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Investigation of Effect of Wet Heating Glycation on Solubility of Sunflower Seed Protein

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ABSTRACT

The wet-heating method was used to make the glycated sunflower seeds protein isolate (GSPI), which was then examined in this work. Galactose and water were added to the sunflower seeds protein isolate (SPI) and *heated* for two hours at 75°C. *Solubility property of GSPI were also* established. *The results illustrated that the heating in wet condition* might speed up glycation. At the same pH levels, GSPI's solubility was considerably *($P < 0.05$)* greater than that of unglycated sunflower seeds protein isolate (SPI).

Keywords: Glycated sunflower seeds protein isolate; Functional properties; Wet-heating method; Solubility property.

1. Introduction

One of the most significant oil crops worldwide is sunflower (*Helianthus annus* L.) seed. A byproduct of extracting oil from sunflower seeds is sunflower seed cake, which has a balanced amino acid composition and is high in protein. In addition to being used as a confectionery component, sunflower is primarily utilized to produce oil from its seeds [1]. De-oiled cake/meal or press cake is a valuable byproduct that is produced when sunflower oil is extracted. Due to the cake's high protein and fiber content, it is frequently utilized as a valued animal feed and for the biofuel production [2]. Due to its high protein content, it's possible that de-oiled sunflower cake can be a cheap source of protein. Sunflower meal has a crude protein level of between 30% and 50% [3].

Additionally, sunflower protein has very low levels of toxic cyanogens and antinutritional substances [4]. It is thought to be a trustworthy supply of high-grade protein fit for human consumption. Sunflower seed meal contains 1-4% polyphenols, primarily chlorogenic acid, according to Pedrosa et al. [5]. In alkaline settings, these polyphenols easily oxidize, resulting in browning and a dark gray extraction of protein [6]. Usually, it is underused or fed to animals. The protein from sunflower seeds has modest functional qualities [7], [8]. To be utilized in the food sector, proteins must undergo modifications to improve their functional qualities [9]. The use of proteins as functional instruments in food systems is encouraged by their functional characteristics [10].

Numerous commercial food items require characteristics like foaming, emulsification, or gelation to be produced. Although animal-based proteins like gelatin, milk proteins, and egg white are still primarily employed because of their high functional capacity, there is growing interest in using plant-based proteins as functional substitutes for animal-based proteins. In the last ten years, there has been a lot of interest in the interaction between reducing sugars and proteins in food. This may be explained by the Maillard reaction's widespread application of glycation processes and certain proteins' changed functioning as a result of their carbohydrate conjugation [11], [12]. Glycated food proteins are used in the food industries with less safety concerns than chemically altered food proteins [10]. The purpose of this investigation was to ascertain how the glycation of galactose by the Maillard reaction affected the functional characteristics of sunflower protein isolate. It is expected that the glycated

sunflower protein isolate may have potential for improving quality of the food products with improved functional properties. Non-enzymatic glycosylation of proteins, called glycation, considered one of the protein modification methods to have them undergo by Maillard reaction [11], [12]. The reducing end of sugars are covalently bonded with amino acid amine groups during the nonenzymatic process of glycation. Schiff bases and their subsequent rearrangement into Amadori products are first glycation products. Advanced glycation end (AGE) products are polymeric aggregates that form when these Amadori products crosslink with other proteins [13], [14]. Consequently, obtaining glycated sunflower seeds protein isolates (GSPI) and characterize them in terms of their solubility were the goals of this investigation.

2. Materials and methods

2.1. Materials

Sunflower seeds were purchased from Al-Dhahabi Market, Brak, southwest Libya. The improved process of Campbell et al. was used to prepare sunflower seed protein isolate (SPI) from defatted sunflower seed flour [15]. Olive oil was obtained from Al-Saraya store. Ortho-phthaldialdehyde (OPA), Bovine serum albumin and other chemicals were kindly provided by Faculty of Food Sciences, Wadi Al-Shatti University, Brack, Libya.

2.2. Methods

2.2.1. Protein Glycation

A 4:1 weight ratio (for protein isolate and galactose respectively) was used to mix sunflower seed protein isolate (SPI) with galactose, in distilled water (dH_2O) at a 5% weight/volume ratio. Since protein is more soluble in alkaline environments, the pH of the solution was adjusted to 10 using a 1N NaOH solution. After 30 minutes of room temperature stirring with a magnetic stirrer, the Solution was heated to 75ºC in a water bath for two hours, and it was then freeze-dried. SPI and galactose were separately maintained for two hours in the identical settings as the control. After the heating treatment, the solution was dialyzed (molecular mass cut off 6–8 kD) against dH_2O for three days at 4ºC to extract the unreacted galactose for further analysis. The sample is known as glycated sunflower seeds protein isolate (GSPI) once it has been freeze-dried.

2.2.2. Measurement of free amino groups

Using the OPA procedure described by Achouri et al. [16] a spectrophotometric assay was used to quantify the free amino groups of protein at pH 7. To create new OPA reagent, 40 mg of OPA (dissolved in 1 ml of ethanol) was mixed with 1.905 g, 0.05 g of SDS (dissolved in 40 ml of dH2O), and disodium tetraborate decahydrate. 2.35 ml of 2mercaptoethanol were added to the dH2O to make the solution's volume reach 50 ml. After adding 2.1ml of OPA reagent, the sample was left at 23 ±2ºC for 5 min. A spectrophotometer was used to measure the absorbance at 340 nm. The calibration curve for leucine was established by preparing standards at values between 0.2 and 5 mM. Each measurement was carried out triplicate.

The following equation was used to determine the glycation degree:

$$\text{Glycation Degree (\%)} = (A_1 - A_2 / A_1) \times 100 \qquad (1)$$

Where A_1 is the control's absorbance; A_2 is the sample's absorbance.

2.2.3. Browning measurement

The browning of protein glycation was measured by spectrophotometric assay with absorbance at 420 nm [18]. Additionally, to reduce light scattering, the samples (1 ml) were diluted in 2 ml of SDS (20% w/v). The results were derived by deducting the blank value from the sample readings and using the unheated sample as the blank.

2.2.4. Statistical analysis

The measurements were done in 3 replications and mean ±standard deviation (SD) were calculated. The data were analyzed using One-way ANOVA, and the least significant difference test (LSD) was performed to compare the means (in triplicate) at a 95% significance level ($p<0.05$). SPSS software was used for conducting the analysis.

3. Results and Discussions

3.1. Free amino group monitoring

Figure 1 illustrates how amino groups change over time as the temperature is heated up further. After two hours of heating, the amount of free amino groups dropped from 2.19 to 1.14µg/µl. Meanwhile, as can also be seen in figure 1 also, when the suspension was heated, the color of the suspension became more brown at 420 nm after a predetermined period of heating.

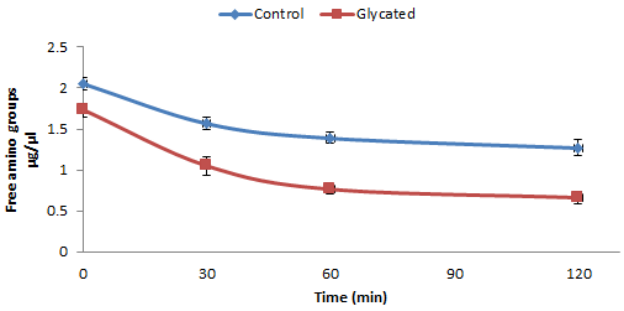

Figure 1. Monitoring of free amino at 75°C

During the glycation, the absorbance increased from 0.237 nm at 15 min to 0.453 nm at 120 min, while some increase was recorded for the control from 0.231 nm at 15 min to 0.331 nm at 120 min.

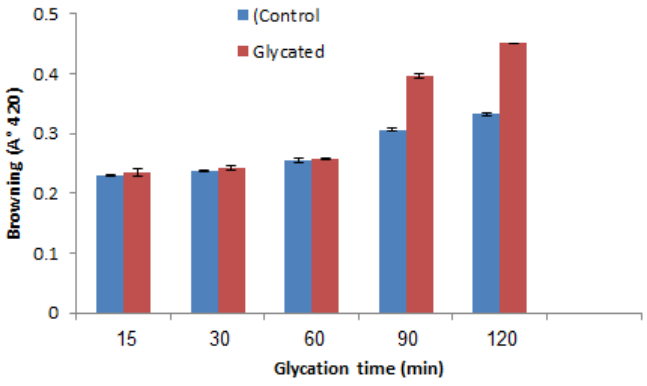

Figure 2. Monitoring of browning of protein

3.2. The glycation degree (GD)

It is anticipated that the Maillard process would increase with higher carbonyl group to amino group molar ratios [18]. However, it was claimed that the molar ratio was less likely to affect the degree of glycation than the overall crowding of the reactants [19]. In that sense, molecular crowding may affect the free amino groups' accessibility as protein concentration increases, hence reducing the rate of glycation. Ideal conditions and the Maillard reaction hypothesis state that the levels of free amino groups should decrease as glycation proceeds since it involves the first stage of the Maillard reaction. Since glycation involves the first step of the Maillard reaction, ideal situations and the theory of the Maillard reaction predict that the amounts of free amino groups should decrease as glycation takes place [20].

As shown in Figure 3, GD increased gradually from 15 to 60 minutes before stabilizing, demonstrating that as SPI and galactose are heated to 75°C in a water bath, more and more SPI and galactose conjugates form.

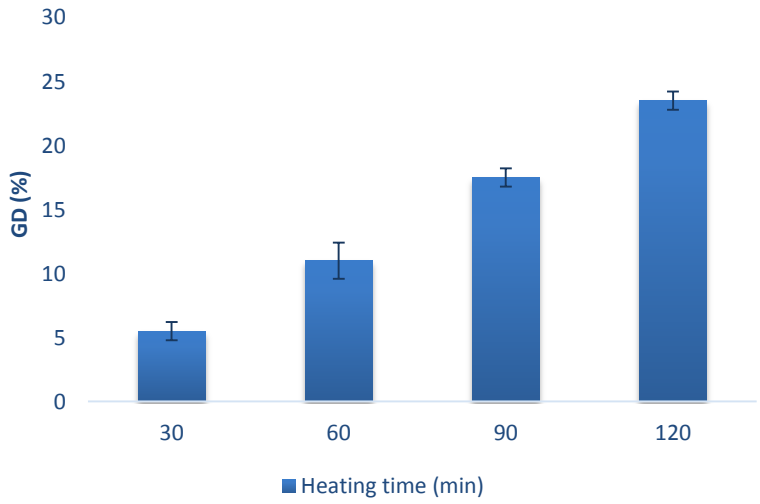

Figure 3. Glycation degree of SPI + galactose at varying reaction time (The SD of measurements made in triplicate is shown by error bars)

3.3. Protein solubility

The solubility in different environments is often crucial in establishing their functional usefulness and in enhancing protein extraction. Because it affects other functional aspects of proteins, solubility is a crucial functional characteristic [21]. The solubility of the protein is frequently required in many formulations based on proteins, such as emulsions [22]. Figure 4 shows how the solubility of SPI and GSPI changes with pH. The solubility of SPI may be significantly increased by glycation, especially when pH levels were between 4.0 and 5.0. Additionally, the GSPI solubility curve did not change much with pH, indicating that GSPI did not have a clear isoelectric point (IP). A protein's solubility typically drops significantly at the pH near its IP, where the protein's net charge is close to zero and it tends to assemble due to the electrostatic interactions brought on by the charge asymmetry of the protein. When a covalent protein-oligosaccharide hybrid forms, it remains intact regardless of pH or salt content [23]. The results showed that GSPI was able to significantly ($P \leq 0.05$) improve solubility across the acid pH range.

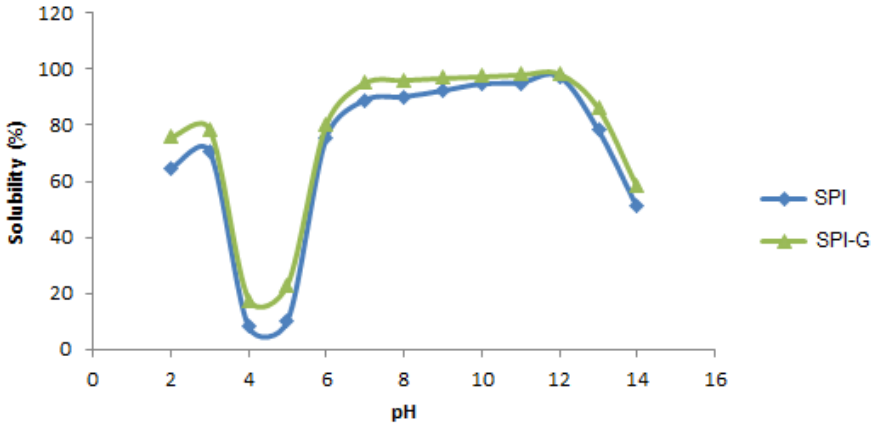

Figure 4. The solubility profiles of SPI and GSPI

4. Conclusion

The solubility property can be improved by glycation, this is what the study showed, the solubility of the glycated protein isolate was higher than the native protein, which makes it promising in many food industries.

Declarations

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The study has not received any funds from any organization.

Competing Interests Statement

The authors have declared no competing interests.

Consent for Publication

The authors declare that they consented to the publication of this study.

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