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Development and Characterization of Saffron Nano-emulsion Incorporated into Coffee Beverage Using Ultrasonic Emulsification and Ionic Gelation

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Abstract

Background and aims: Use of saffron extract is limited by the poor stability of bioactive compounds. Ultrasound-assisted extraction improves recovery efficiency, while encapsulation technologies are being adopted to protect these compounds and enhance their stability in food products. This study presents the development of a novel functional coffee beverage enriched with saffron (*Crocus sativus* L.) bioactives, employing an integrated strategy of ultrasound-assisted extraction, ultrasonic emulsification, and chitosan–alginate ionic gelation to address the inherent instability of saffron phytochemicals.
Material and Method: Spray-dried saffron nanoemulsions (0.1–4% w/v) were successfully formulated and encapsulated via ionotropic gelation, then incorporated into a black coffee matrix. The resulting systems were comprehensively characterized in terms of physicochemical properties, particle size distribution, chromategraphic composition, antioxidant activity, and sensory performance during storage.
Results and Conclusion: 4% saffron nanoemulsion possesses the most highest scores for aroma, taste, and color. HPLC analysis confirmed effective preservation of key bioactives in capsulation, with good levels of picrocrocin and crocin, though safranal. Dynamic light scattering showed successful nanoencapsulation with a small average particle size, but a high polydispersity index and low zeta potential indicated moderate heterogeneity and limited stability. The saffron-enriched coffee exhibited slightly enhanced antioxidant activity compared to the control, suggesting the biopolymer matrix helped protect bioactives. Colorimetric analysis over 35 days revealed a notable decrease in lightness and an increase in redness, confirming continued pigment diffusion into the beverage. Overall, the findings demonstrate promising approach of combined use of ultrasonic emulsification and chitosan–alginate ionic gelation for the development of premium functional coffee beverages. Optimization of colloidal stability could be future trend.

What is "already known":	• Encapsulation technologies overcome problem of poor stability of bioactive compounds in saffron extract. • Ultrasound-assisted extraction improves recovery efficiency of saffron extract.
What this article adds:	• A novel functional coffee beverage enriched with saffron (*Crocus sativus* L.) bioactives • Integrated strategy of ultrasound-assisted extraction, ultrasonic emulsification, and chitosan–alginate ionic gelation • Sensory evaluation was acceptable and HPLC analysis confirmed effective preservation of key bioactives • Optimization of colloidal stability could be future trend.

1.INTRODUCTION

In recent years, growing consumer awareness of the relationship between diet and health has increased the demand for foods and beverages with added functional benefits [1]. Among beverages, coffee is one of the most widely consumed products worldwide and represents a rapidly expanding market segment, particularly in the ready-to-drink category [2]. According to the International Coffee Organization, global coffee consumption reached approximately 177 million 60-kg bags during the 2023/24 coffee year, representing a 2.2% increase compared with the previous year [3]. Beyond its popularity and distinctive sensory characteristics, coffee is increasingly recognized as a functional food due to its rich content of bioactive compounds, including caffeine, chlorogenic acids, catechins, caffeic acid, ferulic acid, tannins, and other phenolic constituents. These compounds contribute not only to the characteristic flavor profile of coffee but also to its antioxidant capacity and potential health-promoting effects [4]. Consequently, consumer demand has shifted from simple stimulation and refreshment toward coffee products offering enhanced nutritional and functional value [5].

Plant-derived ingredients have attracted considerable attention as sources of natural antioxidants and bioactive compounds that can be incorporated into functional foods and beverages [6]. Among these, saffron (Crocus sativus L.) is one of the most valuable medicinal and culinary plants worldwide. The dried stigmas of saffron, commonly known as “red gold,” contain a unique phytochemical profile dominated by crocin, crocetin, picrocrocin, and safranal, which are responsible for its characteristic color, bitterness, and aroma. In addition, saffron contains significant amounts of polyphenols, flavonoids, carotenoids, and terpenoids that exhibit strong antioxidant activity and various biological properties, including anti-inflammatory, antidiabetic, antihyperlipidemic, anticonvulsant, and antitumor effects [6]. These attributes have led to increasing interest in the use of saffron as a functional ingredient in food and beverage formulations.

Despite its considerable bioactive potential, the application of saffron extract in food systems remains limited. The extraction and recovery of bioactive compounds from plant materials are critical processes that directly influence extraction yield, purity, and biological activity. Among emerging extraction technologies, ultrasound-assisted extraction has gained significant attention due to its ability to enhance mass transfer, improve cell permeability, increase extraction efficiency, reduce solvent consumption, and shorten processing time compared with conventional methods [7]. However, the bioactive compounds present in saffron are highly susceptible to degradation during food processing, storage, and digestion. Environmental factors such as temperature, pH, oxygen, light, enzymes, proteins, and metal ions can significantly reduce the stability and bioavailability of saffron constituents, thereby limiting their practical application in food products [8].

To overcome these limitations, encapsulation technologies have emerged as effective strategies for protecting sensitive bioactive compounds and enhancing their stability and functionality in food matrices [9,10]. In particular, nanoencapsulation has attracted increasing attention for the development of functional foods enriched with bioactive ingredients and nutraceuticals [11]. Nanoemulsion-based delivery systems offer several advantages, including high kinetic stability, resistance to creaming and sedimentation, improved protection of encapsulated compounds, and enhanced bioavailability due to their small droplet size, typically ranging from 50 to 200 nm [12]. Such systems provide an efficient platform for incorporating saffron bioactives into food

products while preserving their biological activity and sensory quality.

Among the various encapsulation techniques, ionic gelation is considered a simple, mild, and environmentally friendly method that avoids the use of organic solvents and harsh processing conditions. The technique relies on electrostatic interactions between oppositely charged biopolymers to form stable nanoparticles capable of entrapping bioactive compounds [13]. Chitosan and alginate are particularly attractive materials for ionic gelation due to their biocompatibility, biodegradability, nontoxicity, and ability to entrap and protect sensitive compounds. Furthermore, chitosan exhibits additional functional properties, including antimicrobial, antioxidant, emulsifying, and prebiotic activities, making alginate–chitosan systems promising carriers for food applications [11]. Previous studies have demonstrated that nanoencapsulation of saffron extracts using alginate–chitosan systems can effectively protect their distinctive aroma, flavor, and bioactive constituents [14].

Although saffron nanoencapsulation has been investigated in several food matrices, limited information is available regarding the incorporation of saffron nanoemulsions into coffee beverages using a combination of ultrasonic emulsification and ionic gelation techniques. Furthermore, the effects of such incorporation on the physicochemical characteristics, antioxidant activity, and overall functionality of coffee beverages remain insufficiently explored.

Therefore, the aim of this study was to develop and characterize a saffron nanoemulsion produced by ultrasonic emulsification and stabilized through alginate–chitosan ionic gelation followed by evaluating its physicochemical properties, bioactive retention, antioxidant potential, and sensory performance upon incorporation into a coffee beverage matrix.

2. Materials and Methods

2.1. Materials

Ground roasted Coffee (a blend of 20% Arabica and 80% Robusta coffee varieties, medium dark roasted, fine-grained powder) was purchased from a local supermarket. Alginic acid sodium salt from brown algae (448869-50G/Medium viscosity), Low molecular weight Chitosan (A2033-100G), and 2, 2–diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemical Co. (Germany). Tween 20 was purchased from PanReac Co. Saffron (stigma and root) was purchased from Sahar Khiz saffron Co. (Mashhad, Iran). All other chemicals and solvents which used were of analytical grade.

2.2. Methods:

2.2.1. Preparation of Spray-Dried Saffron Powder

Saffron stigmas and roots were weighed and subsequently converted into powder using a spray dryer. The saffron emulsion (20%) for spray drying was prepared by dissolving the needed quantity of dextrose and saffron in distilled water using a homogenizer and spray dryer (Lab plant, Dorsa, Iran) was used with an inlet temperature 180 °C, with aspiration rate 60% and nozzle opening time of 3 s. The dried powder was collected and stored in laboratory glass plate at 4 °C until further use.

2.2.2. Preparation of Different Concentrations of Saffron Nanoemulsion

In order to prepare different concentrations of saffron nanoemulsion, 0.05, 0.25, 0.5, 1 and 2 g of spray-dried saffron powder were weighed to produce nanoemulsion samples containing 0.1%, 0.5%, 1%, 2%, and 4% saffron, respectively.

Additionally, a 1 g sample of saffron stigma and root powder prepared using an electric grinder (Feller, Germany) was weighed to produce a 2% saffron nanoemulsion. This sample was prepared to compare

the flavor, aroma, and sensory characteristics with those prepared from spray-dried saffron powder.

2.2.3. Preparation of aqueous saffron extracts

A combination of solvent-ultrasonic technology was used to extract saffron stigmas and roots. Saffron powder was prepared using distilled water, in a sample/water ratio (w/v) as a table1. The extracts were shaken for 10 min on a magnetic stirrer at room temperature (MTOPS, South Korea) and then sonicated in ultrasonication probe (Bandelin, Germany) for 5 min at 100 W with TS106 probe under pulse condition of 5 s on-time and 15 s off-time and then centrifuged at 3000 rpm for 10 min (Behsan, Iran). Supernatants were filtered (whatman No. 40, China) and stored at 4 °C [10].

2.2.4. Preparation of Saffron Nanoemulsion

Nanoemulsions were formulated using the technique described by Gahruie et al., (2020) with slight modifications. The emulsion was produced by the slow addition of the oil phase (vitamin D3) to the 50 ml aqueous phase containing the extract. 50,000 IU vitamin D3 were stirred in saffron extract that prepared previous step, at ambient temperature for 5 min. emulsification was carried out by ultrasonication probe at 100 W for 5 min under pulse condition of 5 s on-time and 15 s off-time. One of the reasons for using vitamin D3 was that the presence of lipids in a meal is essential for carotenoid absorption, and saffron contains considerable amounts of carotenoid compounds [15].

Tween 20 solution (0.25%) as an emulsifier for vitamin D3 was added to the prepared saffron extract. The mixture was subsequently treated using an ultrasonication probe (Bandelin, Germany) equipped with a TS104 probe at a power of 100 W for 5 min under pulse conditions of 5 s on-time and 15 s off-time.

2.2.5. Loading of saffron nanoemulsion in chitosan-alginate NPs

Ionotropic gelation technique was used to prepare chitosan-alginate nanoparticles loaded with saffron. Nanocapsules were prepared as described by Rahaiee et al., (2023) with some modifications. Briefly, 25 mL of different saffron nanoemulsion were dissolved into 0.05 g/50 mL of sodium alginate solution (25 mL) and homogenized by mixing on a magnetic stirrer at room temperature for 30 min. Then, 0.04 g of chitosan powder was dissolved in 50 mL of distilled water and the pH was adjusted to 3. Afterward, 25 mL Chitosan solution was incorporated into the mixture. Agitation continued at the same rate for 1 h at room temperature.

Finally, the resultant suspension was treated by an ultrasonication probe at 100 W for 3 min in ice with TS106 probe under pulse condition of 5 s on-time and 15 s off-time followed by stirring for 2 hours to improve curing. Ultrasonic homogenization generates a considerable amount of heat during operation, which may negatively affect the stability of heat-sensitive compounds. Therefore, the use of an ice bath is strongly recommended to maintain the temperature of the sonicated liquid at the lowest possible level throughout the process. In addition to temperature control, other ultrasonication parameters such as probe amplitude, pressure, vessel shape, and container capacity should also be optimized [16,17].

Nanosuspension was subjected to centrifugation at 3000 rpm for 20 min.

After centrifugation, the supernatant phase was carefully separated and transferred into 50 mL Falcon tubes and stored under refrigerated conditions for subsequent analyses.

2.2.6. Preparation of Coffee Beverage and SNE Incorporation

Black coffee brew was prepared by extracting 10 g of medium-dark roasted coffee powder in 150 mL of distilled water at 90 °C. Following extraction, the brewed coffee was filtered through Whatman No. 40 filter paper. To prevent the thermal degradation and volatilization of sensitive aromatic compounds, the freshly prepared coffee base was allowed to cool to 40°C prior to incorporating the saffron nanoemulsion (SNE).

SNE was subsequently added to the coffee beverage at designated volumetric concentrations (Table 1), based on preliminary sensory tolerance thresholds. The fortified coffee mixtures were homogenized under gentle agitation at 50 °C for 15 min, transferred into sterile 50-mL Falcon tubes, and stored under refrigeration (8 °C) for quality assessment [18].

Table 1. experimental formulations of Coffee-Saffron Nanoencapsulation

Sample Name	Saffron Powder (g)	Water for Nanoemulsion (mL)	Coffee drink (mL)	Added Nanoemulsion (mL)	Sample Concentration (w/v%)
SC01	0.05	50	60	3	0.1%
SC02	0.25	50	60	3	0.5%
SC03	0.5	50	60	3	1%
SC04	1.0	50	60	3	2%
SC05	2.0	50	60	3	4%
SC05-1	2.0	50	30	3	4%
SC05-2	2.0	50	30	5	4%
SC06	1.0	50	60	3	2%
SC06-1	1.0	50	30	3	2%
SC06-2	1.0	50	10	3	2%
SC00 (Control)	0	50	60	0	0

2.2.7. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical scavenging activity

The antioxidant capacity was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method as described by Rahaiee et al. (2023). Briefly, 0.5 mL of NPs solution was mixed with 3.5 mL of 0.06 mM ethanolic DPPH reagent. After 30 min of incubation in the dark, the change in absorbance of the solution recorded at 517 nm. The DPPH radical scavenging activity calculated using the Eq.1.

$$[A0 - (A1 - As)]/A0 \times 100 \quad \text{Eq. 1}$$

where A0, A1, and As are the absorbance of the control solution containing only DPPH, the test sample and DPPH, and the test sample free of DPPH, respectively [16].

2.2.8. sensory analysis

Aroma and taste were evaluated using a scoring test, while Multiple comparison test was to determine the comparison between Saffron coffee and control coffee (original coffee, without addition of Saffron Nanoencapsulation). Each panelist was given a sample containing one control sample (SC00) of original coffee and 10 Saffron coffees of various formulations. A total of 10 untrained panelists were asked to evaluate saffron coffees and compare each of them with control of original coffee. The evaluation included saffron coffee assessment was better, equal to, or worse than control of original coffee. The score for aroma and taste in the scoring test was 1-5 (score 5 = typical of saffron, 4 = typical of saffron and a little coffee, 3 = balanced of saffron and coffee, 2 = typical of coffee, a little saffron, 1 = typical of coffee) [14].

2.2.9. Organoleptic evaluation

Assessors were requested to verify the impact of the added saffron nanocapsulation on the instant coffee beverage. They evaluated the coffee aroma quality, the overall taste, and the overall/total sensory

quality) using a hedonic/liking scale from 1 to 9 as well as the acidity intensity using a scale from 1 to 9 [19].

2.2.10. Color index measurement

Three lightness/darkness indicators (L*), red/green (a*), and yellow/blue (b*) were evaluated using the CIELab system to assess the color of the samples (Saffron and coffee drink). The Hunter Lab apparatus was used to identify these signs. For this reason, a specific glass container for Hunter Lab was filled with 20 milliliters of a model drink (the volume of a glass bottle), and the color indications were read [20].

2.2.11. Determination of physicochemical properties of saffron extracts

Measurement of the particle size distribution was carried out using a dynamic light scattering (DLS) instrument (Malvern, Germany). The Z-average mean diameter was determined using a Zetapals instrument (Malvern, Germany) [16].

2.2.12. HPLC analysis for bioactive compounds

The quantification of primary bioactive compounds (crocin, picrocrocin, and safranal) in the optimized saffron nanoemulsion (SNE) was conducted using an HPLC system (KNAUER, Germany) equipped with a binary high-pressure gradient pump and a multiple-wavelength UV–Vis detector (Model 2800). Chromatographic separation was achieved on a Eurospher RP C18 analytical column (4.6 x 250 mm, 5 µm particle size; KNAUER) maintained at room temperature (25℃). Prior to injection, sample extracts were filtered through a 0.45 µm polytetrafluoroethylene (PTFE) syringe filter, and a 20 µL aliquot was injected at a constant flow rate of 1.0 mL/min with a total run time of 35 min.

The mobile phase consisted of ultra-pure water (Solvent A) and HPLC-grade acetonitrile (Solvent B). Elution was carried out in high-pressure gradient mode according to the following optimized program: 0.00--3.00 min, 10% B isocratic; 3.00--21.00 min, linear gradient from 10% to 80% B; 21.00--27.00 min, 80% B isocratic; 27.00--28.00 min, linear gradient to 100% B; 28.00--31.00 min, 100% B isocratic (column wash). Target metabolites were detected at their respective maximum absorbance wavelengths: picrocrocin at 250 nm, safranal at 308 nm, and crocin at 440 nm.

Chromatographic method validation was established in accordance with standard ICH guidelines using reference standard calibration curves ($R^2 > 0.995$). Method precision was verified via relative standard deviation (RSD < 2.5%), and limits of detection (LOD) and quantification (LOQ) were defined based on signal-to-noise ratios of S/N = 3 and S/N = 10, respectively [19,21].

2.2.13. Data and Statistical analysis

All sensory evaluations, organoleptic assessments, and colorimetric analyses were conducted in triplicate (n = 3), and the experimental units (individual 50-mL samples of the coffee beverage matrix) were evaluated using a Completely Randomized Design (CRD). For sensory panels, samples were labeled with random three-digit codes and presented in a randomized order to prevent serving-order bias. Data for these triplicated parameters are expressed as mean ± standard deviation (SD). Physicochemical characterization via Dynamic Light Scattering (DLS; hydrodynamic diameter, polydispersity index, and zeta potential) and chromatographic quantification via HPLC (crocin, picrocrocin, and safranal) were performed as screening/representative analyses (n = 1) on the optimized formulation to assess structural features and main bioactive retention. Consequently, statistical significance testing and SD are reported exclusively for the triplicated attributes.

Statistical analysis was conducted using IBM SPSS Statistics version 20 (IBM Corp., Armonk, NY, USA). Data normality was evaluated using the Shapiro–

Wilk test. Because sensory and organoleptic evaluation scores represent ordinal data (1–5 and 1–9 hedonic scales), non-parametric statistical methods were systematically chosen over parametric ANOVA to maintain statistical rigor. The non-parametric Kruskal–Wallis test was employed to evaluate differences among experimental formulations at individual time points, followed by Dunn–Bonferroni post hoc tests for pairwise comparisons. Temporal variations within each treatment group across the 35-day storage period were analyzed using the non-parametric Friedman test. Effect sizes (Kendall's W for concordance in ordinal sensory testing) and 95% confidence intervals were evaluated to assess the magnitude of temporal and formulation differences. Statistical significance was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Sensory Evaluation

3.1.1. Aroma Assessment

The Kruskal–Wallis test revealed that the formulation of nanoencapsulation saffron with Chitosan-Alginate had a significant effect on the aroma score of the Saffron coffee drink (Table 2). Based on the sensory panelists' feedback, sample SC05-2 (coffee enriched with 4% spray-dried nanoemulsion) achieved the highest score, demonstrating superior overall satisfaction and saffron aroma intensity. Conversely, sample SC01 and the control sample received the lowest ratings, placing them at the bottom of the scale regarding saffron aroma perception.

The aroma of freshly prepared Coffee closely resembled the fruity acidic aroma reported in previous studies. However, after filtration through Whatman filter paper, an additional Aroma was perceived in the Coffee sample, which was most likely associated with the filter paper itself. A similar observation was reported by Santanatoglia et al. [22], a strong paper-like aroma was reported in the balance attribute of Chemex samples, which was likely due to the specific filter material used.

3.1.2. Flavor Assessment

Taste is the primary determinant of consumer preference and acceptance in functional beverage formulation. The non-parametric Kruskal–Wallis test demonstrated a statistically significant difference in taste scores among the experimental treatments ($p < 0.001$, Table 2). Formulation SC05-2 achieved the highest taste score (4.300 ± 0.674), which was comparable to SC05 (2.700 ± 0.674) and SC06-1 (4.200 ± 0.632), but significantly superior to the lower-concentration samples (SC01 at 1.200 ± 0.421) and the control (1.200 ± 0.421). Entrapping the bitter glucoside picrocrocin within the chitosan–alginate biopolymer network effectively mitigated harsh astringency and delivered a harmonious, smooth flavor profile.

3.1.3. Color Assessment

In terms of visual appearance and color attributes, sample SC05-2 was identified as the highest quality sample, receiving a score of 4.6 ± 0.843. Sample SC06-1 followed closely with a score of 4.4 ± 0.843. The lowest ratings were recorded for the control sample and sample SC01, suggesting that lower concentrations of the nanoemulsion are insufficient to produce a significant or desirable visual impact on the final product.

3.1.4. Evaluation of Organoleptic Attributes During Storage

The organoleptic stability of unfortified control coffee (SC00) and the optimized saffron-fortified coffee (SC05-2) was monitored over a 28-day refrigerated storage period across multiple attributes: color, coffee aroma, coffee taste, saffron aroma, saffron taste, acidity, uniformity, and overall acceptability (Tables 3 and 4; Figures 2 and 3).

For the control coffee (Table 3), the non-parametric Friedman test confirmed significant temporal

deterioration across all evaluated attributes over time ($p < 0.05$). Overall acceptance declined progressively from 8.800 ± 0.447 on day 0 to 4.200 ± 0.836 by day 28, driven primarily by the loss of volatile aroma compounds and flavor staling. For the saffron-enriched coffee (SC05-2, Table 4), significant chronological changes were observed in color, saffron aroma, saffron flavor, coffee taste, acidity, uniformity, and overall acceptance ($p < 0.05$). Interestingly, perceived saffron aroma and saffron flavor in coffee increased steadily from day 0 (3.000 ± 0.707 and 5.400 ± 1.140) to reach maximum intensity on day 21 (8.600 ± 0.547 and 7.600 ± 0.547), accompanied by a peak in overall acceptance (8.800 ± 0.447). This progressive enhancement is attributed to the gradual swelling of the chitosan–alginate polymeric shell and the slow diffusion of entrapped bioactives into the beverage matrix.

Table 2. Comparison of Sensory Evaluation Scores (Color, Aroma, and Flavor) of Coffee Drinks Among Experimental Treatments

Treatment	Sensory Evaluation		
	Aroma	Taste	Color
SC01	1.000±0.000	1.200±0.421	1.300±0.483
SC02	1.500±0.527	1.400±0.516	1.700±0.483
SC03	1.700±0.483	1.300±0.483	2.300±0.483
SC04	2.300±0.674	2.000±0.816	2.700±0.823
SC05	2.200±0.632	2.700±0.674	3.000±0.816
SC05-1	3.400±1.173	3.900±0.737	3.900±0.737
SC05-2	4.300±0.948	4.300±0.674	4.600±0.843
SC06	2.600±0.843	3.500±0.707	3.900±0.875
SC06-1	4.100±0.737	4.200±0.632	4.400±0.843
SC06-2	3.800±0.918	3.900±0.567	4.000±0.471
SC00	1.300±0.483	1.200±0.421	1.300±0.483
Test Result	<0.001*	<0.001*	<0.001*

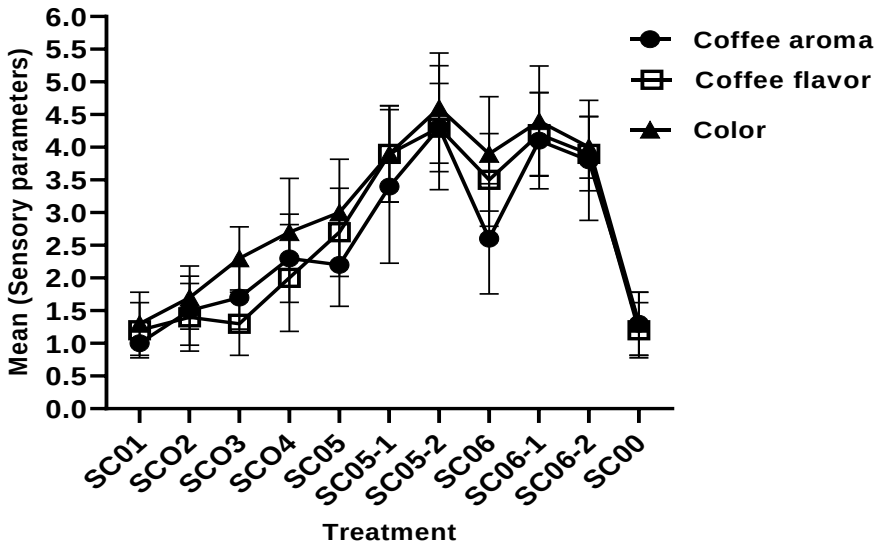

Figure 1. Mean scores of sensory attributes (aroma, flavor, and color) of Coffee drinks across the investigated treatments.

By day 28, a noticeable decrease in acidity (from 7.200 ± 0.836 to 4.200 ± 0.836) coincided with the appearance of structural heterogeneity and partial gel formation (uniformity score of 6.000 ± 0.707). This behavior is linked to the decreased solubility of chitosan as the pH shifts toward neutrality.

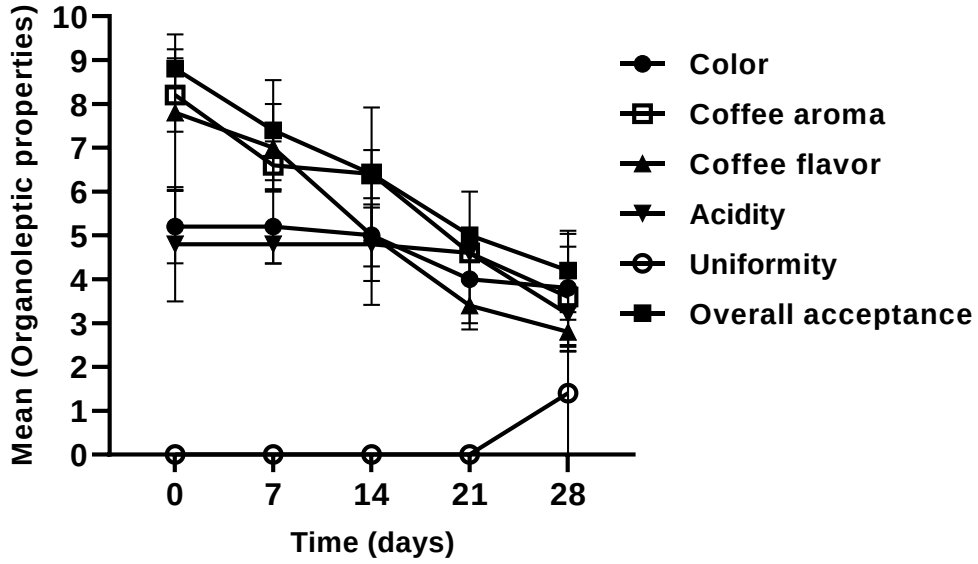

Figure 2. Variation trends in the sensory attributes (color, aroma, flavor, acidity, uniformity, and overall acceptability) of Coffee during the 28-day follow-up period.

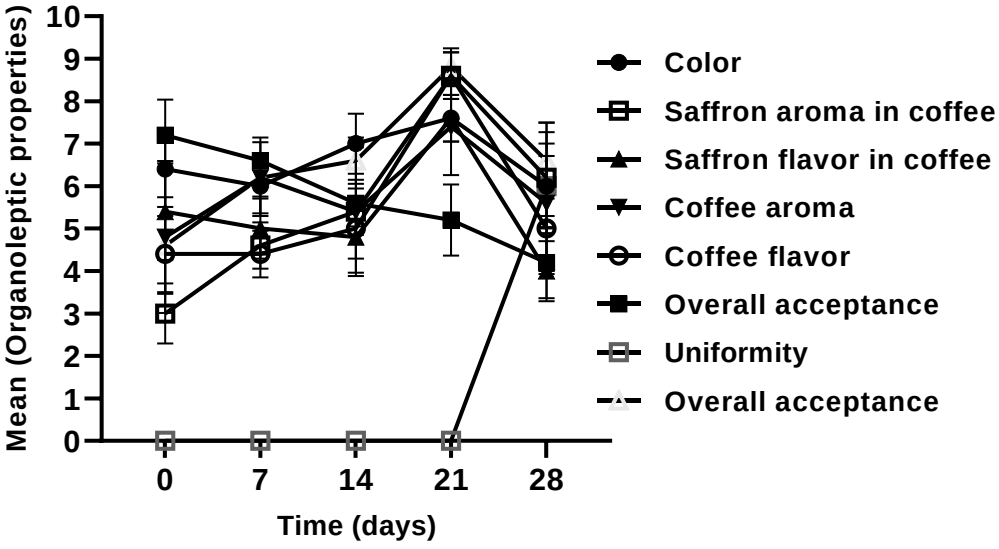

Figure 3. Chronological trends of sensory and saffron-specific organoleptic attributes of saffron-infused Coffee during the 28-day follow-up.

`The SC05-2 formulation treatment was determined as the best treatment because it had a distinctive saffron coffee aroma. Aroma was important because it produced a distinctive aroma of saffron that attracts consumers. The best formulation treatment was applied to produce coffee saffron. The evolution of sensory scores for acidity, taste, aroma and total sensory quality, with storage time for the SC00 and SC05-2 samples, is presented in Figure 2. All tested sensory characteristics exhibited

deterioration over storage time [23]. In coffee beverages, certain features (smell, taste, color, and body) are relevant and highly valued qualitative attributes [24]. The shelf life of both SC05-2 and SC00 instant coffee brew products, determined by the total sensory quality (where a score of 4.3 was set as the total sensory quality limit), was considered, which indicates that the higher the rating of the classic product in terms of overall liking, taste and aroma, the lower the score it will receive in terms of staling, i.e., the fresher it is. According to the results reported by R. Lopes et al. [25], espresso capsules enriched with plant extracts contained the lowest levels of ketones, oxazoles, pyrroles, sulfur-containing compounds, terpenic compounds, norisoprenoids, and thiophene compounds. These findings indicate that although the addition of plant extracts may increase non-volatile constituents such as carbohydrates and caffeine, it can simultaneously reduce the aromatic potential of espresso coffee. This observation is consistent with the sensory evaluations obtained for saffron-enriched coffee.

Table 3. Comparison of Organoleptic Properties (Color, Aroma, Flavor, Acidity, Uniformity, and Overall Acceptability) of Coffee During the Storage Period

Coffee Organoleptic Properties	Time (days)					Test Result
	0	7	14	21	28	
Color	5.200±0.836	5.200±0.836	5.000±0.707	4.000±1.000	3.800±1.303	0.021*
Coffee Aroma	8.200±0.836	6.600±0.547	6.400±0.547	4.600±0.547	3.600±1.140	0.001*
Coffee Taste	7.800±1.788	7.000±1.000	5.000±1.581	3.400±0.547	2.800±0.447	0.002*
Acidity	4.800±1.303	4.800±0.447	4.800±0.836	4.600±0.547	3.200±0.836	0.046*
Uniformity	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	1.400±1.673	0.017*
Overall Acceptance	8.800±0.447	7.400±1.140	6.400±1.516	5.000±1.000	4.200±0.836	0.001*

Table 4. Comparison of Organoleptic Properties (Color, Coffee Aroma, Coffee Flavor, Saffron Aroma, Saffron Flavor, Acidity, Uniformity, and Overall Acceptability) of Saffron-Enriched Coffee During the Storage Period

Coffee Organoleptic Properties	Time (days)					Test Result
	0	7	14	21	28	
Color	6.400±0.894	6.707±0.836	7.000±0.707	7.600±0.547	6.000±1.000	0.046*
Saffron Aroma in Coffee	3.000±0.707	4.600±0.547	5.400±0.547	8.600±0.547	6.200±1.303	0.001*
Saffron Taste in Coffee	5.400±1.140	5.000±0.707	4.800±0.836	7.600±0.547	4.000±0.707	0.002*
Coffee Aroma	4.800±1.788	6.200±0.836	5.400±1.516	7.400±1.140	5.600±1.673	0.063*
Coffee Taste	4.400±0.894	4.400±0.547	5.000±0.707	8.600±0.547	5.000±1.000	0.015*
Acidity	7.200±0.836	6.600±0.547	5.600±0.547	5.200±0.836	4.200±0.836	0.001*
Uniformity	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	6.000±0.707	<0.001*
Overall Acceptance	4.600±1.140	6.200±0.447	6.600±0.547	8.800±0.447	6.600±0.894	0.002*

3.1.5. Evaluation and Comparison of Colorimetric Test Results of Coffee Among the Investigated Treatments Based on Follow-up Time (CIELAB Parameters)

The colorimetric characteristics of the saffron-enriched coffee were evaluated using the CIELAB color space (L*, a*, b*) across the 35-day storage period (Tables 5–7). Although standard baseline

ranges for conventional roasted coffee typically fall between 20 and 40 for L* (lightness), 3 to 12 for a* (redness), and 5 to 20 for b* (yellowness), our optimized formulation (SC05-2) exhibited distinct color coordinates and progressive shifts over time.

Initially, the optimized sample showed an L* value of approximately 55, which gradually decreased to 40 over time, indicating a progressive darkening of the product. This shift towards a lower L* value suggests a maturation process or chemical interaction within the matrix that reduces lightness, bringing the product closer to the typical visual profile of traditional Coffee. Regarding chromaticity, the a* value (redness) increased from 26 to 29 during the storage period. This upward trend is attributed the gradual diffusion and solubilization of saffron pigments within the coffee matrix over time (crocin/crocetin) within the coffee matrix, which significantly enhances the red hue over time. Conversely, the b* value (yellowness) exhibited a decreasing trend, shifting from approximately 90 to 60. The observed deviations from standard commercial coffee ranges—particularly the higher initial a* and b* values—confirm the successful incorporation and subsequent release of the saffron-based nanoemulsion. These fluctuations underscore the dynamic nature of pigment stability in the coffee-saffron blend, which is influenced by factors such as roasting degree, particle size (grind), and the specific composition of the coffee beans.

The CIELAB color space parameters (L*, a*, b*) were evaluated across all eleven experimental formulations over a 35-day storage period (Tables 5–7 and Figures 4–6). The Kruskal–Wallis test revealed statistically significant differences ($p < 0.05$) among the treatments at each evaluation interval. Furthermore, temporal variations within each treatment group were statistically significant over time according to the Friedman test ($p < 0.05$).

For the optimized formulation (SC05-2), lightness (L*) progressively decreased from 55.92 to 41.29, reflecting product darkening and matrix maturation. Concurrently, redness (a*) increased from 26.85 to 28.75, which is attributed to the sustained diffusion of crocin pigments into the beverage matrix. Conversely, yellowness (b*) exhibited a downward trend from 91.16 to 70.53. These dynamic color shifts confirm the active solubilization and migration of saffron bioactive pigments within the coffee environment during storage.

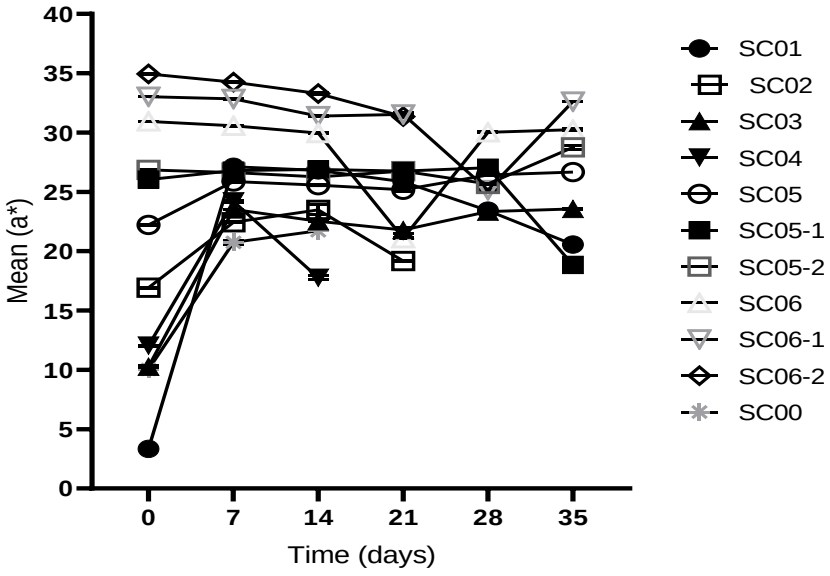

Figure 4. The trend of mean coffee colorimetric a* index values in investigated treatments over the 35-day follow-up

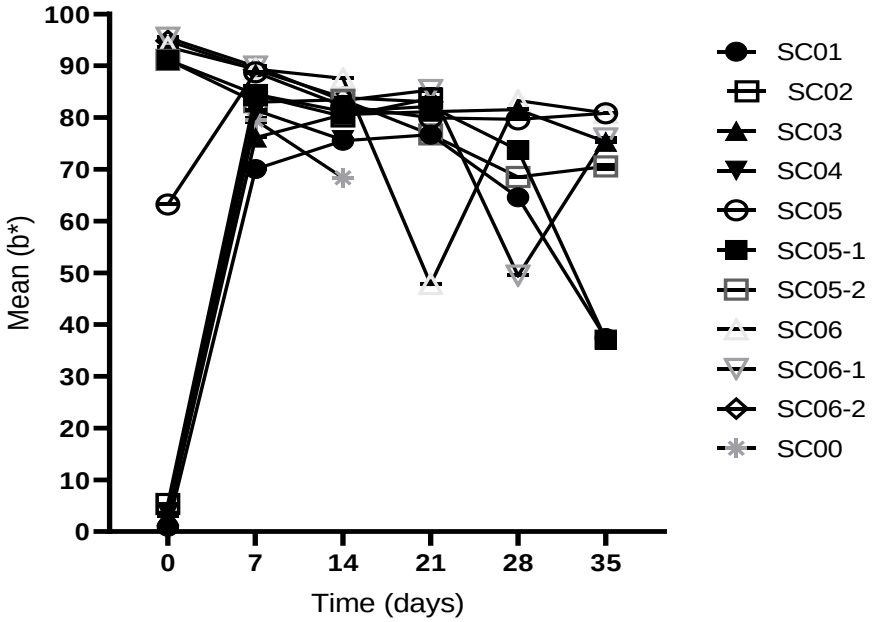

Figure 5. Trend of the mean coffee colorimetric b* index in studied treatments during the 35-day follow-up.

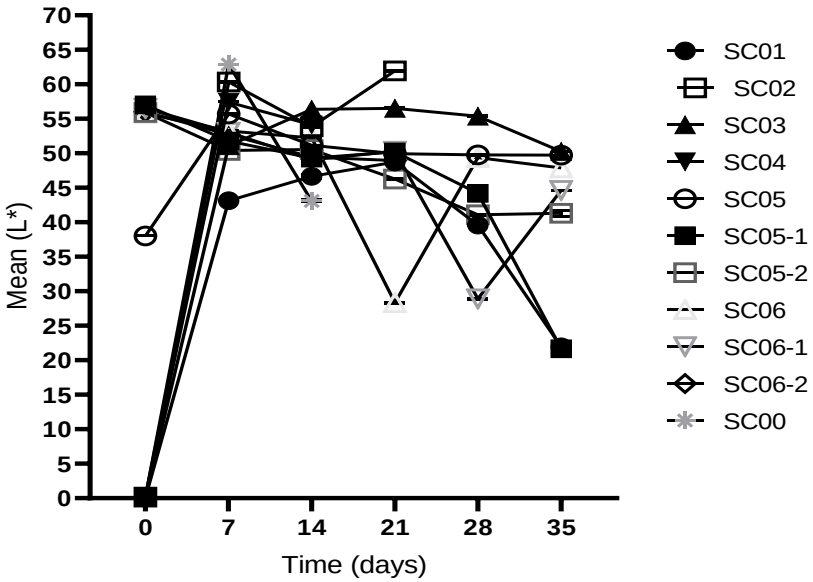

Figure 6. Chronological changes in the mean colorimetric L* index of Coffee across the studied treatments over the 35-day follow-up.

Table 5. Comparison of Mean a* Color Values of Coffee Among Experimental Treatments During the Storage Period

Treatment	Time (days)						Test Result
	0	7	14	21	28	35	
SC01	3.340±0.052	27.116±0.101	26.810±0.230	25.850±0.144	23.426±0.030	20.563±0.028	0.010*
SC02	16.916±0.047	22.433±0.049	23.493±0.433	19.163±0.055	-	-	0.029*
SC03	10.263±0.055	23.516±0.035	22.523±0.025	21.780±0.252	23.333±0.049	23.570±0.026	0.010*
SC04	12.036±0.032	24.196±0.076	17.766±0.152	-	-	-	0.049*
SC05	22.226±0.025	25.880±0.110	25.560±0.019	25.186±0.180	26.420±0.010	26.670±0.0000	0.010*
SC05-1	26.036±0.032	26.800±0.060	26.903±0.051	26.750±0.030	27.046±0.015	18.836±0.051	0.012*
SC05-2	26.850±0.050	26.636±0.047	26.263±0.047	26.743±0.011	25.663±0.051	28.750±0.151	0.010*
SC06	30.936±0.032	30.576±0.025	29.953±0.037	21.100±0.045	30.030±0.043	30.246±0.085	0.010*
SC06-1	33.020±0.026	32.846±0.051	31.383±0.025	31.526±0.152	25.150±0.060	32.620±0.010	0.012*
SC06-2	34.943±0.040	34.250±0.043	33.280±0.072	31.350±0.060	-	-	0.029*
SC00	10.033±0.030	20.743±0.160	21.716±0.015	-	-	-	0.049*
Test Result	<0.001*	<0.001*	<0.001*	<0.001*	<0.003*	<0.003*	

Table 6. Comparison of Mean b* Color Values of Coffee Among Experimental Treatments During the Storage Period

Treatment	Time (days)						Test Result
	0	7	14	21	28	35	
SC01	0.933±0.041	70.060±0.052	75.523±0.015	76.686±0.055	64.556±0.049	37.403±0.055	0.010*
SC02	5.333±0.041	84.326±0.030	80.206±0.096	83.676±0.051	-	-	0.029*
SC03	3.056±0.051	76.130±0.020	80.566±0.416	81.153±0.037	81.553±0.040	75.310±0.065	0.010*
SC04	3.636±0.040	81.433±0.028	75.646±0.130	-	-	-	0.049*
SC05	63.220±0.026	88.746±0.037	82.266±0.376	79.943±0.050	79.663±0.015	80.840±0.000	0.010*
SC05-1	91.133±0.072	84.50±0.435	81.163±0.037	82.136±0.047	73.740±0.026	37.100±0.060	0.011*
SC05-2	91.163±0.030	82.950±0.060	83.436±0.358	76.746±0.005	68.530±0.052	70.536±0.402	0.010*
SC06	93.663±0.015	89.363±0.055	87.583±0.020	47.840±0.026	83.270±0.010	80.850±0.045	0.010*
SC06-1	95.446±0.041	89.860±0.043	83.250±0.010	85.313±0.170	49.466±0.025	75.956±0.041	0.010*
SC06-2	94.800±0.100	89.236±0.310	83.850±0.017	83.036±0.055	-	-	0.027*
SC00	2.966±0.020	79.486±0.401	68.356±0.030	-	-	-	0.047*
Test Result	<0.001*	<0.001*	<0.001*	<0.001*	<0.003*	<0.004*	

Table 7. Comparison of Mean L* Color Values of Coffee Among Experimental Treatments During the Storage Period

Treatment	Time (days)						Test Result
	0	7	14	21	28	35	
SC01	0.090±0.085	43.136±0.032	46.646±0.325	48.736±0.015	39.586±0.025	21.930±0.052	0.010*
SC02	0.150±0.010	60.343±0.047	53.836±0.047	61.940±0.026	-	-	0.027*
SC03	0.146±0.045	51.080±0.075	56.376±0.015	56.533±0.041	55.363±0.055	50.253±0.050	0.010*
SC04	0.150±0.010	57.420±0.026	54.130±0.026	-	-	-	0.049*
SC05	38.030±0.026	55.710±0.072	51.176±0.005	49.943±0.041	49.763±0.030	49.720±0.000	0.010*
SC05-1	57.020±0.026	51.780±0.010	49.320±0.026	50.203±0.176	44.210±0.010	21.653±0.314	0.011*
SC05-2	55.920±0.026	50.423±0.005	50.553±0.297	46.256±0.064	41.076±0.058	41.296±0.438	0.014*
SC06	55.766±0.015	53.226±0.025	52.230±0.026	28.326±0.057	49.370±0.043	47.873±0.005	0.010*
SC06-1	56.520±0.026	53.070±0.062	49.056±0.049	50.296±0.064	28.906±0.097	44.636±0.020	0.010*
SC06-2	56.033±0.030	52.713±0.215	49.446±0.015	48.953±0.055	-	-	0.030*
SC00	0.160±0.010	62.880±0.010	43.093±0.135	-	-	-	0.049*
Test Result	<0.001*	<0.001*	<0.001*	<0.001*	<0.003*	<0.002*	

3.2. Antioxidant Capacity (DPPH)

Antioxidants are compounds capable of inhibiting oxidation processes by scavenging free radicals and preventing oxidative damage. The saffron-enriched coffee exhibited higher DPPH radical scavenging activity (22.5%) than the control coffee (21.8%) Verifying that the primary source of antioxidant capability is the coffee matrix itself because of its high phenolic compound content. The antioxidant activity of the SNE sample was determined to be 45.7%, which can be considered a moderate value compared with those reported in previous studies. The findings suggest that although the ultrasonication process reduced droplet size to the nanoscale and consequently increased the surface area available for bioactive saffron compounds, such as crocin, the encapsulation of these compounds within the chitosan–alginate polymeric matrix may have restricted the direct exposure of encapsulated bioactives to the DPPH radical, resulting in a gradual interaction rather than immediate scavenging. Furthermore, exposure to the elevated temperatures associated with the spray-drying process may have

contributed to the observed antioxidant activity. Nevertheless, the retention of antioxidant capacity at this level indicates the high protective efficiency of the double-layer polymeric wall system in preserving oxidation-sensitive saffron bioactive compounds against environmental and thermal stresses.

Rahaiee et al. [16] demonstrated that chitosan–alginate nanoparticles effectively preserved the antioxidant activity of encapsulated saffron extracts and reported approximately 50% DPPH inhibition for nanoparticles containing aqueous saffron extract, compared with 45.7% inhibition observed for the 4% SNE sample in the present study. The antioxidant activities observed in the present study were generally lower than those reported in previous investigations, which may be attributed to differences in extraction methods, formulation characteristics, and storage conditions. The baseline DPPH radical scavenging activities for control coffee (21.8%) and saffron-fortified coffee (22.5%) were relatively modest. This can be largely attributed to the prolonged storage of the commercial coffee powder (which had been opened approximately six months prior to analysis), leading to partial oxidative degradation of native phenolic constituents. Nevertheless, the significantly higher antioxidant activity observed in the SNE-fortified coffee compared to the control indicates that encapsulated saffron bioactives successfully supplemented the matrix, mitigating antioxidant loss during storage. Similar trends have been reported by Kustyawati et al. [14], who found that the incorporation of cardamom essential oil microcapsules increased the antioxidant activity of coffee relative to the control treatment, highlighting the beneficial effect of plant-derived bioactive compounds on coffee functionality. The slightly lower antioxidant activity obtained in our work may be attributed to differences in extract concentration, encapsulation efficiency, and storage conditions. In contrast, Sharaf [17] reported substantially higher antioxidant activity (77.72%) for coffee extracts prepared under optimized extraction conditions using methanol, emphasizing the strong influence of extraction techniques on antioxidant recovery. Moreover, Erskine et al. [1] demonstrated that coffee can exhibit synergistic antioxidant interactions when combined with plant-derived bioactive ingredients and reported that coffee possesses a relatively high antioxidant potential compared with several other brewing methods. Collectively, these findings support the hypothesis that the incorporation of SNE enhances the antioxidant capacity of coffee beverages and that the chitosan–alginate encapsulation system effectively protects saffron bioactive compounds, thereby contributing to the development of functional coffee products with improved health-promoting properties.

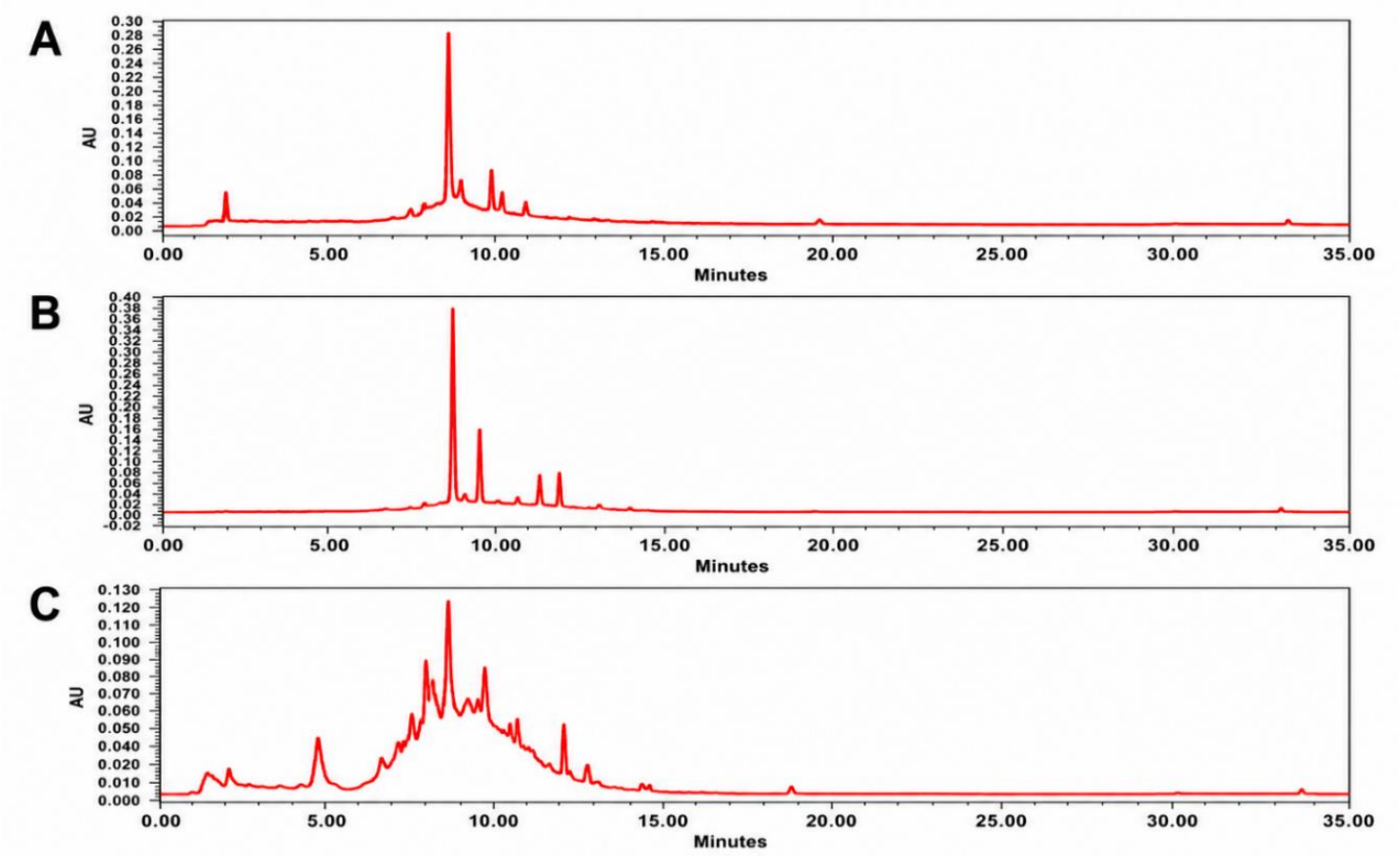

Figure 7. Representative HPLC chromatograms of the major bioactive compounds of saffron: (A) picrocrocin, (B) crocin, and (C) safranal.

3.3. Quantification of Major chemical compounds of SNE by HPLC

Representative HPLC analysis (n = 1) was conducted on the optimized formulation (SNE) to verify the retention and profiles of major saffron non-volatile and volatile bioactives following processing. To evaluate the bioactive compounds, present in the SNE, the samples were analyzed using High-Performance Liquid Chromatography (HPLC). The results revealed that the concentrations of total crocin, picrocrocin, and safranal in the SNE sample were 241.15, 336.58, and 0.11 mg/L, respectively.

According to the obtained results, picrocrocin exhibited the highest concentration among the quantified compounds (336.58 mg/L), whereas safranal was detected at a considerably lower concentration (0.11 mg/L). The substantial levels of crocin and picrocrocin indicate the effective preservation of the major color- and flavor-related constituents of saffron throughout the nanoemulsion preparation process. In contrast, the low concentration of safranal may be attributed to the volatile nature of this compound and its potential loss during sample preparation, homogenization, ultrasonication, and spray-drying processes.

The HPLC chromatogram of the SNE is presented in Figure 7. The presence of well-defined peaks at retention times corresponding to the characteristic saffron constituents confirms the effectiveness of the extraction and nanoemulsion fabrication procedures in retaining a significant proportion of the bioactive compounds of saffron.

The chromatographic profile obtained in the present study was broadly comparable to the saffron bioactive profile reported in other articles [19], indicating successful retention of the principal saffron constituents after processing. The presence of major peaks in the characteristic retention-time window confirms that the nanoemulsion procedure did not destroy the core saffron-derived compounds entirely. Nevertheless, the less resolved and more broadened peak region in our chromatogram suggests that the sample underwent some degree of compositional alteration during processing, most likely due to thermal exposure, ultrasonication, oxidation, or interactions between saffron metabolites and the formulation matrix. This interpretation is in line with the reported low concentration of safranal in the other articles, which the authors attributed to the volatility and instability of this compound during sample preparation and drying. Overall, the chromatogram supports the conclusion that non-volatile saffron bioactives were relatively well preserved, whereas volatile aroma compounds were more prone to loss.

3.4. Particle Size Distribution and Zeta Potential Analysis

According to the results of the particle size analysis, the chitosan–alginate nanoencapsulation system successfully entrapped the saffron extract and produced nanoparticles predominantly within the nanometer range. The particle size distribution exhibited a bimodal pattern, with approximately 99.8% of the particles showing an average diameter of 96.89 nm, confirming the successful formation of nanoscale particles. However, a small fraction of particles (0.2%) presented a diameter of approximately 1010 nm, resulting in an overall average hydrodynamic diameter of 179.6 nm and a polydispersity index (PdI) of 0.462. Although the average particle size was within the desirable range for nanoencapsulation systems, the relatively high PdI value indicates a heterogeneous particle size distribution and moderate instability of the suspension. It should be noted that DLS analysis was performed as a representative screening (n = 1) to establish the baseline particle size distribution and surface charge of the optimized chitosan–alginate saffron nanocapsules.

The small particle size obtained in the present study can be attributed to the application of ultrasonic homogenization, which generates intense shear forces and acoustic cavitation capable of disrupting large polymeric structures and reducing droplet size. Similar observations were reported by Prasetyaningrum et al. [26], who demonstrated that ultrasonication significantly reduced the size of curcumin microcapsules compared with non-sonicated samples. Likewise, Nasrpour [27] reported that ultrasonication was essential for producing alginate–chitosan nanoparticles within the nanometer range, although excessive sonication could disrupt ionic interactions between polymer chains. The particle size obtained in the present study was also comparable to that reported by Siyar et al. [8], who produced liposome-encapsulated saffron extract with particle sizes ranging from 155 to 208 nm. Furthermore, the achieved particle size was substantially smaller than those reported for ionic gelation microparticles produced by Sampaio et al. [28], demonstrating the effectiveness of combining ultrasonication with ionic gelation for particle size reduction.

Despite the favorable particle size, the PdI value obtained in this study (0.462) was considerably higher than values reported for highly homogeneous nanoparticle systems. For example, Mukherjee et al. [29] reported a PdI value of 0.011 for catechin-loaded alginate nanoparticles produced by ionic gelation, indicating excellent uniformity and suspension stability. The higher PdI observed in the present study suggests the presence of particle aggregation and the

coexistence of nano- and microsized populations. This interpretation is supported by the detection of a minor fraction of larger particles and may indicate partial destabilization of the chitosan–alginate network during processing or storage.

A critical consideration arising from the dynamic light scattering (DLS) characterization is the moderate colloidal stability of the system, evidenced by a polydispersity index (PdI) of 0.462 and a surface charge (zeta potential) of −7.87 mV. In stable colloidal suspensions, an absolute zeta potential exceeding ±30 mV is generally required to generate sufficient electrostatic repulsive forces to counteract van der Waals attractions and prevent particle coalescence [8]. The low magnitude of negative surface charge obtained here suggests limited electrostatic stabilization, leaving the system susceptible to flocculation and aggregation during extended storage. This sub-optimal electrokinetic profile indicates that the polyelectrolyte complexation between positively charged chitosan and negatively charged alginate was incomplete. Several mechanistic factors may account for this behavior: 1. pH-dependent charge ionization: Chitosan solubility and cationic charge density (NH_3^+) decrease sharply as the pH approaches neutrality. The pH conditions during or after formulation likely attenuated the electrostatic cross-linking efficiency with carboxylate groups (COO^-) of alginate, leading to an excess of uncomplexed alginate dominating the particle surface (as indicated by the net negative charge) [30].

2. Ultrasonic shear degradation: Although high-intensity ultrasonication successfully disrupted bulk emulsions to sub-100 nm particles, excessive sonication energy can disrupt fragile ionic biopolymer networks, causing premature scission of polymer chains and partial desorption of the chitosan shell from the alginate core.

3. Sub-optimal biopolymer stoichiometric ratio: An imbalance in the alginate-to-chitosan mass ratio can cause charge neutralization rather than forming a core-shell nanoparticle with a dense electrical double layer. Furthermore, the PdI value of 0.462 reflects a moderately broad size distribution, confirmed by the coexistence of a minor secondary population around 1010 nm. This bimodal behavior highlights the partial physical heterogeneity of the nanosuspension. Rather than emphasizing only the successful generation of sub-100 nm droplets, these findings transparently highlight that the present ionic gelation formulation requires structural optimization—specifically regarding polymer stoichiometric ratios, surfactant loading, and controlled pH adjustment—to achieve robust long-term colloidal integrity.

Organoleptic uniformity scoring (Table 4) revealed that the coffee-nanoemulsion system maintained physical macroscopic homogeneity without phase separation or severe sedimentation up to day 21. However, the relatively low absolute zeta potential (-7.87 mV) and moderate PdI (0.462) imply a thermodynamic susceptibility to particle aggregation over extended periods. Furthermore, while the present study focused on characterizing the final bioactive recovery (via HPLC) and functional performance (antioxidant capacity and sensory attributes), direct quantitative parameters such as encapsulation efficiency (EE%), loading capacity (LC%), and in vitro release kinetics were not experimentally measured. Therefore, claims regarding bioactive preservation reflect overall matrix retention rather than controlled release kinetics. Future studies incorporating ultrafiltration/centrifugation protocols and dissolution testing are recommended to elucidate the specific encapsulation efficiency and release mechanisms of this biopolymer system.

4.8. Study Limitations and Future Perspectives

While the present study successfully demonstrates the incorporation of a saffron nanoemulsion into a coffee beverage matrix using ultrasonic

emulsification and ionic gelation, several methodological and structural limitations should be acknowledged: 1. Colloidal Stability Constraints: The low magnitude of negative zeta potential (−7.87 mV) and moderate polydispersity index (PdI = 0.462) indicate sub-optimal electrostatic repulsion, rendering the colloidal suspension susceptible to long-term aggregation and potential sedimentation during extended storage. 2. Lack of Quantitative Release Kinetics and EE: Due to the absence of quantitative determinations of encapsulation efficiency (EE%), loading capacity (LC%), and in vitro gastrointestinal release profiles, the protective performance of the biopolymer shell remains largely inferred from matrix-level bioactive recovery and sensory longevity rather than micro-level release kinetic modeling. 3. Volatile Bioactive Degradation: Thermal and acoustic stress during spray-drying (180 °C) and probe ultrasonication led to significant volatilization losses of safranal (0.11 mg/L), indicating that processing conditions require mild thermal adaptation.

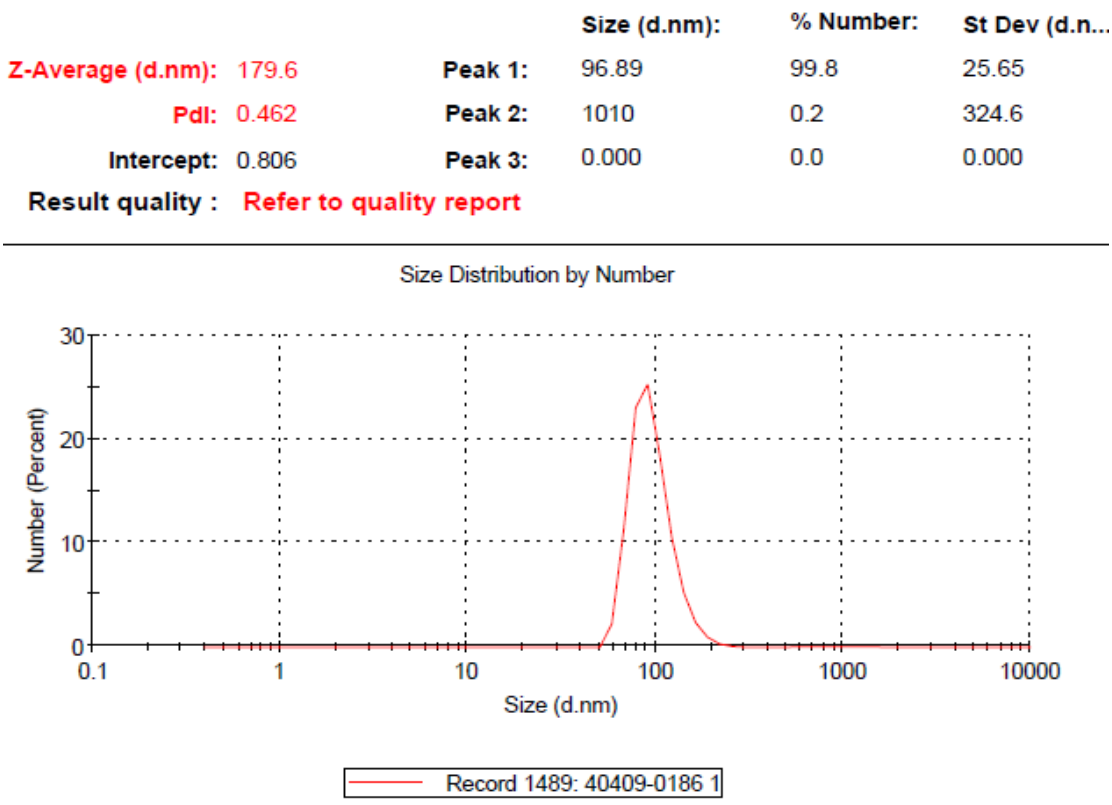

Figure 8. Particle size distribution of the chitosan–alginate saffron nanoemulsion determined by dynamic light scattering (DLS).

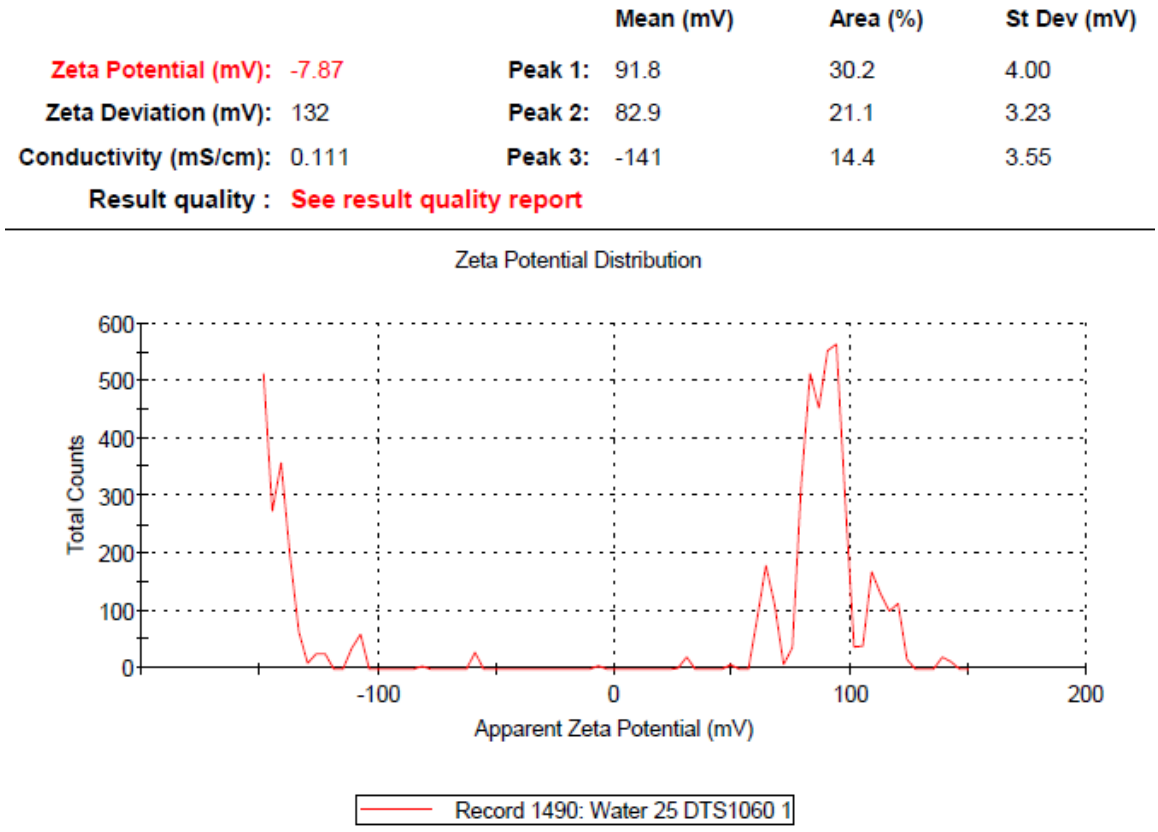

Figure 9. Characterization of nanoparticles. Zeta potential of chitosan-alginate saffron nanoemulsion by DLS method

To build upon these findings, future research should focus on: Optimizing the stoichiometric ratio of chitosan to alginate and incorporating secondary emulsifiers/stabilizers to increase surface charge density (absolute zeta potential > 30 mV) and narrow particle size distribution. Conducting comprehensive in vitro simulated digestion (oral, gastric, and intestinal phases) and release kinetic studies to quantitatively map the bioavailability of crocin and picrocrocin. Evaluating cold-processing encapsulation techniques (e.g., freeze-drying or nano-liposomal entrapment) to minimize volatile loss and retain the characteristic saffron aroma profile.

5-CONCLUSION

This study demonstrated the feasibility of developing a novel functional coffee beverage enriched with saffron bioactives using an integrated approach of ultrasonic emulsification and chitosan–alginate ionic gelation. Sensory evaluation identified formulation SC05-2 (enriched with 4% spray-dried saffron nanoemulsion) as the most acceptable treatment, significantly enhancing aroma, flavor, and visual traits compared to unfortified coffee. HPLC quantification confirmed effective preservation of major non-volatile saffron bioactives (picrocrocin and crocin), whereas the volatile safranal underwent substantial reduction due to processing-induced volatilization.

While dynamic light scattering confirmed the successful generation of a dominant nanoscale subpopulation (~96.9 nm), critical physicochemical evaluation revealed structural limitations in the encapsulation matrix. Specifically, the moderate polydispersity index (PdI = 0.462) and low absolute zeta potential (−7.87 mV) indicate moderate physical heterogeneity and limited electrostatic colloidal stability, leaving the system prone to particle aggregation over time. Furthermore, while matrix incorporation successfully preserved antioxidant

capacity and pigment release during storage, quantitative encapsulation efficiency and in vitro release kinetics remain to be fully established in future research.

Overall, while the chitosan–alginate saffron nanoemulsion offers a promising conceptual framework for functional beverage development, future investigations must focus on optimizing biopolymer stoichiometric ratios, pH conditions, and surfactant systems to overcome colloidal instability, ensure uniform particle distribution, and maximize the long-term commercial shelf life of the product.

6.DECLARATIONS

6.1.Conflict of Interest:

The authors have no relevant financial or non-financial interests to disclose.

6.2 Acknowledgments

The authors are thankful to the Islamic Azad University, Science and ResearchBranch for their partial support of this work.

6.2.Funding:

No funding was received for conducting this study.

6.4Author Contributions

All authors contributed to the study's conception and design.

6.5.Ethical Considerations

All ethical principles were adhered in conducting and writing this article.

6.6Data avaialbility

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

6.7.Using Artificial Intelligent chatbots

No AI chatbot has been used in this study.

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