.67 ± 1.53^{a}	76.67 ± 2.08^{b}	82.33 ± 4.73^{b}

*Table 6: Effect of hydrocolloids on Vitamin D_2 retention during storage

*** Values are presented as mean ± standard deviation (n = 3). Distinct superscript letters within the same row signify significant differences across treatments as determined by Tukey's HSD test ($p < 0.05$), after one-way ANOVA.**

Microbial stability during 0-90 days of storage

Microbial quality is a key factor determining the safety and shelf life of food products. In the present study, total plate count (TPC) and yeast and mold counts increased gradually during storage in all formulations. However, the magnitude of microbial growth differed significantly among treatments (Tables 7, 8, and 9).

The control sample exhibited the highest microbial load, reaching 3.83×10^5 CFU/g after 90 days of storage. In contrast, pectin gummies showed the lowest microbial counts, with a TPC value of 8.00×10^3 CFU/g at the end of storage. Similarly, yeast and mold counts were significantly lower in pectin samples compared with other formulations. The improved microbial stability of pectin gummies may be attributed to their lower water activity and compact gel network, which restricts microbial growth by limiting the availability of free water. Similar observations have been reported in recent studies where hydrocolloid-based gels significantly improved microbial stability of confectionery products during storage [26,27].

Days	Control	Pectin	Agar Agar	Xanthan Gum
0	ND	ND	ND	ND
15	$(5.23 \pm 0.20)\times10^2$	$(2.10 \pm 0.10)\times10^2$	$(4.97 \pm 0.15)\times10^2$	$(6.37 \pm 0.15)\times10^2$
30	$(1.80 \pm 0.10)\times10^4$	$(1.30 \pm 0.10)\times10^3$	$(1.10 \pm 0.10)\times10^4$	$(2.00 \pm 0.10)\times10^4$
60	$(9.33 \pm 0.35)\times10^4$	$(3.20 \pm 0.20)\times10^3$	$(2.80 \pm 0.10)\times10^4$	$(1.20 \pm 0.10)\times10^5$
90	$(3.83 \pm 0.15)\times10^5$	$(8.00 \pm 0.20)\times10^3$	$(4.37 \pm 0.15)\times10^4$	$(2.13 \pm 0.15)\times10^5$

Table 7: Total Plate Count (CFU/g) During Storage (Mean ± SD, n=3)

Days	Control	Pectin	Agar Agar	Xanthan Gum
0	ND	ND	ND	ND
15	$(2.2 \pm 0.1)\times10^2$	$(0.9 \pm 0.1)\times10^2$	$(1.95 \pm 0.05)\times10^2$	$(2.5 \pm 0.1)\times10^2$

30	$(1.45 \pm 0.05)\times10^3$	$(2.8 \pm 0.1)\times10^2$	$(1.2 \pm 0.1)\times10^3$	$(1.7 \pm 0.1)\times10^3$
60	$(3.4 \pm 0.1)\times10^3$	$(7.2 \pm 0.2)\times10^2$	$(2.8 \pm 0.1)\times10^3$	$(3.8 \pm 0.1)\times10^3$
90	$(6.1 \pm 0.2)\times10^3$	$(1.65 \pm 0.05)\times10^3$	$(4.7 \pm 0.1)\times10^3$	$(6.8 \pm 0.1)\times10^3$

Table 8: Yeast and Mould Count (CFU/g) during Storage (Mean ± SD)

Day	Control	Pectin	Agar Agar	Xanthan Gum
0–60 days	ND	ND	ND	ND
90 days	Trace ($<10^2$)	ND	ND	ND

Table 9: Coliform Test; ND(Not Detected)

Regulatory and Economic Feasibility: The market viability of kiwi gummies enhanced with vitamin D_2-enriched *Cordyceps militaris* powder was assessed by reviewing the regulatory frameworks governing functional foods and dietary supplements in India (FSSAI), the United States (FDA CFR Title 21), and the European Union (EFSA).

Regulatory Body	Compliance Status	Remarks
Food Safety and Standards Authority of India (FSSAI, India)	Permissible with appropriate documentation	Vitamin D can be used as a fortification; mushroom powder is permitted as a functional ingredient; labelling is required to include the percentage of the Recommended Dietary Allowance (RDA).
Food and Drug Administration (FDA,United States)	Considered suitable for use as a dietary supplement	Requires Supplement Facts panel and GRAS documentation for mushroom extract are necessary.
European Food Safety Authority (EFSA,Europe)	Permitted with restrictions on claims	Health claims necessitate a pre-approved dossier along with validation of vitamin stability.

Authorised functional claims differ across jurisdictions. General nutrient claims such as "Source of Vitamin D" or "Contributes to bone and immune health" are acceptable provided that the minimum established bioavailability is maintained. The combination of various hydrocolloids significantly influenced the physicochemical stability, sensory characteristics, and nutrient retention of kiwi gummies during storage. Pectin consistently demonstrated superior performance across most metrics. The findings revealed the lowest levels of water activity during storage, suggesting enhanced moisture control and a reduced risk of microbial growth. Pectin-based gummies demonstrated enhanced cohesiveness, springiness, and chewiness, indicating a more favourable elastic and stable matrix when compared to agar-agar, xanthan gum, and the control. The patterns observed in sensory evaluation supported this finding, as the pectin formulations exhibited the highest level of acceptability after 90 days, while the control sample showed a notable decrease. The preservation of Vitamin D_2 showed a similar pattern, with pectin exhibiting the greatest nutrient stability over time, followed by agar-agar and xanthan gum. In contrast, the control experienced the most considerable degradation. The findings demonstrate that pectin emerged as the most effective stabilising component for maintaining structural integrity, sensory quality, and nutritional value in kiwi gummies during prolonged storage.

CONCLUSION

The results of the present study demonstrated that the incorporation of hydrocolloids significantly influences the physicochemical, textural, sensory, nutritional, and microbiological properties of vitamin D_2-fortified gummies during storage. Among the tested hydrocolloids, pectin gummies exhibited superior overall performance, characterized by lower water activity, improved texture, higher sensory acceptability, better vitamin D_2 retention, and enhanced microbial stability.

Therefore, pectin is the most suitable hydrocolloid for developing fortified gummy formulations, as it improves product quality, stability, and consumer acceptability during storage.

DECLARATIONS

- **Ethics Approval:** The sensory evaluation was conducted based on the hedonic scale. The conducted study was non-invasive and involved minimal risk. As certified by the head of the department, formal ethical approval was not required for this study according to institutional practice. In addition, a letter from the Head of the Department confirming that the study was non-invasive and that formal ethical approval was not required has been provided.
- **Consent to Publish:** Not applicable.
- **Author Contributions:**

  Dolli Chauhan[1]: Conceptualisation, methodology, experimentation, data collection, and manuscript writing.

  Dr. Sujata Pandit Sharma: Supervision, review, and final approval of the manuscript.

  Shivani Kohli: Literature review, data collection.

  Nikita Singh: Data collection and manuscript formatting.
- **Clinical Trial Number:** Not applicable.

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