

Article

Alginate–Chitosan Encapsulation of Fenugreek Seed Oil: Effects of Chitosan Molecular Weight on Physicochemical Properties and In Vitro Bioactivity

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Abstract

Fenugreek seed oil contains bioactive constituents of interest for functional-food applications, but its susceptibility to environmental degradation may limit processing and storage. This study developed an alginate–chitosan encapsulation system and examined how chitosan molecular weight affected the physicochemical properties and in vitro bioactivity of the resulting formulations. Fenugreek seed oil was obtained by mechanical pressing and Soxhlet extraction, yielding 2.81% and 5.50% (*v/w*), respectively. The oil was incorporated into calcium-alginate beads (T_1), which were left uncoated or coated with high-molecular-weight chitosan (HMC; T_2) or low-molecular-weight chitosan (LMC; T_3). The formulations were characterized by color measurement, microscopy, scanning electron microscopy, Fourier-transform infrared spectroscopy, thermogravimetric analysis, and particle-size analysis of oil droplets released from the polymer matrix. Free and encapsulated oil samples were also exposed to UV-C irradiation and evaluated using antioxidant assays, an albumin-denaturation inhibition assay, and an α -amylase inhibition assay. Dry-product recovery ranged from 76.72% for T_2 to 94.79% for T_1 , with T_3 showing a higher recovery than T_2 . SEM showed that T_2 had the smoothest and most uniform dried morphology. FTIR spectra were consistent with interactions between alginate and chitosan while retaining characteristic bands associated with the oil. The chitosan-coated formulations also showed altered thermal-degradation profiles compared with alginate alone. After irradiation, T_3 -UV showed DPPH radical-scavenging activity of 63.03% and albumin-denaturation inhibition of 94.95%, whereas T_2 -UV showed the highest α -amylase inhibition. These findings indicate that chitosan molecular weight influences formulation recovery, morphology, thermal behavior, and measured in vitro activity. Further work using matched non-irradiated controls, standardized oil-equivalent concentrations, release testing, and gastrointestinal models is required before functional-food delivery claims can be established.



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Keywords: fenugreek seed oil; encapsulation; sodium alginate; chitosan; polyelectrolyte complex; antioxidant; anti-inflammatory; antidiabetic; UV stability

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual leguminous herb used for various purposes and widely distributed throughout South Asian and North African countries [1]. The seed oil contains many bioactive components, including the terpenoid 4-pentyl-1-(4-propylcyclohexyl)-1-cyclohexane, and methyl esters of linoleic acid, palmitic acid, and linolenic acid. The seed contains the non-proteinogenic amino acid 4-hydroxyisoleucine, which is a well-characterized insulin secretagogue. It stimulates glucose-induced insulin release from pancreatic β -cells and increases peripheral insulin sensitivity [2].

Fenugreek seed oil is a valuable active ingredient in nutraceutical, functional food, and pharmaceutical formulations [3]. Lipid-based bioactive materials are susceptible to several environmental stressors, including UV radiation, oxygen, elevated temperature, and transition-metal catalysis. UV induces the formation of primary and secondary lipid oxidation products, including hydroperoxides, carbonyls, and oxygen-containing degradation products. The photo-oxidative reaction of lipids generates reactive oxygen species (ROS) [4]. Controlled low-dose UV has been shown to induce upregulation of phenolics and flavonoids. The nature of UV radiation is of great practical significance for stability testing and functional characterization of fenugreek oil-based products [5]. Encapsulation is generally recognized as an effective technique to improve the stability of essential oils. Encapsulation prevents direct exposure to environmental factors and protects volatile and oxidation-sensitive components. It significantly decreases the evaporation of the oil and allows controlled release under specific physiological conditions [6]. The alginate–chitosan polyelectrolyte complex has been shown to have attracted continuous research interest due to the complementary physicochemical properties of its polymers and their intrinsic pH-responsive behavior [7]. Sodium alginate is an anionic polysaccharide obtained from brown seaweed. It can form stable hydrogel beads via ionotropic gelation in the presence of divalent cations. It can resist degradation in the acidic gastric environment but gradually swell and dissolve in the neutral-to-alkaline intestinal lumen [8]. Chitosan forms a polyelectrolyte membrane on the surface of the beads due to electrostatic interactions between the amino groups ($-\text{NH}_3^+$) of chitosan and the alginate. It helps increase bead integrity and decrease bead porosity and drug release kinetics. Chitosan bioactivity depends on multiple interacting factors (deacetylation, MW distribution, dose, formulation, assay, administration) [7]. Low-molecular-weight chitosan (LMC) has greater chain mobility and more accessible amino groups. These are correlated with better antioxidant activity and anti-inflammatory activity. High molecular weight chitosan (HMC) has a denser surface coating and higher viscosity, properties that have been associated with better anti-diabetic performance [9]. Alginate–chitosan systems have been used for various kinds of oils. Dobroslavić et al. [10] prepared calcium alginate microbeads containing fennel essential oil by electrostatic extrusion. Abueta et al. [11] coated alginate beads with layer-by-layer (LbL) alginate–chitosan for the enteric protection of sesame oil. The essential oil and plant-extract delivery system was also studied by Essid et al. [12] and Nemickaite et al. [13] using chitosan–alginate carriers. Chitosan is often used at a single molecular weight in these studies. Thus, it would not be possible to conclude on the structural and functional results based on the chitosan chain length, variations in the formulation, and the crosslinking conditions. The effect of chitosan molecular weight on the physicochemical and biological properties of chitosan alone has been reviewed [14], but its effect in a given oil-encapsulation system has not been systematically explored. In the present study, comparative evaluation of high- and low-molecular-weight chitosan has been done on both structural (SEM, FTIR, particle size), thermal (TGA), and functional (antioxidant, anti-inflammatory, antidiabetic) parameters in the context of a similar photo-oxidative stress for fenugreek seed oil encapsulation in a calcium alginate matrix. This integrated approach aims to provide rational evidence for

designing better encapsulation strategies in functional food and nutraceutical applications by providing the structural basis and functional consequences of the alginate–chitosan encapsulation of fenugreek seed oil.

2. Materials and Methods

2.1. Procurement of Raw Materials

Trigonella foenum-graecum seeds were purchased from a local market in Yangling, Shaanxi Province, China. Sodium alginate, calcium chloride, low-molecular-weight chitosan (LMC), high-molecular-weight chitosan (HMC), and other required chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals and reagents used in this study were of analytical grade and used without any additional purification.

2.2. Extraction of Fenugreek Seed Oil

Fenugreek oil was extracted using two different methods: mechanical and solvent extraction. Fenugreek seeds were cleaned and heated in an oven at 50 °C for 24 h to remove moisture. A screw-type mechanical expeller (motor 7.5 hp, 700 W, 3–6 kg/h, VEVO Co., Ltd., Shanghai, China) was used to extract oil from seeds. The expeller was preheated to 40 °C to facilitate the extraction process and enhance oil flow. The fenugreek seeds were loaded in an expeller with an applied pressure (60 MPa). The oil was extracted from the seeds at a screw speed of 40 rpm [15]. The seeds of fenugreek were ground to a fine powder and subjected to a Soxhlet extraction chamber for the extraction utilizing n-hexane for 1 h at 70–80 °C. The solvent-oil mixture was filtered through filter paper (1) Whatman No. 41 (Global Life Sciences Technologies Co., Ltd., Shanghai, China) and heated in a rotary evaporator at 40 °C. The extracted oil was collected and stored at 4 °C for further analysis. The extraction yield of both methods was calculated following the protocol of [16]. The combined oil was used for further analysis.

2.3. Microencapsulation of Fenugreek Oil

Sodium alginate solution (2%) was prepared by stirring (200 rpm) on a hotplate for 40 min at 50–60 °C. The LMC and HMC solutions (0.2%) were prepared for coating treatments. These solutions were agitated (400 rpm) on a hotplate for 1 h at 50–60 °C before blending with oil. Acetic acid (1 mL) was added to the mixture using a syringe to adjust the pH to 4.5 (Table S1). Fenugreek seed oil (1%) was added to the sodium alginate solution (2%) and agitated on a hotplate (60–70 °C, 400 rpm) until fully blended. The alginate and oil mixture was loaded into a syringe needle (26^{1/2} G), with a drop height of ~2 cm, and extruded manually dropwise into calcium chloride solution (0.04 M) while gently stirring at 300–400 rpm. The beads were collected and washed using distilled water. The beads were dipped in chitosan solutions on a hotplate (50–60 °C) at 400 rpm for 10 min. The coated beads were filtered, washed with distilled water, dried in one batch at ambient temperature (25 ± 2 °C), ambient RH (~40–60%), for 48 h, and kept in a zip-lock bag for further analysis (Figure S1) [10].

2.4. Characterization of Encapsulates

2.4.1. Recovery Yield (%)

The percentage recovery yield is the ratio of the weight of dry beads in each batch to the weight of wet beads during the bead preparation process. The following Equation (1) was used to calculate the percentage yield, as described by [17].

$$\text{Recovery yield(\%)} = \frac{W_2}{W_1} \times 100 \quad (1)$$

whereas; W1 = Weight of total solid (sodium alginate + fenugreek oil + chitosan); W2 = Weight of dry beads.

2.4.2. Particle Size Analysis

Particle size analysis of beads was performed using a laser-scattering particle-size distribution analyzer (HORIBA Co., Ltd., Partica LA-960V2, Tulln, Austria). The instrument was calibrated using standard calibration particles of known sizes to ensure accurate measurements. Representative dispersed samples were introduced into the analyzer's sample chamber. The sample was dispersed in a suitable medium (0.1 M citrate) to prevent agglomeration of particles. The analyzer recorded the scattering pattern and calculated particle sizes based on scattering data. The instrument collected data on particle sizes and generated a particle size distribution curve. Results are reported as the volume-weighted diameters D10, D50, and D90, and the distribution width was expressed as $SPAN = (D90 - D10)/D50$ [18].

2.4.3. Color Analysis

The color of the beads was determined using a spectrophotometer (Shenzhen ThreeNH Technology Co., Ltd., 3nh-ys3060, Shenzhen, China). Black and white calibration plates were used to calibrate the colorimeter. A sample container was employed to analyze the encapsulated beads. Color measurement data were recorded, including parameters such as L* representing lightness, a* indicating the red (+ve)—green (−ve) color, and b* representing the yellow (+ve)—blue (−ve) color [19].

2.4.4. Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectrum was analyzed using an FTIR (Alpha II, Bruker, Ettlingen, Germany) in attenuated total reflectance mode with a sample mass of 50 mg. The FTIR instrument was configured for the mid-infrared range, covering a wavelength range of 4000–400 cm^{-1} , with a resolution of 3.9 cm^{-1} , a signal-to-noise ratio achieved by performing 24 scans, and a beam path length of 1 cm^{-1} . The spectra were used to identify characteristic bands of the oil and polymers and to assess shifts consistent with interactions between alginate carboxylate and chitosan amino groups [20].

2.4.5. Microscopic Analysis of Beads

The morphology and surface analysis of the microcapsule were visualized by a light microscope. The dried microcapsules were placed on a clean glass slide, and a cover slip was placed on top to examine the shape of the particle. The structure and surface morphology of alginate–chitosan beads were analyzed using scanning electron microscopy [21] (JEOL, Musashino, Tokyo, Japan) after being coated with a 10 nm gold and palladium layer. A voltage of 20 kV was employed, which allowed for the examination of the beads at a magnification of 1500 $\times$. This range of magnifications allowed the observation of features. The surface features and shapes of the alginate–chitosan beads were captured at an appropriate magnification level for comprehensive analysis [22].

2.4.6. Thermogravimetric Analysis (TGA)

The thermal stability of encapsulated beads was determined using TGA (Linseis, STA PT1000, Selb, Germany). A sample of 10 mg was scanned up to 500 °C with a heating rate of 10 °C/min under normal atmosphere. Thermal stability and decomposition temperatures were determined by analyzing the weight loss data obtained from the TGA thermograms [23].

2.5. UV Radiation Exposure

The effect of UV radiation on free and encapsulated fenugreek oil was evaluated using the method described, with minor modifications. All the treatments of the second phase included fenugreek oil (FO), UV-treated fenugreek oil (FO-UV), UV-treated encapsulated fenugreek oil in sodium alginate (T1-UV), UV-treated encapsulated fenugreek oil in sodium alginate + HMC (T2-UV), and UV-treated encapsulated fenugreek oil in sodium alginate + LMC (T3-UV). Samples (5 g) were spread onto sterile glass Petri dishes and exposed to UV radiation (254 nm) for 50 min. The UV lamp was positioned 20 cm above the samples, the total dose 2.7 J/cm^2 , and the estimated irradiance $\approx 0.9 \text{ mW/cm}$ [24]. The analysis is one-way ANOVA (not treatment $\times$ UV), and the UV effect is shown only via FO vs. FO-UV.

General Condition for Bioactivity Assay

Oil was released from the encapsulated treatments by dissolving 01 g of beads in 10 mL of 0.1 M sodium citrate at 37°C for 30 min with gentle agitation until the matrix had fully dissolved. The resulting dispersion was assayed directly. Free oil (FO, FO-UV) was assayed without this step. Citrate-only and oil-free matrix controls were not included in the assay.

2.6. Antioxidant Potential of Free and Encapsulated Fenugreek Seed Oil

2.6.1. Free Radical Scavenging Activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

DPPH of the oil was measured using the method described by [25]. The oil sample (0.2 mL) was mixed with 0.1 mM DPPH (2 mL) and methanol (0.4 mg/10 mL) in a test tube, shaken well, and kept in the dark for 30 min. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (SPECORD PLUS 200, Jena, Germany). Methanol was used as a blank in the experiment.

2.6.2. Total Phenolic Content (TPC)

TPC of oil was measured using gallic acid as a standard. The oil was combined directly with methanol and the assay reagents; no prior liquid–liquid partition of the polar fraction from the lipid phase was performed. The reported values are therefore operationally defined rather than absolute. Oil (0.1 mL) and methanol (2 mL) were mixed with Folin–Ciocalteu reagent (0.2 mL) in a test tube and allowed to stay in the dark for 5 min. Na_2CO_3 (20% w/v , 1 mL) was added to the solution and kept in the dark for 2 h. A spectrophotometer was used to measure absorbance at 765 nm, with methanol as a blank [25].

2.6.3. Total Flavonoid Content (TFC)

TFC of oil was measured using quercetin (10–100 ppm, w/v) as a standard [26]. Oil (0.1 mL) was mixed well with ethanolic AlCl_3 (2%, 0.1 mL) and kept for 30 min in the dark. The absorbance was measured at 415 nm with methanol as a blank using a spectrophotometer.

2.6.4. Ferric Reducing Power

Ferric reducing power of the oil was measured using ascorbic acid (10–100 ppm, w/v) as a standard [27]. Sodium phosphate buffer (2.5 mL, 0.2 M with pH 6.6) and potassium ferricyanide (2.5 mL, 1%) were added to 1 mL of oil, and incubated at 50°C for 20 min. Trichloroacetic acid (TCA) (10%) (2.5 mL) was added to it, and the mixture was centrifuged at 4000 rpm for 10 min. The 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl_3 (0.1%, w/v) and then kept for 5 min at room temperature. The absorbance was examined at 700 nm using a spectrophotometer, with running distilled water as the blank, and the ferric reducing value was estimated through a standard curve.

2.6.5. Thiobarbituric Acid Reactive Substances Assay (TBARS)

TBARS of oil were determined using Malondialdehyde [28] (2–10 mM) as a standard [29]. Sample (0.1 mL) was mixed with TCA (30 mL, 7.5%) solution and mixed well at 4000 rpm for 1 min, and filtered. Filtrate (5 mL) was blended with TBA (5 mL, 20 mM) and kept in a hot water bath (95 °C for 40 min). The mixture was cooled down, and absorbance was measured at 532 nm using a spectrophotometer, with running water as the blank. The MDA standard curve (2–10 mM) was used as the basis for calculating the TBARS values.

2.7. Anti-Inflammatory Activity (Albumin Denaturation Inhibition Assay)

The anti-inflammatory activity of the oil was determined following the method of [30]. An oil sample (200 µL) was mixed with a bovine serum albumin (1.8 mL, 1% *w/v*, pH 6.5) solution. The resulting mixture was kept at 37 °C for 20 min and heated at 57 °C in a water bath for 10 min. Absorbance was taken at 660 nm using water as a blank. A solution without a sample was used as a control.

2.8. Anti-Diabetic Activity (Alpha-Amylase Inhibition Assay)

Anti-diabetic activity of the oil was determined following the method [31]. A sample volume of 30 µL was added to α -amylase (15 units/5 µL) and left at room temperature for 10 min. Starch solution (0.1%) was added to it and kept at room temperature for 30 min. HCl solution (20 µL, 1 M) and 100 µL of the iodine reagent were added to it. The absorbance was measured at 580 nm using a spectrophotometer. Acarbose (100 to 500 µg/mL) was used as the experimental reference control [32].

2.9. Statistical Analysis

The data were analyzed using analysis of variance, and the means ($n = 3$) were compared at a significance level of $\alpha = 0.05$ using Completely Randomized Design (CRD). Data are presented as mean $\pm$ SD; a one-way ANOVA was applied, with means separated by Fisher's LSD. Statistical analysis was performed using Statistix software (Version 8.1, Tallahassee, FL, USA) [33].

3. Results and Discussion

3.1. Fenugreek Seed Oil Extraction Yield

Mechanical pressing of fenugreek seeds yielded an average oil recovery of 2.81% (*v/w*). It reflected the inherent compositional limitations of this seed rather than a deficiency of the technique itself. Fenugreek seeds contain comparatively low total lipid content and possess small, tightly bound cell structures. Oil-rich cells of conventional oilseeds release better yield under mechanical compression. A maximum oil yield of 5.5% (*v/w*) was observed using Soxhlet extraction with *n*-hexane. The mechanical yield obtained here was notably lower than that of solvent extraction. This difference is attributable to the fundamentally different mechanism of solvent-based extraction. *n*-Hexane penetrates the intact seed matrix and dissolves lipid fractions along with cellular structures. The greater mass-transfer efficiency of solvent extraction has been documented consistently in studies involving low-oil seeds [34]. It is a more effective diffusion-based dissolution than compression-based dissolution. The fenugreek seed is compositionally complex, making oil extraction limited. The seed contains a high amount of dietary fiber, protein, and galactomannan-containing polysaccharides. These nutrients help to absorb applied pressure and consolidate into a dense structural matrix during pressing. Oil is liberated from the seed matrix through solubilization in the solvent and subsequent diffusion-driven mass transfer. Further research into mechanical yield improvement could be directed

toward pre-treatment. It involves seed grinding, enzymatic digestion of seed cell walls, ultrasound-assisted extraction, and controlled thermal conditioning [35].

3.2. Microcapsules/Beads Formation

Microcapsules of fenugreek seed oil were successfully produced using different combinations of sodium alginate and chitosan (Figure 1).

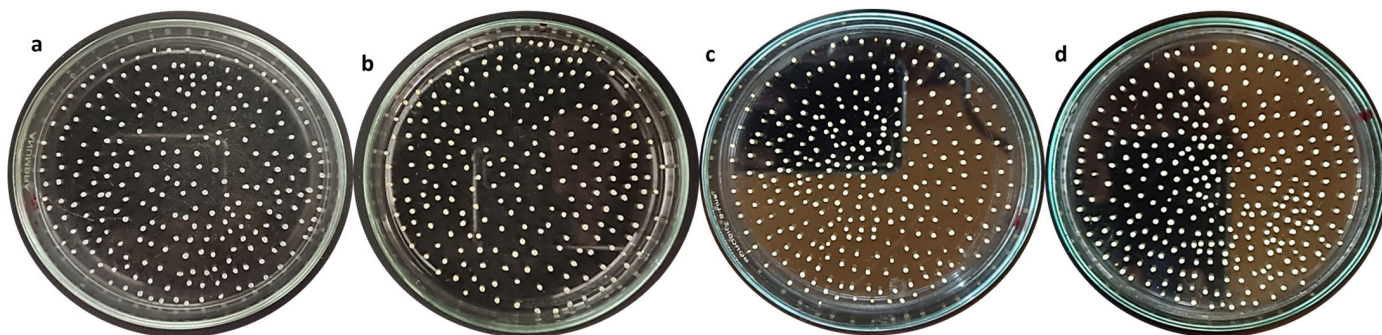


Figure 1. Images of wet beads: (a) $T_0' = 2\%$ Sodium Alginate, (b) $T_1 = 2\%$ Sodium Alginate + 1% Fenugreek Seed Oil, (c) $T_2 = 1\%$ Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% HMC, (d) $T_3 = 1\%$ Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% LMC.

The physical properties of the microcapsules suggested that the main material for bead formation was sodium alginate. With the addition of chitosan, the size of the bead decreased, and the bead color seemed to change (Figure 1). There was an additional size reduction after the drying process compared to the wet beads. The shrinkage of the chitosan-containing beads was greater than that of the beads made of alginate alone, suggesting the importance of chitosan in controlling the dimensional properties of the microcapsules. The size reduction can be partly due to electrostatic interactions between the amino groups ($-\text{NH}_3^+$) of chitosan molecules and the carboxyl groups ($-\text{COO}^-$) of sodium alginate, leading to the formation of a dense polyelectrolyte complex, which physically compresses the bead matrix. This complex also expels water during the drying process, producing beads that are smaller than the wet beads and the beads that are made by the sodium alginate alone. The T_2 microcapsules were darker than the other treatments. The T_2 Microcapsules were darker than the other treatments. This darkening could not be chemically identified during the course of this work, but it may be related to the inherent pigmentation of fenugreek seed oil (deep golden brown color) and its optical effect when treated with alginate–chitosan polyelectrolyte complex, as well as to the concentration of these pigments during drying [36]. The color of the beads thus changed systematically with the wall material composition, which suggests that, in further formulation, it is possible to control the appearance of the microcapsules by adjusting the alginate-to-chitosan ratio. The complexation and alginate–chitosan ratio can be studied in more detail to give control and repeatability in the design of microcapsules [37].

3.3. Recovery Yield%

The yield was calculated by dividing the weight of the capsules after encapsulation by the weight of all the raw materials used. The percentage yield showed significant differences ($p < 0.05$) among the treatments from 76.72% to 100%. For the wall material-only control (T_0'), the highest recovery of 100% was obtained, indicating that no core material was recovered if there is no core material. The addition of fenugreek seed oil combined with sodium alginate (T_1) resulted in 94.79%, which is thought to be the loss of small sections of material that have stuck to the surface and failed to form beads during gelation and drying. When the HMC (T_2) was added, the yield was the lowest at 76.72%,

and when the LMC (T_3) was added, the yield was moderately higher at 82.74%. The chitosan-containing treatments (T_2 and T_3) had a lower yield than T_1 . It is thought to be due to the formation of a polyelectrolyte complex through the interaction of the anionic carboxyl groups of alginate with the cationic amino groups of chitosan. It can cause partial aggregation, material loss during washing steps, and incomplete bead hardening. The lower yield of T_2 relative to T_3 may be due to the higher viscosity of HMC. It resists uniform coating and increases material loss during processing. The LMC chains are more mobile and form a more uniform, tighter complex with alginate. Thus, it results in comparatively better material recovery. The findings are in agreement with previous studies' results, indicating that LMC and HMC influence the recovery yield [12]. Similarly, the wall material, its concentration, and encapsulation conditions play vital roles in the formation of microcapsules [13]. Overall, sodium alginate indicated the highest recovery yield among oil-containing treatments. The incorporation of chitosan (LMC & HMC) resulted in comparatively lower yields. The optimized formulation and processing are required to improve yield in future formulation studies.

3.4. Color Analysis

The microcapsules were analyzed for color using the CIE $L^*a^*b^*$ system. ANOVA results indicated highly significant differences ($p < 0.05$) in all color parameters (L^* , a^* , b^* , C^* , and h^*) across treatments. The highest L^* values were recorded for T_1 (67.18 ± 0.53) and T_0' (65.76 ± 2.09) (Figure 2). This indicates that sodium alginate produced the lightest, whitest beads. The higher lightness of T_0' reflects the white-cream color of dried sodium alginate. The lowest L^* value was observed for T_0 (27.45 ± 1.02), indicating the deep golden-brown natural pigmentation of fenugreek seed oil. T_2 and T_3 indicate that chitosan incorporation reduced lightness. The highest redness value was recorded for T_2 (17.53 ± 0.23), and the lowest for T_0 (0.94 ± 0.19) (Figure 2). The highest yellowness (b^*) was recorded for T_2 (61.16 ± 1.28) (Figure 2). This may be due to the presence of flavonoids and carotenoid-like compounds in fenugreek oil and chitosan. These color attributes are enhanced upon encapsulation. A similar trend was observed for chroma (C^*), with T_2 recording the highest color saturation (63.62 ± 1.29) and T_0 recording the lowest saturation (2.13 ± 0.21). It was consistent with its dark, low-saturation appearance due to brown pigmentation. Hue angle (h^*) values ranged from 1.11 ± 0.05 (T_0) to 1.36 ± 0.01 (T_0') radians. T_2 and T_3 produced beads of the same dominant color tone despite differences in other color parameters (Figure 2).

The chemical mechanism responsible for these color differences was not investigated. Therefore, the observed differences may reflect the combined effects of the intrinsic pigmentation of fenugreek seed oil, the optical properties of the polymer matrix, and changes occurring during drying. Further chemical analysis would be required to determine the mechanism underlying these color differences.

The lowest hue angle reflects its brown-dominated color tone. The alginate-oil-containing treatments indicated a progressive shift toward yellow-orange tones. The color analysis confirmed that fenugreek oil, sodium alginate, and chitosan (LMC, HMC) exerted distinct and statistically significant influences on microcapsule color profile. The interaction of oil pigmentation, polymer whiteness, and browning-type reactions governs the observed variations in lightness, redness, yellowness, saturation, and hue across treatments.

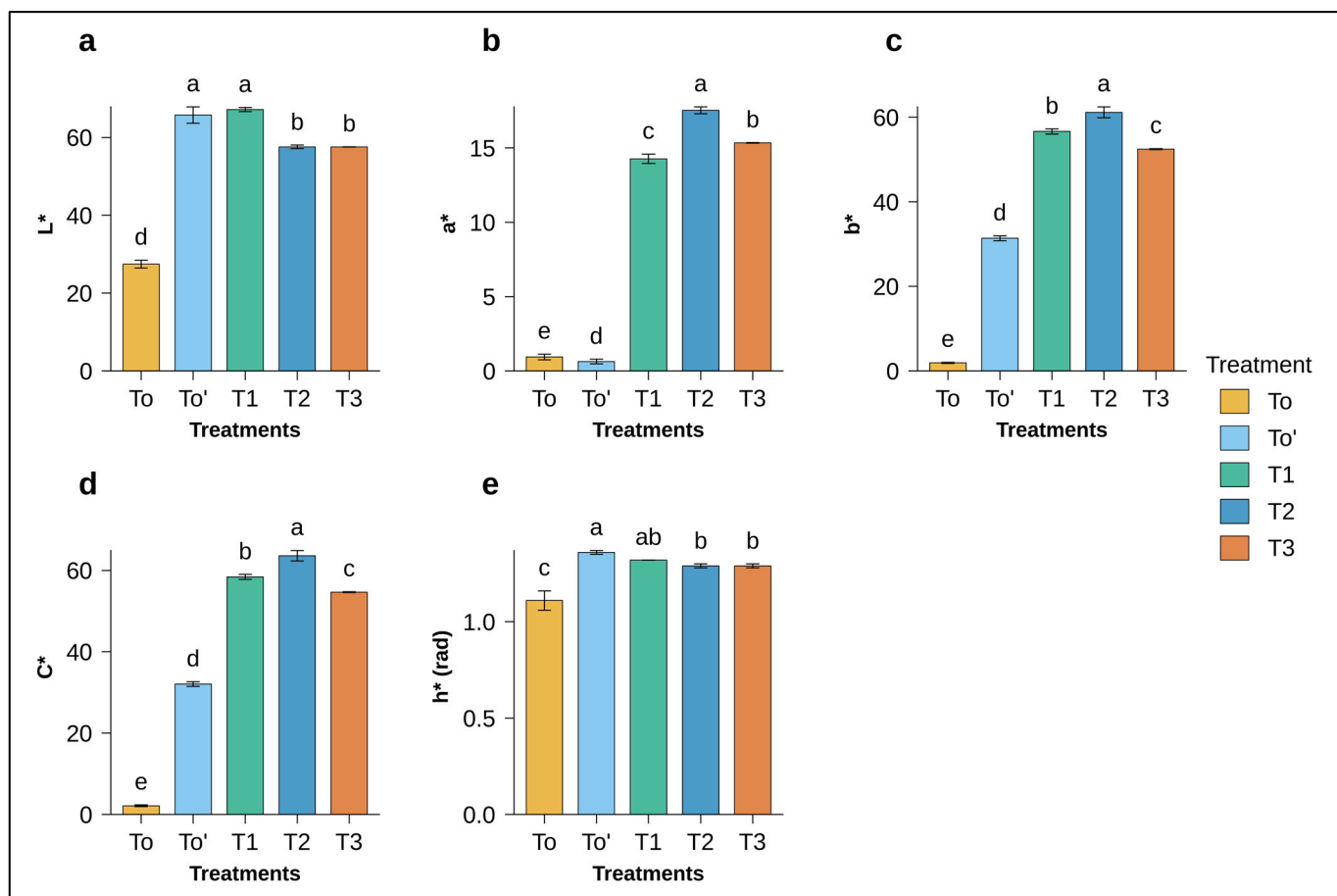


Figure 2. The color characteristics (L^* , a^* and b^*) of the free and encapsulated fenugreek seed oil; T_0 = Fenugreek oil, T_0' = 2% Sodium Alginate, T_1 = 2% Sodium Alginate + 1% Fenugreek Seed Oil, T_2 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% HMC, T_3 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% LMC (a; wet beads & b; dry beads). Where (a) represents “ L^* ”, (b) represents “ a^* ”, (c) represents “ b^* ”, (d) represents “ C^* ”, (e) represents “ h^* ” values. Different lowercase letters above the bars indicate statistically significant differences between groups. Bars sharing the same letter are not significantly different.

3.5. Optical Microscopy Analysis

Optical microscopy was used to evaluate the morphological characteristics of bead shape, surface texture, size, and aggregation behavior. A qualitatively significant difference was noticed across treatments of sodium alginate, fenugreek seed oil, and chitosan (HMC, LMC) in producing microcapsules. T_0 produce round, large, and spherical beads with a transparent periphery and a darker center (Figure 3). This optical gradient is due to the gelation behavior of calcium alginate and its crosslinking network. The addition of fenugreek seed oil to T_1 produced slightly larger and less spherical microcapsules. This morphological disruption is mechanistically due to the hydrophobic nature of the oil. The oil causes the interference of ionic crosslinking between alginate and calcium ions. The aggregation tendency was observed in the oil-alginate interface, which is an insufficiently stabilized T_1 . It suggests that without an additional stabilizing polymer, the beads adhere and coalesce during formation. T_2 (HMC) microcapsules are more uniformly spherical and smaller than both T_0 and T_1 . They have a visibly darker color attributed to a dense chitosan coating surrounding the alginate core. The electrostatic interaction between the amino groups of chitosan and the carboxylate group of alginate helps form a compact polyelectrolyte complex. T_2 microcapsules have a smaller size relative to T_1 due to HMC, which has a high chain length and a greater number of amine groups. It helps form

ionic bonding and forms a more rigid shell that constrains the physical bead expansion. The uniform sphericity of T_2 is consistent with decreased interfacial disruption and may indicate effective oil retention. T_3 treatment indicated an effective encapsulation system with a darker appearance and a smaller bead size. This may be due to the presence of chitosan coating. T_3 beads have a more irregular outline. This morphological difference is mechanistically linked with LMC. LMC shows lower viscosity and higher solubility than HMC [11]. This study holds practical importance for the development of functional foods and nutraceutical delivery systems. This encapsulation system protects the oil from environmental stresses. The comparative use of high- and low-molecular-weight chitosan further provides a practical implementation for improving bead morphology and shell rigidity to meet a targeted product.

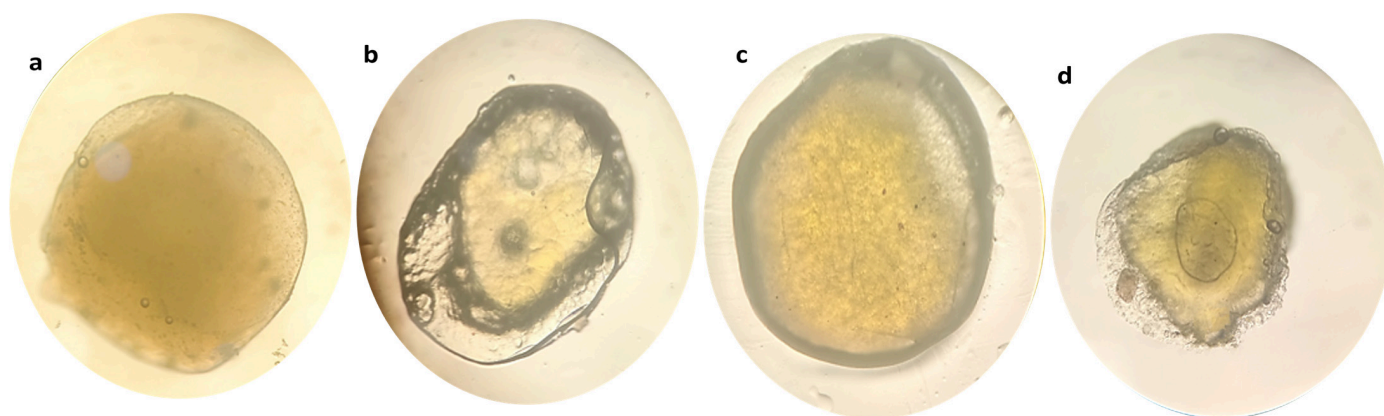


Figure 3. Optical microscopic images of beads; (a) $T_0' = 2\%$ Sodium Alginate, (b) $T_1 = 2\%$ Sodium Alginate + 1% Fenugreek Seed Oil, (c) $T_2 = 1\%$ Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% HMC, (d) $T_3 = 1\%$ Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% LMC.

3.6. Scanning Electron Microscopy Analysis

SEM analysis was conducted to examine the surface topography, microstructural characteristics, and morphological integrity of all four microcapsules. The treatments significantly affected the encapsulation matrix. T_0 micrographs presented a highly irregular, granular, and rough surface texture with a dense and coarse matrix with no spherical particulate structures. This morphology resulted from the behavior of dried calcium alginate hydrogels (Figure 4). The hydrated polymer network collapses during drying, producing a compact, rough surface with no continuous or smooth topography. Calcium-crosslinked alginate shows relatively higher porosity to form the “egg-box” coordination network between Ca^{2+} ions. The guluronate blocks of the alginate chain reflect ionic crosslinking attained during bead preparation. The absence of any spherical or embedded droplet structures in T_0 is expected due to no addition of any encapsulated bioactive core. The heterogeneous and rough surface also highlights an intrinsic limitation of the sole use of sodium alginate as an encapsulation material. Its microporous, irregular matrix offers inadequate physical barriers for retaining hydrophobic bioactive compounds without the addition of any polymer. T_1 presented significant and unfavorable morphological changes characterized by large, deformed, wrinkled, and collapsed bead surfaces relative to T_0 . T_1 showed poor structural integrity and bead deformation, which is consistent with aggregation tendency previously found in optical microscopy [17]. These morphological features are mechanistically due to the hydrophobic nature of fenugreek seed oil. The collapsed, wrinkled morphology of T_1 suggests that sodium alginate alone provides inadequate structural support to fenugreek oil. T_2 presented a highly significant improvement in bead

structure and morphology as compared to T_0 and T_1 . The T_2 bead indicated a well-defined, spherical microcapsule showing a quite smooth and narrow surface.

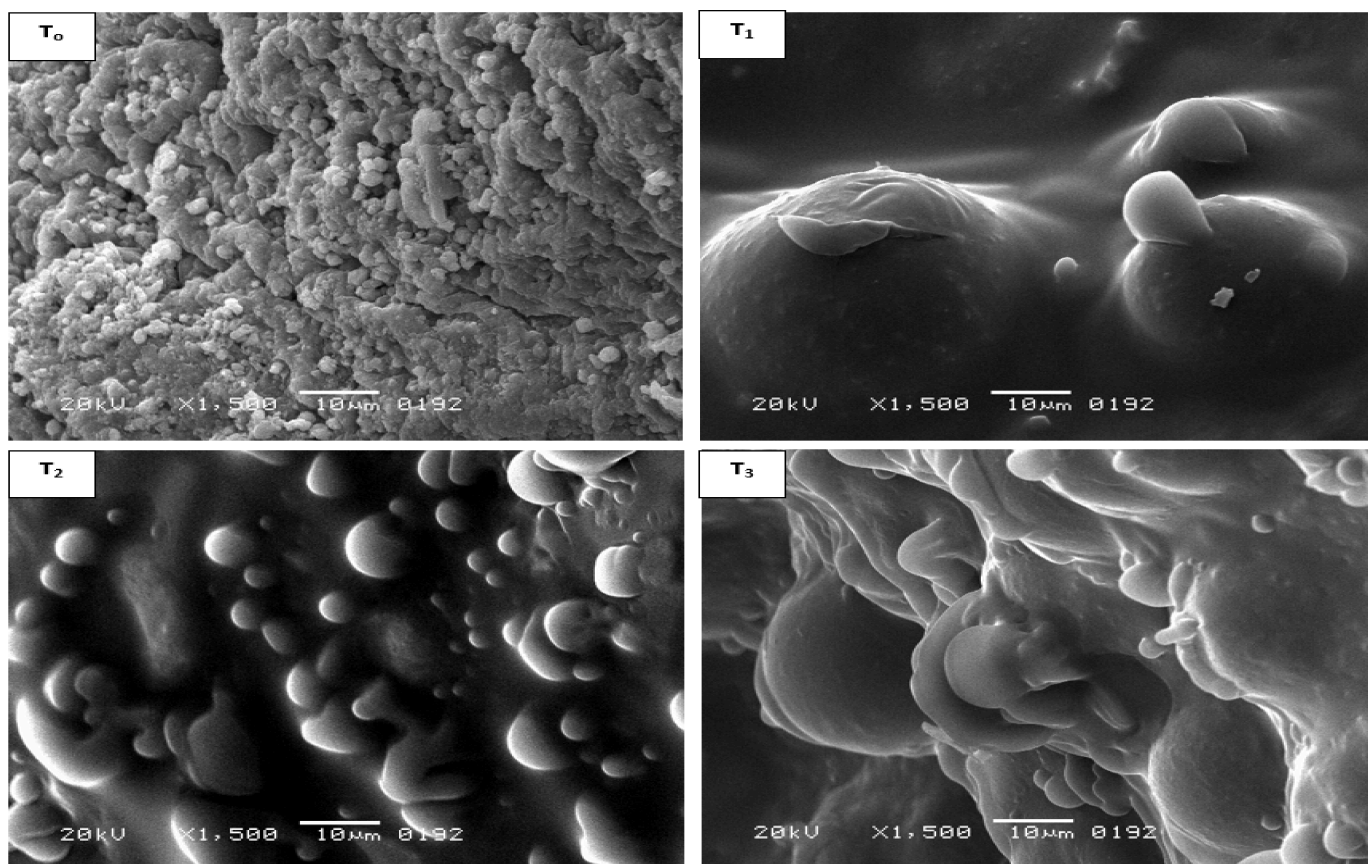


Figure 4. Scanning Electron Microscopic micrographs of beads; Where T_0 = 2% Sodium Alginate, T_1 = 2% Sodium Alginate + 1% Fenugreek Seed Oil, T_2 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% HMC, T_3 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% LMC.

The geometrically regular surface and smooth surface of T_2 occurred through electrostatic interaction between the positively charged amino groups ($-\text{NH}_3^+$) of HMC and negatively charged carboxylate groups ($-\text{COO}^-$) of alginate. This is consistent with a compact polyelectrolyte complex shell and a denser and more continuous outer surface. This observation implies, but is not proof of, better physical retention of the oil in SEM, since only morphological data are available. Similar findings were reported by [38] that only alginate beads showed irregular, rough surface morphology, while chitosan-coated alginate beads showed enhanced structural surface and regularity. Chitosan coating can help to form composite beads with alginate. T_2 appears to be the most structurally satisfactory formulation across all SEM observations. T_3 showed spherical protrusions and vesicular surface structures that may correspond to oil-associated features compared to T_2 . However, these were embedded within an irregularly folded polymer matrix rather than discrete, single spherical microcapsules. Some bead-to-bead fusion and matrix deformation were observed. Pore-like surface features observed in T_3 may be due to drying-induced collapse of the hydrogel polymer network during SEM sample preparation. The morphological difference between T_2 and T_3 is mechanistically explicated by the LMC used in T_3 . LMC has lower viscosity and higher solubility than HMC. T_3 resulted in a less mechanically rigid and structurally constrained polymer shell upon complexation. It allows greater bead deformation before gelation is complete, yielding a less geometrically uniform encapsulate. SEM showed that HMC-coated beads formed spherical microcapsules with a

continuous surface. Furthermore, the SEM-based comparative evaluation of HM and LM chitosan coatings provides formulators with actionable microstructural evidence to guide material selection.

3.7. Fourier-Transform Infrared (FTIR) Analysis

The FTIR spectrum of T_0 revealed absorption bands at 2923 cm^{-1} and 2856 cm^{-1} . It indicates the asymmetric C–H stretching of $-\text{CH}_2-$ in aliphatic chains at 64% transmittance. The medium-intensity absorption at 1455 cm^{-1} (85% transmittance) corresponds to C–H bending vibrations of methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2-$) groups. The strong band at 1743 cm^{-1} corresponds to the C=O ester stretch of triglycerides. The strong absorption at 1157 cm^{-1} (68% transmittance) is consistent with C–O stretching vibrations of aliphatic ester C–O stretching (Figure 5). The 717 cm^{-1} band corresponded to CH_2 rocking of long aliphatic chains. These findings are consistent with previous research on fenugreek seed oil composition [34]. The FTIR spectrum T_0' (Cross-linked with Ca) confirmed the presence of multiple functional groups of sodium alginate. These absorption bands in the $\sim 3200\text{--}3400\text{ cm}^{-1}$ region corresponded to O–H stretching of hydrogen-bonded hydroxyl groups. The strong broad absorption at 3200 cm^{-1} confirmed the presence of carboxylate salt. Absorption near $\sim 2920\text{ cm}^{-1}$ corresponded to C–H stretching. The asymmetric carboxylate (COO^-) stretch appeared at $\sim 1610\text{--}1630\text{ cm}^{-1}$, shifted by Ca^{2+} egg-box coordination, with the symmetric COO^- stretch at $\sim 1410\text{--}1420\text{ cm}^{-1}$. The $\sim 1030\text{--}1080\text{ cm}^{-1}$ region corresponded to C–O–C and C–O stretching of the glycosidic/pyranose ring. Bands at ~ 950 , ~ 880 , and $\sim 810\text{ cm}^{-1}$ corresponded to uronic acid and mannuronic acid vibrations. FTIR analysis of T_1 revealed key interactions between the core and wall materials. The strong broad peak at 3468 cm^{-1} indicated intermolecularly bonded O–H stretching. It suggests hydrogen-bond formation between sodium alginate and the alcohol groups in fenugreek seed oil. Bands near 2360 cm^{-1} arise from atmospheric CO_2 and were excluded from interpretation. Peaks at 2032 cm^{-1} corresponded to the overtone/combination band of carboxylate, showing the alginate COO^- overtone. It indicates the possible interactions between sodium alginate and fenugreek seed oil, leading to the formation of new compounds. Peaks at 756 and 674 cm^{-1} corresponded to C=C bending vibrations of alkene functional groups originating from the fenugreek seed oil. The FTIR spectrum of T_2 retained several characteristic peaks of the constituent materials. The strong peak at 2923 cm^{-1} indicated the asymmetric C–H stretching of $-\text{CH}_2-$ in aliphatic chains. It suggests interaction between the amino groups of chitosan and other compounds in the encapsulated system. Weak features near $2030\text{--}2100\text{ cm}^{-1}$ were treated as overtones that are not assigned. The strong peak at 1742 cm^{-1} corresponded to C=O stretching of esters or δ -lactone groups. The peak at 1163 cm^{-1} indicated C–O stretching of ester compounds originating from the fenugreek seed oil. The peak at 723 cm^{-1} with C–H bending indicated CH_2 rocking of aliphatic chains. The retention of alcohol functional groups confirmed that the encapsulation process did not cause significant chemical alteration of the fenugreek oil. The spectra are consistent with the formation of a polyelectrolyte complex between the carboxyl groups of alginate and the amino groups of HMC, indicated by a shift in the carboxylate stretching band relative to the controls.

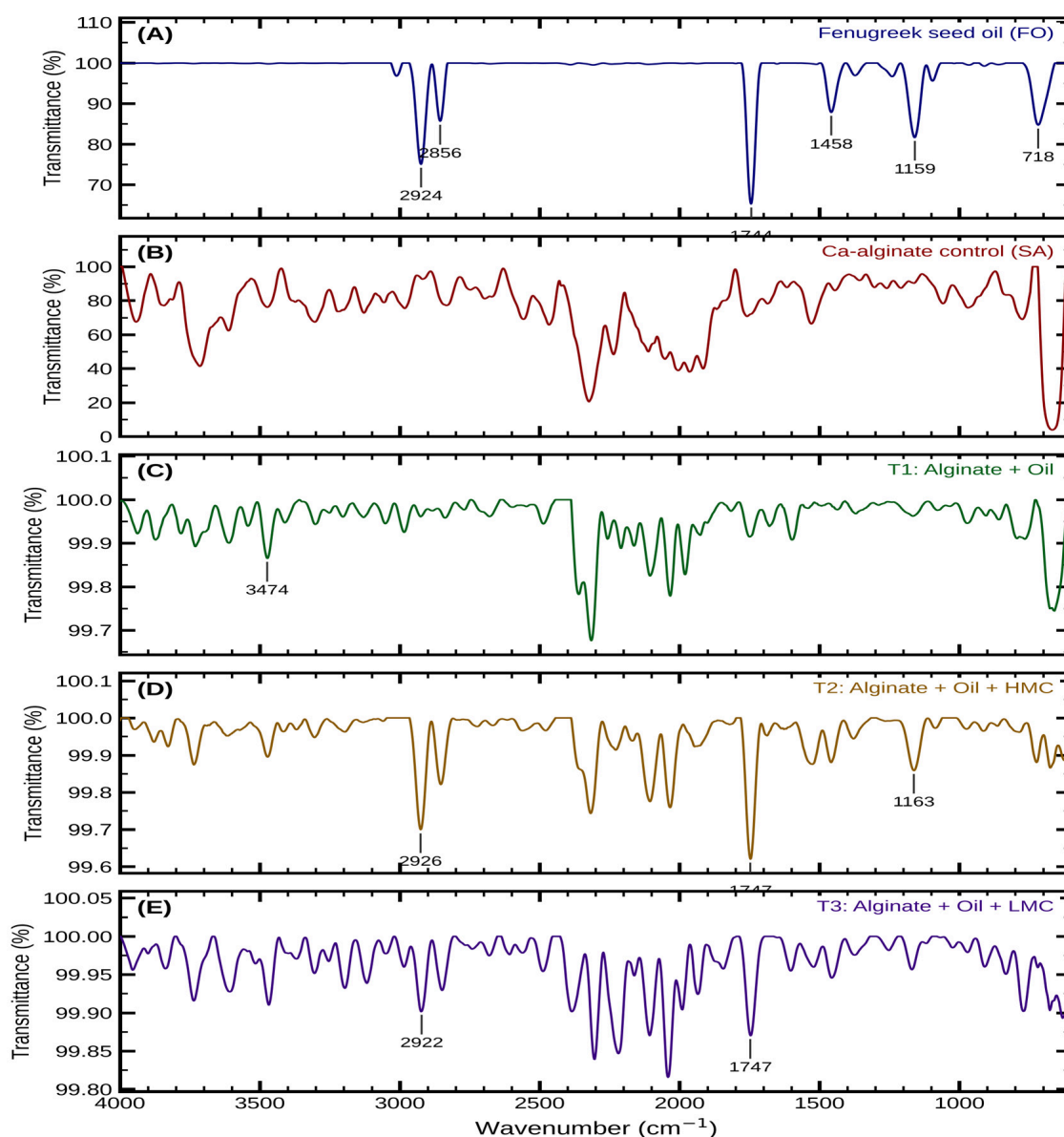


Figure 5. FTIR spectrum of free and encapsulated fenugreek seed oil, where (A) is T_0 = Fenugreek oil, (B) is T_0' = 2% Sodium Alginate, (C) is T_1 = 2% Sodium Alginate + 1% Fenugreek Seed Oil, (D) is T_2 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% HMC, (E) is T_3 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% LMC.

The asymmetric carboxylate band in T_2 and T_3 shifted and broadened, consistent with electrostatic interaction between alginate $-\text{COO}^-$ and chitosan $-\text{NH}_3^+$ superimposed on the existing Ca^{2+} coordination [39]. The FTIR spectrum of T_3 similarly revealed characteristic absorption bands. The strong broad peak at 3470 cm^{-1} indicated intermolecularly bonded hydroxyl groups. The N-H stretching band confirmed the presence of secondary amine groups originating from the LMC coating. It indicates its successful incorporation onto the microcapsule surface. The peak at 673 cm^{-1} indicated C=C bending of disubstituted alkenes in a cis configuration, providing evidence of alkene groups retained within the encapsulated system. The interaction between the $-\text{COO}^-$ groups of alginate and $-\text{NH}_3^+$ groups of chitosan is evidence of polyelectrolyte complex formation. A shift or reduction in the intensity of the carboxylate peak ($\sim 1600\text{ cm}^{-1}$) and amine peak in the chitosan-coated treatments (T_2 and T_3) suggests this interaction [12]. The spectral analysis of T_2 and T_3 indicates differences in their peak intensities. The N-H stretching region ($\sim 3470\text{ cm}^{-1}$).

T₃ may have a higher intensity of the N-H band compared to T₂. It suggests deeper penetration of low-molecular-weight chitosan into the alginate matrix. It affects the degree of alginate–chitosan complexation and the structure of the wall material [40]. The FTIR results support the presence of the characteristic functional groups of fenugreek seed oil within the alginate–chitosan polymer matrix, as well as interaction between the two polymers, which is represented by changes in the carboxylate and amine stretching regions. FTIR suggests the presence of these groups and potential interactions, but does not by itself confirm successful encapsulation and/or encapsulation efficiency. It indicates that the encapsulation process did not cause significant chemical degradation of the oil. These findings validate the integrity and effectiveness of this encapsulation system.

3.8. Thermogravimetric Analysis (TGA)

TGA was performed to evaluate the thermal stability (25–500 °C) and degradation behavior of the microcapsules. It provides insight into the thermal protective capability of sodium alginate and chitosan against thermal decomposition to protect fenugreek oil. The TGA curve of the sodium alginate control (T₀') exhibited three distinct zones of weight loss. The initial weight loss of 18% in the range of 25–168 °C is due to dehydration and evaporation of water molecules. The second zone (168–256 °C) exhibited a 22% weight loss, which was attributed to degradation of the alginate polymer backbone, namely glycosidic bonds, and degradation of primary and secondary alcohol groups. The third zone (256–460 °C) led to a 21% weight loss, which is related to the decarboxylation of the alginate chain and the evolution of CO₂, consistent with the transformation of alginate residues to Na₂CO₃ at high temperature. The results obtained are similar to the known TGA profile of sodium alginate, which usually exhibits three degradation phases: dehydration, breakdown of glycosidic bonds, and final decomposition to Na₂CO₃ [41]. The TGA curve of the oil encapsulated in sodium alginate (T₁) displayed four zones of weight loss. It reflects the additional thermal degradation contribution of the encapsulated oil. The first zone (25–162 °C) showed 16% weight loss due to moisture evaporation. The second zone (162–220 °C) exhibited 12% weight loss corresponding to alginate backbone degradation. The third zone (220–410 °C) yielded the maximum mass reduction of 51%. This is due to the thermal degradation and combustion of fatty acid components of fenugreek oil (polyunsaturated and monounsaturated fatty acids). These typically degrade in an overlapping broad temperature range. The fourth zone (410–500 °C) showed a 21% weight loss, corresponding to complete decarboxylation of alginate residues with CO₂ formation. The appearance of a distinct fourth zone in T₁ compared to T₀' reflects the additional thermal event contributed by the encapsulated oil [42]. The TGA curve of T₂ exhibited three zones of weight loss. The first zone (25–160 °C) showed approximately 12% weight loss due to evaporation of surface and bound water molecules (Figure 6). The second zone (160–460 °C) showed the largest mass reduction of approximately 81%, corresponding to the overlapping thermal degradation of fenugreek seed oil triglycerides and the simultaneous decomposition of the alginate–chitosan polyelectrolyte complex, including the –OH and –NH functional groups of both polymers. The merging of oil and polymer degradation into a single broad zone, compared with the distinct separation observed in T₁, reflects the formation of a denser polyelectrolyte complex between alginate and chitosan that alters the degradation kinetics of the wall material. The third zone (460–500 °C) showed a 7% weight loss, representing complete decarboxylation with CO₂ and Na₂CO₃ formation.

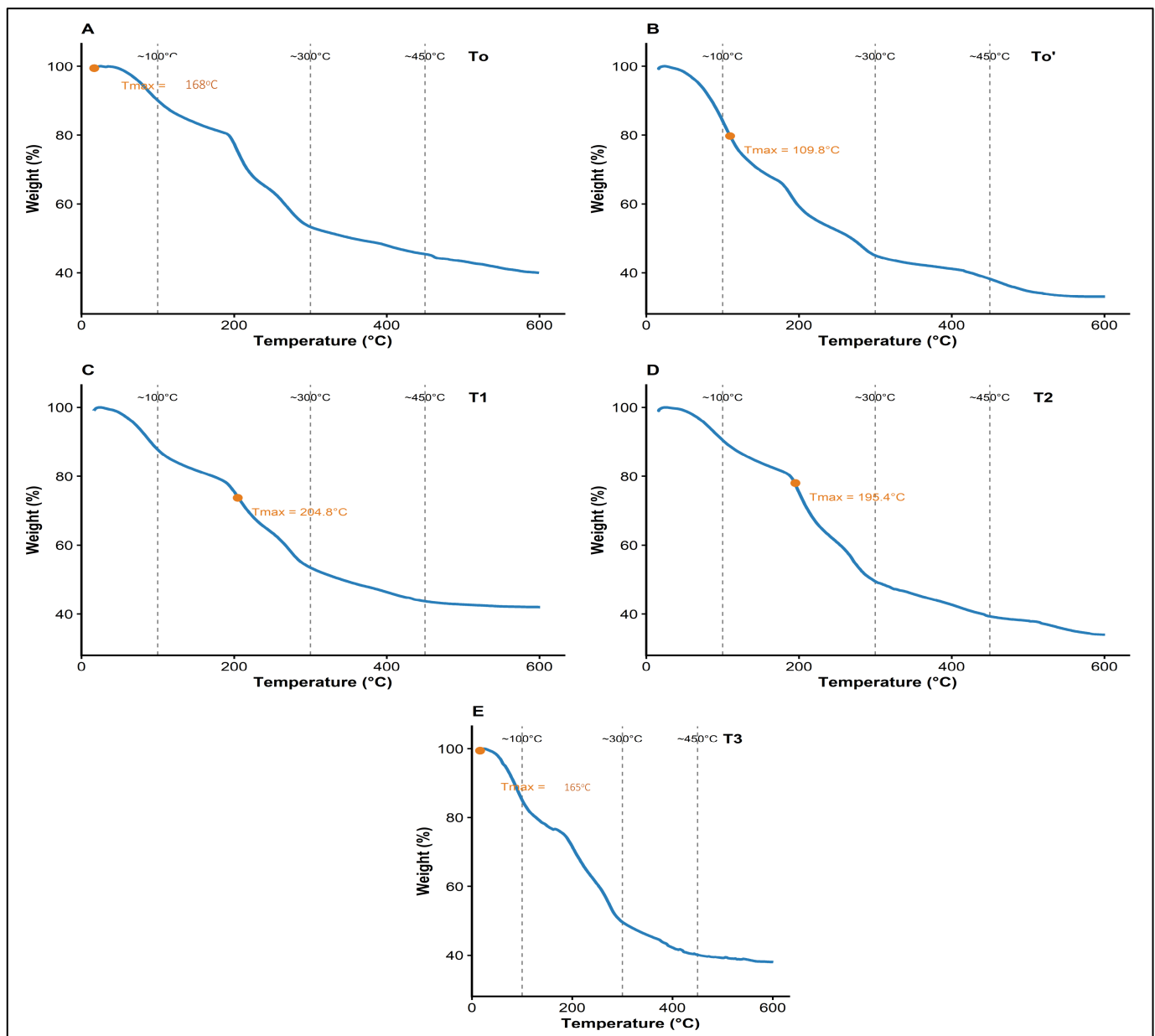


Figure 6. Thermograph of free and encapsulated fenugreek oil, where (A) is T_0 = Fenugreek oil, (B) is T_0' = 2% Sodium Alginate, (C) is T_1 = 2% Sodium Alginate + 1% Fenugreek Seed Oil, (D) is T_2 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% HMC, (E) is T_3 = 1% Fenugreek Seed Oil + 2% Sodium alginate + 0.2% LMC.

The TGA profile of T_3 was broadly similar to T_2 , also exhibiting three degradation zones. The first zone (25–156 °C) showed 12% weight loss due to moisture removal. The second zone (156–464 °C) recorded 80% weight loss from the combined degradation of fenugreek oil, alginate, and LMC polymer matrix. The slight differences in degradation temperatures and zone boundaries between T_2 and T_3 reflect the influence of chitosan molecular weight on the crosslinking density of the wall material. LMC forms a more deeply penetrating but less viscous complex with alginate compared to HMC. This results in marginally different thermal degradation kinetics [43]. The third zone (464–500 °C) showed an 8% weight loss from complete combustion of residual organic material. The TGA data confirm that sodium alginate and chitosan coating may improve the thermal stability of the oil by consolidating the oil and polymer degradation into fewer, broader thermal zones. There was a delayed onset of oil degradation in T_2 and T_3 compared to T_1 .

due to the formation of an alginate–chitosan polyelectrolyte complex. Thus, it provides enhanced thermal protection for fenugreek seed oil. A residual mass of T_0 : 45.1%; T_1 : 44.5%; T_2 : 31.7%; T_3 : 34.1% remained at 500 °C for all, consistent with the inorganic char (Na_2CO_3 and calcium carbonate/oxide) expected from a calcium-crosslinked alginate. Multi-stage thermal decomposition was observed in all of the samples with initial T_{onse6} values ranging from 58.6 °C (T_0) to 68.1 °C (T_2), suggesting that the low-temperature stability of all of the samples is similar. The untreated sample (T_0) had the highest DTG_{max} (0.476% °C^{−1}) and the highest residual mass (45.1%), indicating the best char-forming ability. The degradation stage of T_1 was delayed, with a high intensity at 209.9 °C ($\text{DTG}_{\text{max}} = 0.296\% \text{ °C}^{-1}$), a similar residual mass of 44.5%, and thermal stability similar to T_0 (Table S2). Both T_2 and T_3 had significantly lower residual masses of 31.7% and 34.1%, respectively, even though they had high temperature decomposition points up to 446.2 °C and 448.2 °C, respectively, suggesting that they have low thermal stability. From a general perspective, T_2 and T_3 were found to be less thermally stable than T_0 and T_1 , as reflected in the decreased residual char yield and modified $T_{\text{max}}/\text{DTG}$ curves. The findings suggest that these microcapsules are suitable for applications involving thermal food processing.

3.9. Particle Size Distribution Analysis

Particle size distribution analysis revealed highly significant differences across treatments. The microcapsule size was reduced due to the encapsulation process. The median particle diameter (D50) of fenugreek seed oil (T_0) was found to be $91.88 \pm 0.02 \mu\text{m}$ and the SPAN value 1.499 (Figure 7). It shows a moderately wide size distribution and that the majority of the particles are in the middle of the size range, in the 20–100 μm size range. Particle size analysis was performed on oil droplets released following dissolution of the alginate–chitosan matrix in 0.1 M sodium citrate solution. The mean droplet diameter of encapsulated oil (2.30–2.40 μm) was significantly smaller than free oil droplets (91.88 μm). It indicates that the encapsulation process promoted oil emulsification and reduced droplet size through interaction with the alginate–chitosan polymer network. The median diameter for the sodium alginate control beads (T_0') was nearly the same as that of the control beads, but the SPAN value was greater, 2.555, indicating a greater range of size. This variation is due to the heterogeneous nature of ionotropic gelation and the collapse of the hydrogel network during drying [14]. The measured particle size, D50, and SPAN were significantly reduced with T_1 , with D50 of $2.30 \pm 0.01 \mu\text{m}$ and a narrow SPAN of 1.053. This size reduction is not only due to the encapsulation process but can also be from the dispersion of oil droplets in the alginate matrix. This dispersion produces smaller, oil-laden particles as compared to T_0' . On addition of HMC (T_2), it further reduced the size distribution, with a D50 of $2.28 \pm 0.02 \mu\text{m}$ and a SPAN of 1.002. It is the lowest SPAN among all oil-containing treatments, indicating the most uniform particle-size profile. The large value of SSA (1037 m²/kg). The mechanical system for T_2 is in line with the role of HMC as a compact/hard shell, which is formed by electrostatic interaction with alginate. T_3 (LMC-coated) had the lowest D50 ($2.40 \pm 0.01 \mu\text{m}$) and the smallest SPAN among all the treatments (0.925), and a specific surface area SSA of 978 m²/kg. The slightly larger diameter of T_3 is because the viscosity of LMC is lower and the penetrability is higher. The polymer is less rigid but has a more deeply distributed shell [44]. These results are in agreement with the prior reported particle size characterization of chitosan–alginate microcapsules, which showed that the particle size parameters (D50, size uniformity) were directly influenced by the composition of the wall material and the coating conditions [45]. The reduction in SPAN values from T_0' (2.555) to T_1 (1.053) to T_2 (1.002) to T_3 (0.925) indicates that coating encapsulation and coating with chitosan have a uniform effect on particle size [46].

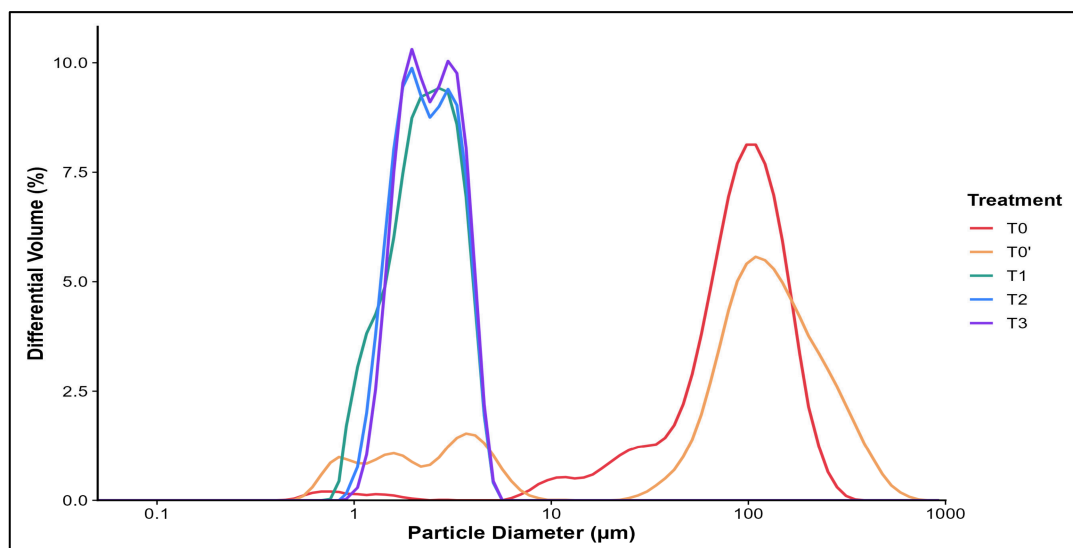


Figure 7. Particle size analysis of free and encapsulated Fenugreek seed Oil; T_0 = Fenugreek oil, T_0' = 2% Sodium Alginate, T_1 = 2% Sodium Alginate + 1% Fenugreek Seed Oil, T_2 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% HMC, T_3 = 1% Fenugreek Seed Oil + 2% Sodium Alginate + 0.2% LMC.

3.10. Antioxidant Potential

3.10.1. DPPH Radical Scavenging Activity

Significant differences were observed among the five tested groups. Because non-irradiated T_1 – T_3 controls were not included, a UV effect can be evaluated directly only by comparing FO with FO-UV. FO-UV recorded the highest scavenging activity ($68.49 \pm 0.45\%$), and FO recorded the lowest ($48.65 \pm 0.69\%$) (Figure 8). The samples exposed to UV showed higher values of DPPH than the untreated sample. It shows that UV irradiation could trigger the formation and activation of radical-scavenging compounds in fenugreek seed oil. This is consistent with [47], who attributed UV-induced increases in antioxidant activity. This was due to the structural modifications in phytochemical constituents under controlled irradiation conditions. T_3 -UV ($63.03 \pm 0.04\%$) indicated higher DPPH scavenging activity than T_2 -UV ($61.96 \pm 0.11\%$). This difference is supported by the very low standard deviations observed in both datasets. The difference between T_3 -UV and T_2 -UV was small, although statistically significant. LMC possesses greater chain mobility, higher aqueous solubility, and more accessible free amino groups. However, HMC favors stronger interactions with reactive oxygen species [48]. The degree of deacetylation further modulates this activity. Higher deacetylation yields a greater density of free $-NH_2$ groups. These are available for hydrogen donation and electron transfer to amplify radical neutralization capacity [9]. Encapsulation rendered functional properties of the oil in the case of DPPH activity, where T_2 -UV and T_3 -UV showed better results than T_1 -UV. The alginate–chitosan blend may help retain the antioxidant components. At the same time, the chitosan shell itself decreases oxygen permeability and the amount of oxygen degradation of sensitive bioactive compounds during processing and storage. Sodium citrate (Release medium) has metal-chelating properties, which bind transition metals, including Fe and Cu. Therefore, it inhibits metal-catalyzed oxidation reactions and reduces the production levels of reactive oxygen species [49]. These findings indicated that LMC-based encapsulation enhanced the oxidative stability and functional attributes.

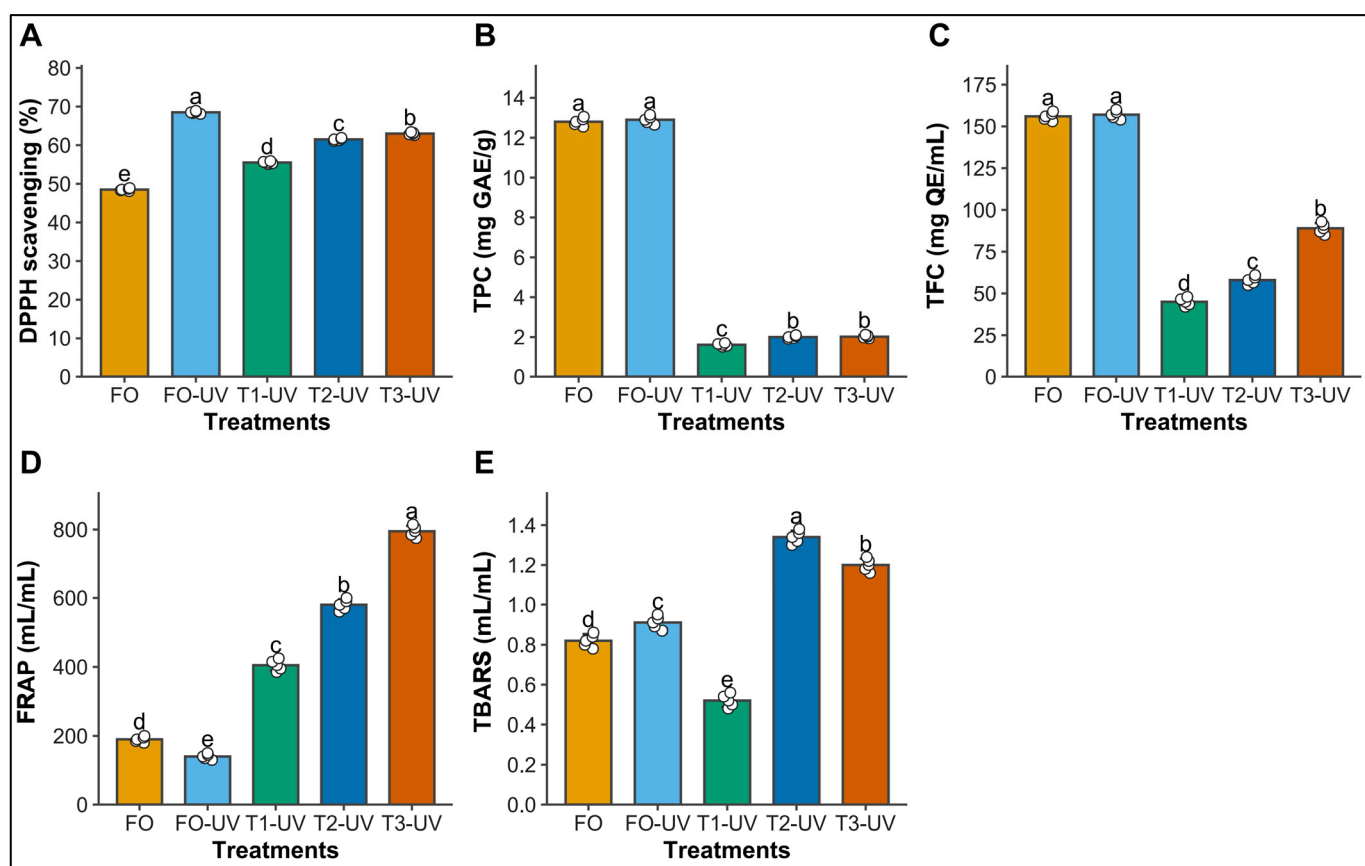


Figure 8. Antioxidant potential of UV-treated free and encapsulated fenugreek oil, Fenugreek oil (FO), UV-treated fenugreek oil (FO-UV), UV-treated encapsulated fenugreek oil in 2% sodium alginate (T1-UV), UV-treated encapsulated fenugreek oil in 2% sodium alginate + 0.2% HMC (T2-UV), and UV-treated encapsulated fenugreek oil in 2% sodium alginate + 0.2% LMC (T3-UV). Values are expressed as means $\pm$ standard deviation ($n = 3$). Where (A) is DPPH, (B) is TPC, (C) is TFC, (D) is FRAP, and (E) is TBARS. Whereas different lowercase letters above the bars indicate statistically significant differences between groups. Bars sharing the same letter are not significantly different.

3.10.2. Total Phenolic Content (TPC)

The ANOVA for TPC showed a highly significant effect among treatments ($p < 0.001$). FO and FO-UV recorded the highest TPC values (12.85 ± 0.05 and 12.84 ± 0.02 mg GAE/g, respectively), with no statistically significant difference between them (Figure 8). The stability of phenolic content in FO-UV following UV exposure aligns with established understanding of phenolic function under photo-oxidative stress: phenolics are recognized as primary contributors to cellular redox balance under such conditions, and their accumulation via the phenylpropanoid pathway is a well-documented UV-protective response in plant-derived materials [47,50]. The encapsulated treatments showed considerably lower TPC values: T1-UV (1.57 ± 0.06 mg GAE/g), T2-UV (1.97 ± 0.29 mg GAE/g), and T3-UV (2.14 ± 0.38 mg GAE/g). This decrease is due to two reasons. The first is that all encapsulated samples were broken up in sodium citrate solution to release the oils before analysis, introducing a dilution effect that was not seen in the pure oil controls. Second, in chitosan-containing treatments, phenols undergo hydrogen-bond cross-linking with the amine and hydroxyl groups in the chitosan polymer chain, thus limiting the availability of these compounds for detection with the Folin–Ciocalteu reagent. UV irradiation further reinforces this effect through photo-crosslinking of the chitosan matrix. Kaczmarek-Szczepańska et al. [51] explained that UV exposure promotes covalent bond formation within chitosan, progressively reducing phenolic release. The slightly higher TPC of T3-UV compared to

T₂-UV indicates that the phenolics are not as well bound in T₃-UV, which is likely due to the lower molecular weight and lower cross-linking density of its structure, allowing for a slightly higher amount of phenolics to be made available during extraction.

3.10.3. Total Flavonoid Content (TFC)

TFC data showed a significant response in all treatments ($p < 0.05$). FO-UV recorded the maximum TFC ($158.30 \pm 2.23 \mu\text{g QE/mL}$), and T₁-UV showed the lowest TFC among all treatments ($45.24 \pm 0.29 \mu\text{g QE/mL}$) (Figure 8). Flavonoids are relatively resistant to UV-induced degradation, which is consistent with the high TFC retained in FO-UV. No increase attributable to synthesis is possible in an extracted oil, and the values reflect retention rather than accumulation [50,52]. The lower TFC values of encapsulated treatments are primarily attributable to dilution in sodium citrate release medium. UV-induced cross-linking of the chitosan wall material, which slows flavonoid diffusion out of the bead matrix [53]. TFC values were higher in T₂-UV and T₃-UV because chitosan provides greater flavonoid retention and protection than sodium alginate alone. The retention reflects chitosan's stronger interaction with polyphenolic compounds through hydrogen bonding and electrostatic attraction.

3.10.4. Ferric Reducing Power

Ferric reducing power values differed significantly across treatments ($p < 0.05$), with T₃-UV recording the highest reducing capacity ($796.08 \pm 0.24 \mu\text{g AAE/mL}$) and FO-UV the lowest ($146.46 \pm 0.76 \mu\text{g AAE/mL}$) (Figure 8). The ferric reducing assay measures the ability of antioxidants to donate electrons and reduce Fe^{3+} to Fe^{2+} [21]. The highest reducing power values were observed in chitosan-containing encapsulated treatments compared to T₁-UV and the unencapsulated controls. Do et al. [54] reported similar results that chitosan protects against UV-induced oxidative damage. The higher reducing power of T₃-UV compared to T₂-UV is yet again in line with the increased accessibility of reducing groups in LMC. Shorter polymer chains have a higher proportion of active hydroxyl and amino functionalities per mass. FO-UV had low ferric reducing power, although the DPPH activity was high, which is probably due to the difference in mechanism between the two assays. Rankings obtained by the two methods may be quite different in the case of plant-derived oils.

3.10.5. Thiobarbituric Acid Reactive Substances (TBARS)

TBARS was significantly affected by treatments ($p < 0.05$). T₁-UV exhibited the lowest TBARS value ($0.52 \pm 0.01 \mu\text{g MDA/mL}$), while T₂-UV recorded the highest ($1.35 \pm 0.01 \mu\text{g MDA/mL}$) (Figure 8). The TBARS assay detects MDA formed when endoperoxides derived from polyunsaturated fatty acid oxidation undergo cleavage. It reacts with thiobarbituric acid to yield a pink chromogen measurable at 532 nm [55]. This result showed that the higher TBARS value in T₂-UV was due to differences in the physical barrier properties of the chitosan type used and the intrinsic antioxidant capacity. HMC forms a thicker, more viscous coating at the bead surface. The coating may not be as even as the other ones because it is more rigid, which causes a less even coating. Therefore, it may cause microscopic defects through which oxygen can leak in and start lipid oxidation. Although it may have a different profile with respect to its radical scavenging activity, it may provide more consistent physical protection against oxidative ingress to LMC. [56]. The TBARS observed for FO-UV is higher than that of FO, indicating that UV irradiation hastens the process of lipid peroxidation in unencapsulated oil. It highlights the protective effect of the chitosan–alginate wall system from oxidative degradation under light stress [57].

3.11. Anti-Diabetic Potential (Alpha-Amylase Inhibition Assay)

Alpha-amylase inhibition was significantly influenced by treatments ($p < 0.05$). T₂-UV recorded the highest inhibition ($71.61 \pm 0.04\%$) and T₁-UV the lowest ($47.66 \pm 0.08\%$) (Figure 9). The increase in the values of FO-UV is due to the UV exposure. Reference [47] indicated that UV irradiation enhances both α -amylase and α -glucosidase inhibitory activity in fenugreek oil. It is attributed to photochemical activation of bioactive terpenoid constituents. The terpene-rich fraction of fenugreek oil has been shown to inhibit pancreatic α -amylase activity by 46% and 52% in pancreatic tissue and plasma, respectively. The amino acid 4-hydroxyisoleucine contributes to antidiabetic activity through stimulation of glucose-induced insulin secretion and improvement of insulin sensitivity [58]. T₂-UV has greater antidiabetic activity than T₃-UV, which stands in contrast to the antioxidant trend observed in DPPH and ferric reducing power assays. It has been reported that HMC acts as a viscous dietary fiber matrix in the gastrointestinal environment. It delays gastric emptying and reduces the rate of glucose absorption by limiting access of intestinal disaccharidases to their substrates. The antidiabetic properties of chitosan more broadly encompass inhibition of α -amylase and α -glucosidase. It enhances glucose metabolism, preserves pancreatic β -cell function, and modulates gut microbiota [59].

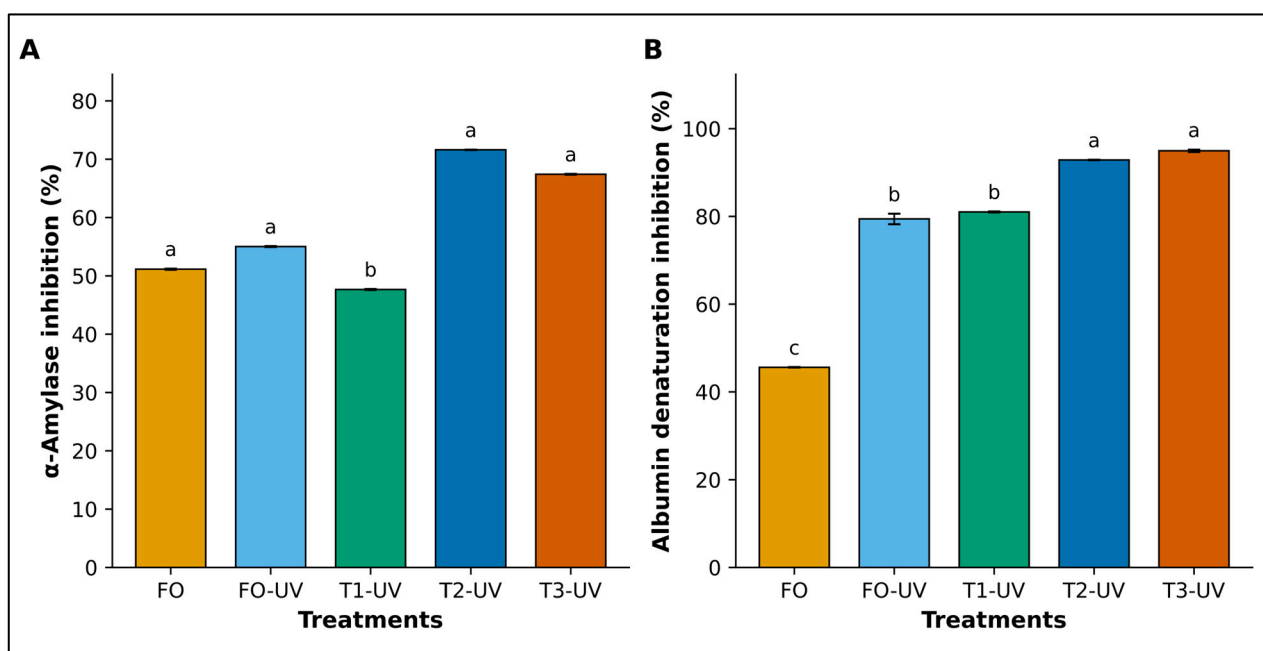


Figure 9. Antidiabetic potential of UV-treated free and encapsulated fenugreek oil. Fenugreek oil (FO), UV-treated fenugreek oil (FO-UV), UV-treated encapsulated fenugreek oil in 2% sodium alginate (T1-UV), UV-treated encapsulated fenugreek oil in 2% sodium alginate + 0.2% HMC (T2-UV), and UV-treated encapsulated fenugreek oil in 2% sodium alginate + 0.2% LMC (T3-UV). Where (A) is α -Amylase inhibition, (B) is Albumin denaturation inhibition. Whereas different lowercase letters above the bars indicate statistically significant differences between groups. Bars sharing the same letter are not significantly different.

3.12. Anti-Inflammatory Activity (Albumin Denaturation Inhibition Assay)

The albumin denaturation inhibition assay showed significant differences across treatments ($p < 0.05$). T₃-UV recorded the highest anti-inflammatory activity ($94.95 \pm 0.27\%$) and FO the lowest ($45.62 \pm 0.05\%$) (Figure 9). The higher activity in the UV-exposed samples may reflect photochemical modification of pre-existing constituents rather than any increase in phytochemical content, which is not possible in extracted oil [60]. The higher activity of

T₃-UV relative to T₂-UV may reflect greater accessibility of the released oil from the more porous LMC matrix, rather than any increase in phytochemical content [47].

The comparatively higher activity of T₃-UV relative to T₂-UV may reflect greater accessibility of the released oil from the more porous LMC matrix. The matrix of LMC is more porous and evenly distributed, allowing the release of anti-inflammatory compounds. But diffusion will be hindered more by the HMC coating. The previously reported results with LMC were slightly more potent as an anti-inflammatory agent than HMC [9]. The chitosan–alginate complex has direct anti-inflammatory activity. The results showed that the anti-inflammatory activity of fenugreek seed oil was also found to be preserved when encapsulated with LMC rather than unencapsulated oil under UV stress conditions [61]. These *in vitro* observations would need further release and bioaccessibility confirmations before functional-food suitability can be claimed.

4. Conclusions

This study successfully developed and characterized a chitosan–sodium alginate encapsulation system for fenugreek seed oil. We evaluated the influence of chitosan molecular weight on the physical, structural, thermal, and biological properties of the resulting microcapsules. Mechanical pressing of fenugreek seeds yielded 2.81% (*v/w*) oil, compared to 5.55% by solvent extraction. Physical and morphological characterization confirmed that chitosan and alginate composite beads are denser microcapsules relative to alginate-only beads. Its particle size analysis confirmed this trend as D50 values progressively narrowed from 91.88 µm for free oil to 2.3–2.4 µm. The process helped to improve size uniformity as reflected by decreasing SPAN values. SEM revealed that HMC-coated beads (T₂) exhibited the most uniform and smooth-surfaced morphology with a continuous shell. Encapsulation recovery was highest for T₁ (94.79%) and declined with chitosan addition. FTIR indicated retention of the characteristic functional groups of the oil in all encapsulated treatments. There was a shift and broadening of the alginate carboxylate band consistent with electrostatic interaction between alginate –COO[−] and chitosan –NH₃⁺. TGA indicated that chitosan coating suggested thermal stability by merging oil and polymer degradation events into a single broad thermal zone. UV irradiation enhanced the antioxidant, anti-inflammatory, and antidiabetic activities in the free oil. T₃-UV (LMC) outperformed T₂-UV (HMC) in DPPH scavenging and anti-inflammatory activity, while T₂-UV indicated superior α-amylase inhibition. Chitosan molecular weight can be selected to influence microcapsule morphology, thermal degradation behavior, and the comparative bioactivity profile of encapsulated fenugreek seed oil. Future work should focus on *in vitro* release kinetics under simulated gastrointestinal conditions. It could be extended to *in vivo* models to validate the translational relevance of these encapsulation strategies for functional food and nutraceutical product development. Encapsulation efficiency and *in vitro* bead behavior are identified as future work.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr14152476/s1>, Table S1: Microcapsule formation of fenugreek seed oil with sodium alginate and chitosan; Table S2: TGA Thermal Decomposition Data for Samples; Figure S1: Encapsulation process for fenugreek seed oil in sodium alginate and chitosan.

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