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Valorization black carrot colorant process liquid waste by clarification and Decolorization: A novel sugar alternative for gummies

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ABSTRACT

The aim of this study was to develop a recycling process for black carrot colorant liquid waste (BCLW) and to investigate the potential use of BCLW as a sugar source for glucose syrup substitution in gummy candy production. Clarification and decolorization were performed using ion exchange and adsorbent resins at three flow rates, followed by evaporation. The highest clarity (88.7 %) was achieved with modified styrene-divinylbenzene resin at 1.0 BV/h. Subsequently, BCLW was incorporated into gummy formulations as a glucose syrup substitute. Higher hardness values were recorded in formulations with over 75 % of BCLW incorporated, compared to the gummy samples produced with 100 % glucose syrup. The brightness remained considerably high when the glucose syrup was replaced with BCLW up to 50 %. Accelerated shelf-life tests showed changes in color and hardness. BCLW presents a sustainable alternative for the confectionery industry, offering a practical solution for waste reduction while contributing to resource efficiency.

1. Introduction

Gummy candies, a type of confectionery, are prepared sugar as the main ingredient, along with gelling agents, acids, aromas, and food colorants to enhance texture and taste (Periche et al., 2014). Sucrose, the most used sweetener, influences gel structure, texture, thermal stability, flavor profile, and sensory characteristics of gummy confections (Wang et al., 2024). Moreover, sucrose serves as a bulking agent and contributes to the total mass of the gummy. It is typically combined with glucose syrup, as glucose syrup enhances sucrose solubility, delays crystallization, reduces water activity and prolongs the product's shelf life. High sugar addition enhances the sweetness of gummies, increasing their appeal for consumption while also influencing their textural properties (Wang et al., 2024). However, the utilization of glucose syrups as a sugar source in gummies raises concerns regarding potential health issues associated with excessive sugar intake. Hence, there is a need to replace glucose syrup with natural and healthier alternatives. Recent studies have focused on partial or complete replacement of glucose syrup with fruit juices, purees, natural sugars, honey, and polyols (Bouphun et al., 2023; Ghodsi & Nouri, 2024; Kurt et al., 2021; Rivero et al., 2020). Kurt et al. (2021) showed that glucose syrup can be substituted with grape, mulberry, and carob molasses in gummies. Kaewpetch et al. (2024) optimized the gummy formulation using honey, xylitol, and gelatin to develop healthier alternatives. Čižauskaite et al. (2019) increased the flexibility of gummies by 27 % with 100 % substitution of glucose syrup using agave syrup.

Color is an important sensory attribute of foods affecting the consumer's perception and the commercial success of food products. In consumers' perception, it links with food quality attributes such as flavor, quality, and nutritional content (Kurt et al., 2021). Food colorants are used to maintain standard color in food products to attract consumers. Although synthetic colorants are more stable in extreme conditions, demands for natural colorants have increased due to health concerns related to synthetic color additives (Sigurdson et al., 2017). Natural colorant pigments are derived from a variety of natural sources such as grape, berry, paprika, beet, saffron, and carrot (Gordillo et al., 2018; Jara-Palacios et al., 2019; Mehrzad et al., 2024). Black carrots are good sources of natural pigments (Kirca et al., 2007). Black carrot

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(Daucus carota ssp. sativus var.) anthocyanins are the most abundant natural colorants in terms of anthocyanin content. Black carrot extracts are highly preferred as natural colorants in the food industry due to their exceptional stability against pH and temperature changes (Akhtar et al., 2017). In the black carrot colorant production process, substantial quantities of liquid waste have been generated. This by-product is rich in anthocyanins and contains high levels of sugars, making it a valuable product (Agcam et al., 2021). However, the studies on the reuse of black carrot liquid waste (BCLW) are limited and mostly focus on residuals of black carrot juice or shalgam production (Kamiloglu et al., 2016). Furthermore, disposal of this waste presents significant challenges for colorant producers due to high constitute content, leading to additional costs and ecological concerns associated with its removal. Generally, it is utilized in the nutritional enrichment of animal feed, but it possesses the potential to be converted to value-added products (Seregelj et al., 2020). Black carrots are rich in macro and micronutrients such as anthocyanins, fibers, proteins, and sugars. Fructose, glucose and sucrose were quantified with a total concentration of 404.1 g/kg in black carrot powder (Kammerer et al., 2004). After natural pigment production, black carrot residuals contain high sugar levels, making the waste a potential sugar

Black carrot liquid waste (BCLW) can be used as a sugar alternative. However, the presence of residual pigments and the waste's high water content pose challenges in using BCLW as a sugar replacement. Additional treatments are necessary to remove pigments and other constituents (e.g., proteins) and lower the water content. The drying process is the most widely used technique to reduce the water content of liquid wastes (Ozer et al., 2024). However, it requires high energy consumption and may not be suitable for heat-sensitive compounds, which may degrade or convert into undesirable forms. Therefore, alternative methods must be developed to increase the potential use of BCLW in food production. BCLW has good sugar levels and can be used in confectionery products. However, its water content is high to maintain desired characteristics in gummies. Kurt et al. (2021) introduced fruits grape, mulberry, and carob molasses with 78-79°Bx into gummy formulations instead of glucose syrup. Each molasses contributed differently to the development of gummies' appearance, texture, and crystallization properties due to their sugar profiles. Pekdogan Goztok et al. (2024) replaced glucose syrup with fruit juice concentrates (pomegranate, grape, and sour cherry) in marshmallow production. They revealed that fruit juice concentrates exhibited a potential alternative for glucose syrup substitution. Additionally, the existence of natural pigments in BCLW leads to visual problems such as color and turbidity and limits the application area of BCLW (Dereli et al., 2015). Sugar syrups are colorless and transparent (Susanto et al., 2016). Generally, ion exchange resins and adsorbent resins are used to remove colorant pigments from liquid products (Liang et al., 2019; Sharma et al., 2011). This improves the visuality of the syrups by eliminating pigments and enables their utilization in various areas. To the best of our knowledge, a new process was developed for the first time for the valorization of BCLW as a glucose syrup alternative for gummy candies. The optimum process conditions for decolorization, clarification, and concentration of BCLW were determined according to the physicochemical properties of the final BCLW product. Subsequently, BCLW was fully or partially substitute glucose syrup in gummies and its effects on the physico-chemical properties, texture, and shelf life of gummy candies were investigated to evaluate its potential as a glucose syrup substitute.

2. Materials and methods

2.1. Materials

In the preparation of gummy samples, water, sucrose (Konya Sugar, Konya, Turkey), 42 DE glucose syrup (Sunar, Adana, Turkey), and 250 bloom gelatin (Gerede Jelatin, Bolu, Turkey) were used. BCLW was

obtained from an industrial coloring agent producer (X Company, Kocaeli, Turkey).

2.2. Study design

BCLW (~12.0°Bx), a by-product from industrial pigment extraction production, was subjected to clarification, decolorization, and evaporation. The process involved passing liquid waste through the anion and cation exchange resins, followed by decolorization with two different adsorbent resins (Crosslinked Polystyrene and Modified Styrene) at three different flow rates (1.0, 1.5, 2 BV/h) and evaporated to concentrate it to over 65.0°Bx. Following treatment, the BCLW samples were characterized, and the optimal conditions for achieving the highest clarification value (T625) of 88.7 % were determined. The BCLW produced under optimal conditions was used to produce gummies. Gummy samples were prepared using formulations established through a Custom mixture design study, with BCLW, glucose syrup, and gelatin as the independent variables. The gummies were then characterized (total soluble solids, pH, water activity, moisture content, and color), and their stability (including color, texture, and water activity) was evaluated under accelerated shelf-life conditions (25 °C, 70 % RH).

2.3. Black carrot extraction process liquid waste

2.3.1. Clarification and Decolorization

The research employed black carrot beet waste (BCLW), a byproduct with $\sim 12.0^{\circ}$ Bx soluble solids (20.0 \pm 5.00 °C, pH <3.50), generated during the pigment extraction phase in black carrot extract powder production (X Company, Turkiye). To clarify and decolorize the BCLW, the fluid waste was loaded into columns with a 4 cm diameter and subjected to procedures optimized through preliminary studies. The BCLW subsequently was passed through a cation exchange resin (Macro-Prep 25S, Bio-Rad, Turkey) at 2 BV/h, and then treated with Macro-Prep High Q anion exchange resin (Bio-Rad, Turkey) under the same flow rate, maintaining a pH of 8.00 \pm 0.20. For samples with a pH of <3.5, the color removal was employed using two types of resins (Modified Styrene-divinylbenzene, 600.0 m²/g, 1.18 g/mL, and Crosslinked Polystyrene, $600.0 \text{ m}^2/\text{g}$, 1.02 g/mL) under three flow rates (1.00, 1.5, and 2.00 BV/h). They were then subjected to a cation exchange using Macro-Prep 25S resin (Bio-Rad, Turkiye) at a flow rate of 2 BV/h. To concentrate the clarified and decolorized samples to a soluble content of at least 65°Bx, evaporation was performed using a pilot-type evaporator (X Company, Kocaeli, Turkiye) operating at 65 °C and 600 mmHg vacuum. In this process, at least 500 mL of each sample was produced for each sample and stored in sterilized glass containers at 4 °C under dark conditions until further analysis. The adsorber type and flow rate yielding the maximum clarification (Transmittance at 625 nm) in BCLW were identified. Furthermore, all samples (n = 6) underwent analysis for clarification efficiency, Brix, pH, total acidity, color attributes (L*, a*, b*, chroma, hue angle), browning index (A420 nm), total phenolic content, protein content, ash, and sugar composition, as detailed in Section 2.3.2. In this study, BCLW with the maximum T625 nm value (88.7 \pm 0.38 %) was utilized in gummy production, designed as a model food and a flow rate of 1.0 BV/h using Modified Styrene-divinylbenzene (DVB) was used as an alternative to glucose syrup (Table 2).

2.3.2. Characterization of BCLW

During preliminary trials, the pH values of BCLW samples were recorded with a digital pH meter (PB-10, Sartorius, Germany) before and after each resin application and the evaporation process for all sample groups. After evaporation, the total solid content of the samples was determined in Brix (°Bx) using a handheld automatic digital refractometer (PAL, Atago, Japan).

The pH values of BCLW samples were measured using a digital pH meter (PB-10, Sartorius, Germany) during preliminary trials for all sample groups before and after each resin application and evaporation.

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In the clarification and color removal studies, the pH values after evaporation were determined. The total solid content of the samples after evaporation was measured in Brix (°Bx) using a handheld automatic digital refractometer (PAL, Atago, Japan). The ash content of the samples before and after evaporation, as an indicator of the inorganic residue remaining after complete combustion, was determined as a percentage (m/m) of ash content. For this purpose, 10 g of the sample was transferred to a pre-weighed and dried crucible and then the organic matter of BCLW was calcined in an ash furnace at 500 °C for 3 h. After combustion, the samples were cooled in a desiccator and weighed to determine the ash content (g/100 g).

The efficiency of the clarification process was evaluated using the spectrophotometric method (Zhao et al., 2014). After the evaporation process, the transmittance and absorbance of BCLW samples were analyzed at 420 nm and 625 nm, respectively using a UV-VIS spectrophotometer (Jasco UV/Vis Spectrophotometer, Japan). Color properties (L*, a*, b*, chroma, and hue angle) were determined using a colorimeter (Chroma Meter CR-400, Konica Minolta, Japan). The Kjeldahl method was used to determine the protein content (Nx6.25) of BCLW (Zhu et al., 2023). To measure the total acidity, BCLW was titrated with a 0.2 M sodium hydroxide solution in the presence of phenolphthalein indicator and results were reported as g citric acid equivalent/L. Total sugar content was determined using a titrimetric method with Fehling solutions, while the sugar profile (sucrose, fructose, glucose contents) of the BCLW samples was analyzed through an HPLC system with a refractive index detector. In sugar profile determination, an Aminex HPX-87H column (300 \times 7.8 mm) (Bio-Rad, Istanbul) was used at 55 $^{\circ}$ C, with a flow rate of 0.3 mL/min and a mobile phase of 6 % acetonitrile and 0.045 N H₂SO₄ (Kelebek et al., 2009). The total phenolic content (TPC) of BCLW was determined by Folin-Ciocalteu method described by (Renaldi et al., 2022) with some modifications. The BCLW samples (40 μL) diluted with methanol were mixed with 3.16 mL of distilled water and 200 μL of Folin-Coicalteau reagent. Later, 600 μL of 20 % Sodium Carbonate solution (Merck, Germany) was added. The mixture was kept at room temperature in the dark for 2 h, and the absorbance was measured at 765 nm wavelength using a spectrophotometer (Jasco UV/ Vis Spectrophotometer, Japan). The results were calculated from the calibration curve of gallic acid and reported as mg of gallic acid equivalent (GAE)/kg. The antioxidant activity potentials of the samples were determined as % inhibition values using the DPPH method (Demirci et al., 2024).

2.4. Gummy samples

2.4.1. Sample preparation

BCLW, with the highest T625 nm value, was used as a substitute for glucose syrup in the gummy formulation. A custom mixture design by a statistical package, Design-Expert, (Stat-Ease Inc. version 13.0, Minneapolis) was used to determine the influence of three independent variables (X1: glucose syrup, X2: BCLW, and X3: gelatin) on the model system consisting of 14 experimental points. Gummy candies were prepared from water, sucrose, gelatin, 42 DE glucose syrup, and/or BCLW according to formulations determined in the experimental design given in Table 1. The ingredients, including water, sucrose, glucose syrup, and/ or BCLW, were heated in a Thermal Mixer (Thermomix TM5, Vorwerk, Germany) to 100 °C with continuous stirring at 200-300 rpm until the Brix value of the mixture reached 85°Bx. The mixture was cooled to 90 °C, and a gelatin solution (250 bloom, 1:2 ratio with water) was introduced to the mixture and stirred for 5 min at 90 $^{\circ}$ C. Then, the prepared mixture was poured into silicone molds and cooled at 20 °C for 24 h. After cooling, demolded gummies were coated with starch to prevent stickiness. Gummy samples were stored in polyethylene bags at room temperature.

Table 1
Custom mixture design study model and gummy sample formulations.

Sample	Glucose Syrup (42 DE) X ₁ , (g/100 g, in dm) *	Clarified and Decolorized BCLW X ₂ , (g/100 g, in dm) *	Gelatin X ₃ , (g/ 100 g in dm)	Sucrose (g/100 g)
1	0.00	41.00	5.70	42.27
2	0.00	41.00	5.70	42.27
3	20.13	20.13	6.45	42.27
4	14.13	27.57	5.00	42.27
5	39.70	0.00	7.00	42.27
6	0.00	39.70	7.00	42.27
7	41.00	0.23	5.47	42.27
8	41.00	0.23	5.47	42.27
9	30.05	10.20	6.45	42.27
10	26.47	13.23	7.00	42.27
11	13.23	26.47	7.00	42.27
12	20.85	20.85	5.00	42.27
13	20.13	20.13	6.45	42.27
14	10.20	30.05	6.45	42.27

BCLW; Black Carrot Liquid Waste, X1: 42 DE Glucose Syrup. X2; Clarified and Deionized, X3; Gelatin. For each sample group, the total input mass was determined based on the dry matter content (in terms of dry matter and including water) and the amount of water used to prepare the gelatin solution (1:2), taking into account the amounts of deionized sugar syrup $(65^{\circ}Bx)$ and glucose syrup $(80^{\circ}Bx)$ for water-soluble dry matter. The feeding amount was based on a total of 120 g per sample.

2.4.2. Characterization of gummies

2.4.2.1. Moisture content. The moisture content of the samples was determined using a gravimetric method with slight modifications (Periche et al., 2015). 10 g of each sample was accurately weighed and placed in an air-ventilated oven at 60 $^{\circ}$ C. The samples were dried until they reached a constant weight, which was defined as no further weight change. The final moisture content (%) was calculated using the following equation:

$$\label{eq:moisture content} \text{Moisture content (\%)} = \frac{m_i - m}{m_i} \! \times \! 100$$

where $m_{\rm i}$ is the initial weight of samples and m is the final weight of samples.

2.4.2.2. Water activity. Water activity was measured using a water activity meter (Aqualab, Addium Inc., USA) with an accuracy of 0.001. Prior to measurement, each gummy jelly sample was cut in half crosswise, and both halves were placed inside the sample holder. The measurement was performed at 25 $^{\circ}$ C, allowing a stabilization period of three minutes before readings were recorded. Each measurement was conducted in triplicate for reliability.

2.4.2.3. pH. The pH of the samples was measured using a Hanna HI2002–02 pH meter (Hanna Instruments, Rhode Island, USA). The pH of BCLW was measured by directly immersing the probe into BCLW solution. For gummies, the samples were thinly sliced, combined with hot water at a 1:3 (w/w) ratio, and continuously stirred until complete dissolution. The solution was then cooled to 25 °C, and pH measurements were taken. All analyses were carried out in triplicate to ensure precision.

2.4.2.4. Color. The surface color of the gummy candies (L*: brightness, $a* \pm red$ –green, $b*:\pm yellow$ –blue,) were determined using a colorimeter device (Chroma Meter CR-400, Konica Minolta, Japan). Chroma and hue angle were calculated using Eq. 1 and Eq. 2 below (Gok et al., 2020);

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{1}$$

$$h^{\circ} = arctan \left(b^{*}/a^{*} \right) \tag{2}$$

2.4.2.5. Texture profile analysis. The gummy candies were prepared with a dimension of 28 mm diameter and 20 mm height. Texture Profile Analysis was conducted for gummies with compression tests using a TATX plus instrument (Stable Micro Systems, Godalming, UK) equipped with a 5 kg load cell and a cylindrical probe with a diameter of 35 mm. The texture variables, including hardness, gumminess, springiness, cohesiveness, chewing, and resilience, were calculated from the forcetime curve. The analyses were conducted with at least five replicates.

2.4.2.6. FTIR analysis. Spectral measurements were performed within the range of 4000–650 cm⁻¹. All measurements were performed in triplicate with a diamond triple-bounce ATR accessory (Bruker Alpha, Germany).

2.4.3. Accelerated shelf-life (ASL) study

The prepared gummies were stored unpacked in a shelf-life cabinet and exposed to accelerated shelf-life testing conditions at 25 °C with 70 % relative humidity (RH) for 7 weeks (Subramaniam, 2016). At 7-day intervals, color analysis (Section 2.4.2.4), texture profile analysis (Section 2.4.2.5), and water activity (Section 2.4.2.2) were conducted. Color change (ΔE^*) was determined based on Eq. 3 below;

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2} \tag{3}$$

 $\Delta L^*,~\Delta a^*,$ and Δb^* values were calculated based on the initial measurement.

To assess Δa_w and $\Delta Hardness,$ the changes between the initial measurements and the final analysis results on day 49 of ASL were analyzed, and the variance percentage (%) was derived from these differences.

2.5. Statistical analysis

The results were expressed as the mean of replication and \pm standard deviation. After clarification and decolorization process, BCLW was characterized and the significance of the difference in the data was obtained through one-way analysis of variance (ANOVA). Significance

was defined at p<0.05 by using Tukey's test. For gummy candies, ANOVA was applied to evaluate the statistical significance of the results, and multiple linear regression was used to identify the most appropriate models with regression coefficients and interactions between linear, quadratic, and cubic terms analyzed via Design Expert software (Stat-Ease Inc., version 13.0, Minneapolis, USA). The analyses were conducted with a 95 % confidence level. The optimum production conditions were determined according to hardness value is 1635–2040 g, elasticity is maximum, critical value for moisture content is lower than 24.0 g/100 g, and water activity is targeted between 0.50 and 0.75. The samples were manufactured under these conditions and validation analysis was carried out to determine the deviations between experimental and predicted values.

3. Results and discussion

3.1. BCLW as a novel sugar

3.1.1. Clarification and Decolorization

The physicochemical properties of the BCLW after processing are summarized in Table 2. After clarification and decolorization, the BCLW samples showed a transmittance value of 625 nm (T625 nm) of 88.7 % \pm 0.38 (Table 2). The T625 value demonstrates the effectiveness of the clarification and decolorization processes in improving the clarity degree of fruit juices by effectively reducing color and suspended particles. In fruit juices, a higher T625 value indicates a clearer sample, highlighting the effectiveness of the treatment in minimizing color and particulate content. The highest clarification was achieved using adsorber resin of Modified Styrene DVB (A1) at a flow rate of 1 BV/h, which proved particularly effective in enhancing clarity. The flow rate of BCLW had an apparent impact on the final product's clarity; as the flow rate increased, the clarity decreased, suggesting that slower flow rates allow for more thorough removal of color and particles. This decrease in clarity at higher flow rates could be attributed to the retention of proteins and polyphenols, which are primary contributors to turbidity in the BCLW (Dıblan & Özkan, 2021). The crude protein content of BCLW treated with adsorber resins A1 and A2 ranged from 2.20 \pm 0.25 to 5.81 \pm 0.46 g/100 g and from 5.05 \pm 0.23 to 6.02 \pm 0.17 g/100 g, respectively, while the TPC was found to range from 10.2 \pm 0.75 to 11.0 \pm 0.23 mg GAE/kg and from 22.3 \pm 0.91 to 24.9 \pm 0.20 mg GAE/kg,

Table 2
Properties clarified and decolorized black carrot process liquid waste (BCLW) prepared by using various adsorber and flow rate.

Adsorber	Flow Rate (BV/h)		pH	TSS (°Bx)		Total Ash Content (g/100 g)	TPC (mg GAE/kg)	Crude Protein (g/100 g)	
	1.0		3.05 ± 0.03^{c}	72.1 ± 0.15		4.57 ± 0.25^{c}	11.0 ± 0.23^{c}	2.20 ± 0.25^{d}	
A1	1.5		$2.81\pm0.05^{\rm c}$	71.8 ± 0.21		4.01 ± 0.31^{d}	$11.0\pm0.15^{\rm c}$	4.18 ± 0.41^{c}	
	2.0		$2.77\pm0.10^{\rm c}$	71.6 ± 0.44		$2.73 \pm 1.59^{\rm e}$	$10.2\pm0.75^{\rm c}$	5.81 ± 0.46^{a}	
	1.0		$3.75\pm0.21^{\rm b}$	70.4 ± 0.96		$5.41 \pm 0.17^{\mathrm{b}}$	22.3 ± 0.91^{b}	$5.05\pm0.23^{\mathrm{b}}$	
A2	1.5		4.12 ± 0.22^a	69.3 ± 0.85		6.01 ± 0.55^{a}	23.1 ± 0.45^{b}	5.67 ± 0.21^a	
	2.0		4.31 ± 0.03^a	71.9 ± 0.50		6.33 ± 0.15^a	24.9 ± 0.20^a	6.02 ± 0.17^a	
Adsorber	Flow Rate (BV/h)	L*	a*	b*	C*	h°	Clarification Degree (T625, %)	Browning Index (A420)	
	1.0	95.7 ± 1.73^{a}	$-1.43\pm0.20^{\rm c}$	2.76 ± 0.41^{d}	$3.13\pm0.26^{\text{d}}$	118 ± 7.08^{a}	88.7 ± 0.38^a	80.8 ± 0.17^a	
A1	1.5	93.4 ± 1.15^a	-0.84 ± 0.16^{c}	$3.15\pm0.33^{\rm d}$	$3.43\pm0.11^{\rm d}$	$105\pm5.61^{\mathrm{b}}$	84.3 ± 0.45^{b}	$35.1\pm1.15^{\mathrm{b}}$	
	2.0	91.8 ± 1.42^a	0.21 ± 0.04^c	$3.53\pm0.39^{\rm d}$	$3.53\pm0.41^{\rm d}$	89.2 ± 3.26^{c}	$80.5\pm0.32^{\rm c}$	6.69 ± 0.10^{c}	
	1.0	$66.2\pm1.22^{\mathrm{b}}$	4.45 ± 0.23^{b}	37.3 ± 2.31^{c}	39.1 ± 2.01^{c}	$75.3\pm4.21^{\rm d}$	$4.14\pm0.05^{\textrm{d}}$	0.12 ± 0.01^{c}	
A2	1.5	$64.1\pm2.22^{\mathrm{b}}$	5.95 ± 0.21^{b}	$43.0\pm1.21^{\rm b}$	$45.2\pm3.00^{\mathrm{b}}$	$77.4\pm2.17^{\rm d}$	$3.11\pm0.04^{\rm d}$	0.11 ± 0.02^{c}	
	2.0	$63.4\pm1.73^{\mathrm{b}}$	8.15 ± 0.20^a	49.8 ± 0.41^a	50.5 ± 0.26^a	$80.9\pm7.08^{\rm d}$	$2.19\pm0.10^{\rm d}$	0.14 ± 0.00^{c}	
Adsorber	Flow Rate	Total acidity		Total sugar	Antioxidant act	ivity	Sucrose	Glucose	Fructose
Ausorbei	(BV/h)	(g/L)		(g/100 g)*	(EC50, %)		(g/100 g)*	(g/100 g)*	(g/100 g)*
	1.0	$0.09\pm0.01^{\mathrm{b}}$		78.2 ± 1.35^a	5.26 ± 0.37^{c}		28.9 ± 1.55^a	21.8 ± 2.35^a	24.9 ± 3.01^a
A1	1.5	$0.11\pm0.01^{\mathrm{b}}$		$72.0\pm2.91^{\mathrm{b}}$	5.45 ± 0.14^{c}		25.4 ± 1.32^{b}	$20.1\pm4.20^{\mathrm{b}}$	$22.1\pm3.20^{\mathrm{b}}$
	2.0	$0.13 \pm 0.00^{\mathrm{b}}$		73.3 ± 3.45^{b}	$5.98\pm1.56^{\rm c}$		$26.7 \pm 2.31^{\mathrm{b}}$	21.0 ± 1.51^a	23.1 ± 2.11^{b}
	1.0	4.15 ± 0.21^a		$71.2 \pm 4.21^{\text{b}}$	$10.2\pm1.11^{\rm b}$		25.7 ± 2.13^{b}	19.3 ± 0.95^{b}	22.1 ± 0.78^{b}
A2	1.5	4.05 ± 0.12^{a}		72.3 ± 5.21^{b}	$12.3\pm0.29^{\rm a}$		24.5 ± 1.11^{b}	19.4 ± 1.22^{b}	21.3 ± 2.01^{b}
	2.0	3.83 ± 0.16^{a}		74.2 ± 3.21^{b}	13.4 ± 0.17^{a}		25.6 ± 0.23^{b}	$21.2\pm0.45^{\rm a}$	$22.2\pm1.15^{\mathrm{b}}$

 $^{^*}$: in dry matter. A1: Modified Stryrene DVB (600 m²/g, 1.18 g/mL), A2: Crosslinked polystyrene (600.0 m²/g, 1.02 g/mL), mean \pm standard deviation, p > 0.05.

respectively. As the flow rate increased, more proteins and polyphenols remained in the BCLW (Table 2), reducing its clarification degree. TPC was significantly lower in the BCLW treated with the A1 resin compared to the A2 resin. This aligns with the clarification results, indicating that the A1 resin adsorbed more phenolic compounds than the A2 resin, resulting in lower TPC but a higher degree of clarification. A considerable decrease in total ash content in BCLW treated with A1 resin confirmed the resin's effectiveness in removing components. This was also in accordance with the liquid product's TPC and crude protein contents. The pH value of BCLW (A1, 1.0 BV/h) was measured at 3.05 \pm 0.03 which was lower than that of many other fruit juices (Dıblan & Özkan, 2021; Orhan Dereli et al., 2023; Turhan Kara et al., 2024). Titratable acidity values were between 0.09 and 4.15, with a notably higher total acidity observed in the BCLW treated with resin A2 than resin A1. It is well known that a higher browning index indicates the presence of browning reactions, which often result from the formation of pigmented compounds. A reduction in the browning index effectively removes these pigments, which are byproducts of browning reactions. The BCLW treated with resin A1 showed a significantly higher browning index than that treated with resin A2; however, this increase did not affect the clarity of the BCLW or color parameter L*.

When the BCLW passed through resin A1 at a flow rate of 1 BV/h, the TSS (72.1 \pm 0.15°Bx), total sugar (78.2 \pm 1.35 g/100 g), sucrose (28.9 \pm 1.55 g/100 g), glucose (21.8 \pm 2.35 g/100 g), and fructose (24.9 \pm 3.01 g/100 g) content reached their maximum levels and comparably higher than A2 resin treatments. As seen in Table 2, the resin used in the decolorization process significantly influenced the color values of the BCLW. Treatment with A1 resin increased the L*and h° values of BCLW while decreasing a*, b*, and C* values. The BCLW decolorized with A1 resin exhibited significantly higher lightness (L*>90), indicating the resin's effectiveness in removing pigments than A2 resin. Khandare et al. (2011) reported lower L* values (L* = 11) in black carrot juice, likely due to the presence of pigments such as anthocyanins. This was also consistent with TPC values as the TPC of A1 resin-treated BCLW was lower compared to A2-treated products, indicating more phenolic compounds like anthocyanins were removed by A1 resin. The reduction in chroma (C*) and intensity of color saturation further support the effective removal of color compounds by the A1 resin. Moreover, a negative value of a* exhibited an increase in greenish tone, and lower b* values indicated increasing blueness in BCLW. The lower a* and b* values proved a loss of color, which was consistent with the findings reported by Albert et al. (2012).

3.2. Substitution of glucose syrup by using BCLW in gummy formulation

3.2.1. Physicochemical properties

Gummy samples were formulated with water, gelatin, sucrose, and

clarified/decolorized BCLW (with the highest T625 nm) or glucose syrup and produced for 14 formulations outlined in Table 1. The physicochemical properties of the gummies, including total soluble solids, water activity, pH, and moisture content, are given in Table 3. Total Soluble Solids (TSS) is an important process control parameter in confectionery, directly affecting the product's stability and texture. In gummy production, the TSS of the mixture is expected to reach a desired value after thermal processing, before the addition of gelatin or gelatin solution. The target TSS value for soft candies is at least 76.0 g/100 g to prevent graining (Hartel et al., 2017). TSS changed from 81.2 \pm 0.01 to 86.7 \pm 0.00°Bx, indicating adequate soluble solid content for gelatin-based gummies. It is important to recognize the limitations of process control as candy samples were prepared under laboratory rather than industrial conditions. For industrial applications and re-formulation studies, one should consider that BCLW has a lower TSS value (72.1 \pm 0.15°Bx) than glucose syrup containing 80.0°Bx of soluble solids. To address this, it may be beneficial to adjust the evaporation conditions for BCLW to reach a TSS level comparable to glucose syrup. The study's primary objective was to develop a material analogous to deionized and clarified fruit juice concentrates, considering the potential reactions that may occur during BCLW evaporation due to its complex composition. TSS value exceeding 65°Bx was targeted for BCLW in this study. However, no significant model emerged for predicting the influence of independent variables on TSS (R^2 , 0,6123).

The pH value of gummies ranged from 4.28 \pm 0.02 to 5.73 \pm 0.01. A significant linear model was determined to assess the impact of independent variables on the pH values of the gummy samples, with an R² value of 0.9434 (Table 4). Adding more BCLW in the gummy mixture decreased the pH value of the final samples (p < 0.05). The gummies produced with the highest BCLW content showed the lowest pH value (4.28). Since gelling agents such as gelatin form gels at their isoelectric point, pH is a critical factor in controlling these agents' gelation and gel stability (Pekdogan Goztok et al., 2024). Moreover, pH variations influence the intensity of flavors perceived in gummies. To manage this, acid regulators are widely incorporated into gummy production, for example, citric acid being the most used. However, no citric acid was incorporated into the main gummy mixture to observe the effect of BCLW on gel formation and strength. The pH of fruit juices or concentrates typically varies between 2.0 and 6.7 (Basak et al., 2023). Adding juice or concentrate could serve as an alternative to acid regulators for lowering the pH in gummies while supporting gel stability (Pekdogan Goztok et al., 2024). In our study, the findings suggest that BCLW can serve as a viable alternative to glucose syrup in candy production, as it can reduce the pH of the gummy mixture required for stable gel network formation due to its low pH (3.05 \pm 0.03), yielding results comparable to those achieved with fruit juice concentrates. Although a higher pH was observed in the gummies than in conventional formulations, this

Table 3 Physico-chemical and color properties of BCLW.

Sample	TSS (°Bx)	Water Activity	pН	Moisture Content (g/100 g)	L*	a*	b*	Chroma	Hue Angle
1	84.5 ± 0.02	0.690 ± 0.002	4.28 ± 0.02	20.19 ± 0.11	79.6 ± 1.45	-1.64 ± 0.04	11.0 ± 0.4	11.1 ± 0.40	98.6 ± 0.22
2	83.9 ± 0.01	0.690 ± 0.000	4.33 ± 0.00	19.66 ± 0.52	80.0 ± 2.22	-1.62 ± 0.04	10.8 ± 0.56	10.9 ± 0.56	98.5 ± 0.47
3	86.7 ± 0.00	0.680 ± 0.001	4.88 ± 0.01	15.91 ± 0.22	95.2 ± 0.26	-0.97 ± 0.04	5.51 ± 0.42	5.59 ± 0.41	100.0 ± 1.01
4	81.2 ± 0.01	0.680 ± 0.003	4.54 ± 0.00	14.75 ± 0.66	76.5 ± 0.74	-1.41 ± 0.07	9.29 ± 0.53	9.40 ± 0.54	97.9 ± 1.37
5	81.5 ± 0.02	0.670 ± 0.003	5.68 ± 0.01	12.37 ± 0.12	95.5 ± 0.32	-0.99 ± 0.05	6.04 ± 0.04	6.12 ± 0.03	99.3 ± 0.51
6	86.0 ± 0.01	0.690 ± 0.000	4.87 ± 0.47	18.50 ± 0.08	72.3 ± 1.89	-1.57 ± 0.02	11.0 ± 0.37	11.0 ± 0.39	98.2 ± 0.29
7	86.6 ± 0.01	0.660 ± 0.004	5.66 ± 0.01	12.21 ± 0.23	96.2 ± 0.47	-1.05 ± 0.08	4.81 ± 0.03	4.92 ± 0.03	102.4 ± 0.98
8	86.7 ± 0.01	0.680 ± 0.002	5.73 ± 0.01	11.71 ± 0.28	97.0 ± 0.14	-0.96 ± 0.12	4.86 ± 0.24	5.63 ± 0.23	101.4 ± 1.13
9	84.8 ± 0.01	0.690 ± 0.000	5.26 ± 0.01	14.80 ± 0.04	97.9 ± 0.31	-0.45 ± 0.84	6.56 ± 0.25	6.62 ± 0.25	98.2 ± 0.37
10	88.7 ± 0.01	0.680 ± 0.001	5.20 ± 0.01	14.44 ± 0.01	96.7 ± 1.21	-1.03 ± 0.05	7.92 ± 0.52	7.98 ± 0.53	97.5 ± 0.12
11	85.5 ± 0.01	0.680 ± 0.003	4.80 ± 0.01	17.02 ± 0.15	93.3 ± 0.64	-1.22 ± 0.06	9.11 ± 0.49	9.19 ± 0.49	97.6 ± 0.23
12	87.7 ± 0.03	0.670 ± 0.003	4.73 ± 0.00	16.51 ± 0.09	85.8 ± 0.89	-1.06 ± 0.12	7.42 ± 0.90	7.50 ± 0.91	98.1 ± 0.20
13	85.1 ± 0.02	0.690 ± 0.000	4.89 ± 0.00	14.85 ± 0.18	94.3 ± 0.27	-1.25 ± 0.10	8.22 ± 0.59	8.32 ± 0.59	98.7 ± 0.14
14	$\textbf{85.8} \pm \textbf{0.01}$	0.660 ± 0.004	4.66 ± 0.00	17.35 ± 0.04	91.5 ± 0.19	-1.18 ± 0.04	8.26 ± 0.15	8.35 ± 0.16	98.1 ± 0.14

Mean \pm standard deviation, TSS; Total soluble solids.

Table 4ANOVA results for physico-chemical properties of clarified and deionized BCLW including gummy samples.

	TSS (°Bx)		Water Activity	y pH			Moisture Content (g/100 g)		
	SS	P value	SS	P value	SS	P value	SS	P value	
Model	23,37	0,1171	0,0122	0,0204	0,3369	< 0.0001	135,94	< 0.0001	
Lineer Mix	18,93	0,0370	0,0070	0,0058	0,3221	< 0.0001	130,89	< 0.0001	
X_1X_2	3,21	0,2243	0,0007	0,1155	0,0083	0,1084	4,61	0,0050	
X_1X_3	2,04	0,3241	0,0011	0,0696	0,0013	0,4901	0,5727	0,2145	
X_2X_3	2,04	0,3247	0,0011	0,0692	0,0011	0,5230	0,6245	0,1968	
Lof*	8,54	0,6063		0,3933	0,0068	0,8846	0,4636	0,9725	
P.E	6,25				0,0134		2,06		
Total	38,16				0,3571		138,46		
R^2		0,6123	R^2	0,9224	R^2	0,9434	R^2	0,9818	
Adj- R ²		0,3700	Adj- R ²	0,7982	Adj- R ²	0,9080	Adj- R ²	0,9704	
Pred- R ²		-0,4558	Pred- R ²	-79,8999	Pred- R ²	0,8135	Pred- R ²	0,9514	
Adeq.Prec.		51,404	Adeq.Prec.	85,827	Adeq.Prec.	14,4724	Adeq.Prec.	25,3793	

TSS; Total soluble solids, BCLW; Black Carrot Liquid Waste; X_1 : 42 DE Glucose Syrup. X_2 ; Clarified and Deionized, X_3 ; Gelatin. *; Linear mixture. The test for the linear mixture terms if the Scheffé polynomial model compares the linear coefficient estimates to each other rather than comparing the coefficients to zero. If the linear coefficients are the same. There is no linear effect. Even though the coefficient estimates may be very large. p < 0.05. Adeq. Precission.

outcome was regarded as beneficial, indicating that BCLW may offer favorable effects in gummy candy production.

Moisture is crucial in the production of sugar confections as it influences the product's quality, texture, stability, and shelf life. In general, a decrease in moisture content leads to changes in the texture of the confection, making it shift from soft to firm. The moisture content of BCLW-containing gummies changed from 10.71 ± 0.28 to 20.19 ± 0.11 g/100 g. The results agree with previous studies that investigated the effects of incorporating fruit juices or concentrates on the moisture content of gummies (Pekdogan Goztok et al., 2024; Teixeira-Lemos et al., 2021). It was found that the independent variables had a significant impact, and the model representing these effects achieved a high R² value (Table 4). The increase in BCLW content in gummies elevated the moisture content. The variations in the moisture content of gummies could be related to substituting glucose syrup with BCLW, which had a different sugar profile. BCLW contains 28.9 \pm 1.55 g/100 g of sucrose, 24.9 \pm 3.01 g/100 g of fructose and 21.8 \pm 2.35 g/100 g of glucose, respectively (Table 2). Monosaccharides are hygroscopic compounds that can interact with and bind water molecules through their hydroxyl groups. Fructose is the main factor contributing to the increased moisture content in gummies due to its strong affinity for water, leading to more moisture retention. Glucose shows lesser water-binding capacity, balancing moisture retention and texture stability. At the same time, sucrose is the least hygroscopic component but helps maintain the structure and overall water content of gummies. It should also be noted that high fructose content may influence the crystallization behavior of sucrose, texture, stability, and shelf life of gummies.

The water activity (aw) of gummies was measured as an indicator of the stability of the product since the aw is affected by their moisture content and sugar profile. The fitted model, depending on the use of glucose syrup (X1), clarified and deionized BCLW (X2), and gelatin (X3) were identified as linear. The R² value of the model was found to be 0.9224 (Table 4). The water activity (aw) of gummies was found to be 0.66–0.69 (Table 3), which was in the ranges for gummies (0.50–0.75) reported previously (Ergun et al., 2010). The addition of BCLW had a significant (p < 0.05) effect on aw values. However, this can vary significantly depending on the sugar composition, moisture content, or hydrocolloids presented in the gummy. Substituting glucose syrup with fruit juices or concentrates increased aw (Hani et al., 2015; Periche et al., 2014; Tarahi et al., 2023). For gummies, exceeding the critical aw value of 0.6 reduces stability for crystallization, moisture migration, and mold growth (Fan et al., 2017). In general, adding sugars tends to reduce aw values, enhancing the gel's structural integrity and increasing its melting point (Wang & Hartel, 2022). This also helps prevent crystallization, minimizes moisture migration, and reduces the risk of mold growth, contributing to improved preservation and quality in gummy

products (Tireki et al., 2021; Fan et al., 2017; Stępień et al., 2023). BCLW affected the sugar composition of gummies and consequently changed the water activity.

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Color is a crucial factor in determining consumer acceptance and evaluating the quality of confectionery products (Konar et al., 2022). In response to consumer demand for natural ingredients, natural colorants are widely incorporated in confectioneries. BCLW, rich in natural pigments like anthocyanins, provides an appealing color that enhances gummy products (Kumar et al., 2022). In addition to moisture content, total soluble solids, and texture, the incorporation of BCLW affects color while also influencing the appearance and aroma of gummies, further contributing to overall sensory appeal. To analyze color variations in gummies, the color properties were examined and are summarized in Table 3, while the statistical evaluation of these results is shown in Table 4. A significant linear model (p < 0.05) was identified for L*, a*, b*, Chroma, and Hue angle, highlighting the significance of all color parameters. The R² values of these models for L*, a*, b*, Chroma, and Hue angle were 0.8363, 0.8966, 0.7147, 0.7387, and 0.9173, respectively (Table 5). The L*, a*, b*, Chroma, and Hue angle of gummy candies were measured, ranging from 72.3 to 97.9, -1.64 to -0.45, 4.81 to 11.0, 4.92 to 11.1, and 97.5 to 100.0, respectively. For all gummy formulations, gelatin showed no impact on color development. The use of glucose syrup led to a significant increase in brightness, whereas the addition of BCLW negatively affected the brightness of the gummies (Supplementary File 2). Although L^* value decreased with increasing amount of BCLW, the brightness of the gummies was still significantly higher than that of gummy samples prepared with fruit concentrates (Moghaddas Kia et al., 2020; Pekdogan Goztok et al., 2024). The glucose syrup significantly contributes to the lightness of gummies, likely due to its lack of intense pigments or colors. However, when the glucose syrup was replaced with BCLW up to 50 %, the brightness remained considerably high (L*>95). The a* value was negative for all gummy candies representing a greenish color. The color changes were significantly (*p* < 0.05) dependent on the interaction effect of glucose syrup and BCLW concentration (Table 5) in the gummies. The gummies added with BCLW showed a descending trend in the redness parameter with increased BCLW content in the formulation. Chroma measures color intensity or purity and correlates with the degree of anthocyanin content (Khandare et al., 2011). The results suggest that the Chroma value of gummies increased as the proportion of BCLW in the formulation rose. The maximum Chroma value was observed in samples where glucose syrup (0 g/100 g) was replaced entirely by BCLW (41 g/100 g), indicating that BCLW contributed to color intensity. This trend suggests that BCLW's color-enhancing properties are more effective at higher concentrations

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Table 5ANOVA results for color properties of clarified and deionized BCLW including gummy samples.

	L*		a*		b*		Chroma		Hue Angle	ue Angle	
	SS	P value	SS	P value	SS	P value	SS	P value	SS	P value	
Model	2276,24	0,0053	70,05	0,0009	79,93	0,0406	107,62	0,0296	1952,77	0,0004	
Lineer Mix	2043,38	0,0010	60,54	0,0002	58,26	0,0157	84,24	0,0094	1446,36	0,0001	
X_1X_2	78,60	0,2690	9,19	0,0167	17,93	0,0668	20,36	0,0724	502,33	0,0014	
X_1X_3	79,14	0,2675	0,0382	0,8507	5,92	0,2577	5,03	0,3340	7,28	0,5811	
X_2X_3	73,84	0,2829	0,0386	0,8499	5,80	0,2624	4,92	0,3390	7,77	0,5689	
Lof*	310,44	0,4214	6,17	0,3113	23,85	0,3379	28,53	0,3345	71,76	0,8186	
P.E	135,27		1,91		8,07		9,55		104,38		
Total	2721,95		78,13		111,85		145,70		2128,91		
R^2		0,8363	R^2	0,8966	R^2	0,7147	R^2	0,7387	R^2	0,9173	
Adj- R ²		0,7339	Adj- R ²	0,8320	Adj- R ²	0,5363	Adj- R ²	0,5753	Adj- R ²	0,8656	
Pred- R ²		0,3295	Pred- R ²	0,6869	Pred- R ²	0,0896	Pred- R ²	0,1424	Pred- R ²	0,7687	
Adeq.Prec.		85,274	Adeq.Prec.	98,397	Adeq.Prec.	59,234	Adeq.Prec.	62,793	Adeq.Prec.	10,8733	

BCLW; Black Carrot Liquid Waste; X_1 : 42 DE Glucose Syrup. X_2 ; Clarified and Deionized, X_3 ; Gelatin. *; Linear mixture. The test for the linear mixture terms if the Scheffé polynomial model compares the linear coefficient estimates to each other rather than comparing the coefficients to zero. If the linear coefficients are the same. There is no linear effect. Even though the coefficient estimates may be very large. p < 0.05. Adeq. Precission.

due to probably anthocyanin content, giving the gummies a more saturated appearance. The hue angle, derived from a* and b* values, reflects changes in color associated with pigment variations. The hue angles were expressed on a 360° color wheel where red color is represented with hue value $<\!20^\circ$ and $>330^\circ$, yellow from 20° to 80° , and green from 80° to 160° (Cömert et al., 2020). In gummy samples formulated with BCLW, a statistically significant increase in hue angle (p <0.05) shifted towards green due to the interaction between glucose syrup and BCLW.

3.2.3. Texture analysis

Texture profile analysis was conducted for 14 formulations prepared using a mixture design to evaluate the influence of variables on the textural properties of gummy candies, including hardness, resilience, cohesiveness, gumminess, chewiness, and springiness. The results of the TPA analysis are shown in Table 6. The results suggested that the interaction of glucose syrup, BCLW, and gelatin had no significant impact on hardness (785–2099.3 g), springiness (50.8–86.6 %), cohesiveness (91.3–96.6 %), gumminess (753.0–2084 g), while significant fitted linear models (p < 0.05) were identified for chewiness (347.2–988.4 g), and resilience (38.1–67.6 %). $\rm R^2$ values of chewiness and springiness were calculated as 0.7324 and 0.9901, respectively. (See Table 7.)

Hardness and gumminess are key texture parameters used for classification in confectionery products, influenced by factors such as the type and concentration of hydrocolloids, process conditions, and overall composition (Dalabasmaz et al., 2024; Wang & Hartel, 2022). Hydrocolloids contribute significantly to the structural integrity of gummies, with changes in their interaction within the formulation affecting

hardness properties (Aidat et al., 2023). In our study, although no significant correlation was observed for the hardness and gumminess of gummy samples and the interaction of variables (glucose syrup, BCLW extract, and gelatin), substantially higher hardness values were recorded in formulations where over 75 % of BCLW was incorporated, compared to the gummy samples produced with 100 % of glucose syrup. These results are consistent with previous findings suggesting that changes in the structural composition of hydrocolloids and sugar profiles can significantly alter hardness (Ghodsi & Nouri, 2024; Pekdogan Goztok et al., 2022). BCLW, rich in sucrose (28.9 g/100 g, Table 2), likely contributed to the firmer texture by reinforcing gelatin network binding points. Sucrose is known to promote network formation of gels and enhance their gel strength (Tireki et al., 2021). Although BCLW contained high monosaccharides (21.8 g/100 g, glucose, and 24.9 g/100 g, fructose), the high sucrose content may have reduced the potential softening effect of fructose, which has a high moisture affinity (Ergun et al., 2010). Pekdogan Goztok et al. (2022) investigated the impact of replacing glucose syrup with fruit juice concentrates in marshmallow products. Findings revealed that the substitution significantly influenced hardness properties due to the differences in the sugar profile of fruit juice concentrates. Increasing the gelatin content in gummy samples containing over 75 % BCLW had a more pronounced effect on hardness development than formulations with lower BCLW substitutions (Supplementary File 3). This highlights the crucial role of both sugar and gelatin in forming and reinforcing the gel structure. Another potential explanation for the increase in hardness with the addition of BCLW could be related to its influence on the pH of the gummy mixture. The pH level is critical for establishing a stable gel network in gummy formulations. In standard gummy production, citric acid is often incorporated

Table 6Texture properties.

Sample	Hardness (g)	Springiness (%)	Cohesiveness (%)	Gumminess (g)	Chewiness (g)	Resilience
1	1567.1 ± 10.5	42.0 ± 3.25	98.6 ± 0.05	1552.3 ± 38.7	648.1 ± 16.1	84.3 ± 0.61
2	1669.6 ± 13.7	38.1 ± 0.91	99.2 ± 0.56	1655.4 ± 96.3	630.5 ± 36.3	82.7 ± 0.56
3	1169.9 ± 62.8	49.7 ± 4.72	97.8 ± 0.41	1144.0 ± 57.2	565.6 ± 24.2	77.7 ± 1.58
4	1328.3 ± 99.7	40.3 ± 4.24	99.2 ± 0.48	1316.8 ± 95.3	529.9 ± 22.8	81.3 ± 2.32
5	1620.1 ± 40.1	61.3 ± 4.03	96.0 ± 1.22	1554.9 ± 26.6	952.5 ± 32.6	66.1 ± 2.53
6	1984.5 ± 28.4	46.5 ± 0.97	99.6 ± 0.27	1976.0 ± 61.7	918.4 ± 12.9	86.6 ± 0.64
7	919.2 ± 7.98	65.6 ± 3.73	96.6 ± 1.45	873.6 ± 11.4	580.1 ± 23.0	50.8 ± 3.29
8	881.6 ± 65.2	67.6 ± 7.91	93.7 ± 2.74	819.2 ± 22.1	543.4 ± 23.8	51.8 ± 1.21
9	1698.2 ± 66.2	44.0 ± 5.95	95.6 ± 1.62	1621.6 ± 36.1	713.4 ± 16.4	64.2 ± 2.10
10	1941.4 ± 30.8	57.4 ± 4.95	91.3 ± 4.11	1756.2 ± 44.4	988.4 ± 12.0	64.7 ± 4.13
11	1417.0 ± 97.7	41.0 ± 2.15	98.8 ± 0.66	1400.1 ± 54.9	576.5 ± 21.5	78.1 ± 3.56
12	785.7 ± 73.9	48.5 ± 11.7	96.0 ± 0.93	753.0 ± 19.8	347.2 ± 15.1	66.1 ± 4.51
13	1489.2 ± 35.7	41.0 ± 7.63	99.3 ± 0.05	1478.3 ± 34.8	601.9 ± 18.7	78.3 ± 2.03
14	2099.3 ± 40.5	38.7 ± 6.81	99.2 ± 0.89	2084.1 ± 55.0	790.9 ± 27.9	77.8 ± 1.02

Mean \pm standard deviation.

Table 7ANOVA results for texture properties of clarified and deionized BCLW including samples.

	Hardness (g)		Springiness	(%)	Cohesion (%)		Gumminess (g) Chewiness		imminess (g) Chewiness(g		Resilience (%)	
	SS	P value	SS	P value	SS	P value	SS	P value	SS	P value	SS	P value
Model	1081E + 06	0,2443	522,79	0,2553	40,18	0,2595	1055E+06	0,2337	3277E+05	0,0323	1731,94	0,0001
L.Karışım	1069E + 06	0,0578	348,81	0,1250	37,89	0,0686	1040E + 06	0,0549	2710E+05	0,0088	1514,16	< 0.0001
X_1X_2	8078,13	0,8084	157,67	0,1550	0,0137	0,9594	8935,17	0,7935	45,984,23	0,1177	64,03	0,0077
X_1X_3	1198,77	0,9254	35,31	0,4787	1,40	0,6094	3254,16	0,8743	10,417,24	0,4283	82,13	0,0046
$X_{2}X_{3}$	1081,64	0,9292	37,00	0,4687	1,49	0,5988	3221,22	0,8749	11,329,74	0,4096	82,84	0,0045
Lof*	7001E + 05	0,4469	135,11	0,9343	34,22	0,1540	6347E + 05	0,4953	1150E+05	0,0257	15,34	0,0381
P.E	3282E + 05		376,45		5,51		3410E + 05		4727,17		1,96	
Total	2109E+06		1034,34		79,91		2031E+06		4474E+05		1749,24	
R^2		0,5125	R^2	0,5054	R^2	0,5028	R^2	0,5195	R^2	0,7324	R^2	0,9901
Adj- R ²		0,2078	Adj- R ²	0,1963	Adj- R ²	0,1920	Adj- R ²	0,2192	Adj- R ²	0,5652	Adj- R ²	0,9743
Pred- R ²		-0,3635	Pred- R ²	-0,5933	Pred- R ²	-15,368	Pred- R ²	-0,3386	Pred- R ²	0,1641	Pred- R ²	-399,0505
Adeq.Pred	:.	38,050	Adeq.Prec	41,121	Adeq.Prec	39,667	Adeq.Prec	39,965	Adeq.Prec	72,552	Adeq.Prec	23,5620

BCLW; Black Carrot Liquid Waste, X_1 : 42 DE Glucose Syrup. X_2 ; Clarified and Deionized, X_3 ; Gelatin. *; Linear mixture. The test for the linear mixture terms if the Scheffé polynomial model compares the linear coefficient estimates to each other rather than comparing the coefficients to zero. If the linear coefficients are the same. There is no linear effect. Even though the coefficient estimates may be very large. p < 0.05. Adeq. Precission.

to lower the pH, which facilitates the formation of robust gelatin gel networks. Similarly, the natural acidity of BCLW may enhance the gel structure by lowering the pH of gummy products without adding citric acid (Table 3).

Chewiness is closely linked to sensory perception as it reflects the product's behavior in the mouth during mastication and the effort required to chew the food into a swallowable state. Chewiness is calculated by multiplying hardness, cohesiveness, and springiness. This makes chewiness valuable for gummy candies. Higher chewiness values are typically associated with gummies with greater hardness and reduced springiness. Table 6 shows chewiness also tended to rise when hardness increased, indicating a firmer, more substantial texture requiring additional chewing energy. In contrast, springiness remained lower at higher chewing values, as expected. The resilience of gummy samples ranged from 51.8 % to 86.6 %. Gummies with 75-100 % BCLW substitution demonstrated higher resilience than those with lower BCLW levels. Statistical analysis indicated that all variables (glucose syrup, BCLW, and gelatin) significantly influenced the resilience of the samples. These findings suggest that substituting more than 75 % of glucose syrup with BCLW enhances the gummies' ability to maintain their structural integrity.

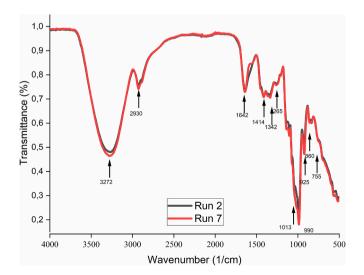


Fig. 1. Variation of glucose syrup and BCLW amounts investigated by FT-IR spectroscopy (Run 2: 0 g/100 g Glucose Syrup (42 DE); 41.00 g/100 g BCLW; 5.70 g/100 g Gelatine; Run 7: 41.00 g/100 g Glucose Syrup (42 DE); 0.23 g/100 g BCLW; 5.47 g/100 g Gelatine).

3.2.4. FT-IR spectra

Fourier transform-infrared (FT-IR) spectroscopy monitors the structural changes in the samples. In Fig. 1, changing the glucose syrup and BCLW amounts in the formulation was analyzed with FT-IR spectroscopy. The band around 3272 cm⁻¹ is mainly related to the OH stretching vibrations of water. Although moisture content of the samples increased with addition of BCLW, the intensity of this band was interestingly decreased with the use of BCLW. The peaks around 2930 cm⁻¹ correspond to the C-H deformation vibration of carboxylic acids and NH3 stretching vibrations of free amino acids. The most significant band (Amide I) related to the gelatin is observed in the 1700–1600 cm⁻¹ spectral range (Cebi et al., 2019). The intensity of 1642 cm⁻¹ band (amide group of proteins) did not change with changing the syrup type. Gummy contains sugar (sucrose) and corn syrup; therefore, it is expected to see sugar-based peaks in wavenumbers between 1500 and 750 cm⁻¹. The peaks at 925, 1265, and 1414 cm⁻¹ can be attributed to the C—H bending, C-O stretching and O-H stretching/bending vibrations of bending modes of CH2 and CH3 groups in proteins and carbohydrates, respectively (Tewari & Irudayaraj, 2004). These bands' intensity was decreased by using BCLW in the formulation of gummy samples. The band observed around 993 cm⁻¹ corresponds to the vibrational band of glycosidic links of sucrose. The peak around 925 cm⁻¹ corresponds to the α -anomeric glycosidic linkages (C–O–C bonds) between glucose and fructose in sucrose (Nizamlioglu et al., 2022). This finding aligns with the higher TSS content observed in Run 7 compared to Run 2 (Table 3). The slightly higher transmittance values in sugar-based peaks were obtained from glucose syrup using gummy formulations.

3.2.5. Accelerated shelf life

The gummy samples were subjected to accelerated shelf-life testing for 7 weeks at 25 °C and 70 % relative humidity. Samples were periodically evaluated for alterations in texture, focusing on hardness, color variation (ΔE), and water activity values (Table 8). It was observed that the least color differences occurred in gummies prepared with 50 % or less BCLW as glucose substitute. On the other hand, the color stability was poor when BCLW over 50 % was incorporated in gummies. At higher BCLW substitution, colored pigments presented in gummies were high. This indicates that more pigments were degraded, increasing color differences in gummies under accelerated storage conditions. Anthocyanins, the primary natural pigments in black carrots, are highly susceptible to environmental factors such as temperature, pH, and oxygen, which can cause their degradation into colorless or brown compounds (Kamiloglu et al., 2018). Kirca et al. (2006) reported a faster degradation rate of anthocyanins in pineapple nectar stored at 37 $^{\circ}\text{C}$ and 20 $^{\circ}\text{C}$ compared to storage at 4 °C. This aligns with previous findings, suggesting that anthocyanin degradation contributed significantly to color differences (ΔE) observed in gummy products despite the presence of

Table 8

Zero-order rate constant under accelerated shelf-life conditions for water activity values of gummy samples including clarified and decolorized BCLW.

BCLW	GS	G	Water Act	ivity (aw)		Color Differ	ence (ΔE)		Hardness		
			Δa _w (%)	k ₀	R ²	ΔE_{Day49}	k ₀	R ²	ΔSertlik (%)	k ₀	R ²
41.00	0.00	5.70	9.49	0.0012	0.6652	4.34	-0.043	0.5666	30.90	127.99	0.5542
41.00	0.00	5.70	9.00	0.0011	0.6946	4.36	-0.042	0.3091	31.45	116.55	0.4045
20.13	20.13	6.45	22.29	0.0022	0.9220	2.34	-0.072	0.7337	36.70	97.39	0.4245
27.57	14.13	5.00	8.75	0.0012	0.8032	6.14	-0.122	0.2239	-7.53	100.34	0.3526
0.00	39.70	7.00	14.87	0.0017	0.8941	2.13	0.192	0.3853	147.17	195.70	0.5673
39.70	0.00	7.00	5.38	0.0007	0.7748	4.99	-0.060	0.4354	-7.73	184.60	0.4053
0.23	41.00	5.47	17.10	0.0020	0.5959	5.15	0.693	0.5217	-6.18	172.03	0.3214
0.23	41.00	5.47	16.64	0.0018	0.5539	5.63	0.705	0.8047	0.66	127.06	0.4080
10.20	30.05	6.45	8.00	0.0024	0.7943	1.77	-0.047	0.8504	126.89	181.92	0.4377
13.23	26.47	7.00	22.29	0.0020	0.8120	2.28	-0.059	0.6898	246.32	223.60	0.4954
26.47	13.23	7.00	8.75	0.0015	0.8546	6.68	-0.152	0.5893	214.14	190.56	0.5141
20.85	20.85	5.00	14.87	0.0012	0.7586	2.93	-0.052	0.4336	149.85	93.04	0.5489
20.13	20.13	6.45	5.38	0.0012	0.7962	4.16	-0.170	0.6976	94.69	155.89	0.5614
30.05	10.20	6.45	1710	0.0012	0.7852	13.85	-0.110	0.2287	103.69	164.66	0.5413

Mean \pm Standart Deviation; BCLW; Clarified and Decolorized Black Carrot Liquid Waste, (g/100 g, in dm); dm; Dry Matter; GC, Glucose Syrup (42 DE) (g/100 g, in dm), G; Gelatin, (g/100 g in dm).

small amounts of pigments remaining in BCLW after the decolorization process. When the substitution of BCLW was over 50 %, the change in water activity rate was slow. The gel network formation was likely enhanced in samples with higher gelatin concentrations, and an increase in hardness was observed over time. Tireki et al. (2021) observed an increase in the hardness of gummies with the incorporation of higher gelatin concentrations in the formulation, attributed to enhanced intermolecular interactions between gelling agent molecules. In contrast, formulations with lower gelatin concentrations and higher BCLW substitutions exhibited limited enhancement in gel network strength or resulted in weaker network formation or deformation of the network. The decreasing effect of gelatin concentration on hardness might have been compensated with approximately 50 % substitution with BCLW. This is consistent with the previous findings that highlighted the ratio of glucose syrup to sucrose as a critical factor influencing changes in gummy hardness over time.

3.2.6. Validation study

For the optimization study, the analysis results of the samples obtained from the market were used as target values, and for the validation of the optimum compositions, samples with this composition were prepared and analysis were carried out. The parameters used for gummy candies: hardness value is 1635-2040 g, elasticity is maximum, critical value for moisture content is lower than 24.0 g/100 g, and water activity is targeted between 0.50 and 0.75. The optimum points were determined as 24.97 g/100 g glucose syrup, 14.73 g/100 g BCLW and 7.00 g/100 g gelatin (Table 9). The deviations of predicted and experimental results of the responses were between -5.67 % and +5.08 %, and desirability was found to be 0.577.

4. Conclusion

Waste management is a significant challenge in the natural colorant industry. The extraction process's inefficiency and the residuals' high organic content complicate the disposal of process waste. A new process was developed and optimized for black carrot liquid waste, involving clarification, decolorization and concentration steps to transform it into a value-added product. After clarification, decolorization, and concentration, BCLW was successfully recycled and evaluated as a potential sugar syrup for the food industry. Subsequently, BCLW was incorporated into gummy formulations as a glucose substitute based on the study design. The results showed that substituting glucose syrup with BCLW significantly influenced the physicochemical properties of gummies, including total soluble solids, pH, water activity, and moisture content. Notable changes were observed in the texture of gummies, particularly in chewiness and resilience, while the color parameters were also affected by the substitution. The accelerated shelf-life evaluation revealed changes in the color and hardness of gummies under high relative humidity conditions, depending on the varying proportions of glucose syrup, BCLW, and gelatin in their formulations. Overall, these results demonstrate the potential of BCLW as a sustainable substitute in the confectionery industry, providing a solution to waste reduction while contributing to the development of natural products in food production.

CRediT authorship contribution statement

İlyas Atalar: Writing – review & editing, Methodology, Formal analysis. Burcu Tüzün: Methodology, Formal analysis. Ibrahim Palabiyik: Writing – review & editing, Conceptualization. Omer Said Toker: Writing – review & editing, Conceptualization. Suzan Uzun:

Table 9 Composition optimization and validation for BCLW group gummy samples (Desirability = 0.577).

Factor A	Factor B	Factor C	Response 1	Response 2	Response 3	Response 4
Glucose Syrup (g/100 g)	BCLW (g/100 g)	Gelatin (g/100 g)	Hardness (g)	Springiness (%)	Moisture Content (g/100 g)	Water Activity
24.97	14.73	7.00	1688	62.93	11.19	0.64
Experimental Results						
	Optimum		Observed		% Deviation	
Hardness (g)	1712		1655.56		-5,67	
Springiness (%)	49.75		52.36		5,25	
Moisture Content (g/100 g)	12.91		12.33		-4,49	
Water Activity	0.59		0.62		5,08	

^{*} CI: Confidential Intervals, BCLW; Black Carrot Liquid Waste.

Writing – review & editing. **Tahra ElObeid:** Writing – review & editing. **Nevzat Konar:** Writing – review & editing.

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.fochx.2025.102362.

Data availability

Data will be made available on request.

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