



Physicochemical Properties of Glycated Chickpea (*Cicer Arietinum L.*) Protein

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Abstract Glycated chickpea protein isolate (GCPI) was prepared by the wet-heating method and studied in the present work. The chickpea protein isolate extraction was heated at 80°C, for 120 min with the endogenous reducing sugars and oligosaccharides present in the protein extraction (solution). Physicochemical properties of GCPI were also determined. The results showed that the wet-heating method could improve the rate of the glycation. The solubility of GCPI was significantly ($P \leq 0.05$) higher than that of unglycated chickpea protein isolate (CPI) at the same pH values. The emulsion activity index (EAI) of the GCPI increased remarkably. Furthermore, a significant ($P \leq 0.05$) improvement on the emulsifying stability index (ESI) was observed. Little visible flocculation during extended storage (two weeks) was detected.

Keywords: Glycated chickpea protein isolate; Physicochemical properties.

الخصائص الفيزيوكيميائية لبروتينات الحمص المجكزة

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المخلص معزول بروتين الحمص المجكز تم تحضيره بطريقة التسخين الرطبة وتمت دراسته في هذا البحث، أخضع مستخلص معزول بروتين الحمص للتسخين على درجة حرارة 80°م ولمدة ساعتين مع السكريات الموجودة بشكل طبيعي في المستخلص (السكريات المختزلة والأوليغوسكاريد الموجودة في الحمص). ولقد تمت أيضاً دراسة الخصائص الفيزيوكيميائية لبروتين الحمص المجكز (GCPI). لقد بينت النتائج إمكانية التطوير الذي يمكن أن تضيفه طريقة التسخين الرطبة إلى الجلكزة. كان هناك ارتفاع ملحوظ ($P \leq 0.05$) في نوبانية معزول بروتين الحمص المجكز (GCPI) مقارنة بمعزول البروتين الذي لم يُعرض للجلكزة (CPI) عند نفس قيم الأس الهيدروجيني (pH). كان هناك ارتفاعاً ملحوظاً لمؤشر نشاط المستحلب لمعزول بروتين الحمص المجكز. وعلاوة على ذلك، كان هناك تحسناً معنوياً ($P \leq 0.05$) لمؤشر ثابتيّة الاستحلاب (EAI). كما أن التلبد المرئي للمستحلب خلال فترة التخزين لمدة أسبوعين كان بسيطاً.

الكلمات المفتاحية: بروتينات الحمص المجكزة، الخصائص الوظيفية.

Introduction

Paper must Among the current needs of food industry, we can mention the search for less expensive proteins for use as ingredients in many food processes. This search has increased the use of legumes. Most legume proteins are relatively rich in lysine, so they are considered as high quality protein compared to that of cereals and other plant proteins. Even though these plants are poorer than animal protein in phytates and methionine, these essential amino acids could be supplemented. Vegetable protein sources are many and varied, including leaf tissues, legumes, cereals and oilseeds. Globally, particularly in the developing world, roughly 90% of the protein intake and 88% of the human energy needs come from vegetable sources. [1].

Chickpea (*Cicer arietinum L.*) an important staple food in the world, which considered the 5th valuable legume in terms of worldwide economical standpoint. Chickpea is planted in Asia and Mediterranean. India is the principal, chickpea provider, realizing 75% of the world gross production [2]. Increased interest in legume

proteins in food has led to the evaluation of chickpea as a source of high protein crop. Chickpea is considered a good source of carbohydrates and proteins. Similar to other legumes, chickpea's albumins and globulins represent the two major fractions found in beans. In legumes, the albumin fraction, less abundant, represents up to 15-25% out of the beans' proteins whereas the globulins, represented mainly by vicinin and legumin, reach up to 60-80% out of the extractable proteins of the beans [3]. Albumins display a higher nutritive value compared to the globulins due to their high content in lysine and sulfur aminoacids. So, they play an important role in chickpea since they contain most of the proteins and enzymes with metabolic significance. The proteins from chickpea are better appreciated compared to the proteins from greengram, blackgram and pigmelon peas [4] because of their high biodisponibility, high biological value, well balanced amino acids content and low content in antinutritional factors [5]. The low fat content and

the special characteristics of chickpea beans give reason for the nowadays concerns for chickpea protein concentrates and isolates obtaining and their functional properties. Sánchez-Vioque et al. [6] studied the chickpea protein recovery yield in different conditions, the composition and functional properties of protein isolates in direct relations with the possibility of using this protein in food applications. According to their results, the chickpea protein isolates characterized by a high water absorption capacity (WAC) and oil absorption capacity (OAC) are adequate for obtaining bakery, cheese and meat products. On the contrary, the protein isolates with a fine emulsifying capacity can be well used for obtaining frankfurter and cream-like products. A small number of scientific data on the topic of the freeze-dried chickpea protein concentrate obtaining and characterization, from a chemical and functional viewpoint are reported. The interaction between proteins and reducing sugars in foods has attracted considerable attention during the past decade. This may be attributed to the ubiquitous nature of protein glycation reactions due to the Maillard reaction and the modification of some functional properties of proteins after their conjugation with carbohydrates [7], [8], [9]. Also, the application of glycated food proteins in the food industry presents fewer safety issues when compared with chemically modified food proteins (Kato, et al., 1996). The positive characteristics that occur due to the Maillard reaction are mainly comprehended in food applications. These characteristics are divided into two groups, sensorial and textural. The first group, sensory characteristics, includes the development of food colour, flavour and aroma compounds during food production. On the other hand, the textural characteristics include the improvement in the solubility, water absorption capacity, gelling, thermal stability and emulsifying properties of protein and until the present, most glycation reactions of proteins and reducing sugars to improve functional properties have occurred in the dry state [10-16]. The functionality of the conjugated proteins improved with increasing chain length and content of polysaccharide [16]. Accordingly, the objectives of this study were to obtain glycated protein isolate (GCPI) and to characterize these isolates from the physicochemical properties viewpoint. Regarding the physicochemical properties of the isolates, emulsion capability, solubility, and surface hydrophobicity of the GCPI were investigated. The creaming behaviour was also measured before and after the glycation.

Materials and methods

Materials

Chickpea flour was from a local store in Brack, Libya. Chickpea protein isolate (CPI) with protein content of 90.11% was prepared from defatted chickpea flour according to the improved method of Campbell et al. [17]. Sunflower oil (grade I) was obtained from a local supermarket. Bovine serum albumin (BSA), Ortho-phthaldialdehyde (OPA) and other chemicals were of the highest commercial grade and were kindly provided by Department of

Food Sciences and Technology, Faculty of Engineering Sciences and Technology, Sebha University (Sebha, Libya).

Methods

Preparation of Glycated Chickpea Protein Isolate

As outlined in Figure. 1, a 2 litre dispersion of defatted chickpea flour sample in distilled water (5% w/v) was adjusted to pH 10.0 with 2N NaOH, and stirred for 1 hour at RT. One 500 ml sample was removed to serve as unglycated (control). The suspension was heated for two hours in a shaking water bath at $80^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 120 min, followed by cooling to RT by immersion of the flask in cold water. The samples were centrifuged (5000 x g) for 30 min at RT to remove the starch and fibre fractions. The pH of the different supernatants was adjusted with 2N HCl to 4.5 to precipitate the proteins; the precipitated proteins were centrifuged at 5000 x g for 30 min at RT, washed twice with dH₂O, resuspended in water, neutralized to pH7.0 with 0.1N NaOH, dialysed (cut off 10 KDa) overnight at 4°C against dH₂O and freeze-dried. The sample is referred to as glycated chickpea protein isolate.

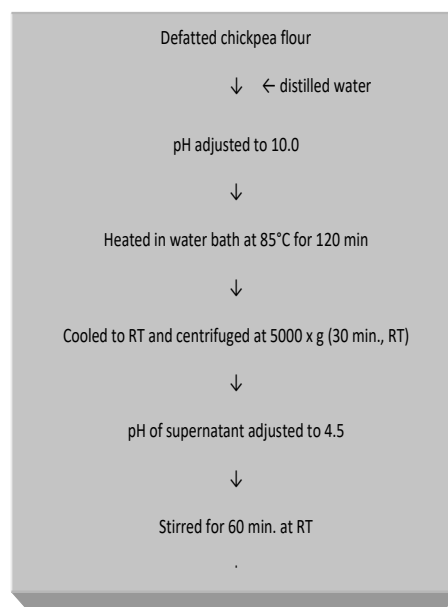


Fig. 1: Procedure for modification of chickpea protein (GCPI)

Determination of free amino groups

A spectrophotometric assay was used to measure the free amino groups of 5% (w/v) solutions of protein samples (2 hours of heat treatment at 80°C) at pH7 by the orthophthaldialdehyde (OPA) method described by Achouri et al., 2005. The OPA reagent was freshly prepared before use by mixing 40 mg of OPA (dissolved in 1ml of ethanol), 1.905 g disodium tetraborate decahydrate and 0.05g of SDS (dissolved in 40ml of dH₂O). The volume of solution was brought to 50 ml with dH₂O and 2.35ml of 2mercaptoethanol was added. 100µl of the sample was added to 1.8ml of OPA reagent and allowed to stand for 5 min at room temperature. The absorbance was measured at

340 nm using a Genesys 6 spectrophotometer (Thermospectronic, USA). A calibration curve of leucine was obtained by preparing standards with concentrations of 0.25-2 mM. Three replicates were performed for each measurement.

The glycation degree (GD) was calculated using the following equation:

$$GD\% = (A_0 - A_t / A_0) \times 100$$

Where A_t = absorbance of the sample; A_0 = absorbance of the control.

Monitoring of browning

Browning of chickpea protein extraction during modification treatment (2 hours of heat treatment at 80°C) was measured in the present by using a spectrophotometric assay as absorbance at 420 nm as described by Brands et al. [18]. Moreover the samples (1ml) were diluted in 20% (w/v) SDS (2ml) to reduce light scattering. The unheated sample was used as blank and the sample results were calculated by subtracting blank value from the sample readings.

Emulsifying properties

The emulsions were prepared to measure the emulsifying activity index (EAI) and the emulsifying stability index (ESI) of CPI and GCPI. The pure sunflower oil/water emulsions, stabilized with chickpea protein samples were prepared from 50 g of 1% aqueous protein suspension and 30 ml of sunflower oil by vigorous stirring for 5 min at RT. The mixture was then homogenised at 13,000 rpm for 2 min. 50 μ L portions of the emulsions were transferred by pipette at 0 and 10 min after homogenization from the bottom of each container. The portions were diluted with 10 mL of 0.1% SDS solution. Absorbance of each diluted sample was measured at 500 nm using a Genesys 6 Spectrophotometer (Thermospectronic, USA). The absorbances measured immediately and at 10 min after emulsion formation were then used to calculate the EAI and the ESI according to Klompong et al. (2007). The EAI was expressed as:

$$EAI (m^2/g) = 2T A_0 \times \text{dilution factor} / C \times \phi \times 10000$$

Where $T = 2.303$; dilution factor = 200; A_0 = absorbance measured immediately (at 0 min) after emulsion formation; C = weight of protein/unit volume ($g mL^{-1}$) of aqueous phase before emulsion formation; Φ = oil volume fraction of the emulsion. And the ESI was expressed as:

$$ESI (min) = A_0 \times \Delta t / \Delta A$$

Where $\Delta t = 10$ min and $\Delta A = A_0 - A_{10}$.

Each sample was prepared in triplicate for EAI and ESI measurement.

Emulsion stability against creaming was determined visually in the serum separated from the samples stored in glass containers, for 0, 1, and 14 days.

Statistical analysis

The determinations were performed in triplicate ($n=3$) and mean \pm standard deviation (SD) values were calculated. Data obtained in each chapter were analysed by one-way ANOVA. The comparison between means (3 replications) was performed at the 95% significance level ($p \leq 0.05$) by the least significant difference test (LSD). The

analyses were carried out using SPSS version 10 for Windows (SPSS Inc., NY, USA).

Results and discussion

Monitoring of free amino groups and browning

Figure 2 shows the changes in free amino groups during the extended heating duration. It can be seen from Figure 2 that free amino groups decreased from 2.18 to 1.12 μ g/ μ L after 120 min of heating time. Meanwhile, as shown in the same figure also, when suspension was heated, a brown colour increase occurred at 420 nm after a certain duration of heating time.

The glycation degree (GD)

Glycation degree (GD) reflects the protein-polysaccharide reaction degree, while the decrease in free amino groups during an extended heating time is due to the graft reaction [17], [19],[20], [21].

As seen from Figure 3, glycation degree (GD) increased slowly from 15 min to 60 min and after that reached a steady state, indicating that more and more CPI and endogenous reducing sugars and/or oligosaccharides present in the solution formed conjugates when heated in a water bath at 80°C.

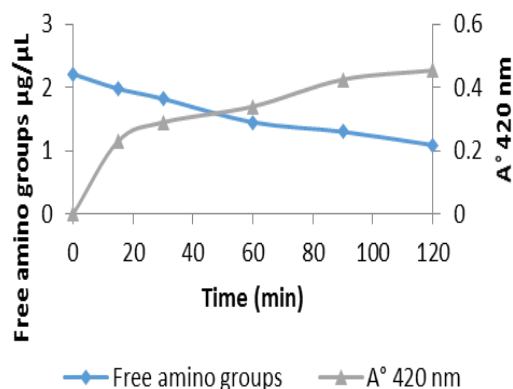


Fig. 2: Monitoring of free amino groups and browning of protein extraction heated in a water bath at 80°C.

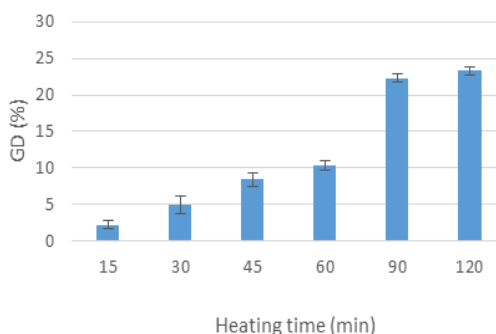


Fig. 3: Glycation degrees (GD) of chickpea protein extraction at different reaction time. Error bars indicate the standard deviation of triplicate measurements.

Protein solubility

Solubility characteristics of proteins under different conditions are often important in determining their functional applicability and in

the improvement of protein extraction. Solubility is an important functional property, as it has effects on other functional properties of proteins [22].

In lots of protein-based formulations, for instance emulsions, solubility of the protein is commonly required [23]. Figure 4 depicts the changes in solubility of CPI and GCPI at different pH values. The glycation could remarkably improve the solubility of CPI, especially when pH values were between 3.0 and 5.0. Furthermore, the solubility curve of GCPI did not vary rapidly with pH, which indicated that there was no obvious isoelectric point (IP) for GCPI. In general, the solubility of a protein decreases remarkably at the pH around its IP where the net charge of the protein is about zero and the protein tends to aggregate for the reason that the electrostatic interactions caused by the charge asymmetry of the protein. A covalent protein-polysaccharide hybrid, once formed, does not dissociate, irrespective of the pH or salt concentration. Moreover, glycation may exhibit a higher solubility than simple protein-polysaccharide mixtures [24]. The results exhibited that CPI modified by the glycation could significantly ($P \leq 0.05$) increase solubility in acid pH range.

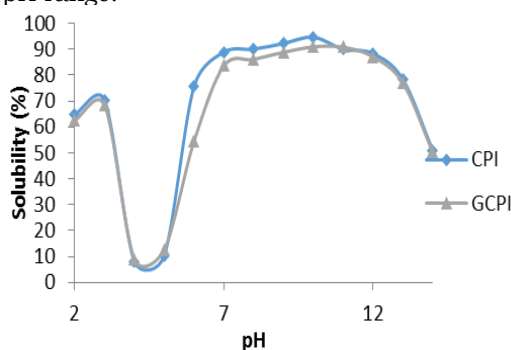


Fig. 4: The solubility profiles of CPI and GCPI.

Emulsifying properties

Emulsions are, from the physicochemical point of view, thermodynamically unstable systems rapidly or slowly separating into two immiscible phases according to the kinetic stability. Mechanisms of physical destabilisation of emulsions include oil droplets size variation processes such as flocculation, and coalescence and particle migration phenomena like sedimentation and creaming [25]. In the present study, emulsifying activity, emulsifying stability, and droplets distribution were measured to study the emulsion properties in detail. The EAI and ESI of CPI and GCPI at different reaction times are presented in Figure 5.

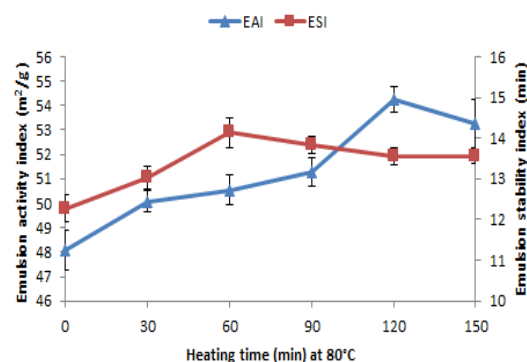


Fig. 5: EAI and ESI values of chickpea protein extraction heated for a given time (pH 7.5)

During extended reaction time, the glycation of protein and with the endogenous reducing sugars and oligosaccharides resulted in a gradual and significant ($P \leq 0.05$) increase in EAI and reached the highest value after 120 min. These results suggested that glycation via Maillard reaction could evidently improve the EAI of CPI. Consequently, the sample glycated for 120 min was chosen for further study. Moreover, ESI of CPI was also enhanced to some extent by glycation modification. The EAI of CPI was higher than that of GCPI, being possibly related with the calculating formula. Where A_{10} of the conjugates is higher, $(A_0 - A_{10})$ is not. Compared with the control, the EAI of the glycated chickpea protein isolate at neutral and acidic pH values was significantly ($P \leq 0.05$) increased (Figure 6). The influence of the glycation reaction on the EAI at pH 4.0 was more remarkable than that at pH 7.5. EAI of GCPI increased by 22.50% and 89.23% at pH 7.5 and pH 4.0, respectively. This might be mainly attributed to the difference between the solubility at these pH values. The EAI of all the samples (Figure 6) showed higher values at pH 7.5 than were those at pH 4.0. Similar results were also found in other protein-polysaccharide conjugates [13], [26], [27]. It was concluded that EAI was coincidental with the solubility at these pH values. The solubility of protein is an important prerequisite for the film formation because rapid migration and adsorption on the oil-water interface is critical. A positive correlation between the solubility and emulsifying capacity of proteins has been reported [28].

Creaming behaviour of GCPI (A) and CPI (B) emulsions

As shown in Figure 8, no creaming occurred after one day but after 14 days there was some creaming of GCPI, which was significantly less than that of CPI. This indicated that chickpea protein-saccharide glycation could produce stable emulsions. The presence of GCPI in the continuous phase at the time of the formation of emulsions (oil-in-water) enhanced the stability of emulsions. This result was established by the increased creaming stability and viscosity [26].

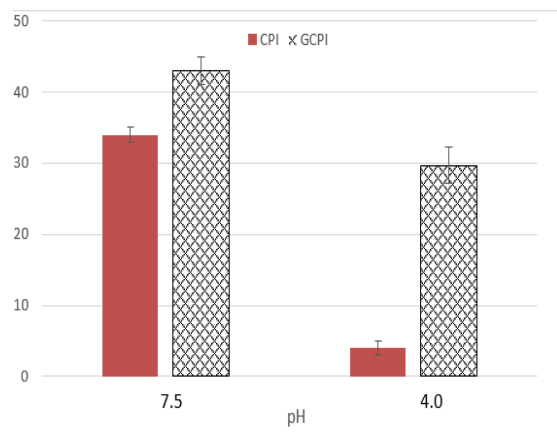


Fig. 6: EAI of CPI and GCPI at pH 7.5 and pH 4.0

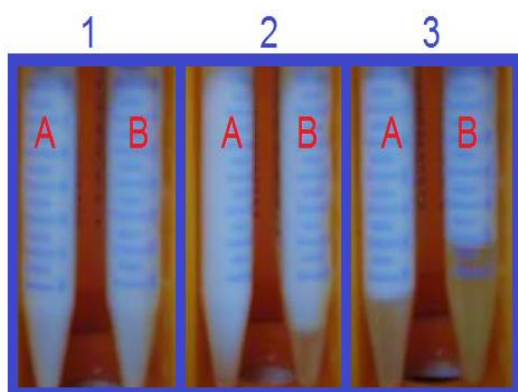


Fig. 7: Creaming behaviour of GCPI (A) and CPI (B) emulsions after different storage duration time: 0 day (1), 1 day (2) and 14 days (3), at pH 7.0

Conclusion

In the present study, the functional characteristics of chickpea protein were greatly improved by glycation via the Maillard reaction. Glycation appears to result in better functional properties of chickpea protein including solubility, emulsifying activity and stability.

In conclusion, the wet-heating method was a faster way for the preparation of more homogeneous GCPI. The present study clearly demonstrated that the via the Maillard reaction under wet-heating condition could significantly improve the solubility and emulsifying properties of CPI. The solubility of GCPI was significantly ($P \leq 0.05$) higher than that of CPI (unglycated) at the same pH values. The emulsion activity index (EAI) of the GCPI increased remarkably. The visible flocculation occurred during extended storage (14 days) was significantly little. Furthermore, the solubility and emulsifying properties of CPI were closely correlated in the acid condition. Compared with the dry-heating method, heating in a water bath at 80°C of chickpea protein extraction (under wet-heating condition) was a more efficient method for shortening the reaction time and lowering the brown colour of the reaction mixtures.

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