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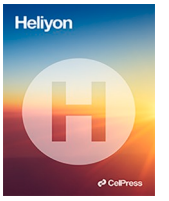

Research article

Formulation of functional gummy candies containing natural antioxidants and stevia

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ABSTRACT

The research aimed to enhance the nutritional value of gummy candies by incorporating pistachio green hull extract (PGHE), stevia, and starch into the formulations. The gummy candies formulations were optimized using PGHE (1–5 %), stevia (0.013–0.040 %) and gelatin-to-starch ratio (9:1, 2:8, and 3:7) by response surface methodology (RSM), central composite design (CCD), with six center points. The physicochemical and textural properties of the gummy candies were assessed. Three optimal formulations were determined, which were preferred by the majority of panelists. One of them was selected for testing total phenolic content (680.31 ± 0.6 mg GAE/100g gummy candy), antioxidant activity (IC_{50} = 277 μg/mL), FTIR analysis, morphology examination, and storage stability. This study resulted in the development of gummy candies that not only offer a reduced-sugar product (50 %; equal to 12 % of sucrose) with high antioxidant activity but also eliminate the need for artificial flavors and synthetic colorants in the formulation.

1. Introduction

In recent years, there has been an increasing demand for functional foods that promote healthy lifestyle and improve overall well-being. This growing interest from consumers in enhancing their quality of life has had a significant impact on the confectionery market. To meet the expectations of discerning consumers, confectionary manufacturers have been incorporating new ingredients into their products that cater to these needs [1]. Gummy candies, being hugely popular across all age groups, are suitable matrix for the addition of functional ingredients [2]. Traditionally, gummy candies are made using a combination of sugar, water, and gelatin [3]. 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 [4]. Gelatin is often used in gummy candies because it provides a strong and transparent texture to the final product that is desirable to consumers. But since pigskin is the primary source of gelatin in the global market, it is often controversial for religious believers [5]. Therefore, it is imperative to use a suitable substitute for gelatin in the production of gummy candies. Modified starch is widely used, alone or in combination with other gelling agents, for making gummy candies due to their good gelling properties [6]. They can be added in varying amounts to achieve the desired texture. Sugar is another key ingredient in gummy candies formulations, but it may not be

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suitable for individuals with diabetes or other health conditions that require them to limit their sugar consumption.

Stevia, as a natural, zero-calorie sweetener, can be used as a sugar substitute in food formulations. It is a suitable option for individuals looking to reduce their sugar intake [7]. Recently, plant extracts have been used to add flavor, aroma, and nutritional value to various food products [8]. By formulating gummy candies with plant extracts, manufacturers can offer consumers a tasty snack with added health benefits. The use of plant extracts in gummy candies formulation has been the subject of numerous studies. For example, a study in 2019 investigated the use of eggplant peel extract in gummy candies. It found that adding 1.5 % of free extract and spray dried powder improves the color properties and acceptability of the product [9]. Another study published in 2023 looked at the use of red onion extract in gummy candies. The study found that red onion extract can increase antioxidant activity and improves sensory properties [10]. Teixeira-Lemos et al. showed that gummy jellies formulated with orange juice, berry puree, and honey can produce healthier formulations with antioxidant properties while also improving organoleptic properties [2]. Charoen et al. (2015) reported that *Psidium guajava* leaf extract can be used as an antioxidant supplement in gummy jellies [11]. Various studies have examined the potential inclusion of phenolic compounds from sage [12], ginger [13], bougainvillea [14], and rosemary [15] as antioxidants or nutraceutical ingredients in gummy candies.

Pistachio green hull (PGH) is a by-product of pistachio processing that contains numerous antioxidant-active phytochemicals such as phenolic acids, flavonoids, and tannins [16,17]. Previous research has demonstrated the significant antioxidant, antimicrobial, and health benefits of PGH, including its potential to prevent hypertension, mutagenesis, and diabetes [18]. However, as far as we know, no research performed on the application of PGH extract as a natural antioxidant source and colorant in gummy candies.

Hence, the objective of this study was to investigate the effects of replacing gelatin with starch, substituting sugar with stevia, and incorporating PGH extract on the texture, color, antioxidant properties, and storage stability of gummy candies. One limitation of our study was the potential alterations in the composition of new ingredients and their interactions within the gummy candies formulations, which were investigated by establishing optimal conditions through the RSM methodology. The importance of this research lies in the production of functional gummy candies that not only improve its overall nutritional profile but also enhance its antioxidant properties.

2. Materials and methods

2.1. Materials

The pistachio green hull used in this study was obtained from Kerman Agricultural Research Center in Kerman Province, Iran. Gelatin (Bloom number 220, Pooran powder, Tabriz, Iran) and modified starch potato (Acetylated Starch, E1420, KMC, Denmark) were utilized as gelling agents. Stevia powder (food grade, purity 98 %, Germany) was provided by Techfa Co. Glucose syrup with a dextrose equivalent (DE) of 42 and a Brix value of 80 was obtained from Zar Fructose Co. (Alborz, Iran). In addition, Folin-Ciocalteu reagent, gallic acid, 1, 1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), and sodium carbonate, were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Preparation of pistachio green hull extract

Pistachio green hull powder (mesh 40) was mixed with distilled water in a ratio of 1:15 and stirred for 8 h at 25 °C. The mixture was then centrifuged for 10 min at 3000 g, and the supernatant was filtered through Whatman No. 42 filter. The extract was subsequently dried using a spray dryer. The resulting powder was stored in a sealed container at −20 °C until the test was conducted [19].

2.3. Total phenolic content (TPC)

TPC was determined by the Folin-Ciocalteau method [20]. A 20 μL aliquot of aqueous PGHE was mixed with 100 μL of Folin-Ciocalteau reagent and 300 μL of Na_2CO_3 (20 % w/v). The mixed solution was allowed to stand for 45 min in the dark. The absorbance of the solution was measured at 760 nm. Results were expressed as mg gallic acid equivalent (GAE) per g dry weight of extract.

2.4. $DPPH^{\bullet}$ assay

DPPH radical scavenging activity was determined according to the method of Lalegani et al. [20]. Different concentrations of the extract (0.3 mL) were mixed with 2.7 mL of $DPPH^{\bullet}$ in methanol (0.1 mM). The reaction mixture was shaken and incubated for 45 min at room temperature in a dark place. The absorbance was read at 517 nm against the blank. The scavenging activity was calculated using Eq. (1). The IC_{50} value was used to evaluate antioxidant activity. Ac and As are absorbance of control and sample, respectively.

$$\text{Free radical scavenging activity } (\%) = \frac{\text{As} - \text{Ac}}{\text{Ac}} \times 100 \quad (1)$$

2.5. LC/MS determination of phenolic compounds of PGH extract

The analysis of phenolic compounds in PGHE was carried out using a LC/MS system, specifically an agilent 6150 single quad mass

spectra, equipped with an agilent1260 binary pump, degasser, column heater (40 °C) and 1367C autosampler. To achieve chromatographic separation, a waters XBridge C18 column (150 × 4.6 mm, 5 μm) was utilized. Solvent A consisted of H_2O + 0.1 % (v/v) formic acid, and solvent B consisted of acetonitrile + 0.1 % (v/v) formic acid. The following gradient conditions were applied: 0–3 min, 0 % B; 3–9 min, 3 % B; 9–24 min, 12 % B; 24–30 min, 20 % B; 30–33 min, 20 % B; 33–43 min, 30 % B; 43–63 min, 40 % B; 63–67 min, 100 % B; 67–72 min, 100 % B. The system was then equilibrated for 7 min, resulting in a total run time of 72 min. The flow rate was 1.0 mL/min, and the injection volume was 5 μL. UV–Vis spectra of phenolic compounds were recorded at 260 nm. Identification of the phenolic compounds was accomplished by comparing retention times, UV–Vis spectra, scan mass spectra (50–1350 amu), and MS fragmentation patterns with their corresponding standards analyzed under identical conditions, as well as previous literature reports [21].

2.6. Gummy candies production

The aqueous gelatin solution was prepared by dissolving gelatin in distilled water and heating it in a water bath at 80 °C for 20 min, with regular manual stirring. Similarly, a starch solution was prepared using the same procedure, but this time the water bath was kept at 90 °C, and the mixture was stirred for 10 min. Glucose syrup and sugar were mixed and heated in a water bath at approximately 100 °C until fully dissolved. The mixture was cooled to 90 °C. Next, the syrup and starch solution were added to the gelatin solution, and the entire mixture was stirred manually for 1 min. Stevia and PGHE were also added to the mixture and stirred for 2 min. The mixture was then heated in a water bath at 80 °C until it became clear. Afterward, the final formulation was subjected to pH measurement. The mixture was then transferred into starch molds and left at 25 °C for 24 h. Finally, the molds were removed, and the product was packed [6].

2.7. Moisture content and pH of samples

The moisture content of gummy candies samples was measured using a oven made by Memmert, Germany. The pH values were measured using the Metrohm-780 model made in Herisau, Switzerland. Both measurements were conducted according to the previously reported method [22].

2.8. Color parameters

The color parameters of the gummy candies, including L*, a*, and b*, were measured using a Hunter Colorflex Colorimeter (Hunter Lab, Reston, Virginia, USA). The L* value represents the luminance or lightness component and ranges from 0 (black) to 100 (white). The positive and negative a* values indicate red and green, respectively, while the positive and negative b* values indicate yellow and blue, respectively. Color changes in the gummy candies were calculated using Eq. (2) [23]:

$$\Delta E = [(L^{*c} - L^*)^2 + (a^{*c} - a^*)^2 + (b^{*c} - b^*)^2]^{(1/2)} \quad (2)$$

where superscript "c" refers to the color reading of the control sample that was used as the reference, and a larger ΔE indicates that the color has changed more than the reference sample.

2.9. Instrumental textural profile analysis (TPA)

The texture properties of the gummy candies were evaluated using a Brookfield Texture Analyzer equipped with a cylindrical probe with a diameter of 35 mm. The gummy candies were compressed at a cross-head speed of 1 mm/s, with a load cell capacity of 5 kg, a trigger force of 5 g, and a delay of 60 s between compressions. Hardness (g), cohesiveness, and springiness (mm) were measured according to the method described by Mutlu et al. [22].

2.10. Total phenolic content and antioxidant activity assessments of produced samples

For each gummy candy sample, 2 g was combined with 10 mL of 80 % v/v methanol. The mixture was homogenized using a stirrer at a speed of 100 rpm for 8 h. The homogenate was then solubilized by heating at 40 °C for approximately 10 min under stable magnetic stirring and subsequently centrifuged at 10,000 g for 10 min. The resulting supernatant was collected to determine the total phenolic content (TPC) and the $DPPH^{\bullet}$ radical-scavenging activity, as described in sections 2.3 and 2.4, respectively.

2.11. Sensory evaluation

A total of twenty-five panelists, selected from Tarbiat Modares University due to their familiarity with the overall acceptability characteristics of gummy products, participated in a sensory test using a 5-point Hedonic scale (Table S1). The appearance, taste, chewiness, adhesiveness, texture, color, and overall preference were assessed, with higher scores indicating higher preference [22].

2.12. FTIR

The functional groups and bonding arrangement of the ingredients in the optimal formulation were examined by ATR technique in the region from 4000 to 600 cm^{-1}. Additionally, FTIR spectra of the extract and stevia were recorded in the range of 4000 to 400 cm^{-1} using a FTIR spectrometer (Thermo Nicolet, USA) [24].

2.12.1. Scanning electron microscopy (SEM)

The morphology of the optimal sample, both in the presence and absence of PGHE, was studied using scanning electron microscopy (FESEM, MIRA III, Czech Republic). To prepare for imaging, the powders were mounted on an aluminum plate using double-sided adhesive tape, followed by coating with a thin layer of gold.

2.13. Microbiological analysis

Gummy candies (1 g of optimized formulation) was homogenized with a solution ringer (9 mL) to achieve a final dilution of 10^{-1}. Serial decimal dilutions were prepared using the same diluent. Mold and yeast counts were determined by incubating on Yeast Glucose Chloramphenicol Agar (YGC) at 28 °C for 5 days [25].

2.14. Experimental design

For optimization of gummy candies formulations, response surface methodology (RSM, central composite design (CCD)) was employed, consisting of three independent variables (Table S2). In total, thirty-four experimental trials were carried out, including six trials at the center point to assess the method's repeatability. The selected responses for analysis include ΔE, hardness, cohesiveness, springiness, pH, and moisture content. The experimental design of the gummy candies was conducted using Design Expert (version 7.0, Stat-Ease Inc., Minneapolis, MN, USA). Statistical analysis tables were generated using analysis of variance, and the significance of all variables was determined by calculating the F value. The p criterion was set at ≤ 0.05. Numerical optimization based on the multiple response (desirability) function was performed. To validate the predicted optimal concentration of the mixture and confirm compliance with the predicted response, experiments were conducted using the determined concentrations of variables under the same experimental conditions.

2.15. Statistical analysis

All experiments were performed in triplicate to determine the statistical significance of the results. Mean values and standard deviations were calculated and reported. Statistical analysis was conducted using one-way ANOVA followed by Tukey's test using SPSS software. Additionally, the Kruskal–Wallis test, implemented in SPSS, was employed for sensory evaluation. A p-value < 0.05 was considered in all experimental data.

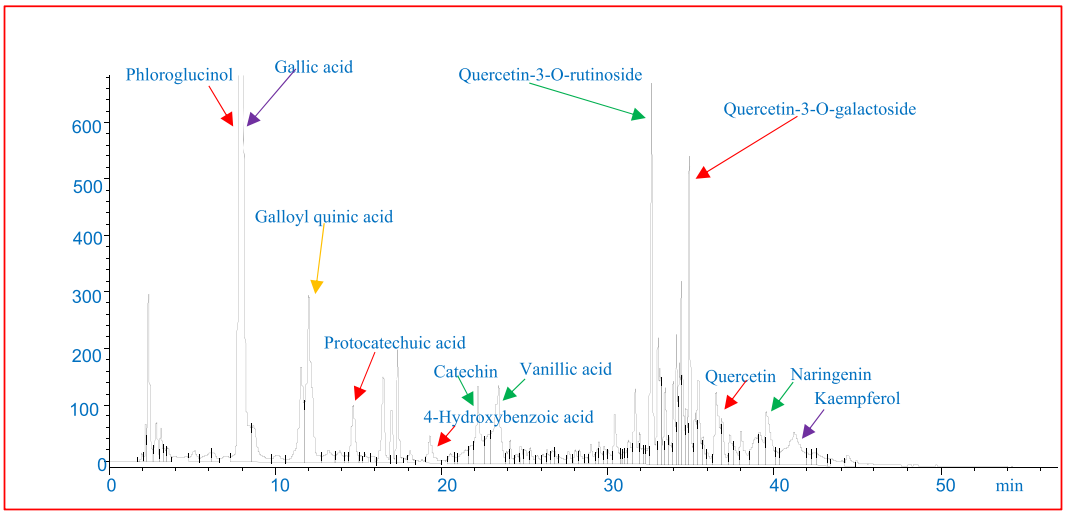

Fig. 1. Representative LC/MS chromatogram of phenolic compounds of pistachio green hull aqueous extract. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results and discussion

3.1. Total phenolic content, antioxidant activity, and identification of phenolic compounds of PGHE

The total phenolic content (TPC) and IC_{50} of the PGHE were found to be 522.9 ± 0.3 mg GAE/gdw and 130.0 ± 0.0 ppm,

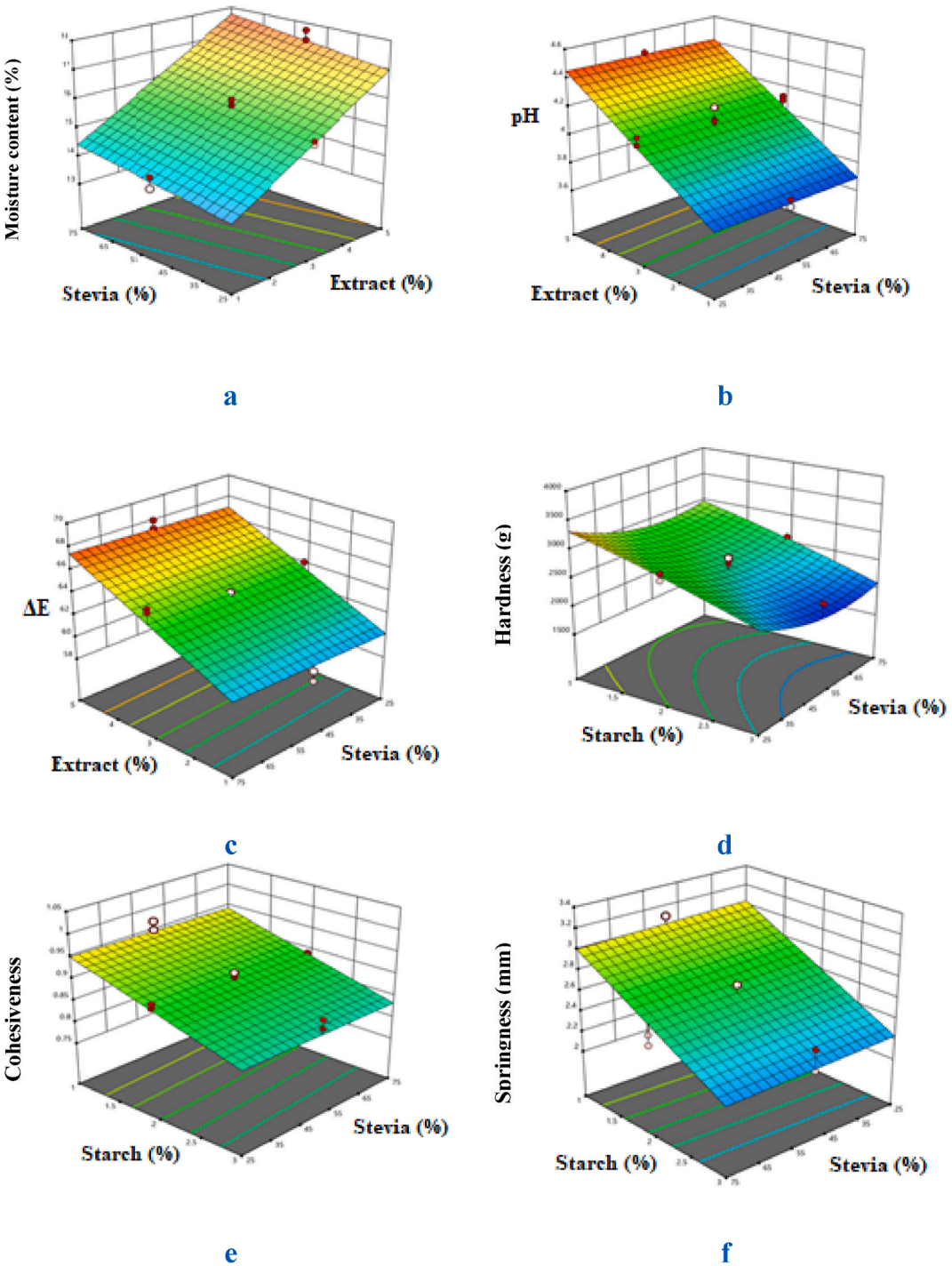

Fig. 2. The influence of variables (starch, stevia, and extract) on moisture content (a), pH (b), color change (c) and textural properties (hardness (d); cohesiveness (e); springiness (f)) of produced gummy candies. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

respectively. Previous studies have shown that PGH is a valuable source of phenolic compounds as natural antioxidants [17,20,23]. To determine the phenolic profile of the PGHE, its retention times and MS spectra were compared with pure standards and previous literature data. As seen from Fig. 1, the presence of 11 phenolic compounds in the aqueous PGHE, including four phenolic acids (gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, and vanillic acid), five flavonoids (quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin, naringenin, and kaempferol) and two tannins (galloyl quinic acid and phloroglucinol) is confirmed. Among these compounds, phloroglucinol, gallic acid, and quercetin-3-*O*-rutinoside were found to be the most abundant polyphenols in the extract, which is consistent with previous studies [18,20,21]. It is worth noting that catechin, quercetin, gallic acid, and protocatechuic acid exhibited the strongest free radical scavenging activity among the identified phenolic compounds [21].

3.2. Determination of moisture content and pH

Moisture content of gummy candies ranged from 13.00 to 17.95 % (w/w) and the pH ranged from 3.5 to 6.5 for the samples. The fitted models for stevia (X1), starch (X2), and extract (X3) were determined as linear (Fig. 2a and b), with R^2 values of 0.9625 and 0.9105, respectively. These models showed significant effects of stevia, starch, and extract on moisture content (Eq. (3)) and pH (Eq. (4)) ($p < 0.05$).

$$\text{Moisture content} = +11.915 + 0.015\ X_1 + 0.268\ X_2 + 0.836\ X_3 \quad (3)$$

$$\text{pH} = +3.55697 - 0.001\ X_1 - 0.007 X_2 + 0.184\ X_3 \quad (4)$$

All of the samples had an acceptable level of moisture content. The formulation with the highest moisture content (17.95 %) contained a high concentration of extract, stevia (low sugar, high glucose syrup), and starch. On the other hand, formulations with a low content of extract, stevia, and starch had the lowest moisture content (13.00 %). Increasing the amount of starch in candy formulations led to an increase in moisture content, as starch can bind more water due to its water-soluble hydroxyl groups. This increase in moisture content resulted in gummy candies with a softer texture [26]. Similarly, increasing the amount of stevia, which means adding more glucose syrup to achieve the desired soluble solid content, can also affect the moisture content of gummy candies. Glucose syrup has a higher moisture content than sugar, helping to maintain the moisture content of gummy candies. The incorporation of different concentrations of extract in the formulation of gummy candies can significantly affect the moisture content [27].

As shown in Fig. 2b, the pH of the gummy candies was only affected by the addition of extract, possibly due to the pH level of the PGHE (measured to be 6.0). However, the pH range of the tested samples (3.5–4.6) was suitable for gummies made with gelatin and starch. Maintaining the pH level of gummy candies is crucial to prevent instability or hinder the formation of a gel [28].

3.3. Color properties

This research investigated the color changes (ΔE) of gummy candies and their relationship to different combinations of extract, starch, and stevia (refer to Fig. 2c). A linear model used to analyze the color changes (refer to Eq. (5)), and a good correlation ($R^2 = 0.9084$) was observed between the experimental and predicted values for color changes.

$$\Delta E = +59.879 + 0.008\ X_1 - 0.795\ X_2 + 1.709\ X_3 \quad (5)$$

The color changes (Fig. 2c) observed in the gummy candies could be attributed to the varying levels of extract and starch used in their formulations. Increasing the level of PGHE in the gummy candies samples resulted in decreased lightness (L*) and increased redness (a*) and yellowness (b*) (data not shown). Consequently, the ΔE value, which indicates the magnitude of color changes in the samples, significantly increased ($p < 0.001$) with higher levels of PGHE. These color changes are likely caused by the presence of natural colorants in the extract, such as chlorophylls, carotenoids, and other pigments [29], which are sensitive to changes in pH and heat. The inclusion of PGHE in the production process of candies can lead to a color change, resulting in a greenish-brown or brownish hue. Similar effects on the color properties of ice cream due to the addition of PGHE have been reported by Ghandehari Yazdi et al. [23]. Furthermore, the addition of extract to gummy candies resulted in opacity. Additionally, the results indicate that the lightness (L*) value of the samples increased with higher level of starch, while the red/green (a*) and yellow/blue (b*) values decreased (Data not shown). Starch, being a white, turbid substance that reflects light, thereby causing the samples to appear lighter in color. Compared to the extract, the effect of starch on the color changes was relatively minor.

3.4. Textural properties

The hardness (1967–3572 g), cohesiveness (0.75–1.02), and springiness (2.0–3.4 mm) values of produced gummy candies containing different levels of starch, extract, and stevia were determined. The fitted models for these parameters were quadratic, linear, and linear respectively (Fig. 2d, e, and 2f), with R^2 values ranging from 0.88 to 0.95. The models for the effects of starch, extract, and stevia on hardness (Eq. (6)), cohesiveness (Eq. (7)), and springiness (Eq. (8) were found to be significant ($p < 0.001$).

$$\text{Hardness} = +4463.591 - 50.653\ X_1 - 111.623\ X_2 - 52.676\ X_3 - 0.049\ X_1 X_2 + 1.887\ X_1 X_3 - 57.797\ X_2 X_3 + 0.384\ X_1^2 - 39.314\ X_2^2 + 24.621\ X_3^2 \quad (6)$$

$$\text{Cohesiveness} = +1.096 - 0.001 X_1 - 0.055\ X_2 - 0.0288\ X_3 \quad (7)$$

$$\text{Springiness} = +3.806 - 0.001\ X_1 - 0.403\ X_2 - 0.115\ X_3 \quad (8)$$

The influence on hardness was observed to follow the order of extract > starch > stevia content (by changing the ratio of sugar and liquid glucose), indicating significant effects of these three factors ($p < 0.001$). Among the produced gummy candies, those containing 9 % gelatin, 1 % extract, and 0.013 % stevia exhibited the highest hardness, while the lowest hardness was observed in samples with 7 % gelatin, 5 % extract, and 0.04 % stevia. The addition of starch to the samples tended to decrease the hardness, likely due to starch interacting with water molecules and sugar, forming a weaker network compared to gelatin and resulting in a softer texture. Furthermore, the inclusion of glucose syrup in the gummy candies led to a soft texture, possibly due to the moisture-absorbing property of glucose syrup, which acts as a humectant depending on its degree of hydrolysis (DE), water content, and low molecular weight of sugars. Consequently, the increase in stevia and glucose syrup contents affected the total soluble solid, resulting in a reduction in the magnitude of the three-dimensional structure, as demonstrated by Fig. 2d. These findings align with previous research by Sęczyk et al. [30], supporting the concept that the phenolic compounds presented in the PGHE interact with proteins and starch, contributing to the hardness changes.

Cohesiveness, representing the internal resistance within the food structure, depended on the intramolecular interactions of the formula′s components. Increasing the gelatin and extract contents in gummy candies increased the texture cohesion.

The springiness of the samples increased as the ratio of gelatin to starch increased. Both starch and extract had a similar impact on springiness. It was observed that the springiness of the samples decreased when the PGHE increased to 5 %. It was discovered that altering the concentration of *Psidium guajava* extract resulted in a decrease in springiness. Additionally, the amount of herbal extract directly affects the structure of gummy jellies [11].

3.5. Optimization

The numerical optimization technique was employed to simultaneously optimize multiple responses. Our primary aim was to determine the optimal composition of the gummy candies, incorporating extract, stevia, and starch, which would yield desirable texture (hardness, springiness, and cohesiveness). To achieve this, three distinct types of gummy candies were produced, as outlined in Table 1. The extract concentration remained constant and unchanged throughout the entirety of the experiment. By utilizing an experimental design matrix and specifying desired conditions, a solution with a desirability value of 0.93. The evaluation of the empirical predicted gummy candies results revealed a close agreement between the experimental values and the predicted values ($p < 0.05$) (Table 1).

3.6. Sensory evaluation

To assess the preferences and overall acceptability of different optimum formulations of gummy candies, various characteristics such as appearance, taste, chewiness, adhesiveness, texture, color, and overall preference were evaluated. Based on Fig. 3, it was observed that gummy candies No. 1, No. 2, and No. 3 exhibited similar attributes in terms of appearance, color, and texture, with no significant differences detected ($p < 0.05$). The preference for chewiness and adhesion increased when higher gelatin ratios were used, along with a 50 % reduction in sugar content compared to a 75 % increase. Furthermore, no statistical differences in taste was observed between formulations No. 1 and No. 2, indicating that the extract did not impart any aftertaste in the gummy candies. However, adjustment in the level of stevia, as well as reduction in sugar content and increase in glucose syrup amount, could slightly affect the taste and adhesion of gummy candies, although these effects were not statistically significant. Consequently, out of the three suggested formulations, No. 2 was found to have a superior score compared to the others, warranting further analysis through TPC, $DPPH^{\bullet}$, SEM, FTIR, and storage stability.

3.7. Total phenolic content and antioxidant activity of optimal formulation

Based on the data analysis, the addition of 2 % extract to the gummy candies formulation led to a significant increase in the total phenolic content. The values for the total phenolic content were 680.31 ± 0.6 mg GAE/100g gummy candies. This increase can be attributed to the presence of phenolic compounds found in the PGHE and stevia [16,23,31,23,18].

Table 1
Three different optimized gummy candies formulations and their predicted and observed responses.

No	Variables					Responses		
	Predicted level (g)	Optimized formulation	Criteria		Importance	Predicted mean	Observed Mean	Error (%)
1	8:2 (gelatin/starch), 0.026 % (stevia)		hardness	maximize	++++	2803.75	2445	12.8
2	9:1(gelatin/starch), 0.026 % (stevia)	2	springiness	maximize	++	3.13	2.97	5.11
3	9:1 (gelatin/starch), 0.040 % (stevia)		cohesiveness	maximize	+++	0.97	0.88	9.28

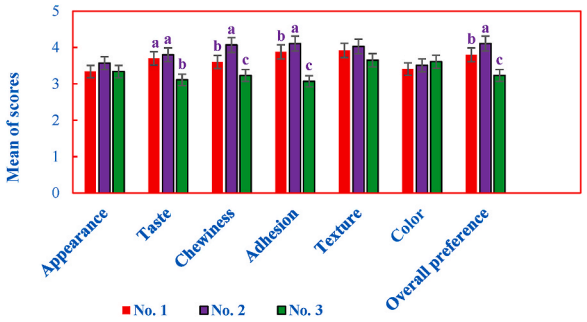

Fig. 3. Sensory analysis of gummy candies with optimum formulation. Columns without lower case letters are not significantly different (p<0.05).

The antioxidant activity of the samples was measured using an IC_{50} value, which represents the concentration of the substance required to scavenge 50 % of free radicals in a DPPH$^{\bullet}$assay. The results of the DPPH$^{\bullet}$assay showed that the PGHE significantly affected the antioxidant activity of the experimental gummy candies. The IC_{50} value for gummy candies containing 2 % extract was found to be at a concentration of 277 μg/mL. It is obvious that fortified gummy candies have high total phenolic content and strongly antioxidant activity (Table 2). Arjeh et al. have reported that PGHE contain significant amount of phenolic compounds, including gallic acid, quercetin, phloroglucinol, theogallin, galloyl derivatives catechin, and pyrogallol, which possess antioxidative properties [18]. In addition, stevia contains compounds called steviol glycosides that have been found to have antioxidant properties [31].

3.8. Infrared analysis

Fig. 4a, b, 4c and 4d presents FTIR spectra of the extract, stevia, control gummy candies and optimal gummy candies (No. 2), respectively. The extract exhibited characteristic peaks at the following wavenumbers: 3443.72 cm^{-1}(O–H stretching band of phenolic and hydroxyl groups), 2926.99 cm^{-1} (C–H bonding), 1578.09 cm^{-1} (C═C aromatic ring), 1412.46 cm^{-1} (bending vibrations of methyl (CH_3) groups), 1045.04 cm^{-1} (primary O–H groups), and 1016.56 cm^{-1} (the stretching vibrations of C–O alcohols). Additionally, peaks at 927.09 $cm^{-1}$and 651.09 cm^{-1} were attributed to C–H bending and ring puckering, respectively. These findings are consistent with existing literature [19].

The presence of functional groups in the stevia (Fig. 4b) is indicated by the peak at 3420.85 cm^{-1} to C–*O*–H bending vibrations, and the peak at 2936.05 cm^{-1}, which corresponds to the asymmetric and symmetric stretching vibrations of –CH, CH_2, CH_3. Additionally, peaks at 1727.15 cm^{-1} and 1577.21 cm^{-1} are assigned to the stretching vibration of the –C═O band, which is due to the presence of steviol glycosides and is also a characteristic band [32]. The FTIR spectra of the sample also showed bands around 1205.58 cm^{-1}, 1076.52 cm^{-1}, and 1037.94 cm^{-1}, which are characteristic absorption bands of the steviol glycosides [33]. The bands at 1417.7 cm^{-1} and 1337.66 cm^{-1} correspond to the bending vibration of the CH bond.

The FTIR spectrum of control gummy candies, shown in Fig. 4c, reveals that the major components of the gummy candies are water, sugar, glucose syrup and gelatin. The peak at 3283.34 cm^{-1} corresponds to the stretching vibrations of hydroxyl groups of phenolic and water. The peak at 2922.16 cm^{-1} is attributed to the stretching vibrations of C–H bonds in carboxylic acids and NH_4^+ bonds in free amino acids. The spectral range from 1500 to 1200 cm^{-1} is known to be a mixed region influenced by the bending of the –CH_2 and

Table 2
Comparison of enhanced bioactivity of extract studied with some recent reports.

Source of phenolic compounds	Results	Ref.
Sage (*salvia lavandulifolia* vahl) by-product extracts into novel jelly candies	Addition of 0.75 g of sage extract results in a TPC of 30–38 mg GAE/100 g	[12]
Red onion peel extract into gummy candies	Addition of 50 g of red onion extract resulted in a DPPH radical scavenging activity of 29.75 %.	[10]
Encapsulated *Melodorum fruticosum* Lour. in gummy jelly	FRAP antioxidant activity was ~6 μmol Fe^{2+}/g gummy jellies contain 30 ppm of Encapsulated *Melodorum fruticosum*.	[37]
Barberry extract in the confectionery products	TPC of chewing gum, jelly, and marshmallow containing 10 % of barberry extract was 392, 109.15, and 536.29 mg GAE/kg, respectively.	[38]
Natural fruits in gummy jellies	Antioxidant activities of the gummy jellies containing red fruit puree (54.5 %) and orange juice (86.2 %) were 83.7 and 50.4 mg TE/100 g, respectively.	[2]
Propolis extract, raspberry powder, and orange juice in two gelatin candies	Addition of 7.5 %, 5.2 %, and 10.9 % of propolis extract, raspberry powder, and orange juice, respectively to two candies showed TPC of 491.9 and 550.8 mg GAE/100 g, respectively.	[39]
Strawberry and red beetroot in jelly candies	Addition of 75 % strawberry + 25 % beetroot resulted in an AoA of 52.55 %.	[40]
Rosemary extract, fructan fiber, and stevia in jelly candies	Addition of 0.26 g rosemary extracts increased TPC and AoA to 410.79 μg GAE/g and 4.14 μmol Trolox/g, respectively.	[15]
Red beet extract in gelatin/gellan based gummy candy	Addition of red beet extract (0.3 %) increased the AoA upto 50 %.	[41]
Pistachio green hull extract and stevia in gummy candies	Addition of 2 % pistachio green hull extract resulted in a TPC and IC_{50} of 680.31 ± 0.6 mg GAE/100g and 277 μg/mL gummy candies.	This study

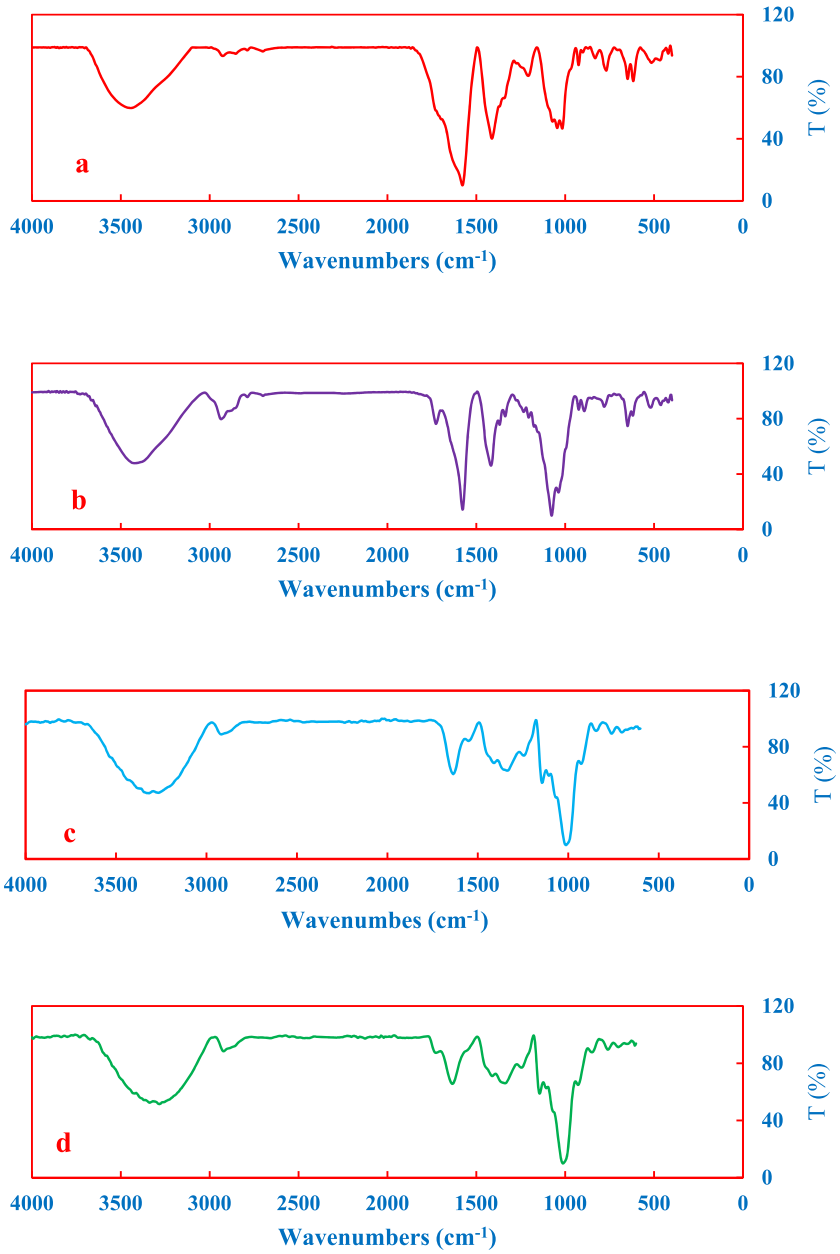

Fig. 4. IR spectra of green hull aqueous extract (a), stevia (b), control gummu candies (c), optimal formulation of gummy candies (d). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

–CH_3 groups in proteins and by the C–H bending vibrations of carbohydrates. Additionally, the observed peak at 1000 cm^{-1} has been known as the characteristic vibrational band of glycosidic bond of sucrose [34]. The peak at 760 cm^{-1} corresponds to the (C–O) bonds in sucrose, indicating the presence of saccharides. The FTIR spectrum of the optimal gummy candies formulation (Fig. 4d, with PGHE and stevia) closely resembled the spectrum of the control gummy candy (without PGHE and stevia), with the bands of PGHE and stevia being largely overlapped. This observation suggests a physical entrapment of phenolic compounds within the gummy candies matrix. These findings align with previous reports on PGH encapsulation [35]. Notably, the absence of a peak around 1750 cm^{-1} in the spectrum of the control gummy candies, as compared to the spectra of stevia and the optimal gummy candies, indicates the stretching vibration of the –C═O band, which is due to the presence of steviol glycosides.

3.9. Morphology of samples

Fig. 5a and b illustrate the morphological structures of the gummy candies with and without PGHE. In the control sample (without

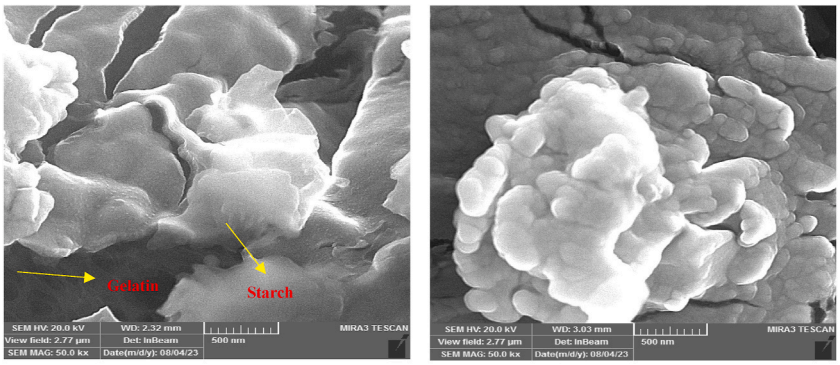

Fig. 5. SEM micrograph of (a) gummy candies without PGHE, (b) gummy candies containing green hull aqueous extract. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

extract), the gelatin displayed a spongy and regular appearance, while the swollen starch granules had disconnected from the gelatin network (Fig. 5a). When two proteins and/or polysaccharides are mixed together, phase separation can occur, leading to a less compact appearance [6]. The gummy candies containing PGHE had rough surfaces with granular protrusions (Fig. 5b). These structures are likely attributed to the extract used in this formulation. Consequently, the addition of PGHE led to a denser texture, which aligns with the observed hardness in the texture profile analysis (TPA) tests. It is worth noting that phenolic compounds have the ability to interact with other ingredients presented in the food matrix, potentially reducing the bioavailability of these compounds. It was reported that interactions between phenolic compounds and white bean components resulted in a significant decrease in the free phenolic content in the white bean paste [30]. However,it is important to note that these interactions can also have a positive effect. They can contribute to the release of phenolic compounds and enhance oxidative stability during the gastrointestinal passage [36].

3.10. Storage stability

The shelf life study revealed that, when exposed to temperature 35 °C over a storage period of 90 days, the TPC of the gummy candies gradually decreased (Fig. S1). Although statistically significant differences were observed during the study, but at the end of the storage period, the gummy candies still contained approximately 600 mg GAE/100g. It is worth noting that the stability of the free PGHE was found to be lower than of the microencapsulated PGHE after 60 days of storage [35]. In addition, it has been demonestrated that the anthocyanin content of gummy jellies decreased over an eight-week storage period [37].

The data obtained from the microbial analysis of functional gummy candies revealed that there was no presence of yeast and mold throughout the 90-day storage period at 35 °C. Consequently, this study provides evidence that gummy candies containing PGHE and stevia are safe for consumption by humans.

4. Conclusions

This study successfully optimized the formulation of gummy candies by employing a RSM combined with a CCD, focusing on the ratio of gelatin to starch, PGH extract, and stevia. The incorporation of 2 % of PGH extract and 0.026 % of stevia (~12 % sucrose) allowed for the production of functional gummy candies. The resulting samples exhibited desirable physicochemical properties, enhanced bioactivity, and a reduced sugar content. Additionally, the utilization of this by-product provided a natural color and an acceptable taste to the gummy candies. Consequently, the addition PGH extract, which is rich in polyphenolic compounds, to the gummy candies offers a natural approach to improving the nutritional of such foods that typically possess low nutritional content. The results of this study have been presented and compared with other reports in Table 2, indicating the relative advantage of this research. Finally, further *in-vivo* tests are necessary to validate these findings. However, *in-vivo* tests are imperative to prove our findings. Furthermore, the encapsulation of PGHE is advised prior to its incorporation in food product formulations. The encapsulation of the extract could significantly bolster the stability of phenolic compounds within the product matrix.

Ethics statement

Our experiments were conducted according to established ethical guidelines, and informed consent obtained from the participants. Also, we provide confirmation that the study complies with all regulations and confirmation that informed CONSENT was obtained.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable

request.

CRediT authorship contribution statement

Mozhgan Roudbari: Writing – original draft, Investigation, Formal analysis. **Mohsen Barzegar:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Mohammad Ali Sahari:** Writing – review & editing, Investigation. **Hassan Ahmadi Gavlighi:** Writing – review & editing, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31581.

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