Zamorano University

Food Science and Technology Department

B.S. in Food Science and Technology

Special Graduation Project

Synthesis and characterization of casein-based films with decanoic acid, ethanol and glycerol

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Abstract

This study investigated the effect of glycerol, decanoic acid, and ethanol concentration on the mechanical, optical, and rheological properties of casein-based films. Edible films, a sustainable alternative to plastics, were developed from casein due to its exceptional film-forming and barrier properties. However, casein-only films are typically brittle, necessitating the use of plasticizers like glycerol. It was hypothesized that incorporating ethanol and decanoic acid, would improve the mechanical properties of the films while reducing the amount of glycerol necessary to reach desirable mechanical properties. Film-forming solutions were prepared using two different solvent systems: distilled water (0% ethanol) and 50% ethanol, and different concentrations of decanoic acid (0, 10 and 20%). The film's mechanical properties (tensile strength and elongation at break), optical properties (absorbance), and the film-forming dispersions' rheological properties (apparent viscosity) were characterized. Results showed that increasing ethanol content generally led to higher absorbance and reduced transparency. In contrast, higher concentrations of decanoic acid increased the apparent viscosity of the film-forming dispersions without ethanol. The study concluded that formulations with 20% decanoic acid combined with 20% glycerol, with or without ethanol, showed potential for use as packaging materials, as they balanced strength and flexibility. Although these films were classified as opaque, their suitability depends on the specific food application.

Keywords: Casein films, decanoic acid, edible packaging, ethanol, glycerol, mechanical properties.

Resumen

El presente estudio investigó el efecto de las concentraciones de glicerol, ácido decanoico y etanol en las propiedades mecánicas, ópticas y reológicas de películas a base de caseína. Se desarrollaron películas comestibles a partir de caseína como una alternativa sostenible a los plásticos, debido a sus excepcionales propiedades formadoras de películas y barreras. Sin embargo, las películas de caseína solas suelen ser quebradizas, lo que requiere el uso de plastificantes como el glicerol. Se planteó la hipótesis de que la incorporación de etanol y ácido decanoico mejoraría las propiedades mecánicas de las películas al tiempo que reduciría la cantidad de glicerol necesaria para alcanzar las propiedades deseadas. Se prepararon soluciones formadoras de película con dos sistemas solventes diferentes: una solución con 50% de etanol y una solución con 0% de etanol. Se caracterizaron las propiedades mecánicas (resistencia a la tracción y elongación a la rotura), las propiedades ópticas (absorbancia) y las propiedades reológicas (viscosidad aparente) de las soluciones. Los resultados mostraron que el aumento del contenido de etanol generalmente condujo a una mayor absorbancia y a una menor transparencia. En contraste, concentraciones más altas de ácido decanoico aumentaron la viscosidad aparente de las soluciones sin etanol. El estudio concluyó que las formulaciones con 20% de ácido decanoico combinado con 20% de glicerol, con o sin etanol, mostraron potencial para su uso como materiales de empaque, ya que equilibraban la resistencia y la flexibilidad. Aunque estas películas se clasificaron como opacas, su idoneidad dependerá de la aplicación alimentaria específica.

Palabras clave: Ácido decanoico, empaque comestible, etanol, glicerol, películas de caseína, propiedades mecánicas.

Introduction

Plastic waste pollution has emerged as one of the most urgent environmental crises of the 21st century and packaging is one of the biggest contributors accountable. Food packaging has evolved to be a key aspect to successfully safeguard food from external factors grouped into physical, chemical and biological. Approximately 40% of all plastic produced is used for packaging, and about 60% of that is intended for food and beverage products (Ceballos et al., 2024). The problem lies in the fact that fossil fuels are the main raw material used in the manufacture of conventional non-degradable plastics, for instance, in 2009 it was reported that up to 8% of world oil is channeled towards their production with 50% of it serving as feedstock and the other 50% as fuel for the conversion process (Hopewell et al., 2009).

Growing environmental consciousness has spurred interest in edible packaging films as a biodegradable and sustainable alternative to synthetic plastics. These films, composed of edible polymers, offer mechanical protection and serve as barriers against physical, chemical, and biological elements between the food and the environment (Huber & Embuscado, 2009). There is a wide range of edible polymers that could be used to create edible packaging films, but out of all the available options proteins possess outstanding film-forming properties while also providing nutritional value to potentially packaged food and function as carriers for bio-actives (antimicrobial or antioxidants) to extend shelf life (Lacroix & Vu, 2014).

Casein, the predominant protein in bovine milk, comprising approximately 80% of its total protein content (Bhat et al., 2016), is particularly well-suited for this application. In milk, casein exists as large colloidal particles known as casein micelles. These micelles can be processed into a biopolymer for creating edible packaging films with remarkable barrier properties against oxygen and other nonpolar molecules. The high efficacy of casein as a barrier material stems from its molecular structure, rich in polar groups like hydroxyl and amino groups that promotes strong hydrogen bonding (Tomasula et al., 2003). Unfortunately, casein only films tend to shrink during drying and become brittle, limiting their application as coating/packaging materials (Longares et al., 2005). Edible

plasticizers such as glycerol or sorbitol are often used to create films with good tensile strength and moderate elasticity (Wagh et al., 2014). Although glycerol has proven to improve the mechanical properties of casein/glycerol films, the presence of glycerol reduces the barrier properties of casein films by increasing the hydrophilicity of films (Vieira et al., 2011).

While previous research has focused on the synthesis and characterization of casein-based films, particularly those with glycerol (Bora & Mishra, 2016; Cardoso et al., 2011), the impact of ethanol concentration on these films remains unexplored. Given that ethanol has been shown to alter the self-associating properties of casein micelles (Lewis et al., 2022), it is hypothesized that incorporating ethanol into the film-forming dispersion will enhance the mechanical properties of the resulting films.

Preliminary casein based films preparation was conducted in Prof. Harte's research lab to determine a specific fatty acid to be use as a plasticizer, distinct fatty acids like stearic acid (C 18:0) oleic acid (C 18:1), linoleic acid (C 18:2), myristic acid (C 14:0) decanoic acid (C 10:0) and caprylic acid (C 8:0) where first used in preliminary experiments to produce films at a specific concentration (10% w/w in respect to casein). After film synthesis, all samples were visually evaluated for homogeneity and brittleness. The film synthetized with decanoic acid had better qualitative properties and its melting point (30.5 °C) makes it processable, flexible and functional within the casein matrix. After defining the specific fatty acid to work with, another preliminary experiment was carried out to determine an appropriate decanoic acid concentration. The concentration range synthesized was from 10-50% decanoic acid w/w in respect to casein in intervals of 10%. Samples were retrieved and visually evaluated for homogeneity and brittleness, only the samples with concentrations of 10% and 20% decanoic acid showed a homogenous film matrix. Unfortunately, none of the samples showed promising qualitative flexibility.

For the reasons mentioned above, this research looked at decanoic acid as a possible plasticizer in conjunct with glycerol to reduce the amount of glycerol necessary to reach desirable mechanical properties. Decanoic acid is a straight-chain saturated fatty acid that has previously been

used to enhance the properties of zein films by preventing the rapid aggregation of zein by disrupting its intermolecular interactions while also improving the transparency, compactness, and flexibility (Xu et al., 2024). The objective of the project is to study the effect of ethanol, decanoic acid and glycerol concentrations on the mechanical properties of casein-based films and determine a formulation with potential use as a packaging material in the food industry.

Materials and Methods

Study Location

This study was developed in Dr. Harte's research lab at the Department of Food Science, The Pennsylvania State University, in the Rodney A. Erickson Food Science Building, State College, Pennsylvania 16802, USA.

Materials

Casein from bovine milk, sodium hydroxide, and decanoic acid were purchased from Sigma - Aldrich (St. Louis, MO, USA). Ethanol was acquired from Koptec (200-proof, Decon Labs Inc., PA, USA). Glycerol (BDH1172-1LP) was purchased from VWR Chemicals (VWR-BDH, Radnor, PA, USA).

Methods

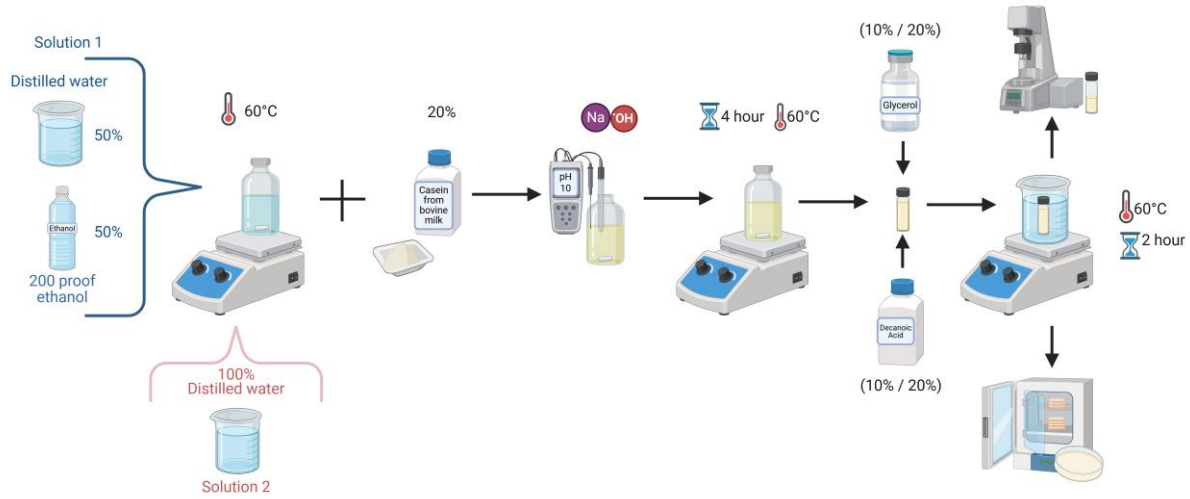
Preparation of Casein Films

As shown in Figure 1, two different bulk casein dispersions were prepared, one using distilled water as the solvent and the other using a combination of distilled water and ethanol at a ratio of 1:1 relation by volume, both dispersions were stirred at 60 °C, 20% (w/v) casein was added to both solvents. Then, the pH of both caseins dispersion was adjusted at 10 with NaOH and held under constant agitation at 60 °C for four hours. The reason behind these specific pH, time, temperature and ethanol percentage is to dissociate casein micelles and to form monomers and smaller aggregates as it was demonstrated by Sharma et al. (2024) and Vyas & Harte (2025), promoting more interactions between casein, decanoic acid and glycerol.

20 mL of each bulk casein dispersion was transferred with a syringe to individual flasks, then glycerol and decanoic acid were weighed and added at concentrations of 0%, 10% and 20% relative to casein, according to the respective treatments. These films forming dispersions were left under constant agitation at 60 °C for two hours after which 10 mL of each flask were used for the rheological analysis and the other 10 mL were cast on polystyrene petri dishes and placed in an electric oven to dry at 30 °C for 72 h.

Figure 1

Preparation of casein films.



Solubility of Decanoic Acid

Solubility was determined by a gravimetric procedure adapted from OECD Test Guideline 105: Water Solubility (Organization for Economic Cooperation and Development [OECD], 1995). Briefly, 10 mL solutions were prepared with a concentration ranged from 0% to 100% ethanol in water, at 10% intervals. Then, 1 g of decanoic acid was added and the mixture was kept at 60 °C under constant stirring for three hours. Then, the mixture was centrifuged at 1000 RPM for 10 minutes, and 1 mL of the supernatant was added to previously weighed glass tubes. Finally, the tubes were dried in an oven at 60 °C for three days and reweighed to compare the two measurements and obtain the solubility results following equation [1].

$$\text{Solubility (g/mL)} = \frac{\text{Mass of decanoic acid}}{\text{Volume of supernatant}} \quad [1]$$

Mechanical Properties

To measure the mechanical properties of the films, samples were cut using a TA cutter with a 5 mm width and a 30 mm length, the thickness of the samples was measured using a digital caliper. This prepared sample was characterized by the TA-XT2i texture analyzer (TA. XT2i Stable Micro

Systems, Surrey, UK) using the test mode for force measurement in tension, at a test speed of 0.5 mm/s recompiling the data in Newtons and the distance before breaking in millimeters.

Tensile strength for each sample was calculated by dividing the load at break (in Newtons) by the original minimum cross-sectional area (in square meters), with the result being reported in megapascal given by equation [2]:

$$\text{Tensile Strength} = \frac{(\text{Load at break})}{(\text{Original width})\ (\text{Original thickness})} \quad [2]$$

While elongation at break for each sample was calculated by dividing the elongation at rupture by the initial gauge length and multiplying by 100, given in equation [3]:

$$\text{Elongation at break } \% = \frac{(\text{Elongation at rupture})}{(\text{Initial gage length})} * 100 \quad [3]$$

Absorbance

For the absorbance measurement an Ocean Optics Spectrophotometer was used in conjunction with the Logger Pro 3 software. The absorbance of a sample is given by equation [4]:

$$\text{Absorbance} = \log\left(\frac{I_0}{I}\right) \quad [4]$$

Where I_0 is the intensity without a sample, and I is the intensity with the sample in the light path of the spectrophotometer. This calculation was done at the 400 nm wavelength for all the samples.

Rheological Analysis

The rheological properties of the solutions before drying was conducted using the Discovery HR-3 hybrid rheometer (TA Instruments, New Castle, DE, USA) , a 40 mm parallel plate of the Peltier plate stainless steel series was used as geometry, with a rheogram with a shear range (1/s) from 0 to 100 for 60 seconds at 20 °C collecting the apparent viscosity data point at 100 s^{-1} shear rate.

Statistical Analysis

A Completely Randomized Design with a factorial arrangement was followed. Twelve treatments were evaluated with three repetitions per treatment for a total of 36 experimental units

as shown in Table 1, using the statistical analysis software SAS®, through a two-way analysis of variance (ANOVA), and mean separations with the Duncan test to find significant differences among samples ($P < 0.05$).

Table 1

Description of the treatments.

Treatment	Decanoic acid (%)	Glycerol (%)	Ethanol (%)
D0G10E0	0	10	0
D0G10E50	0	10	50
D0G20E0	0	20	0
D0G20E50	0	20	50
D10G10E0	10	10	0
D10G10E50	10	10	50
D10G20E0	10	20	0
D10G20E50	10	20	50
D20G10E0	20	10	0
D20G10E50	20	10	50
D20G20E0	20	20	0
D20G20E50	20	20	50

Note. D: Decanoic acid (0%, 10%, 20%), G: Glycerol (10%, 20%), E: Ethanol (0%, 50%)

Results and Discussion

Solubility

Decanoic acid is an aliphatic chain of ten carbons and a single carboxyl group, making it an amphiphilic compound (Sepulveda et al., 2023). However, its overall behavior is dominated by the non-polar part because of its long hydrophobic tail. This hydrophobic character causes decanoic acid to interact more readily with solvents that share similar properties, according to Oliveira et al. (2009) fatty acids with a 10-carbon chain show very low solubility in water.

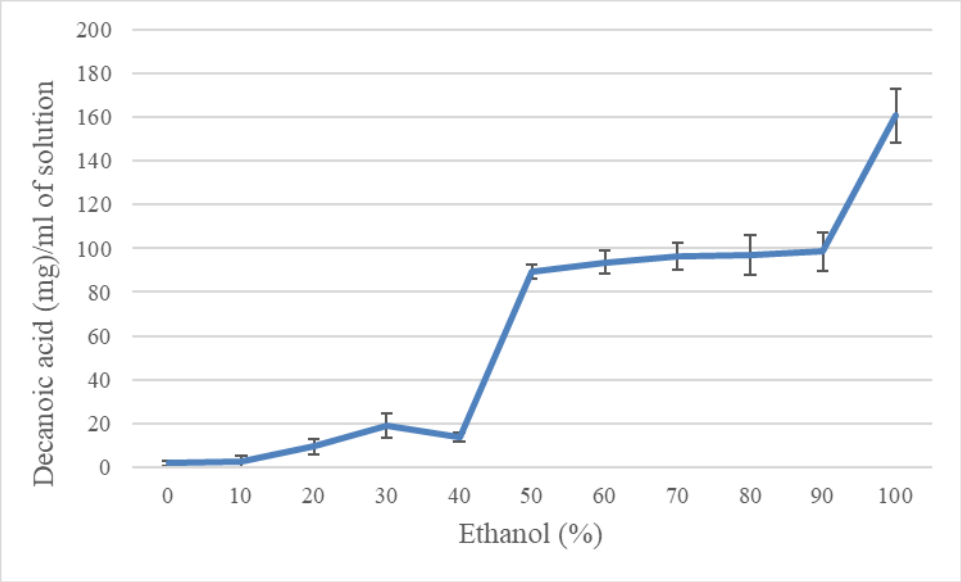
The dielectric constant is the number that indicates how polar a medium is, at 60 °C, water and ethanol have dielectric constants of 66.81 and 18, respectively (Malmberg & Maryott, 1956; Raspo & Neau, 2020). The intermediate dielectric constant of ethanol, indicating a less polar environment, explains why the solubility of decanoic acid increases as the percentage of ethanol increases.

Ethanol is also an amphiphilic molecule, its polar hydroxyl group stabilizes the carboxyl group of decanoic acid through hydrogen bonding, while its ethyl group interacts with the hydrophobic tail via dispersion forces. This dual affinity reduces the free energy needed to keep the molecules dispersed in solutions and allows concentrations up to 160 mg/mL in 100 % ethanol, as shown in Figure 2. By contrast, dissolving decanoic acid only in water yields a very low concentration, like the 0.027 g /100 mL H_2O at 60 °C reported by Ralston & Hoerr (1942).

The sharp rise in solubility between 40% and 50% ethanol, almost a 70 mg increase, is due to a change in the solvent's microstructure. These results were consistent with reports by Ralston & Hoerr (1942) showed that the solubility of C10–C12 fatty acids increases five to seven fold when the ethanol fraction surpasses 45%. This phenomenon is attributed to the breakdown of water's hydrogen bond network and the formation of ethanol rich domains that can host hydrophobic chains. Altogether, the intermediate dielectric constant, preferential solvation by ethanol, and reduced free energy of aggregation account; for both the macroscopic transparency of the suspension and the solubility, jump from 20 to 90 mg mL^{-1} observed experimentally in Figure 2.

Figure 2

Solubility capacity of decanoic acid according to the percentage of ethanol.



Absorbance

While lower absorbance values at specific wavelengths are generally associated with greater light transmission and transparency (Le Akpabio et al., 2004), it is critical to note that absorbance alone is not the industry-standard metric for assessing the optical properties of packaging materials. The accepted standard for quantifying the transparency of plastic sheeting in food packaging is the ASTM D1746-15 test method (Zhao et al., 2022).

Although not an industry standard for assessing optical properties, absorbance provides a consistent and reliable metric for comparative analysis. Standardizing the measurement format and conditions allows for a direct comparison of the relative impact of each variable within the experimental design.

As shown in Table 2, samples with ethanol generally had a higher absorbance than their non-ethanol counterparts. This was true for all samples except the treatments with 20% decanoic acid and 20% glycerol. Research conducted by Ye and Harte (2013), demonstrated that ethanol at 50% concentration, combined with elevated temperature and alkaline conditions (pH > 8), can cause casein micelle dissociation into smaller, less light-scattering particles. Since these conditions were met during

the film synthesis, the increased absorbance in ethanol-based films suggests that a different phenomenon, rather than micelle dissociation, is responsible for the increased light scattering.

Table 2

Absorbance of casein films prepared at 0 and 50% ethanol and 0-20% decanoic acid and glycerol concentration.

Treatment	Absorbance
D0G10E0	0.11 ± 0.01^{A}
D20G10E0	0.15 ± 0.01^{AB}
D10G20E0	0.18 ± 0.00^{BC}
D10G10E0	0.22 ± 0.03^{C}
D10G10E50	0.42 ± 0.01^{D}
D0G20E0	0.58 ± 0.03^{E}
D0G10E50	0.78 ± 0.01^{F}
D10G20E50	0.79 ± 0.02^{F}
D20G20E50	0.82 ± 0.05^{FG}
D0G20E50	0.87 ± 0.00^{G}
D20G20E0	1.31 ± 0.05^{H}
D20G10E50	1.57 ± 0.01^{I}
CV (%)	4.84
Probability	P (<.0001)

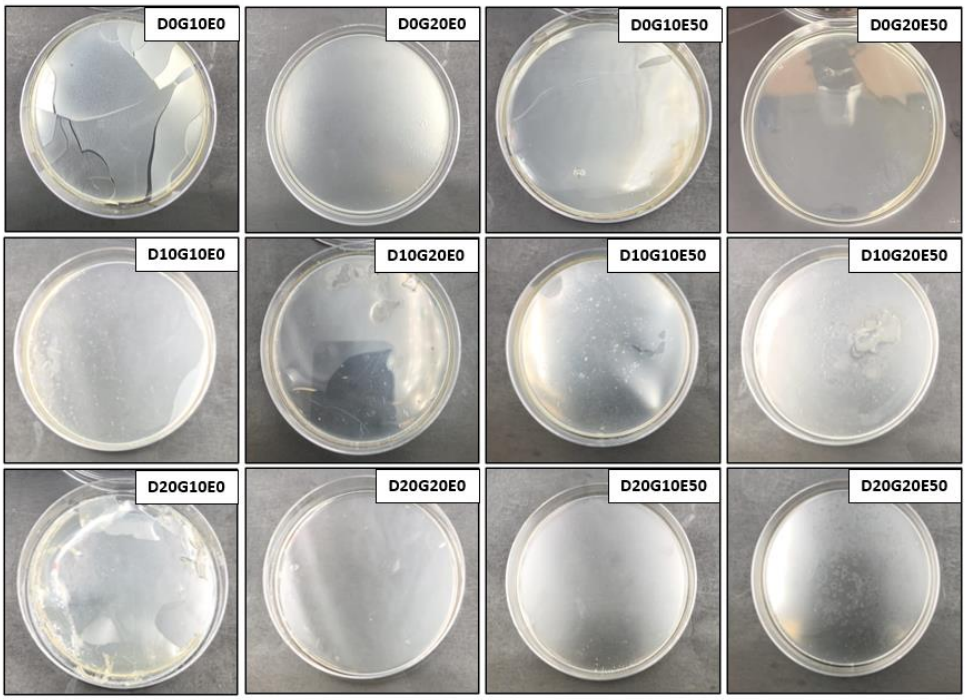
Note. Data point at 400 nm wavelength; A-I: Different letters indicate significant difference ($P < 0.05$); CV (%): Coefficient of variation; nm: nanometer; D: Decanoic acid (0%, 10%, 20%), G: Glycerol (10%, 20%), E: Ethanol (0%, 50%)

The difference in absorbance between the ethanol-water and water only films, is most likely due to the kinetics of the solvent evaporation as the samples with 50% ethanol solutions have very different volatilities (Hu & Larson, 2005). During the drying process, the more volatile ethanol escapes first, rapidly changing the solvent environment from a 50:50 ethanol-water mix to a progressively more water-rich solution in which micelle dissociation into smaller particles is likely occurring in the initial solution phase, but the rapid removal of ethanol forces the proteins into a state of supersaturation and aggregation (Spinozzi et al., 2016). This uncontrolled aggregation results in the formation of larger, irregularly shaped protein clusters leading to a highly heterogeneous film structure that is highly effective at scattering light, which directly leads to an increase in absorbance and a decrease in transparency (Ghosh et al., 2016).

According to Guzman-Puyol et al. (2022) transparent packaging materials have an absorbance value between 0-0.1, translucent materials are in a range of 0.1-1, and opaque materials have an absorbance value higher than 1. Based on the experimental data in Table 1, all the films classify as translucent packaging material, except for treatments D20G20E0 and D20G10E50, as they are considered opaque. This suggests that at high concentrations of both decanoic acid and glycerol, the hydrophobic and hydrophilic balance of the casein micelles is disrupted (Fabra et al., 2009). This disruption leads to a highly heterogeneous film matrix, with phase separation resulting in visible fat blooming in the films, which is not a desirable characteristic for packaging applications as shown by Figure 3.

Figure 3

Photographs of casein films prepared at 0 and 50% ethanol and 0-20% decanoic acid and glycerol concentration.



Note. D: Decanoic acid (0%, 10%, 20%), G: Glycerol (10%, 20%), E: Ethanol (0%, 50%)

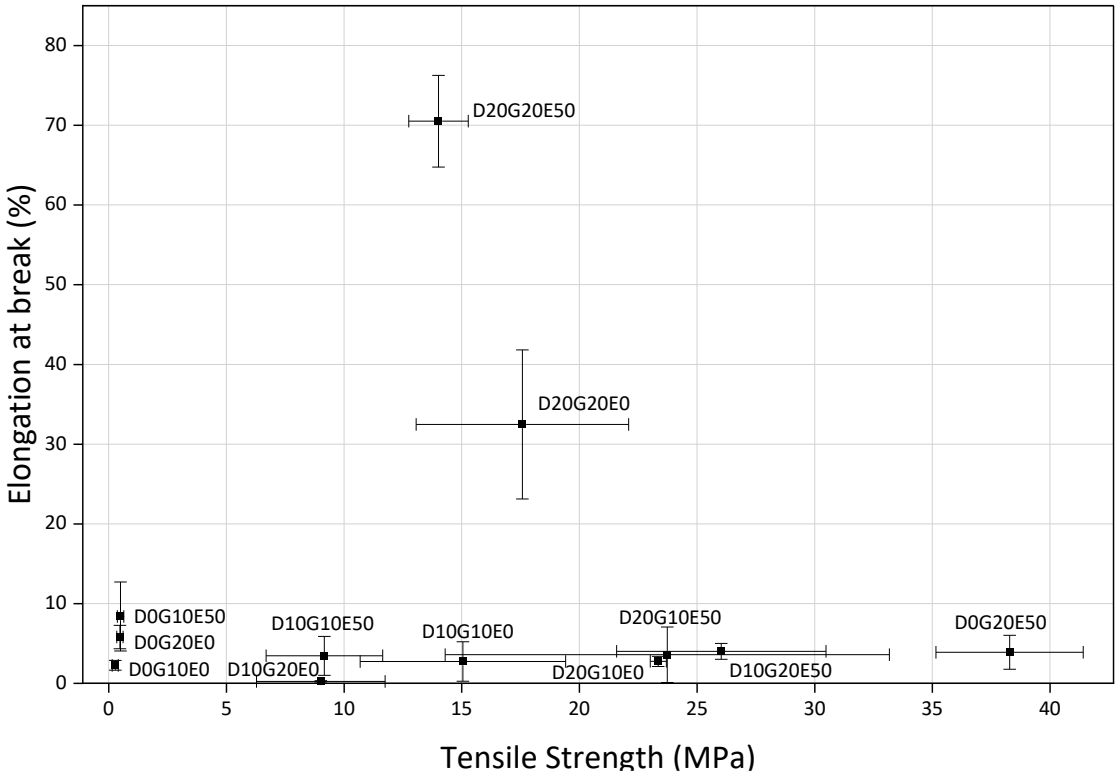
Mechanical Properties

The mechanical properties of the films are mainly evaluated based on tensile strength (TS) and elongation at break (EB). TS is defined as the maximum stress the film can withstand before breaking, is a direct measure of the cohesion and structural integrity of the polymer network (Chaudhary et al., 2022). On the other hand, EB is the percentage of stretching the film undergoes before fracture, serving as an indicator of the material's flexibility and deformation capacity (Pires et al., 2024).

Based on the results presented in Figure 4, it is observed that the treatments that demonstrate higher TS are not the ones with greater flexibility. In the case of EB, films with a high content of decanoic acid and glycerol (D20G20E0, D20G20E50) are the only ones showing significant differences. This is because glycerol, as a hydrophilic plasticizer, increases the free space between protein chains and facilitates their mobility under stress. Meanwhile, decanoic acid with its hydrophobic tail, introduces hydrophobic domains that decrease protein cross-linking and increase molecular mobility. This behavior is consistent with that reported by Jongjareonrak et al. (2006), who described that incorporating medium-chain lipids into protein films increases elongation but reduces strength due to the disruption of the protein bond network. The combination of these two variables achieves greater flexibility, since reducing the concentration or removing one of them, as in the case of treatments D0G20E0 and D0G20E50, greatly decreases flexibility.

Figure 4

Relationship between elongation at break and tensile strength.



Note. D: Decanoic acid (0%, 10%, 20%), G: Glycerol (10%, 20%), E: Ethanol (0%, 50%); CV tensile strength: 31.48%; CV elongation at break: 39.71%

Based on the results presented in Figure 4, the D0G20E50 formulation exhibited the highest tensile strength (38.28 MPa) but low elongation (3.88%), resulting in a film capable of withstanding stress but with limited flexibility. This is because ethanol, during film formation process promotes casein aggregation and compaction, leading to a cohesive matrix that the glycerol present cannot disrupt. However, for packaging applications, a balance between strength and flexibility is desired, which can be achieved with a high content of decanoic acid and glycerol (D20G20E0, D20G20E50). These treatments have a great EB that shown sufficient flexibility to prevent fractures caused by localized stress, and their TS remains above the minimum threshold reported for functional protein-based packaging (around 20 MPa) (Bonnaillie et al., 2014).

The results presented in Figure 4 are matched with photographs in Figure 3, which illustrate the structural differences among treatments. Films containing only 10% glycerol exhibited a brittle

behavior. However, the incorporation of decanoic acid and ethanol, or an increase in glycerol concentration to 20%, resulted in improved cohesion and enhanced structural integrity, and thus enhanced films' flexibility.

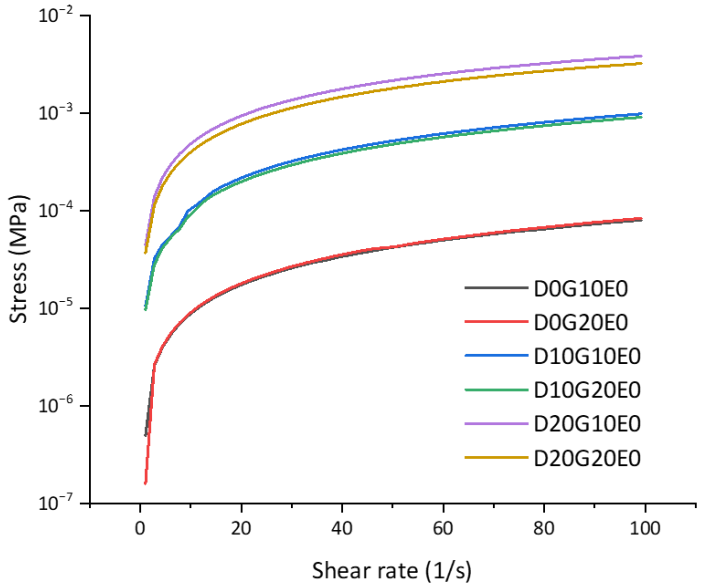
The high coefficient of variation for tensile strength and elongation at break is attributed to the heterogeneous nature of the films as some films were highly brittle and made it impossible to obtain three perfectly uniform rectangles from the same treatment.

Apparent Viscosity

Viscosity is a key rheological property that expresses a fluid's internal friction and therefore its resistance to flow. It is defined as the coefficient (μ) that couples applied shear stress (τ) to the resulting shear rate (γ) through the simple relation $\tau = \mu \cdot \gamma$ (Wilson, 2018). However, when the viscosity changes with the applied shear rate the fluid is classified as non-Newtonian. According to Hemar et al (2001) casein-based solutions exhibit non-Newtonian pseudoplastic behavior due to the alignment and disruption of micellar and protein networks under shear, this pseudoplastic behavior corresponds to the shear-thinning properties found in the treatments as shown by Figures 5 and 6.

Figure 5

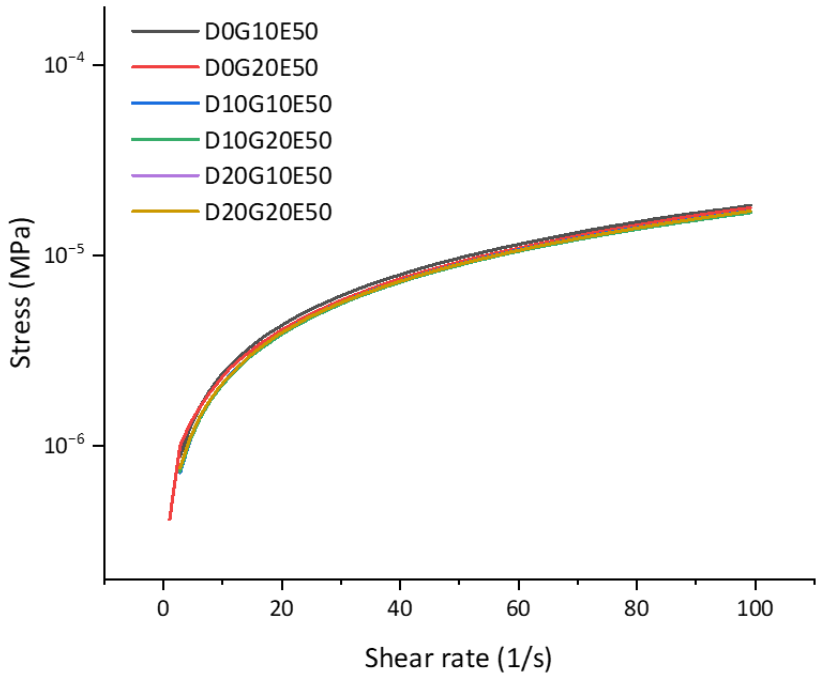
Rheogram of 0% ethanol film-forming dispersions.



Note. D: Decanoic acid (0%, 10%, 20%), G: Glycerol (10%, 20%), E: Ethanol (0%, 50%)

Figure 6

Rheogram of 50% ethanol film-forming dispersions.



Note. D: Decanoic acid (0%, 10%, 20%), G: Glycerol (10%, 20%), E: Ethanol (0%, 50%)

Because casein solutions are non-Newtonian fluids, their viscosity changes with the applied shear rate, consequently, apparent viscosity is used instead of a single viscosity value. This approach, which reports a specific apparent viscosity at a corresponding shear rate, provides a standardized method for comparing the rheological properties of these shear-dependent materials (Moller et al., 2009).

Apparent viscosity was analyzed as a practical criterion for predicting how easily the casein solution used to cast biodegradable films will spread, level and solidify. As illustrated in Table 3, all treatments that contain 50 % ethanol show a low apparent viscosity compared with their counterparts without ethanol, indicating that adding ethanol to the mixture has a decisive effect on viscosity, regardless of the amounts of decanoic acid and glycerol. This effect occurs because ethanol lowers the dielectric constant and weakens the hydrogen bonds and phosphate–salt bridges that connect the casein subunits, thereby loosening the micelle's electrostatic cohesion.

Table 3

Apparent viscosity of casein film forming dispersions prepared at 0 and 50% ethanol and 0, 10 and 20% decanoic acid and glycerol concentration.

Treatment	Apparent viscosity (Pa·s)
D0G20E50	0.15 ± 0.00^{A}
D10G10E50	0.17 ± 0.00^{A}
D10G20E50	0.17 ± 0.01^{A}
D20G10E50	0.17 ± 0.01^{A}
D0G10E50	0.18 ± 0.00^{A}
D20G20E50	0.19 ± 0.01^{A}
D0G10E0	0.81 ± 0.01^{B}
D0G20E0	0.83 ± 0.02^{B}
D10G10E0	9.95 ± 0.13^{C}
D10G20E0	12.49 ± 0.41^{D}
D20G20E0	32.76 ± 0.74^{E}
D20G10E0	38.21 ± 0.56^{F}
CV (%)	3.69
Probability	P (<.0001)

Note. Data point at 100 shear rate; A-F: Different letters indicate the significant difference ($P < 0.05$); CV (%): Coefficient of variation; Pa·s: pascals per second; D: Decanoic acid (0%, 10%, 20%), G: Glycerol (10%, 20%), E: Ethanol (0%, 50%)

Moreover, casein micelles dissociate when both temperature and ethanol are present (O'Connell et al., 2001), which might be expected to increase the solution's hydrophobic tendency by exposing hydrophobic interaction sites located inside the casein proteins. However, ethanol content above 30 %, together with a temperature higher than 40 °C, causes ethanol to block these hydrophobic pockets, reducing hydrophobicity and, consequently, protein re-aggregation (Trejo & Harte, 2010). This decrease in hydrophobicity results in low apparent viscosity because the hydrophobic bridges are lost in the fragmented micelles.

In treatments without ethanol, decanoic acid clearly acts as a thickening agent as shows in Table 3 that increasing its level almost triple the viscosity, because the fatty-acid aliphatic tails insert themselves between casein chains and create hydrophobic bridges that stiffen the network. In contrast, glycerol on its own, whether at 10 % or 20 %, hardly changes viscosity, since its main role is as a plasticizer, not a gelling agent.

Combining glycerol with decanoic acid produces nonlinear behavior. With 10 % decanoic acid, glycerol raises the total solids and liquid friction, so viscosity increases. With 20 % decanoic acid, the matrix is already highly cross-linked by fatty-acid tails, glycerol then penetrates the hydrophobic domains and hydrates the chains, which lowers the effective number of proteins bridges and thus reduces viscosity.

According to Rossman (2009), the optimal viscosity for edible films lies between 1 and 10 Pa·s; values outside this range give uneven or perforated films. Therefore, treatment D10G10E0, with a viscosity of 9.95 Pa·s, is taken as the formulation that flows best without wrinkling and producing layers of sufficient thickness, however this treatment did not show good elongation at break, which rules out its potential as packaging.

Conclusions

The concentrations of ethanol, decanoic acid, and glycerol significantly affected the mechanical, optical, and rheological properties of casein-based films. Increasing ethanol content generally led to higher absorbance values, indicating reduced transparency, whereas, higher concentrations of decanoic acid increased the apparent viscosity of the film-forming dispersions. In contrast, elevated ethanol concentrations exerted an opposite effect on viscosity.

Formulations containing decanoic acid combined with 20% glycerol relative to casein, both with and without ethanol, showed promising potential as packaging materials. These treatments enhanced film flexibility while maintaining adequate tensile strength. Although such films exhibited reduced transparency that could limit their applicability, their suitability ultimately depends on the specific requirements of the target food matrix.

Recommendations

Adjust the amount of decanoic acid, glycerol and ethanol conducting experiments with a wider range of concentrations with more intervals to improve the formulation and achieve better mechanical and barrier properties.

Conduct additional studies on barrier properties, such as oxygen and water vapor permeability, in the optimized formulations to confirm their suitability under real food packaging conditions. Moreover, sensory tests can be conducted to ensure that the films do not alter the sensory quality of the packaged products.

The use of more replicates is recommended when evaluating the mechanical properties in order to reduce variability in the results, as mechanical properties measurements are influenced by many factors, including the film thickness, width, and the cutting tools used to prepare the films.

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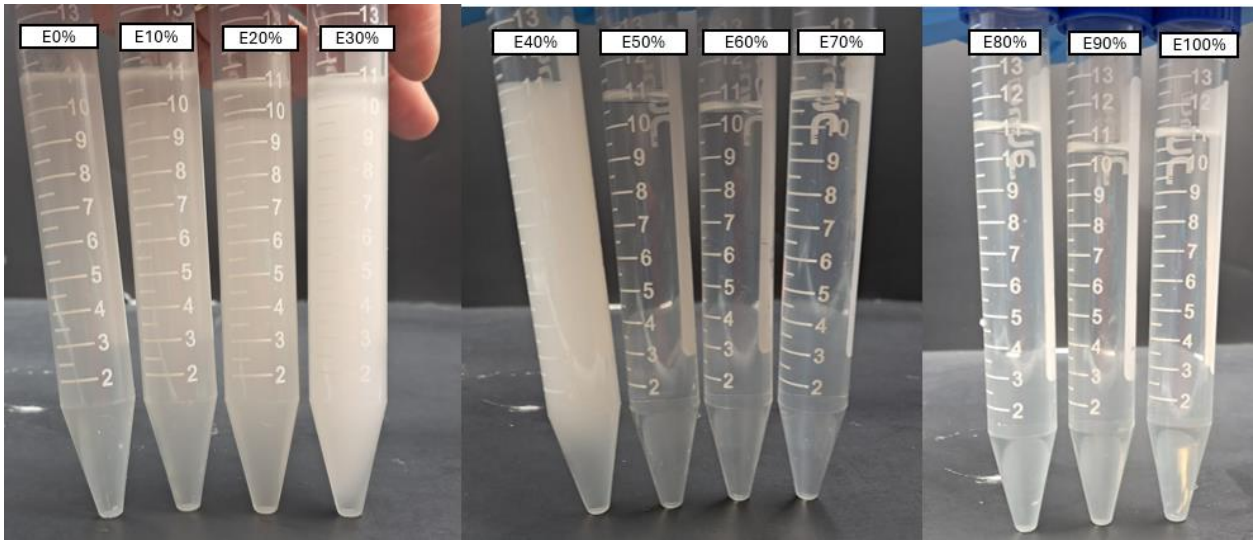
Appendices

Appendix A

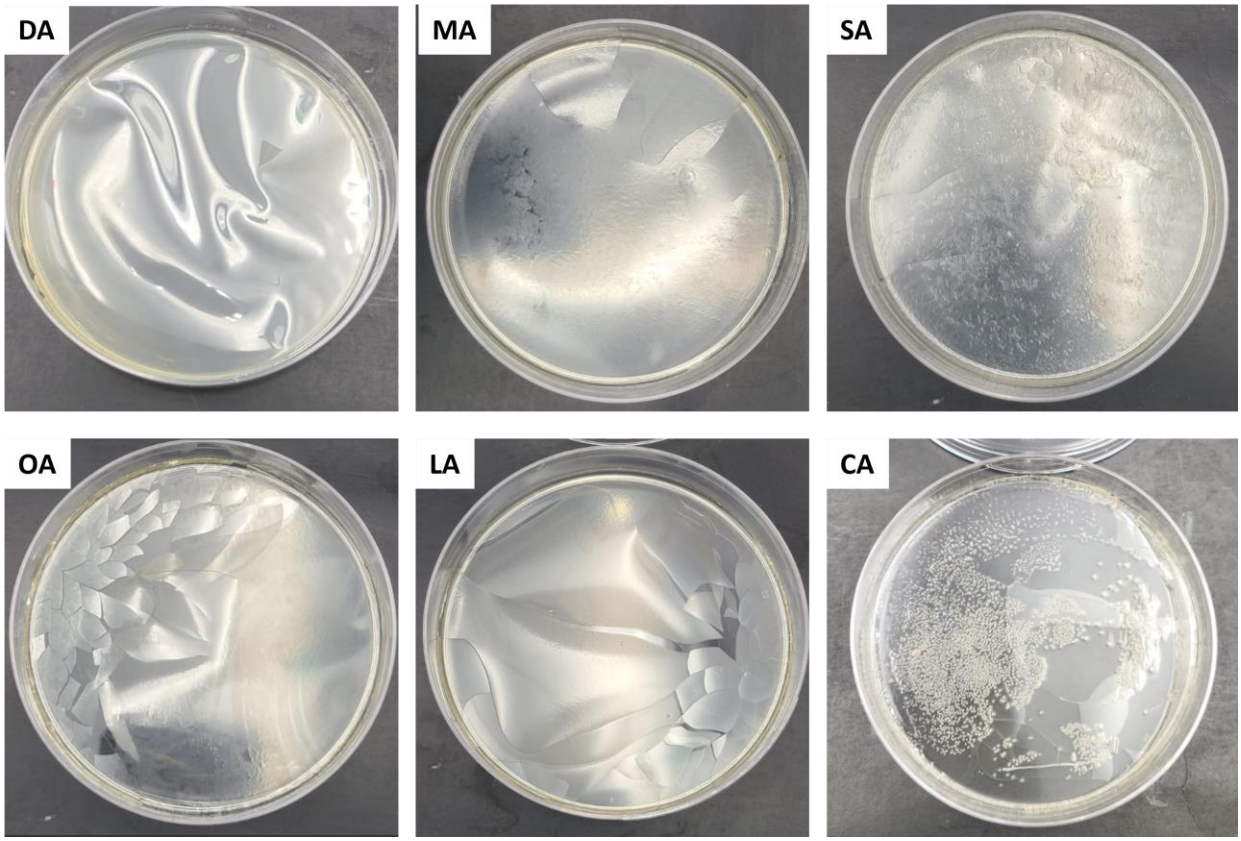
Photograph of solubility of decanoic acid



Note. E: Percentage of ethanol in the solution

Appendix B

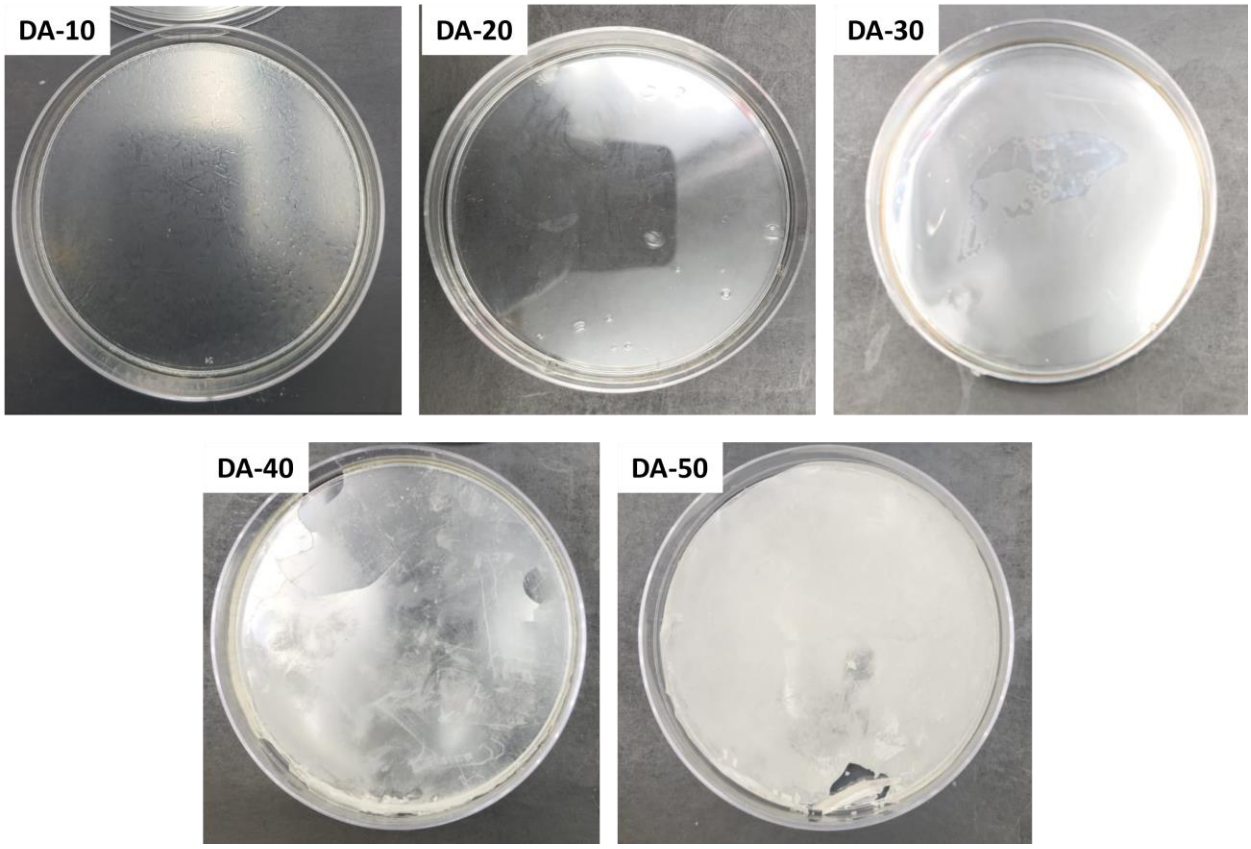
Photographs of first preliminary casein-based films preparation



Note. DA: Decanoic Acid; MA: Myristic Acid; SA: Stearic Acid; OA: Oleic Acid; LA: Linoleic Acid; CA: Caprylic Acid.

Appendix C

Photographs of second preliminary casein-based films preparation



Note. DA: Decanoic Acid; 10-50: % of decanoic acid in the film in respect to casein.