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Functional properties of native and succinylated lentil (*Lens culinaris*) globulins

Pushkar Singh Bora*

Departamento de Tecnologia Química e de Alimentos, Universidade Federal da Paraíba, 58059-970 João Pessoa-Pb Brazil

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Abstract

The functional properties of native and succinylated lentil globulins were evaluated. Succinylation caused a shift in the isoelectric pH of native globulins from 4.5 to 3.5 and improved the solubility above pH 4.0. However, below this pH the solubility of succinylated globulins was reduced. The water absorption and the viscosity of the sucinylated globulins were increased by almost 100%, while there was a decrease in the oil absorption capacity. The extent of succinylation used in this study did not show any significant relationships to these functional properties. Emulsion activity was also increased by succinylation; being 54.1% for the native globulins, 60% for the 57.9% succinylated globulins and 62.7% for the 87.2% succinylated globulins. Similarly, the emulsion stability was also improved. Foaming capacity of the succinylated globulins was decreased slightly, while foam stability, except at pH 2.5, was considerably reduced. Native and succinylated globulins showed maximum foam stabilities at pH 3.5 and 2.5, respectively.

Keywords: Lentil globulins; Succinylation; Functional properties

1. Introduction

The use of vegetable protein as an ingredient in food formulations is dependent on their functional properties. For their application it is necessary that their functional properties should be investigated and, if necessary, desirable functional properties should be incorporated in the protein through modification. Acylation of amino acid residues of protein, with anhydrides, positively affects their functionality. Canella, Castriotta, and Bernardi (1979) suggested the use of modified protein as an ingredient for a series of food formulations. During the last decade extensive literature has been published on the different modification methods of various proteins, for example by physical (Heinzelmann, Hoene, Muschliolik, & Rawel, 1994; Muschiolik, Rowel, Hoene, & Heinzelmann, 1994), genetic (Creamer, 1994), enzymatic (Hajos et al. 1989; Thomas & Leoffler, 1994) and chemical (Wagner & Guéguen, 1999a, 1999b; Guéguen, Bollecker, Schwenke, & Raab, 1990; Schwenke et al., 1990; Ponnampolam, Goulet, Amiot, Chamberland, & Brisson, 1988 etc.) means.

Lentil (*Lens culinaris*) proteins, have been studied for their composition (Bhatty, 1986; Bhatty, Slinkard, & Sosulki, 1976), nutritional quality (Bhatty & Christison, 1984; McCurdy, Scheier, & Jocobson, 1978) and effect of germination on their functional properties (Hsu, Leung, Morad, Finny, & Leung, 1982). However, no report is so far available on the functionality of chemically modified lentil proteins. The present work was therefore, undertaken to study the effect of succinylation on the functional properties of lentil globulins.

2. Materials and methods

2.1. Materials

Lentils (*Lens culinaris*) of the Brewer variety, were used in the present study. The lentils were ground in a cyclone sample mill with 0.5 mm screen (UD Corporation, USA). The flour was used for the preparation of protein isolate.

* Tel.: +55-83-216-7576; fax: +55-83-216-7179.
E-mail address: pradesh@uol.com.br

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2.2. Preparation of globulin isolate

Lentil globulin isolate was prepared following the method of Koyoro (1985). Lentil flour was extracted with 0.5 M NaCl, 50 mM potassium phosphate buffer (pH 7.2) in a ratio of 5 ml buffer/g of flour. The slurry was stirred for 1 h at room temperature, then centrifuged at 3000 rpm at 5 °C for 30 min, in a RC-3B refrigerated centrifuge (Sorvall Instrument). The pH of the supernatant was adjusted to 4.5 and the pellets were recovered by centrifugation at 19,000 ***g*** for 15 min at 5 °C. The pellets were re-extracted with extraction buffer, centrifuged (19, 000 ***g*** for 15 min at 5 °C) and precipitated again at pH 4.5. The pellets (globulins) were recovered by centrifugation under the same conditions and freeze-dried.

2.3. Succinylation of globulins

Succinylation of lentil globulins was carried out according to the method described by Groninger (l973). The pH of the globulin solution was adjusted in the range of 8.0–8.5 with 1 N NaOH and cooled in an ice bath to reduce the temperature to 2–5 °C. Succinic anhydride was added to the globulin solution at a concentration of 0.1, 0.25, 0.5 and 1.0 g/g of protein. The pH of the solution was maintained at about 8.0 to 8.5 with constant agitation. The reaction was considered to be complete when the pH of the solution was stabilized. Succinylated globulins were isoelectrically precipitated, centrifuged at 19,200 ***g*** at 5 °C for 20 min, lyophilized and stored in glass vials at −20 °C.

The extent of succinylation was measured as coloured lysine ninhydrin derivatives, produced in a dimethylsulfoxide system. The difference in absorbance between the dimethyl sulfoxide derivatives of native and modified globulin at 580 nm was used as an index of the extent of chemical derivatization (Beckwith, Bonder, & Ciegler, 1975).

The protein content was determined by the Biuret method. Bovine serum albumin (Sigma) was used as a standard for calibration.

2.4. Functional properties

2.4.1. Solubility

One hundred milligrammes of lyophilized globulins (native and succinylated) were suspended in 20 ml distilled water and the pH of the suspensions was adjusted from 2.0 to 8.0 using 0.1 N HCl or NaOH solution. The suspensions were agitated with a magnetic stirrer for 30 min at room temperature, the pH was checked and adjusted, then centrifuged at 4300 ***g*** for 30 min. The quantity of protein dissolved in supernatant was determined.

2.4.2. Water and oil absorption

Water and oil absorption of the native and succinylated globulins were determined according to the method described by Beuchat (1977). Five hundred milligramme samples were dispersed in 5 ml distilled water or corn oil and placed in 10 ml graduated centrifuge tubes. The dispersions were stirred occasionally with a glass rod. After a holding period of 30 min, dispersions were centrifuged at 3000 ***g*** for 10 min and the volume of the released fluid was measured. Water and oil absorptions were expressed as ml of water or oil retained per g of protein.

2.4.3. Viscosity

The relative viscosities of native and modified globulin solutions at pH6.5, 20 °C, and at concentration of 40 mg/ml in distilled water were determined before and after heating to 90 °C for 15 min in an Oswald viscometer.

2.4.4. Emulsifying properties

Emulsifying activity and stability were determined by the method of Yasumatsu et al. (1972). Ten millilitre portions of protein solutions (15 mg/ml) of varying pH (3, 5, 7 and 8) were homogenized with 10 ml corn oil at a speed of 5: in a scale varying from 1 to 10 of an Omni Sorvall mixer for 1 min. The emulsions were centrifuged at 1100 ***g*** for 5 min. The height of emulsified layer and that of the total contents in the tube was measured. The emulsifying activity were calculated as:

$$\frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total contents in the tube}} \times 100$$

To determine the emulsion stability, the emulsions were heated to 80 °C for 30 min and centrifuged again. Emulsion stability was calculated as:

$$\frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100$$

2.4.5. Foaming properties

Determination of foaming properties was performed in a water-jacketed apparatus, a modification of the foaming apparatus of Waniska and Kinsella (1979). The water-jacketed glass column (100 cm long; 0.98 cm i.d.) was calibrated to one tenth of a ml. The bottom of the column was equipped with a removable rubber stopper, which permitted the introduction of protein solution through a hypodermic needle and compressed air through a sintered glass frit of medium porosity. The pH of the protein solution (5 mg/ml) to be foamed was adjusted to 3, 5, 7 and 8. Fifteen millilitre solutions, at each pH ,were injected into the column via a hypodermic needle, using a syringe. The temperature of water in the column was maintained at 25 °C. Air was sparged for 1 min at a rate of 60 ml/min. The foam volume at the end of the sparging period was considered

as foam capacity. The time required for one half of the volume of liquid in the foam to drain was used as a measure of foam stability.

Five repetitions were carried out for the determination of functional properties, except that of solubility, for which three repetitions were made.

2.5. Statistical analysis

Statistical analysis of the results was done with Statistics for Windows 5.0 (1995) and the *t*-test was used to determine significance of differences between means. Trends were considered significant when means of compared sets differed at $P < 0.05$.

3. Results and discussion

The extent of succinylation of lentil globulins obtained with different concentrations of succinic anhydride was as follows:

Succinic anhydride (g/g of protein)	Succinylation of α-amino groups of lysine (%)
0.00	0.00 (NG)
0.10	57.9 (SG1)
0.25	78.6 (SG2)
0.50	87.2 (SG3)
1.00	90.3 (SG4)

The solubility profiles of native and succinylated lentil globulins, in the pH range 2 to 8, are shown in Fig. 1. The isoelectric pH of the native globulins was 4.5, which is in agreement with Vani and Zayas (1995) who reported that most of the plant proteins have isoelectric points at pH 4–5, while for succinylated globulins it was at pH 3.5. Succinylated proteins exhibit a shift in their isoelectric pH, thus resulting in an enhanced solubility at neutral to alkaline pH (Dua, Manajan, & Mahajan, 1996; Gruener & Ismond, 1997; Sheen, 1991; Sitohy, Sharobeem, & Abdel-Ghany, 1992). The shift reflects an increase in the negative charge, as a result of replacing the α-amino groups of lysine with negatively charged carboxyl groups. In comparison with native globulins, succinylated globulins show better solubility in the pH range of 4.5–8.0 but the solubility is greatly reduced below pH 4.0. The greater the extent of succinylation, the higher was the effect of pH on their solubility. Similar observation was also made by Sheen (1991) for the succinylated tobacco leaf proteins.

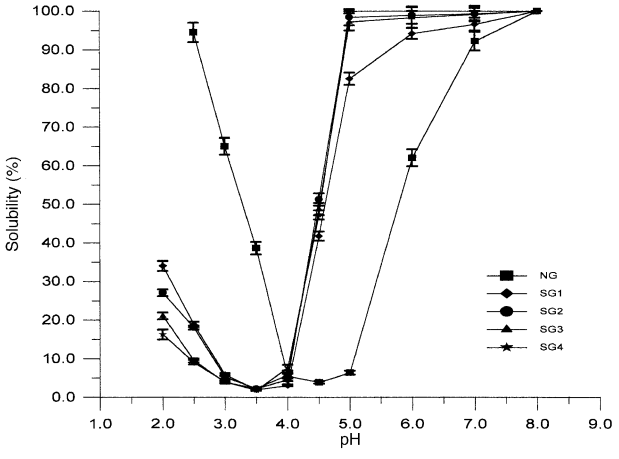

Fig. 1. Protein solubility behavior of native and succinylated lentil globulins at different pH values.

The data on the water absorption capacities of succinylated globulins are shown in Table 1. Acylation causes unfolding of protein, due to the electrostatic repulsion between the added carboxyl groups and the neighboring native carboxyl groups, exposing buried amino acid residues, and making them available for interactions with the aqueous medium (Chou & Morr, l979). Succinylation improves the water absorption capacity of native globulins from 1.1 ml/g to about 2.3 ml/g for 57.9% succinylated globulins. However, beyond this level of succinylation, the water absorption capacity remains almost unaltered. Improvement in the water absorption capacity of proteins by succinylation has also been reported for yeast (Geic et al., 1989), tobacco leaf (Sheen, 1991) and rapeseed (Dua et al., 1996) proteins, though Sheen (1991) observed increase in water absorption with increase in the extent of succinylation.

The oil absorption behaviour of the succinylated globulins was different from the water absorption. The native globulins showed an oil absorption of 2.6 ml/g protein. Succinylation caused a decrease in the oil absorption capacity of the native globulins. No significant difference was observed in the oil absorption capacity of the lentil globulin samples with different extent of sucinylation. The decrease in the oil absorption capacity of succinylated globulins could possibly be attributed to the increase in the hydrophilic groups at the oil-water interface (Morr, l979). However, Dua et al. (1996), Monteiro and Prakash (1996), Poonampalam et al. (1988) and Sheen (1991) have reported improvement in the oil absorption capacities of various succinylated protein isolates. Sheen (1991) also observed that the oil absorption increased with the extent of modification of leaf proteins, while Dua et al. (1996) reported maximum oil absorption in samples with minimum modification.

The relative viscosity of the lentil globulins is also shown in Table 1. Succinylation caused an increase in relative viscosity of globulins. However, statistically, the increase in the viscosity was independent of the extent

of succinylation. The viscosity of the succinylated globulins was also higher than the native globulins after heating at 90 °C for 15 min. Significant difference in the viscosities of 87.3 and 90.3% succinylated samples was observed before and after heating. Increase in the viscosity of succinylated rapeseed protein was also reported by Dua et al. (1996). During succinylation the protein molecule unfolds, due to the increase in net negative charge, resulting in an increase in the viscosity.

Emulsion activity and emulsion stability of native and modified globulins are shown in Table 2. Succinylation improves the emulsion activity and emulsion stability of lentil globulins. Succinylated globulins, with 78.6 and 87.2% modification, showed the best emulsion activities, while all the succinylated samples, except that of 90.3%, showed high emulsion stabilities when compared to native globulins. However, statistically no significant difference in the emulsion stability was observed among these samples. The native globulins showed 54.1% emulsion activity which was increased to 60.0, 62.2, 62.8 and 60.6% for 57.9, 78.7, 87.2 and 90.3% succinylated globulins, respectively. Corresponding emulsion stabilities of these samples were 52.2, 57.9, 58.8, 57.5 and 54.4%, respectively. Difference in the behavior of different proteins, with respect to the effect of sucinylation on the emulsifying properties has been reported: for example, Giec et al. (1989), Gueguen et al. (1990), Monteiro and Prakash (1996), and Sheen (1991) have observed an improvement, while Murphy and Howell (1990) and Sitohy et al. (1992) reported a decrease.

Table 3 shows the foaming capacities of native and succinylated globulins at different pH. In the range of pH values (2.5 to 8.0) studied, except at pH 3.5, succinylation at a level of 57.9% gave a similar foaming capacity to that of the native globulins. However, above this level of succinylation a small decrease in the foaming capacity of the samples was observed. The stability of the foams was considerably reduced by the succinylation at all pH values, except at pH 2.5, at which the stability of succinylated globulins was better than that of native globulins. At their isoelectric pH (3.5), succinylated globulins gave an extremely unstable foam, which collapsed the moment it was formed. Decrease in the foam stability of the succinylated proteins was also reported by Murphy and Howell (1990) and Phillip and Kinsella (1990) for β-lacto-globulin, and bovine albumin serum, respectively. Sheen (1991) and Sitohy et al. (1992) did not observe any change in the foam stabilities of succinylated wheat gluten, egg albumin, corn zein and leaf protein. According to Kinsella (l977), succinylation improves the solubility of proteins but has an ambiguous effect on foaming.

Table 1
Water and oil absorption capacities, and viscosities of native and succinylated globulins

Sample	Water absorption (ml/g protein)	Oil absorption (ml/g protein)	Relative viscosity	
			At 20 °C	After heating at 90 °C for 30 min
Native globulins (NG)	1.1±0.1a	2.6±0.2a	1.43±0.16aA	1.75±0.16aA
57.9% Succinylated globulins (SG1)	2.3±0.1b	2.0±0.2b	3.15±0.32bA	3.59±0.28bA
78.6% Succinylated globulins (SG2)	2.3±0.2b	2.2±0.1b	3.15±0.24bA	3.55±0.12bA
87.2% Succinylated globulins (SG3)	2.2±0.2b	2.2±0.1b	2.92±0.13bA	3.40±0.10bB
90.3% Succinylated globulins (SG4)	2.3±0.1b	2.2±0.1b	2.94±0.10bA	3.40±0.14bB

No. of replicates 5. Means in each row and column followed by different letters were significantly different ($P<0.05$ level). Capital and small letters show statistical differences for data in rows and columns, respectively.

Table 2
Emulsion activity and stability of native and succinylated globulins

Sample	Emulsion activity (%)	Emulsion Stability (%)
Native globulins (NG)	54.1±0.3a	52.2±0.8a
57.9% Succinylated globulins (SG1)	60.0±0.9a,b	57.9±1.1b
78.6% Succinylated globulins (SG2)	62.2±1.2b,c	58.8±1.2b
87.2% Succinylated globulins (SG3)	62.8±0.8c,d	57.5±0.7b
90.3% Succinylated globulins (SG4)	60.6±0.8b,d	54.4±0.3a

No. of replicates 5. Means in each row and column followed by different letters were significantly different ($P<0.05$ level). Capital and small letters show statistical differences for data in rows and columns, respectively.

Table 3
Foam capacities and foam stabilities of native and succinylated globulins at different pH

pH	Foam capacity (ml)					Foam stability (s)				
	Native globulins	57.9% Succinylated	78.7% Succinylated	87.2% Succinylated	90.3% Succinylated	Native globulins	57.9% Succinylated	78.7% Succinylated	87.2% Succinylated	90.3% Succinylated
2.5	87.0±0.5 AB,ab	89.0±1.4 A,b	86.0±1.6 B,a	74.5±0.5 C,a	81.0±0.8 D,ab	34.0±0.3 A,a	30.0±0.8 B,a	56.0±0.8 C,a	54.0±0.3 C,a	34.0±1.4 A,a
3.5	88.0±1.7	0.0	0.0	0.0	0.0	77.0±1.2	0.0	0.0	0.0	0.0
4.0	83.5±2.0 AB,b	87.2±0.6 A,bc	85.0±0.9 AB,a	82.0±1.9 B,bc	79.3±1.2 C,bc	42.0±0.7 A,b	18.1±0.5 B,c	17.0±0.4 B,b	8.0±08 C,b	8.6±2.4 C,b
5.0	84.5±0.9 AC,ab	86.6±1.2 AB,bc	87.5±1.5 B,a	83.0±1.1 C,bc	78.0±0.6 D,c	53.0±0.7 A,c	22.0±0.3 B,d	21.5±0.4 B,c	30.0±1.0 C,c	33.0±0.3 D,a
6.0	83.6±1.1 A,b	82.0±0.7 A,a	82.3±0.4 A,b	87.0±2.1 B,c	78.5±1.2 C,bc	57.0±1.3 A,d	21.0±0.2 B,d	19.5±0.2 B,d	25.0±0.7 C,d	25.0±0.7 C,c
7.0	85.3±0.8 A,ab	85.0±1.0 A,ac	80.0±0.6 A,b	80.0±0.2 A,bc	83.0±1.2 A,a	63.7±1.2 A,e	18.3±0.6 B,c	18.0±0.3 B,b	17.0±0.3 B,e	11.0±0.2 C,b
8.0	86.0±1.3 A,ab	85.6±1.2 A,c	80.0±0.5 B,b	78.0±0.7 B,ab	79.5±1.1 B,bc	60.3±0.9 Af	16.0±1.1 Be	11.0±0.7 Ce	12.0±1.0 Cf	11.0±0.1 Cb

No. of replicates 5. Means in each row and column followed by different letters were significantly different ($P < 0.05$ level). Capital and small letters show statistical differences for data in rows and columns, respectively.

4. Conclusions

Succinylation modified the functional properties of the lentil globulins. In comparison with the native globulins, succinylated globulins showed a shift towards the acidic side of pH and showed an increase in solubility in the pH range 4 to 7. Water absorption capacities of the succinylated globulins were enhanced while the oil absorptions were reduced. Increases in the apparent viscosity, emulsifying activity and stability, and a pronounced decrease in the foam stability of the succinylated globulins, were also observed. There were no significant differences in the functional properties of the modified globulins with different extents of succinylation.

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