# EVALUATION OF THE EFFICIENCY OF ENZYMATIC CROSSLINKING IN BIODEGRADABLE FILMS BASED ON WHEY AND CORN STARCH

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## ABSTRACT

Research on sustainable alternatives in packaging has guided investigations towards the development of biodegradable materials that combine functional efficacy with low environmental impact. Within the scope of this study, transglutaminase stands out as an enzyme that can induce cross-linking between proteins, proving promising in improving the properties of biodegradable films. This study examines the feasibility of transglutaminase as a cross-linking agent in biodegradable films made from whey, corn starch, and glycerol, focusing on application in the food industry. The ideal formulation for film production was defined in the initial phase, with 1.5% whey, 3.5% corn starch, and 3% glycerol, chosen for its ability to form a uniform, flexible film without structural imperfections. Various concentrations of transglutaminase (0%, 0.5%, 1.5%, and 2.5%) were tested in the whey protein composition, assessing properties such as thickness, tensile strength, elasticity, water solubility, and water vapor permeability. The results show that the addition of 2.5% transglutaminase resulted in significant improvements in the film's properties, especially in elasticity and reduction of water solubility, highlighting the crucial role of this enzyme in enhancing biodegradable packaging. Transglutaminase not only strengthens the cross-links within the polymeric matrix but also increases the film's resistance in humid environments, thus contributing to sustainable progress in the field of food packaging.

**Keywords:** Cross-linking. Elongation. Water Solubility. Sustainability.

## 1 INTRODUCTION

Global concern over the environmental impact caused by the disposal of synthetic polymer packaging has been increasingly evidenced by the significant increase in the production of these materials, which rose from 270 million tons in 2010 to 370 million in 2020. With projections indicating a growth to 445 million tons by 2025, the urgent need to explore and implement sustainable alternatives that can mitigate the adverse effects of these wastes on the environment emerges.1", 2".

In this context, whey stands out as a promising raw material for the manufacture of biodegradable films, valuing its abundance and underutilization in the dairy industry, as well as its nutritional value.3". Research has shown that whey-based films, combined with other biopolymers or with the incorporation of additives, including plasticizing agents, bioactive compounds, and cross-linking agents, display distinct properties such as antimicrobial action and immunomodulatory capacity, standing out among other biopolymers.4", 5". These packaging solutions have a variety of applications, particularly in the preservation of foods such as meats, cheeses, and fruits, highlighting their potential as a sustainable solution in mitigating the issue of plastic waste.6".

Furthermore, the combination of corn starch with whey has been shown to improve the flexibility and mechanical strength of the resulting films. This integration presents a good interaction, as biocomposite films tend to have greater stability during their application.7",8". In this context, the enzyme transglutaminase, known for catalyzing the formation of cross-links between protein molecules, has been the subject of interest for its capabilities to enhance both the structural integrity and functional properties of materials based on biopolymers of protein origin, such as whey.9", 5".

Additionally, the synergy between whey and corn starch in enhancing the flexibility and mechanical strength of films demonstrates a positive interaction, suggesting improved stability in biocomposite films' application.7",8". Incorporating transglutaminase, extensively studied for its ability to catalyze cross-links between protein molecules, further elevates these materials by bolstering both their structural integrity and functional properties. This approach underscores a multifaceted strategy to improve biopolymer-based materials performance.9",5".

Research shows transglutaminase boosts film strength and elasticity, decreases oxygen permeability, and improves mechanical and thermal properties, while lessening water interaction. These findings underscore its role in improving packaging and food preservation.10",11",12".

In this context, this study aims to evaluate the effect of transglutaminase on enhancing the physical and mechanical properties of biodegradable films based on whey and corn starch, aiming to provide an innovative and sustainable alternative for the food packaging industry, in line with global efforts to mitigate the environmental impacts associated with plastic packaging waste.

## 2 MATERIAL & METHODS

### 2.1 Materials

The materials used to create the films were corn starch (Refeisucos), whey protein concentrate at 80% protein - WPC 80 (Sooro Renner Nutrition S.A), both as the polymeric matrix, glycerol (Dinâmica, Brazil) as the plasticizing agent, transglutaminase enzyme (YG - Ajinomoto, Brazil) as the cross-linking agent, and water as the solvent. When pH adjustment was necessary, it was done with 0.1 N potassium hydroxide (Dinâmica, Brazil).

### 2.2 Film production

The methodology implemented for the development of biodegradable films was structured in two distinct phases, starting with the formulation of blends based on a specific combination of starch, whey, glycerol, and water. This initial approach was guided by the recommendations proposed by [13]" detailed in Table 1 are the proportions of the ingredients used to create biodegradable films from corn starch and whey protein concentrate (WPC 80).

**Table 1** Composition of the suspension for the development of corn starch and whey protein concentrate (WPC 80) films.

| Identification | Water (%) | Whey Protein (%) | Corn Starch (%) | Glycerol (%) |
|---|---|---|---|---|
| W1:CS4:G2 | 93 | 1 | 4 | 2 |
| W1:CS4:G2.5 | 92.5 | 1 | 4 | 2.5 |
| W1:CS4:G3 | 92 | 1 | 4 | 3 |
| W1.5:CS3.5:G2 | 93 | 1.5 | 3.5 | 2 |
| W1.5:CS3.5:G2.5 | 92.5 | 1.5 | 3.5 | 2.5 |
| W1.5:CS3.5:G3 | 92 | 1.5 | 3.5 | 3 |
| W2:CS3:G2 | 93 | 2 | 3 | 2 |
| W2:CS3:G2.5 | 92.5 | 2 | 3 | 2.5 |
| W2:CS3:G3 | 92 | 2 | 3 | 3 |

Source: Adapted from Izzi et al. (2023)

After preparing the formulations, the solutions underwent heating at 80°C for 10 minutes in a water bath to induce starch gelatinization, following Rosseto methodology. Upon completion of the heating process, the solutions were cooled to room temperature, and 11g of each formulation were poured into Petri dishes. These dishes were then placed in an oven (Sinergia, SSDic, Brazil) at 40°C for 48 hours. Once dried, the films were exposed to controlled relative humidity conditions of 59% using a saturated sodium bromide (NaBr) solution for three days, in preparation for subsequent characterizations. [12]".

The next step focused on evaluating the macroscopic properties of the film (overall appearance). Following this, the most promising formulation was selected for further studies involving different concentrations of transglutaminase enzyme applied to the whey mass. The enzymatic cross-linking technique, adapted from Rosseto .12"., involved diluting the whey in water, adjusting the pH to 7, incorporating the transglutaminase enzyme, and then stirring at 130 rpm at 37°C for 20 minutes in an orbital shaker (Marconi, MA-420, Brazil). Enzyme concentrations of 0.5%, 1.5%, and 2.5% were explored, compared to a control sample without enzyme addition.

After enzyme incorporation and action, the previously described steps for film development were followed, now with the mixture incorporated with starch. In this context, the films were characterized for water solubility, water vapor permeability, mechanical strength, elongation, and thickness, allowing for a detailed assessment of the impact of different enzyme concentrations on the final properties of the films.

### 2.3 Characterization of the films

The evaluation of the films' appearance will be conducted according to the methodology suggested by Gontard.[14]". In this regard, the films will be examined through a macroscopic analysis, considering criteria such as homogeneity (absence of insoluble particles and bubbles, uniform coloration), continuity (lack of breaks or fragile regions), and handling (ease of removing the films from the support).

The measurement of the film thickness will be conducted using a digital micrometer (Digimess, 110.284, Brazil). This analysis will cover the entire length of the film, with approximately 6 measurement points, aiming to determine if the thickness remains uniform.

The evaluation of tensile strength and percentage elongation at break will be conducted using a texture analyzer (Stable Micro Systems, TA.XTplus Texture Analyser, Reino Unido), following the guidelines established in ASTM D882 standard.[15]". The test will be conducted with an initial separation of the grips of 40 mm and a test speed of 0.8 mm/s. The samples used will have standardized dimensions of 85 x 25 mm.

The water vapor permeability was determined according to the method adapted from ASTM E9600.[16]". The films were placed in thermoplastic polyurethane resin capsules with an exposure area of 2,545 cm², containing approximately 2 g of silica gel beads with a diameter of 4-8 mm. The capsules were positioned in a desiccator with controlled relative humidity of 50 ± 2%, maintained by a saturated sodium chloride solution, at a temperature of 25 ± 1°C. The weight of water absorbed by the silica gel, and consequently transferred through the film, was determined by weighing it after 24 hours.

The water solubility was evaluated according to the methodology proposed by Gómez-Estaca.[17]". Samples of 4 cm² were inserted into aluminum capsules containing 15 ml of distilled water and gently stirred at 22 °C for 15 hours. Then, the solution was filtered to recover the undissolved portion of the film, which was subjected to drying at 105 °C for 24 hours.

The statistical analysis was conducted using Tukey's test with a confidence interval of 95%. The software used to perform these procedures was Statistica 7.0 (StatSoft Inc., USA).

## 3 RESULTS & DISCUSSION

The results regarding the initial phase of the study, which encompass the macroscopic analysis of variations in starch, whey, and glycerol concentrations, are presented in Figure 1.

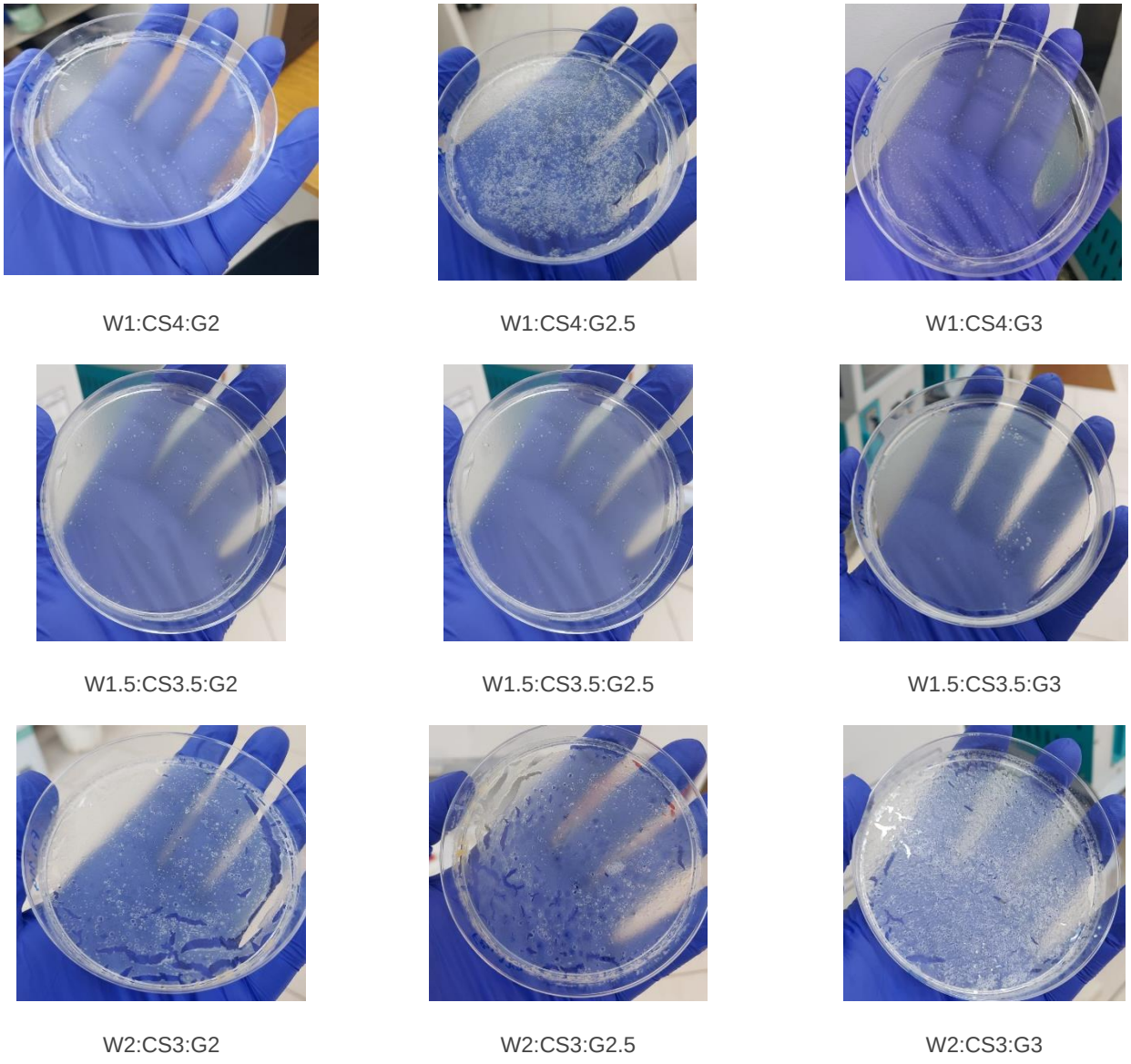


**Figure 1** Macroscopic visualization of films composed of whey protein concentrate (WPC 80), corn starch, and glycerol.

Among the formulations tested, the film with 1.5% whey protein, 3.5% starch, and 3% glycerol stands out for its homogeneity, demonstrated by the absence of insoluble particles and bubbles, and uniform coloration, suggesting an ideal blend and favorable

chemical interaction between the components. This composition also proved superior in terms of continuity, showing no breaks or fragile regions, indicating a balanced distribution of molecular interactions that may confer greater mechanical strength to the material. Furthermore, the observed ease of handling, particularly the easy removal from the support, evidence desirable flexibility and cohesion for practical applications. In contrast, the other films showed vulnerabilities such as fragility at the edges and bubble formation, as well as a tendency to break during handling, compromising their functionality and further highlighting the qualities of the chosen film.

After the initial evaluation, an additional procedure was conducted as part of the study, which involved the inclusion of transglutaminase enzyme with the aim of investigating the effect of this additive on the properties of the film previously identified as having the greatest potential for application. The subsequent analysis focused on critical parameters such as thickness, tensile strength, elongation, water solubility, and water vapor permeability, presented in Table 2.

**Table 2** The effect of different concentrations of transglutaminase enzyme on whey protein and corn starch-based films.

| Enzyme (% of protein mass) | Thickness (mm) | Tensile Strength (MPa) | Elongation (%) | Water Solubility at 25°C (%) | Water Vapor Permeability (g.mm/d.m². kPa) |
|---|---|---|---|---|---|
| 0 | 0.08$^{a}$ | 0.67$^{a}$ | 113.85$^{a}$ | 97.59$^{c}$ | 14.30$^{a}$ |
| 0.5 | 0.07$^{a}$ | 0.54$^{a}$ | 114.47$^{ab}$ | 96.43$^{b}$ | 14.03$^{a}$ |
| 1.5 | 0.08$^{ab}$ | 0.64$^{a}$ | 120.35$^{b}$ | 96.23$^{b}$ | 13.30$^{a}$ |
| 2.5 | 0.11$^{b}$ | 0.56$^{a}$ | 118.97$^{ab}$ | 94.81$^{c}$ | 12.18$^{a}$ |

Different letters in the same column indicate a significant difference according to the Tukey test, with a confidence level of 95%.

The addition of transglutaminase to protein films does not alter their appearance, preserving their flexibility, integrity, and absence of bubbles, essential characteristics for various applications. This stability is the result of the enzyme's ability to establish cross-links between proteins without negatively affecting the properties of the film.[18”]. Therefore, transglutaminase proves to be an effective additive for enhancing the properties of protein films, suitable for use in food packaging and biodegradable materials, among others.[19”,20”,12”,21”].

Incorporating 2.5% transglutaminase into the mix leads to thicker films, indicative of a densification process fueled by the enhancement of protein cross-links. This underlines the enzyme's prowess in fostering a more compacted structural arrangement. This development is tied to transglutaminase's knack for facilitating protein interactions, effectively bridging protein chains to fortify the film's structure, hinting at a more systematically arranged molecular framework.[19”,20”].

However, the tensile strength of the films does not show significant changes with the addition of transglutaminase, indicating that the cross-links formed by the enzyme, at the tested concentrations, do not directly impact the mechanical strength of the films. This is due to the specificity of the enzyme's cross-links, which, although strengthen the structure at a molecular level, do not reorganize the macroscopic structure in a way to enhance this property.[22”,12”].

The analysis of film elongation in response to the variation of enzyme concentration reveals a detailed overview of protein interactions and their impact on the mechanical properties of the material. With an enzyme concentration of 1.5%, a noticeable increase in film flexibility is observed. This phenomenon can be attributed to the formation of more elaborate protein structures, which endow the material with an increased capacity to stretch without breaking, enhancing its ductility.[24”]. In contrast, when increasing the enzyme concentration to 2.5%, a reduction in the elongation capacity of the films is observed. This effect is possibly due to an excess of cross-links or protein aggregation, which imposes significant rigidity on the film, compromising its elasticity and flexibility.[25”].

Combining the mechanical properties, the enzyme's ability to catalyze the formation of covalent cross-links between polypeptide chains results in a more cross-linked and complex protein matrix, imparting greater flexibility to the film.[26”]. This cross-linking allows the film to absorb and dissipate energy under tension, stretching further before reaching the breaking point, directly reflecting on the film's elasticity. On the other hand, the strength of the films is less affected, as it is more related to the density and alignment of the polypeptide chains, as well as the interaction between different components of the film, aspects that are not significantly altered by the bonds promoted by transglutaminase. [25”].

The water solubility of the films decreases with increasing enzyme concentration, aligning with the needs of applications that require lower solubility in moist environments. This phenomenon is attributed to the formation of less hydrophilic protein structures, resulting from the intensification of cross-links between the proteins.[12”,28”]. In contrast, the water vapor permeability of the films is not significantly influenced by transglutaminase, suggesting that the regulation of this property is governed by structural and physical factors independent of enzymatic modifications in the film proteins. This may include the density and molecular arrangement of the film matrix, as well as the presence of other components or additives in the film that may affect its permeability.[29”,30”]. Therefore, while transglutaminase is effective in strengthening the film structure by increasing cross-links, its influences on water vapor barrier characteristics appear to be limited, indicating the need for complementary strategies to optimize this property in specific applications.

## 4 CONCLUSION

The study identified the optimal formulation for film production and demonstrated that the inclusion of 2.5% transglutaminase significantly enhances the film's characteristics, especially in terms of increased elongation and reduced water solubility. This result highlights the enzyme's ability to strengthen cross-links within the polymeric matrix and increase the film's resistance in humid environments, emphasizing the importance of transglutaminase for the development of biodegradable and sustainable packaging materials for the food industry.

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