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DEVELOPMENT OF SUSTAINABLE INSECT PROTEIN HYDROGELS FROM *TENEBRIO MOLITOR*

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ABSTRACT

The increasing incidence of chronic diet-related disorders, such as diabetes and obesity, is attributed to the consumption of high-calorie diets. In response, food industries are seeking healthier products with reduced calories, fats, and digestible carbohydrates. However, creating low-calorie products that maintain similar physical and sensory properties as their high-calorie counterparts is challenging. Protein hydrogels appear as an alternative, as they have low toxicity, high biocompatibility, and gelling capacity. The growing demand for alternative proteins leads to the exploration of sustainable sources, such as insect proteins. This research aims to produce hydrogels from the insect protein *Tenebrio Molitor* (IPTM) at different pH (3.5 and 7) for use as texture modifiers, fat substitutes, and delivery systems for bioactive compounds. IPMT hydrogels exhibit uniform structure at pH 3, demonstrating pseudoplastic non-Newtonian behavior and robust mechanical properties, making them suitable for industrial applications.

Keywords: *Tenebrio Molitor*. Protein hydrogels. Functional foods.

1 INTRODUCTION

The increasing incidence of chronic diet-related disorders, such as diabetes and obesity, has been attributed to the consumption of high-calorie diets. In response, the food industry has developed a variety of healthier food products with reduced levels of total calories, fats, and digestible carbohydrates, such as starches.[1,2] However, developing low-calorie products that maintain similar physical and sensory properties to their full-calorie counterparts is challenging. This is because fats and starch granules play important roles in determining the desirable appearance, taste, and texture of many foods.[1]

Therefore, there is a need to develop new ingredients or structural approaches that can reduce the calories of foods while maintaining their desirable physicochemical and sensory properties.[3] Protein hydrogels are an alternative in this case, as they exhibit low toxicity, are easily processed by the body, and have a high biocompatibility.[4] Besides these advantages, an important functional property of the proteins in these hydrogels is their gelling capacity. Hydrogels can also be used in bioengineering, facilitating the formation of cultured meat. Given the global trends in climate change and the increasing consumption of meat, there is an urgent need to complement animal meat production with alternative protein sources in the diet.[5]

As the demand for proteins grows, the search for alternative proteins becomes more intense, aiming to address not only nutritional needs but also environmental concerns.[6] In this context, exploring alternative and more sustainable raw materials becomes desirable. In addition to plant-derived proteins, insects are gaining relevance as an environmentally sustainable source of proteins.[7] In response to this trend, this research aims to produce hydrogels from the insect protein *Tenebrio Molitor* (IPTM) to be used as texture modifiers, fat replacers, starch substitutes, and compound delivery systems.

2 MATERIAL & METHODS

2.1 Chemicals and materials

The IPTM protein concentrate (73% purity) was obtained from *Tenebrio Molitor* flour provided by Kreca (Netherlands) following the method of Ranasinghe et al.[8] Analytical grade reagents such as Sodium Hydroxide, Hydrochloric acid, and Coomassie Brilliant Blue G-250 were purchased from Sigma-Aldrich. 8-Anilino-1-naphthalenesulfonic acid (ANS) was purchased from AmBeed (USA).

2.2 Preparation of insect protein hydrogels

An appropriate amount of IPTM was completely dissolved in buffer solutions prepared with deionized water, with pH adjusted to 3, 5, and 7 using NaOH and HCl. This resulted in three protein solutions with a concentration of 0.2 g/mL at different pH levels. The protein solutions were transferred to 5 mL clear glass vials and heated in a water bath at 100 °C for 60 minutes, followed by cooling in an ice-water bath to form the hydrogel.

2.3 Determination of appearance

The appearance of the samples was analyzed according to the methodology described by Wang et al. [9], with some modifications. For this, the samples in glass vials were positioned on a black background. The photographs were taken when the glass vials were placed vertically and tilted at approximately 180°, allowing the appearance of the hydrogels to be observed. Based on the observations made, the samples were classified into three categories: hydrogel that flows when tilted, uniform hydrogel that does not flow when tilted, and protein precipitation with consequent dehydration contraction phenomenon.

2.4 Rheology

The samples were tested using an MCR302 rheometer (Anton Paar, Austria) equipped with a 25 mm diameter stainless steel plate, maintaining a gap of 1 mm. The sample was placed between the flat plate and the platform, and the steady-state shear test was conducted at 25 °C, varying the shear rate from 0.1 to 100 s^{-1}. Changes in viscosity were recorded at different shear rates. A strain sweep was performed before the oscillatory sweep test to determine the linear viscoelastic range.

2.5 Chemical force

Chemical forces were analyzed following the method described by Dan et al. [10], with some adaptations. Initially, the samples were weighed in four portions (approximately 0.25 g each) and placed in 50 mL centrifuge tubes. These samples were dissolved in 5 mL of solutions of 0.05 M NaCl (PA), 0.6 M NaCl (PB), 0.6 M NaCl + 1.5 M urea (PC), and 0.6 M NaCl + 1.5 M urea + 0.6 M NaCl + 8 M urea (PD), being stirred for 2 min each. Then, the solutions were refrigerated at 4 °C for 60 min, and the supernatant was obtained by centrifugation (6791×g, 10 min). Finally, the protein concentration of the supernatant was determined using Bradford protein analysis.

3 RESULTS & DISCUSSION

The states of the hydrogels, as shown in Figure 1, reveal the effectiveness of the cross-linking process at a temperature of 100 °C and pH 3, leading to the formation of a uniform, dense, and immobile gel, providing a promising perspective on its stability. However, when prepared at 100 °C and pH 5, the hydrogels exhibited fluidity and signs of stratification between the protein and aqueous layers. This behavior can be attributed to the fact that pH 5 is closer to the isoelectric point of proteins, resulting in the formation of aggregates that cause phase separation. [11] At pH 7, protein precipitation occurred. Heating to 100 °C at this pH may have destabilized the protein structure. This response to different pH conditions not only highlights the sensitivity of the system but also emphasizes the importance of carefully optimizing preparation conditions to achieve desired results. As highlighted by Wang et al.[9], pH plays a crucial role in the gelation of proteins, influencing the balance between the gravitational and repulsive forces between them. This behavior observed in the pH change with *Tenebrio Molitor* protein is similar to that described for whey proteins.

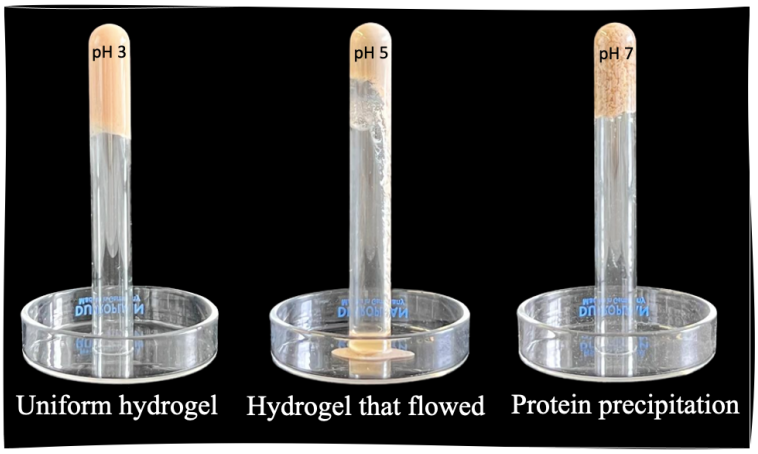

Figure 1 Appearance of IPTM hydrogels prepared at 100 °C and different pH values.

The evaluation of appearance showed that the IPTM hydrogel prepared at pH 3 showed more favorable results in terms of stability. According to Kim et al.[12], a lower pH can be strategically applied to improve the quality of insect proteins in the gel matrix, resulting in greater elasticity and gumminess. This observation highlights the benefits of low pH conditions. Therefore, the hydrogel prepared at pH 3 was chosen for further rheological analyses.

In Figure 2 (A), the shear viscosity decreases proportionally to the increase in shear rate. This rheological response reveals a non-Newtonian pseudoplastic behavior of the samples, a common characteristic in semisolid foods.[13] Analysis of the hydrogels' linear viscoelastic region (LVR) was also performed. Parameters were set with a strain range of 0.1% to 100% and a frequency of 1 Hz, and changes in moduli were recorded. During the frequency sweep, the strain was maintained at 10% (in the linear viscoelastic region), and the storage (G′) and loss (G″) moduli were recorded as a function of frequency (Figure 2 (B)). The storage (G′) and loss (G″) moduli are representative of the solid and liquid properties of the viscoelastic material, respectively.[14] When analyzing the storage (G′) and loss (G″) moduli of the IPTM hydrogels, it was observed that G′ was higher than G″, indicating that the IPTM hydrogel is predominantly solid under these conditions and possesses suitable mechanical properties for various applications.

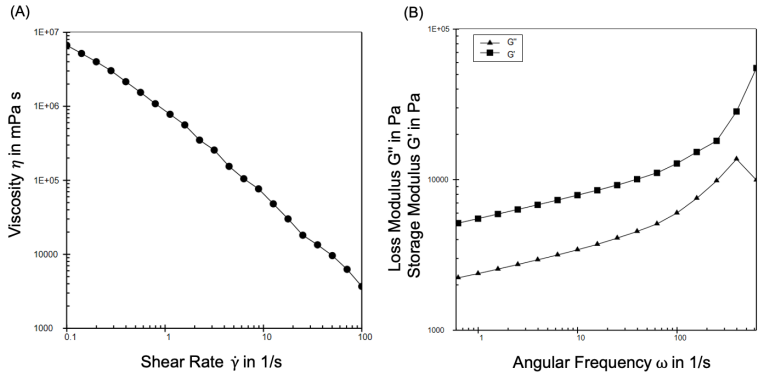

Figure 2 (A) Apparent viscosity and shear rate (B) Modulus (G′ and G″) and frequency

To measure the intermolecular forces maintaining the structure of the IPMT hydrogel and determine the mechanism of hydrogel formation, we used different denaturants, as described in section 2.5. The analysis of the intermolecular forces (Table 1) revealed that hydrophobic interactions predominate, while hydrogen bonding interactions are minimal, and electrostatic interactions showed negative values. These negative values indicate that the electrostatic repulsive force within a molecule, caused by a net negative charge, leads to the swelling and expansion of protein molecules. [15] These results are consistent with previous studies performed at low pH, highlighting the importance of optimizing pH conditions in the formation of protein hydrogels. [9]

Table 1 Chemical interactions of IPTM hydrogels.

Analysis	Hydrogel (mg/mL)
Electrostatic interactions (g/L)	- 0.902 ± 0.0
Hydrogen Bond (g/L)	0.254 ± 0.0
Hydrophobic Interaction (g/L)	1.052 ± 0.0

4 CONCLUSION

The IPMT hydrogels are more stable at pH 3, exhibiting a uniform structure and non-Newtonian pseudoplastic behavior. Rheological analysis showed that G′ is greater than G″, indicating a solid material with suitable mechanical properties. The predominant intermolecular forces are hydrophobic interactions. These hydrogels are promising for industrial applications, including texture modifiers and delivery systems for bioactive compounds.

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