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# Incorporation of Chitosan-Stabilized Green Tea Pickering Nanoemulsion into Hybrid Semi-Refined Carrageenan-Gelatin Films: Stability, Antioxidant Activity, and Performance in Active Food Packaging

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The environmental impact of plastic packaging calls for innovative, sustainable alternatives for food preservation. This study addresses this critical need by developing novel hybrid semi-refined carrageenan-gelatin films enriched with chitosan-stabilized green tea extract and pickering oil nanoemulsions (PN-GTE/O) with the aim of improving active food packaging. PN-GTE was produced by incorporating green tea extract and soybean oil in a 1:1 ratio. GTE, which was stored for 63 days, was stable in terms of particle size and PDI value at both refrigeration and room temperature. Plasticized carrageenan and gelatin were prepared with different PN-GTE concentrations for the formulation of active food packaging. The films were characterized by FTIR analysis, which confirmed the incorporation of the bioactive GTE compounds by hydrogen bonding between chitosan and polyphenols. The mechanical properties were significantly affected by the PN-GTE concentration in the active films, with 10% PN-GTE showing a remarkably high tensile strength of the film of 4.58 MPa, indicating a transition from interfering inclusions to reinforcing fillers. Physical properties such as film thickness, opacity, solubility and moisture content were also evaluated, demonstrating the suitability of the films for packaging applications. In addition, the films exhibited strong antioxidant activity. 10% of the PN-GTE films achieved up to 60% scavenging of DPPH radicals, confirming their potential for long-lasting oxidative protection. By integrating stable, antioxidant-rich green tea nanoemulsions into biodegradable biopolymer films, a practical and environmentally conscious solution is offered to extend the shelf life of food and ultimately contribute to less food waste and a healthier planet.

**Keywords**: Pickering nanoemulsion, semi-refined carrageenan, gelatin active packaging, antioxidant activity

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The growing environmental impact of plastic packaging waste has intensified the search for sustainable alternatives for food packaging. Currently, more than a third of food packaging is made from petroleum-based plastics [1]. However, less than 5% of these plastics are recycled, leading to an accumulation of persistent waste and microplastics in ecosystems [1]. In response, bio-based and biodegradable packaging materials are gaining increasing attention due to their low environmental impact. Such materials can often safely biodegrade within a few weeks and offer an abundant, non-toxic resource base compared to conventional plastics [2-3]. Importantly, next-generation packaging is not only environmentally friendly but also actively preserves food quality, for example by containing natural additives that extend shelf life. This active packaging approach can reduce the need for synthetic preservatives using embedded antioxidants or antimicrobials that inhibit food spoilage [2-4].

Carrageenan is a sulfated polysaccharide where semi-refined carrageenan (SRC) offers greater sustainability than refined carrageenan owing to fewer purification steps that reduce raw-material cost and processing footprint [3]. Despite its simpler processing history, studies showed SRC consistently preserves film-forming capability and functional performance comparable to carrageenan derivatives [4-5]. Combination of polysaccharides SRC and gelatine have proven to be promising substrates due to their excellent film-forming properties. The blend of carrageenan, whose rigid polysaccharide backbone provides a high tensile modulus, with protein-based gelatin, known for its extensibility and elastic recovery, forms an interpenetrating biopolymer matrix that balances strength and

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mechanical integrity [5]. In addition, carrageenan and gelatine are biodegradable, GRAS-listed biopolymers derived from low-cost, renewable raw materials. Therefore, films made from their blends provide an easy-to-manufacture, cost-effective platform for sustainable food packaging that also meets stringent safety requirements [6].

To further improve food preservation, researchers are incorporating bioactive compounds into biopolymer films to create active, biodegradable packaging. *Camellia sinensis* extract is rich in phenolic constituents, such as catechin and its derivatives, which exhibit strong antimicrobial and antioxidant functions by effectively suppressing microbial proliferation and mitigating oxidative deterioration in food matrices. Green tea extract (GTE) is hydrophilic and rich in polyphenols confirmed by previous studies [8-9]. Green tea oil (GTO) is lipophilic and dominated by fatty and several active compounds related to antioxidant and antimicrobe activity, including n-Hexylcinnamaldehyde, (15.21%), L-α-Terpineol (10.02%), 2-Propanol, 1,1′-oxybis- (8.5%), and D-Limonene (8.42%) reported by Perumal et al. (2021) [10]. These contrasting constituents are significant for Pickering emulsion design and film integration due to differences in polarity, volatility, and interfacial affinity. Studies have shown that integrating GTE into chitosan results in stronger mechanical interactions with hydrophilic matrices but may increase film solubility due to altered water interactions [8]. In addition, Siripatrawan et al. (2010) [11] reported that chitosan films enriched with green tea extract exhibited increased free radical protection, improved tensile strength, and enhanced water vapor barrier properties. Zhao et al. [12] found that GTO enhances oxidative stability, provides terpene-driven antimicrobial and antioxidant effects, and its hydrophobic properties improve the water barrier of films. Thus, establishing appropriate formulations for Pickering stability, improved matrix adhesion, and effective dispersion for releasing active compounds in food is essential to develop sustainable active packaging.

A key challenge in formulating active functional films is achieving a uniform dispersion and sustained stability of bioactive compounds within the hydrophilic polymer matrix. Pickering emulsions offer an innovative solution where active particles irreversibly adsorb at the oil–water interfaces and stabilise the dispersed oil droplets without the need for synthetic surfactants. Chitosan nanoparticles (CNPs) have proven to be suitable food-grade emulsifying particles for Pickering stabilisations reported from many studies [10-11]. When processed into nanoscale particles, chitosan can effectively anchor to oil–water interfaces, forming a stable pickering emulsion while imparting antimicrobial functionality to the system. The use of chitosan nanoparticles to stabilize emulsions of bioactive oils is therefore an innovative strategy to create highly effective antimicrobial delivery systems in packaging demonstrated in recent studies [2]. Nevertheless, it is still uncertain whether Pickering emulsions that pair chitosan nanoparticles with green-tea extract can be dispersed uniformly throughout biodegradable carrageenan/gelatin films in a way that optimises both distribution and functional performance.

Therefore, this study aims to incorporate green tea extract into a carrageenan-gelatin matrix through a Pickering emulsion stabilized with chitosan nanoparticles. The ration of GTE/soybean oil was prepared in CNPs in a low pH Pickering emulsion and stability of the antioxidants during the storage period and conditions were analysed. Subsequently, carrageenan/gelatin films were synthesized with GTE-loaded Pickering to achieve the desired mechanical and barrier properties of the films. The overall goal is to demonstrate a novel, fully bio-based packaging material that meets sustainability goals and improves food shelf life through the synergistic use of biopolymers, Pickering emulsion technology and natural bioactive compounds.

## EXPERIMENTAL

### Materials

All chemicals and reagents used in this study were of analytical grade. Medium molecular weight chitosan (190–310 kDa) with a deacetylation degree of 75–85% was obtained from commercial suppliers. Green tea leaves were purchased from a Bharat Tea Plantations Sdn. Bhd., Pahang, Malaysia. by Perumal et al. (2021) [10]. Semi-refined carrageenan (SRC) was sourced from Carrageen Sdn. Bhd. (Indonesia). Food-grade gelatin was acquired from a local market. Soybean oil was purchased from a local supermarket and used as received. Glycerol (purity 99%), ethanol (95% and 80%), acetic acid, sodium hydroxide (NaOH), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied by Sigma-Aldrich. Ultrapure water was used for the preparation of all solutions throughout the experiments.

### Extraction of Green Tea Leaves

Green tea extract was prepared using a modified solvent extraction method [1]. Green tea leaves were first ground in a miller and mixed with an 80:20 v/v ethanol:water solvent mixture, stirred at 150 rpm for 4 hours at room temperature. The mixture was then centrifuged at 10,000 rpm for 15 minutes and the mixture was filtered to separate the clear solution from the residue. After filtration, the pooled filtrate was concentrated by rotary evaporation (≤40 °C water bath, in vacuum) to green tea extract concentrate (GTE). The concentrate was protected from light and stored at 4 °C until use. Total soluble

solids were determined by aliquot of the concentrate GTE and transferred into a pre-weighed vial, dried under reduced pressure at ≤40–45 °C to constant mass and reweighed to obtain the dry mass The soluble solid green tea extract concentrate was calculated as GTE solid concentration (mg/mL) = $Wa/Va$, where $Va$ is volume aliquot of GTE and $Wa$ is dry mass of GTE.

**Preparation of Chitosan-Stabilized Pickering Nanoemulsion**

The preparation of chitosan-stabilized Pickering nanoemulsion containing green tea extract (PN-GTE) was conducted in two sequential steps [2]. First, chitosan nanoparticles were prepared according to a modified ionic gelation method. Chitosan (2 mg/mL) was dissolved in 1% (v/v) acetic acid solution by stirring at 1200 rpm for 3 hours at room temperature until a clear solution was obtained. The pH of the chitosan solution was adjusted from approximately 4.1 to 6.0–6.5 using 5 M NaOH; this pH range is critical for forming stable nanoparticles with optimal surface charge. Subsequently, a Pickering nanoemulsion was formulated by mixing 50 mL of the chitosan nanoparticle suspension (2 mg/mL) with green tea extract and soybean oil in equal proportions (1:1, mg/mL), forming a coarse emulsion. The coarse emulsion was then homogenized at 12,000 rpm for 5 minutes using a high-speed homogenizer to produce a preliminary emulsion. Finally, the preliminary emulsion was subjected to ultrasonic treatment using an ultrasonic cell crusher at 40% power for 2 minutes, with 3-second on/5-second off cycles to prevent overheating. This sonication yielded a stable nanoemulsion with a uniform droplet size distribution. This ultrasonic treatment resulted in the formation of a stable nanoemulsion with uniform droplet size distribution. Control samples were prepared without the addition of green tea components. The formulations are summarized in Table 1.

**Droplet Size and Polydispersity Index (PDI)**

Droplet size and polydispersity index (PDI) of the Pickering nanoemulsions were determined using dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments, UK). Samples were appropriately diluted with ultrapure water to avoid multiple scattering effects. Stability of the nanoemulsions was assessed by conducting measurements at various intervals under room temperature (room) and refrigerated 4°C (chiller) conditions. All measurements were conducted in triplicate, and the results presented as mean ± standard deviation.

**Preparation of Hybrid Semi-Refined Carrageenan-Gelatin Films**

Hybrid semi-refined carrageenan-gelatin films were prepared using a solution casting method with modifications to incorporate the Pickering nanoemulsion [3]. Initially, SRC powder (2% w/v) was dissolved in 100 mL of distilled water under continuous magnetic stirring for 30 minutes. The solution was then heated to 70°C while maintaining constant stirring. Glycerol, used as a plasticizer (40% v/v based on the dry weight of SRC powder), was added to the solution, and the temperature was gradually increased to 80°C with constant stirring to achieve gelation. Once the SRC-glycerol solution was homogeneous, gelatin (4% w/v) was introduced into the mixture. The solution was allowed to cool to 50°C at room temperature while maintaining constant stirring to ensure uniform dispersion of the gelatin. At this stage, different volumes of the previously prepared chitosan-stabilized green tea Pickering nanoemulsion (1, 3, 5, 7, and 10 mL) were incorporated into separate batches of the film-forming solution. The mixtures were then homogenized using an Ultra Turrax T25 homogenizer (IKA, Germany) at 1300 rpm for 20 minutes to ensure even dispersion of the nanoemulsion within the polymer matrix. Control films were prepared without the addition of Pickering nanoemulsion. Each 100 mL portion of the film-forming solution was carefully poured into non-stick casting plates and dried at 100 ± 2°C for 24 hours. Once completely dried, the films were carefully peeled off from the casting plates and conditioned at 25°C and 50% relative humidity for 48 hours before characterization. The film formulations are summarized in Table 2.

**Table 1**: Formulation of Green Tea Pickering Emulsion.

| Sample | Green tea (v/v) | Soybean oil (v/v) |
|---|---|---|
| Control (SO) | - | 5.0 |
| Green Tea Oil (GTO) | 2.5 | 2.5 |
| Green Tea Extract (GTE) | 2.5 | 2.5 |

**Table 2.** Classification of SRC-based Films.

| Sample | Materials |
|---|---|
| Control | SRC (2% w/v) + Gly (40% v/v) + Ge (4% w/v) |
| 1%GTE | SRC (2% w/v) + Gly (40% v/v) + Ge (4% w/v) + 1% (v/v) PN-GTE |
| 3%GTE | SRC (2% w/v) + Gly (40% v/v) + Ge (4% w/v) + 3% (v/v) PN-GTE |
| 5%GTE | SRC (2% w/v) + Gly (40% v/v) + Ge (4% w/v) + 5% (v/v) PN-GTE |
| 7%GTE | SRC (2% w/v) + Gly (40% v/v) + Ge (4% w/v) + 7% (v/v) PN-GTE |
| 10%GTE | SRC (2% w/v) + Gly (40% v/v) + Ge (4% w/v) + 10% (v/v) PN-GTE |

**FTIR Analysis**

Fourier transform infrared (FTIR) spectroscopy was utilized to investigate functional groups and potential interactions within the Pickering nanoemulsions. Spectra were acquired using a Spectrum 100 FTIR spectrometer (PerkinElmer, USA) fitted with an attenuated total reflectance (ATR) accessory. Samples were applied directly onto the ATR crystal, and spectra were recorded over the range 4000–600 $cm^{-1}$ at a resolution of 4 $cm^{-1}$, averaging 32 scans per sample. Background scans were taken prior to each measurement and automatically subtracted from the sample spectra.

**Characterization of Hybrid Films**

**Mechanical Properties**

Mechanical properties, including tensile strength (TS) and elongation at break (EAB), of hybrid films were evaluated using a universal testing machine (Zwick BZ2.5/TN1S, Zwick GmbH & Co. KG, Ulm, Germany) according to ASTM D882. Film specimens (1.5 cm × 10 cm) were conditioned at 25°C and 50% relative humidity for 48 hours before testing. The initial grip separation was set to 50 mm, and crosshead speed maintained at 50 mm/min. Tensile strength values were computed by dividing maximum load by initial cross-sectional area (expressed in MPa), and elongation at break was reported as percentage length increase at failure relative to initial length. Measurements were replicated at least five times per film formulation, and data expressed as mean ± standard deviation.

**Physical Properties**

*Thickness Measurement*

Film thickness was determined using a digital micrometer (Mitutoyo Co., Tokyo, Japan) with 0.001 mm precision. Measurements were taken at five random positions on each film sample, and the average thickness was used in subsequent analyses for opacity and mechanical properties.

*Opacity Measurement*

Film opacity was assessed using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). Rectangular film samples were directly placed in the spectrophotometer cell, and absorbance was measured at 600 nm. Opacity was calculated by dividing absorbance at 600 nm by film thickness (mm), with higher values indicating decreased transparency. Tests were performed in triplicate per formulation.

*Film Solubility*

Film solubility was evaluated through a modified standard procedure. Film squares (2 cm × 2 cm) were dried at 100°C for 24 hours to obtain initial dry weights ($W_0$). These dried films were immersed in 30 mL distilled water for 24 hours at room temperature with gentle stirring. After this period, undissolved films were recovered, dried again at 100°C for 24 hours, and weighed (Wf). Film solubility percentage was calculated as $[(W_0 – Wf)/W_0] \times 100$. All measurements were performed in triplicate.

*Moisture Content*

Moisture content was determined by assessing weight loss upon drying. Film samples (4 cm × 4 cm) were weighed initially (Wi), dried at 100°C for 24 hours, and reweighed (Wf). Moisture content percentage was computed using $[(Wi – Wf)/Wi] \times 100$. Triplicate measurements were taken per film formulation.

**Antioxidant Activity Assessment**

*DPPH Radical Scavenging Assay for Nanoemulsion*

Antioxidant activity of nanoemulsions was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method [11]. A DPPH solution (0.1 mM in methanol) was prepared, and 0.1 mL of diluted nanoemulsion sample was mixed with 3.9 mL of DPPH solution. The mixture was vigorously shaken and incubated at room temperature in darkness for 30 minutes. Absorbance was measured

at 517 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). A methanol-only solution served as the control. DPPH scavenging activity (%) was calculated based on absorbance changes.

DPPH Scavenging Activity (%) = [(Abscontrol - Abssample)/Abscontrol] × 100

where Abscontrol is the absorbance of the control and Abssample is the absorbance of the sample. Measurements were performed at regular intervals (0, 7, 14, 28, 42, and 67 days) during storage to monitor changes in antioxidant activity over time. Each measurement was performed in triplicate, and the results were expressed as mean values ± standard deviation.

**Statistical Analysis**

All experiments were performed in triplicate unless otherwise specified, and the results were expressed as mean values ± standard deviation. Statistical analysis was conducted using SPSS software (version 25.0, IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to determine significant differences among samples at a significance level of $p < 0.05$. Pearson correlation analysis was conducted to evaluate relationships between different parameters.

## RESULTS AND DISCUSSION

**Particle Size and Polydispersity Index (PDI) of Pickering Emulsions**

Figure 1 shows that the particle size profiles of Pickering emulsions depend strongly on both the dispersed phase (green tea extract, green tea oil, or soybean-oil as control) and the storage temperature (25°C and 4°C). Chitosan, a cationic polysaccharide, imparts a positive surface charge and thus contributes to the stability of the emulsion [10]. The GTE emulsions showed remarkable stability at both refrigeration and room temperatures, maintaining a constant particle size of 15-18 nm throughout the 63-day period. This suggests that the GTE components, likely polyphenols, interact synergistically with chitosan to form a robust interfacial emulsion that effectively prevents particle aggregation and maintains colloidal stability [2]. In contrast, the green tea essential oil (GTO) emulsions exhibited significant destabilization at room temperature, where the particle size increased from about 13 nm to 77 nm. GTO emulsions showed a gradual increase in particle size, indicating progressive instability in cold condition-chiller. The control samples with soybean oil (SO) consistently exhibited very low particle size (below 5 nm) in room and cold temperature, indicating their inherent high stability, which is likely due to optimal wettability of the particles and strong adsorption at the interface by chitosan. The pronounced instability of GTO emulsions which consists mainly of lipophilic and volatile compounds, may not bind effectively with the hydrophilic chitosan at the interface, or its volatile components could degrade over time and compromise the integrity of the interface [13]. This leads to increased particle aggregation and an increase in particle size, indicating a breakdown of the emulsion. Conversely, GTE is rich in water-soluble polyphenols, likely enhances the ability of chitosan to form a stable interfacial barrier, as these compounds are known to interact with polysaccharides and proteins [14]. Figure 1 shows that water-soluble bioactives such as green tea extract can act as interfacial co-stabilizers that reinforce the chitosan nanoparticle shell and significantly improve the stability of the Pickering emulsion.

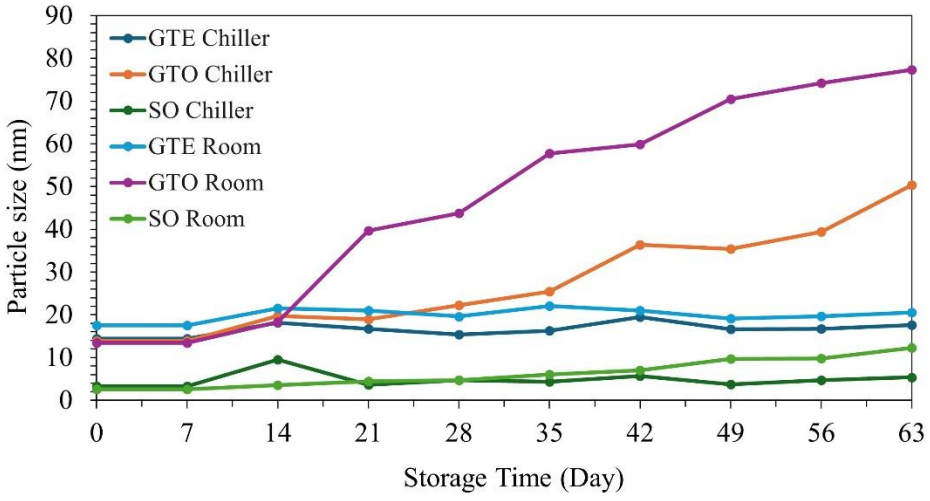


**Figure 1**. Particle size of the Pickering nanoemulsion over storage days.

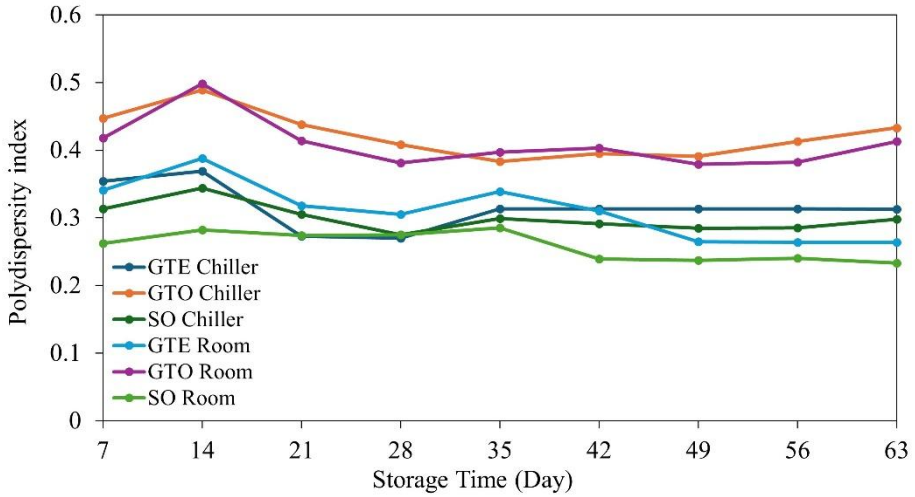


**Figure 2.** Polydispersity index of each sample from week 1 to week 9.

The polydispersity index (PDI) is a measure of the width of the droplet size distribution where values below 0.5 indicate reasonably monodisperse systems. Figure 2 illustrated all samples met 0.5 nm over nine weeks storage period for cold and room environment. In average for SO emulsion had the highest average PDI (~0.30), indicating a slightly broader distribution than the green tea formulations. GTO emulsions exhibited a transient increase in PDI (~0.50) at week 2, which coincided with the onset of droplet growth. This indicates an early stage of coalescence, where droplets enlarge and destabilisation became more uniform at 49 days. GTE-based Pickering emulsions exhibited the narrowest droplet size distribution, with temperature-independent polydispersity indices of 0.20–0.25 at both refrigerated (4°C) and room temperature (25°C). This consistency confirms the dual effect of the catechin-rich polyphenols formulation in pickering emulsion reduce interfacial tension during high shear homogenization, promoting efficient droplet fragmentation, and subsequently reinforce the interfacial layer between chitosan and particles through hydrogen bonding and electrostatic attraction, preventing coalescence after processing [15]. The consistently low PDI values in GTE systems favour downstream film casting and ensure homogeneous dispersion in carrageenan/ gelatin matrices as well as more predictable release of the encapsulated active ingredients in foods.

**FTIR Analysis**

Figure 3 showed FTIR spectra revealed characteristic absorption bands of structural composition and interfacial interactions within films incorporated with Pickering emulsion samples. Peaks were observed in the regions of 2870-2920 $cm^{-1}$ (C-H stretching), 1650-1660 $cm^{-1}$ (C=O stretching of amide I), 1590-1600 $cm^{-1}$ (N-H bending of amide II), and 1070-1100 $cm^{-1}$ (C- O-C stretching in the glycosidic linkage) [16]. Absorption intensity in the 3200-3500 $cm^{-1}$ region indicating of hydroxyl groups contributed by the polyphenolic compounds in carrageenan and gelatins biopolymer. A particularly significant observation was the appearance of a new absorption band in the 1600-1650 $cm^{-1}$ region in the green tea-containing nanoemulsions. This band corresponds to the carbonyl (C=O) groups present in flavonoids, polyphenols, and catechins, confirming the successful incorporation of these bioactive compounds into the nanoemulsion structure [17,18]. The intensity of this band was notably higher in the green tea extract formulation compared to the green tea oil formulation, reflecting the higher concentration of polyphenolic compounds in the extract. Furthermore, slight shifts were observed in the characteristic peaks of chitosan upon incorporation of green tea components. The N-H bending vibration of chitosan shifted from approximately 1590 $cm^{-1}$ to 1585 $cm^{-1}$ in the green tea-containing nanoemulsions, suggesting hydrogen bonding interactions between the amino groups of chitosan and the hydroxyl groups of polyphenols [19]. The FTIR analysis also confirms the presence of the bioactive compounds from green tea within the nanoemulsion structure, which is essential for their subsequent functionality in active packaging applications suggesting that their functional properties, including antioxidant activity, are likely preserved as well.

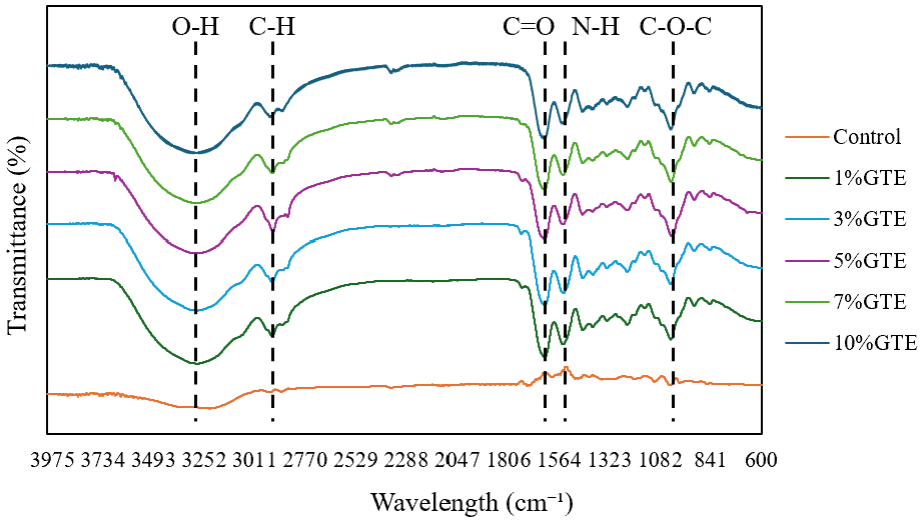


**Figure 3.** FTIR analysis of hybrid films.

**Antioxidant Activity**

Figure 4 depicted GTE-stabilized chitosans in Pickering nanoemulsions exhibit DPPH radical scavenging activity, highlighting their ability to act as antioxidant in film samples. Control film consist without Pickering emulsion exhibited minimal DPPH radical scavenging activity with values ranging from 0% to 20%. The marginal results attributed to the inherent antioxidant properties of carrageenan polysaccharide, which contains amino and hydroxyl groups capable of donating hydrogen atoms to free radicals [20]. Pickering nanoemulsion active films containing green tea extract demonstrated strong antioxidant performance, with DPPH radical scavenging efficiencies of approximately 60% and 54% for the higher (10% PN-GTE) and lower (5% PN-GTE) extract concentrations, respectively. These values emphasize the effective retention of catechin-rich polyphenols in the film matrix and confirm their suitability as active components to improve oxidative stability [21]. Polyphenol and their derivatives in GTE possess multiple hydroxyl groups that can donate hydrogen atoms to free radicals, effectively neutralizing them and terminating chain reactions involved in oxidative processes [22]. Overall, these findings confirm that the antioxidant properties observed are derived from the hydrophilic polyphenolic constituents of green tea extract (GTE) rather than from lipophilic components found in green tea oil. Incorporating chitosan-stabilized GTE Pickering nanoemulsions into biopolymer films thus offers a promising strategy for developing active packaging materials capable of providing prolonged protection against oxidative degradation and extending the shelf life of perishable foods.

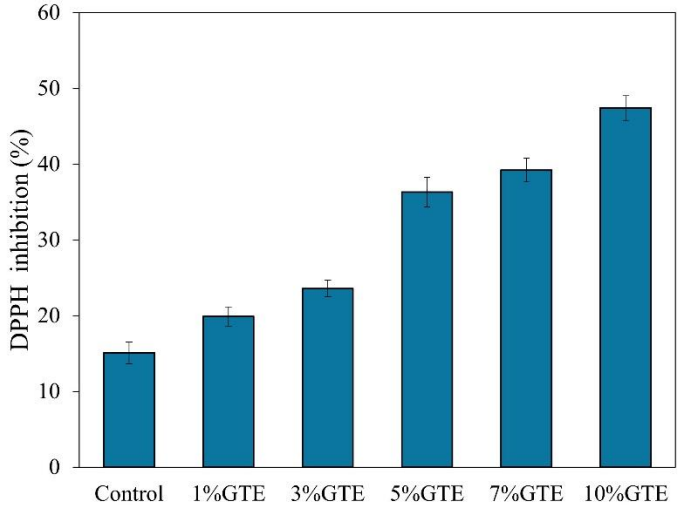


**Figure 4.** DPPH inhibition of hybrid films.

**Mechanical Properties of Hybrid Films**

Incorporating chitosan-stabilised green-tea Pickering nanoemulsions (PN-GTE) into semi-refined carrageenan–gelatin (SRC-Ge) films altered their mechanical behaviour in a concentration-dependent manner, as illustrated in Figure 5. The control film exhibited the highest tensile strength (4.63 ± 0.21 MPa), attributed to the complementary interactions between SRC and gelatin, where the anionic sulfate groups of carrageenan interact electrostatically with the positively charged amino groups of gelatin, forming a robust network structure [23]. The incorporation of PN-GTE at low concentrations (1–3%) resulted in a significant decrease ($p < 0.05$) in tensile strength, with values of 2.54 ± 0.15 MPa and 2.72 ± 0.18 MPa for 1% and 3% PN-GTE films, respectively. This reduction likely arose from the disruption of polymer matrix continuity by the nanoemulsion droplets, which interfered with the cohesive interactions between SRC and gelatin. However, as the PN-GTE concentration increased further, the tensile strength gradually improved, reaching 3.33 ± 0.17 MPa (5%), 3.58 ± 0.14 MPa (7%), and 4.58 ± 0.19 MPa (10%), with the 10% PN-GTE film showing no significant difference ($p > 0.05$) compared to the control film. At higher concentrations, the nanoemulsion droplets appear to transition from being disruptive inclusions to reinforcing fillers fill voids in the polymer matrix and interact with both SRC and gelatin through electrostatic and hydrogen bonding interactions, creating additional crosslinking points that strengthen the overall structure [24].

The elongation at break (EAB) values exhibited an inverse relationship with tensile strength across the different film formulations. The control film showed the lowest EAB of 33.91%, reflecting its relatively rigid and brittle nature despite its high tensile strength (Figure 5). This limited flexibility can be attributed to the dense network structure formed by SRC-gelatin interactions, which restricts polymer chain mobility under stress [25].Upon incorporation of PN-GTE, a significant enhancement in EAB was observed, with values increasing to 57.52%, 53.37%, 50.42%, 48.83%, and 46.89% for films containing 1, 3, 5, 7, and 10% of nanoemulsion, respectively. This substantial improvement in flexibility, particularly at lower nanoemulsion concentrations, indicates that the nanoemulsion droplets act as plasticizing agents within the polymer matrix [26]. Nanoemulsion droplets may disrupt the dense packing of polymer chains, increasing the free volume and facilitating chain mobility under stress [27]. Moreover, the gradual decrease in EAB with increasing nano-emulsion concentration, while still maintaining values significantly higher than the control, suggests a transition from predominantly plasticizing effects at low concentrations to a more balanced combination of plasticizing and reinforcing effects at higher concentrations. This transition aligns with the observed recovery in tensile strength, indicating that the structural role of nanoemulsion particles evolves with concentration which supports from Almasi et al. (2020) [28]. The simultaneous enhancement of EAB relative to the control film, even at the highest nanoemulsion concentration where tensile strength is largely recovered, represents an optimal combination of mechanical properties for food packaging applications. This balance between functional properties and mechanical performance is essential for developing commercially viable active packaging materials.

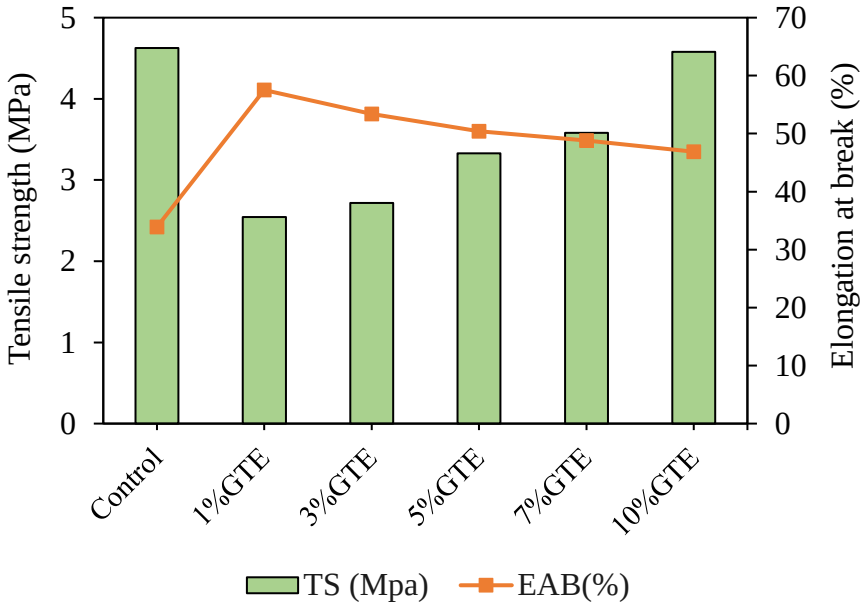


**Figure 5.** Tensile strength (MPa) and elongation at break (%) of hybrid films.

**Table 3.** Result on physical properties of hybrid films.

| Sample | Physical properties | | | |
|---|---|---|---|---|
| | Thickness (mm) | Opacity ($mm^{-1}$) | Film Solubility (%) | Moisture Content (%) |
| Control | 0.081 | $0.005 \pm 0.0034^{a}$ | $44.578 \pm 0.630^{a}$ | $22.188 \pm 0.370^{a}$ |
| 1%GTE | 0.083 | $0.007 \pm 0.0035^{b}$ | $51.530 \pm 0.867^{b}$ | $13.931 \pm 0.407^{b}$ |
| 3%GTE | 0.093 | $0.012 \pm 0.0042^{c}$ | $55.209 \pm 0.888^{c}$ | $14.621 \pm 0.589^{c}$ |
| 5%GTE | 0.099 | $0.013 \pm 0.0062^{d}$ | $57.931 \pm 0.940^{d}$ | $15.774 \pm 0.858^{d}$ |
| 7%GTE | 0.099 | $0.017 \pm 0.0085^{e}$ | $59.378 \pm 0.973^{e}$ | $16.348 \pm 0.339^{e}$ |
| 10%GTE | 0.101 | $0.020 \pm 0.0096^{f}$ | $63.067 \pm 0.356^{f}$ | $18.166 \pm 0.826^{f}$ |

**Physical Properties of Hybrid Films**

The physical properties of packaging films, including thickness, opacity, water solubility, and moisture content, significantly influence their functionality and applicability in food preservation. The incorporation of chitosan-stabilized green tea Pickering nanoemulsion (PN-GTE) into semi-refined carrageenan-gelatin (SRC-Ge) films resulted in systematic changes to these properties, providing insights into structure-function relationships and potential applications.

The thickness of the hybrid films increased progressively with higher concentrations of PN-GTE, ranging from 0.081 mm for the control film to 0.101 mm for the film containing 10% of nanoemulsion (Table 3). This increase can be attributed to the additional solid content contributed by the nanoemulsion particles, which include chitosan nanoparticles and encapsulated green tea components [7]. The relationship between nanoemulsion concentration and film thickness was not strictly linear, with more pronounced increases observed at lower concentrations (1-5%) and a plateau effect at higher concentrations (7-10%). This non-linear relationship suggests that the incorporation of nanoemulsion affects not only the total solid content but also the packing density and microstructure of the film matrix. At lower nanoemulsion concentrations, the particles may primarily occupy void spaces within the polymer network, leading to a more efficient packing arrangement and moderate increases in thickness. As concentration increases, the nanoemulsion particles may begin to disrupt the original polymer network structure, potentially creating a more open and less densely packed arrangement that results in greater thickness increases per unit of added nanoemulsion [25]. Studies reported the average range of 0.081-0.101 mm remains within the practical limits for flexible packaging materials, ensuring that the films maintain good handling properties and can be effectively sealed and processed using conventional packaging equipment [29].

The opacity of the hybrid films, which reflects their light barrier properties, increased systematically with higher concentrations of PN-GTE (Table 3). The control film exhibited the lowest opacity value of 0.005 $mm^{-1}$, indicating high transparency, while the film containing 10% of nanoemulsion showed the highest opacity of 0.020 $mm^{-1}$, representing a four-fold increase. This progressive increase in opacity can be attributed to several factors related to the nanoemulsion components. The natural pigments present in green tea extract, including chlorophyll, lutein, and β-carotene, absorb and scatter visible light, reducing transparency [30] and nanoemulsion droplets themselves act as light-scattering centers due to the difference in refractive index between the oil phase and the surrounding polymer matrix [31]. The enhanced opacity of films containing PN-GTE is beneficial for food packaging applications, particularly for products sensitive to light-induced oxidation including lipids, proteins, vitamins that are susceptible to photodegradation, which can lead to quality deterioration, off-flavor development, and nutrient loss [32].

Water solubility is a critical property for food packaging materials, as it influences their integrity and functionality in high-moisture environments. The incorporation of PN-GTE resulted in a significant increase in film solubility, with values ranging from 44.58% for the control film to 63.07% for the film containing 10% of nanoemulsion (Table 3). This increase in solubility can be primarily attributed to the hydrophilic nature of the green tea polyphenolic compounds encapsulated within the nanoemulsion [33]. These compounds contain numerous hydroxyl groups that can form hydrogen bonds with water molecules, enhancing the overall hydrophilicity of the film matrix [34]. Additionally, the nanoemulsion structure may create more accessible pathways for water penetration into the film, facilitating faster dissolution of water-soluble components [35]. Studies observed by Wan Yahaya et al. (2020) [4], high water solubility can be beneficial to facilitate composting as the packaging formulation breakdown of the material in natural environments. This property aligns with the sustainability goals of developing environmentally friendly alternatives to conventional plastic packaging materials.

The control film exhibited the highest moisture content of 22.19%, while the incorporation of 1% PN-GTE resulted in a significant decrease to 13.93%. As nanoemulsion concentration increased further, the moisture content gradually increased, reaching 18.17% at 10% PN-GTE, though still remaining below the control value. The high moisture content of the control film can be attributed to the hydrophilic nature of both SRC and gelatin, which contain numerous hydroxyl and amino groups capable of binding water molecules [36]. Additionally, the glycerol used as a plasticizer is highly hygroscopic, further contributing to water retention within the film matrix [37]. The initial decrease in moisture content upon incorporation of PN-GTE suggests the formation of an inclusion complex between the film matrix and nanoemulsion components. This complex likely involves interactions between the hydrophilic groups of the polymer matrix and the chitosan shell of the nanoemulsion, reducing the number of sites available for water binding [38]. Additionally, the oil phase of the nanoemulsion introduces hydrophobic domains within the film structure, which can displace water molecules and reduce overall moisture content. The subsequent gradual increase in moisture content with higher nanoemulsion concentrations can be explained by the additional hydroxyl groups introduced by the green tea polyphenolic compounds. As nanoemulsion concentration increases, these hydroxyl-rich compounds become more abundant within the film matrix, providing additional sites for water binding through hydrogen bonding [39]. Lower moisture content generally correlates with improved barrier properties against water vapor and gases, which is beneficial for protecting moisture-sensitive and oxidation-prone foods [40]. Meanwhile, hydrophilic and hydrophobic components achieved through nanoemulsion incorporation in films allows adequate moisture content for diverse food packaging applications.

## CONCLUSION

The development of chitosan-stabilized Pickering nanoemulsions incorporating green tea extract (PN-GTE) demonstrates their superior colloidal stability and enhanced functional properties. Particle size and polydispersity index analyses consistently showed that GTE-based emulsions maintained remarkable stability over extended periods, attributed to the synergistic interactions between green tea polyphenols and chitosan. These interactions, further elucidated by FTIR spectroscopy and understanding underscores the critical role of water-soluble green tea extracts as interfacial co-stabilizers with CNPs, significantly improving the emulsion's robustness throughout storage condition.

Furthermore, incorporation of PN-GTE significantly improved the mechanical and antioxidant properties of hybrid films. The films exhibited enhanced tensile strength and comparable EAB properties at PN-GTE. This dual improvement in mechanical performance, coupled with a concentration-dependent increase in film thickness and opacity, highlights the potential for PN-GTE to create robust and protective packaging materials. Furthermore, the substantial DPPH radical scavenging activity demonstrated by the PN-GTE films confirms the effective retention and functional integrity of the catechin-rich polyphenols, offering prolonged protection against oxidative degradation for packaged food products. These findings collectively establish PN-GTE as a promising candidate for developing advanced active packaging solutions with superior stability, mechanical integrity, and antioxidant efficacy.

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