doi: 10.17221/154/2015-CJFS

Modification of Structural and Functional Properties of Sunflower 11S Globulin Hydrolysates

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Abstract

Ren J., Song Ch.-L., Wang P., Li S., Kopparapu N.-K., Zheng X.-Q. (2015): **Modification of structural and functional properties of sunflower 11S globulin hydrolysates**. Czech J. Food Sci., 33: 474–478.

The structural and functional properties such as solubility, emulsifying properties, foaming properties, oil binding capacity, and surface hydrophobicity of sunflower 11S globulin hydrolysates generated by Alcalase at hydrolysis time of 30, 60, 90, and 120 min were evaluated. Circular dichroism analysis showed the hydrolysates possessed a decreased α-helix and β-structure. The hydrolysates exhibited lower surface hydrophobicity. Hydrolysates with shorter hydrolysis time showed the higher emulsifying activity index, but the same emulsion stability and oil binding capacity compared to the original 11S globulin. The longer hydrolysis resulted in lower foaming and emulsion stability. Thus it was demonstrated that by controlling the hydrolysis time of sunflower 11S globulin, hydrolysate with a desirable functional properties can be obtained.

Keywords: sunflower protein hydrolysates; structure; functionality

Vegetable proteins are an economic and good alternative for animal proteins as a functional ingredient in food formulations. Oilseeds are the most important source of vegetable protein ingredients. Till now, soy protein has been the main oilseed protein used as a functional ingredient in foods such as bakery products, milk substitutes and meat products. However, sunflower proteins might be a good alternative. Sunflower is one of the four most important oil crops globally. Its seeds contain about 20% of protein, some of the defatted sunflower meals such as oil press cakes and extraction residues range from 30% to 50% (Pickardt *et al.* 2009). Proteins from sunflower press cake are low in or devoid of antinutritional factors (ANFs), e.g. protease inhibitors, cyanogens, glucosinolates, hence they are considered to be a valuable alternative as food ingredients (Gonzalez-Perez & Vereijken 2007). The sunflower proteins consist of four fractions (1.7, 7.8, 11.9, and 18.1 S) designated by sedimentation coefficients (Schwenke *et al.* 1979), of which sunflower 11S globulin is the major protein fraction, ranging from 40% to 90% (Gonzalez-Perez & Vereijken 2007). The properties of sunflower 11S globulin are important.

Enzymatic proteolysis is a well-known and valuable method to enhance the functional properties of the original protein. Protein hydrolysates with better solubility have a wide range of applications such as food ingredients, hypoallergenic infant formulas, dietetic food, sport drinks, and bioactive peptides (Clemente 2000; Hartmann & Meisel 2007). The functional property of a hydrolysate is related to the molecular composition and properties of peptides generated during hydrolysis. Understanding of the relationship between the physico-chemical, structural characteristics and functional properties of protein hydrolysates confers a benefit on improvements in the quality and stability of hydrolysate as a food ingredient

Supported by the National Natural Science Foundation of China, Grant No. 31271982, and of Heilongjiang Province Natural Science Foundation China, Grant No. B201211.

doi: 10.17221/154/2015-CJFS

(Frokjaer 1994; Mahmoud 1994). Moreover, many potential applications of hydrolysates require well defined and reproducible peptides. It is reported that hydrolytic treatment of the sunflower protein isolates to produce protein hydrolysates is a useful method to improve its functional and nutritional properties (Conde *et al.* 2005a,b; Conde & Patino 2007; Patino *et al.* 2007). However, to our knowledge, there are no reports concerning the precise characterization of peptides produced in the course of enzymatic hydrolysis of sunflower 11S globulin proteins in order to obtain required functional properties, and how this modification results in different structural and functional properties of sunflower 11S globulin has not been investigated so far.

In the present study, sunflower 11S globulin extracted from the defatted sunflower flour was hydrolysed by Alcalase at different hydrolysis time. Modifications of the structural and functional properties of the hydrolysates were investigated, including circular dichroism, surface hydrophobicity, protein solubility, water and oil binding capacities, foaming and emulsifying properties by characterisation. The present study aimed to reveal the impact of hydrolysis time on the structure and some functional properties of the sunflower 11S globulin.

MATERIAL AND METHODS

Material and chemicals. Sunflower (variety of high linoleic acid, grown in Heilongjiang Province, China) was obtained from Qiqihar local market. Alcalase (2.94×10^2 U/mg) was obtained from Novozymes (Bagsvaerd, Denmark). All chemicals used in the experiments were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, USA).

Preparation and physicochemical characterisation of sunflower 11S globulin. Sunflower 11S globulin was extracted from defatted sunflower flour according to the method described by (Sastry & Rao 1990). The protein extract was dialysed in 0.025 mol/l Tris-glycine buffer (pH 8.3), lyophilised and ground to powder. Crude protein content of the sample was determined by the Kjeldahl method and the value was calculated by subtracting the mineral nitrogen from the total nitrogen and multiplying the result by 5.5, and protein content was 92.0%.

Preparation of sunflower 11S globulin hydrolysates. Sunflower 11S globulin solution [7% (w/v)] was incubated with Alcalase at an enzyme to substrate (E : S) ratio of 2% (w/w, weight of enzyme powder : weight of sunflower 11S globulin) at 60°C and pH 8.5. The hydrolysates were produced by varying the hydrolysis time to 30, 60, 90, and 120 minutes.

After hydrolysis, the solution was heated at 90°C for 5 min to inactivate the enzyme, centrifuged at 4000 rpm for 15 minutes. The supernatant was collected and pH was adjusted to 7.0, and lyophilised. The degree of hydrolysis (DH) was defined using the equation described by Adler-Nissen (1986), and the total number of peptide bonds per weight unit (h_{tot}) was evaluated according to the equations described by Adler-Nissen (1982), where h_{tot} = total number of peptide bonds in a protein (mmol/g of protein), and h_{tot} was calculated from the amino acid composition of the protein and was 7.5 mmol/g for sunflower protein.

Far-ultraviolet circular dichroism analysis. Far-ultraviolet circular dichroism (CD) spectra (190 to 240 nm) of the samples were obtained from a Jasco J-815 CD spectrometer (Jasco Corp., Tokyo, Japan) by scanning the solutions of 0.15 mg/ml (in 10 mmol/l phosphate buffer of pH 7.0) in a 0.1 cm path length cuvette. The data were expressed as mean residue ellipticity deg cm^2/dmol.

Evaluation of functional properties. Surface hydrophobicity of sunflower 11S globulin and its hydrolysates was measured according to the method of (Song & Zhao 2014) using 1-anilino-8-naphthalene sulphonate (ANS) as a probe. The sample solution was diluted to obtain various concentrations (0.025–0.4 g/l) by the phosphate buffer, and was thoroughly mixed with 25 µl 8 mmol/l ANS (10 mM phosphate buffer, pH 7.0). The fluorescence intensity was measured at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of the relative fluorescence intensity versus the protein concentration plot was regressed and used as an index of surface hydrophobicity.

Foaming properties of samples were determined by the high speed agitation method described by Motoi *et al.* (2004). Foaming properties were evaluated by the relative overrun of protein (0.1% in 50 mmol/l phosphate buffer, pH 7.0) at a speed of 12 000 rpm for 1 min, and the total volume of foam in the measuring cylinder was measured immediately, and the relative overrun was computed according to Motoi *et al.* (2004). Foam stability was determined by comparing the foam volume after 30 min with the initial foam volume (0 min) and their ratio was used as the indicator of foam stability.

Emulsifying activity index (EAI) and emulsion stability index (ESI) of the protein samples were evaluated

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by turbidimetric methods as described by Pearce and Kinsella (1978). Oil binding capacities of sunflower 11S globulin and its hydrolysates was determined by the method described by Saetae *et al.* (2011).

Statistical analysis. All experiments or analyses were carried out three times. All reported data were expressed as means or means ± standard deviations. Differences between the means of multiple groups were analysed by one-way analysis of variance (ANOVA) with Duncan's multiple range tests. SPSS v. 13.0 software (SPSS Inc., Chicago, USA) and MS Excel 2003 software (Microsoft Corporation, Redmond, USA) were used to analyse and report the results.

RESULTS AND DISCUSSION

Structural characteristics of sunflower 11S globulin hydrolysates. By applying the above-mentioned preparation methods, four types of sunflower 11S globulin hydrolysates generated by Alcalase were obtained. DH of the hydrolysates was 15.2, 20.2, 22.3, and 23.2%, respectively. CD analysis was used to reveal the structural modification of sunflower 11S globulin hydrolysates, and the result is shown in Figure 1. The characteristic features of the sunflower 11S globulin are a minimum at about 210 nm and a zero-crossing around 200 nm, typical absorbance of the b-structure and random coil (Johnson 1990). This result showed that the dominant secondary structure of sunflower 11S globulin is b-structure and random coil, and similar to an earlier reported study (Gonzalez-Perez & Vereijken 2007). However, the far-UV CD spectra of the hydrolysates are totally altered. The spectra showed only negative ellipticity, and the spectra have a minimum at about 200 nm, a typical region to reflect the random coil structure. In comparison with sunflower 11S globulin, its hydrolysates seem to consist of higher random coil structures. Using curve-fitting procedures, the secondary structure content was estimated, confirming the high content of random coil structures, which was increased from 42% to 62%. It is thus concluded that the sunflower 11S globulin hydrolysates have more random coiled structures than the native one.

Functional properties of sunflower 11S globulin hydrolysates. Surface hydrophobicity of sunflower 11S globulin and its hydrolysates was 16.2, 3.5, 3.3, 2.7, and 2.6, respectively (Table 1). This result showed that the hydrolysis of sunflower 11S globulin led to decreased surface hydrophobicity, i.e. hydrolytic treatment conferred the protein hydrophilic surface. This might be so because peptides released from a network structure of the sunflower protein reveal great flexibility, which helps them to adopt a conformation with hydrophilic groups more exposed outward. This kind of results was earlier observed for Alcalase-treated peanut protein isolate (Zhao *et al.* 2011) and soybean protein concentrate (Surowka *et al.* 2004).

Foaming properties of the sunflower 11S globulin and its hydrolysates are shown in Table 1. Relative overrun of the hydrolysates generated by Alcalase was increased in comparison with original sunflower 11S globulin. This phenomenon may be due to the fact that the increase of the hydrolysate solubility confers more protein available to adsorb at the interface (Patino *et al.* 2007). The relative overrun and foam stability of sunflower hydrolysates were also affected by hydrolysis time, and their maximum values were obtained at 30 minutes. The structure of the sunflower protein was excessively destroyed by longer hydrolysis, which adversely affected the relative overrun and

Table 1. Sunflower 11S globulin hydrolysates generated with Alcalase and characterised at different hydrolysis time (30, 60, 90, and 120 min)

Fucntional properties	Sunflower 11S globulin	Hydrolysis time (min)			
		30	60	90	120
Surface hydrophobicity	1623 ± 41^{c}	350 ± 24^{b}	328 ± 23.4^{b}	265 ± 30^{a}	255 ± 21^{a}
Oil binding capacity (ml/g protein)	2.3 ± 0.1^{a}	2.4 ± 0.1^{a}	2.6 ± 0.1^{b}	2.8 ± 0.1^{c}	2.4 ± 0.1^{a}
Emulsifying activity index (m^2/g protein)	32.5 ± 2.2^{c}	42.3 ± 1.7^{d}	39.7 ± 3.1^{d}	27.6 ± 0.6^{b}	24.4 ± 1.6^{a}
Emulsion stability index (%)	23.0 ± 2.1^{c}	22.8 ± 1.8^{c}	17.5 ± 2.4^{b}	18.5 ± 0.8^{b}	14.1 ± 1.4^{a}
Foaming capacity (%)	133 ± 5.6^{a}	181 ± 6.7^{d}	175 ± 9.3^{c}	155 ± 3.2^{b}	157 ± 6.4^{b}
Foaming stability (%)	54.8 ± 5.5^{c}	65.9 ± 4.6^{d}	48.9 ± 3.6^{b}	44.8 ± 3.3^{a}	43.8 ± 4.8^{a}

Different lowercase letters as the superscripts after the values in same row indicate that one-way ANOVA of the means is significantly different ($P < 0.05$)

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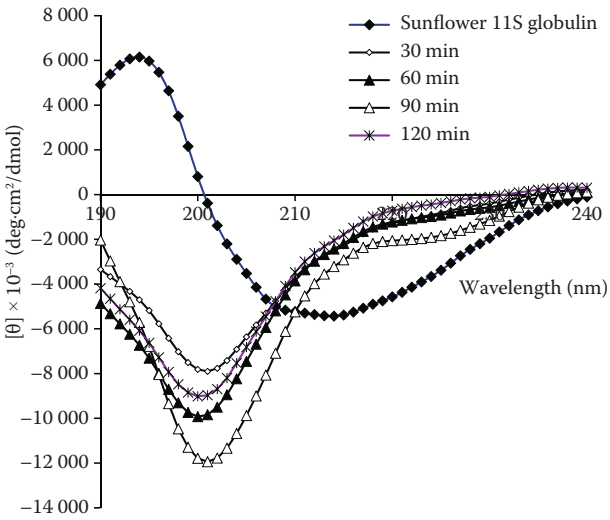

Figure 1. Circular dichroism (CD) spectra of sunflower 11S globulin and its hydrolysates generated by Alcalase at 30, 60, 90, and 120 minutes. The samples were dispersed in 0.01 mol/l phosphate buffer at pH 7.0. The CD spectra were measured at 25°C

foam stability. Thus, prolonged hydrolysis should be avoided as it leads to undesirable properties.

EAI and ESI of sunflower 11S globulin and its hydrolysates are shown in Table 1. When proteins were hydrolysed for 30 min to 60 min, hydrolysates exhibited strong emulsifying properties. Nevertheless, EAI and ESI decreased with increasing hydrolysis time from 90 min to 120 min, i.e. excessive hydrolysis brings about the loss of emulsifying properties (Kristinsson & Rasco 2000; Gbogouri *et al.* 2004). The peptides with low molecular weight may not be amphiphilic enough to exhibit good emulsifying properties (Chobert *et al.* 1988). Hydrolysates with a longer hydrolysis time had poorer EAI and ESI due to their small peptide size (Figure 1). Small peptides migrate rapidly and adsorb at the interface, but show less efficiency in decreasing the interface tension since they cannot unfold and reorient at the interface like large peptides to stabilise emulsions (Kristinsson & Rasco 2000; Gbogouri *et al.* 2004). The flexibility of protein or peptide structure may also be a vital factor governing the emulsifying properties (Kato *et al.* 1985). Both the flexibility of the hydrolysate and the large peptide size conferred the hydrolysate generated at 30 min and 60 min that showed better emulsifying properties than the original sunflower 11S globulin.

Oil binding capacity of sunflower 11S globulin and its hydrolysates is shown in Table 1. The obtained hydrolysates exhibited better oil binding capacity than the original 11S globulin protein, and the hydrolysate generated by Alcalase at 90 min showed the highest oil binding capacity (2.8 vs. 2.3 ml/g protein). Rapeseed globulin substrate hydrolysates exhibited higher oil binding capacity than their original globulins and indicated that the improvement of the fat absorption of proteins may be due to exposed hydrophobic groups initially buried in the core of the protein (Chabanon *et al.* 2007). The hydrolysis decreased the surface hydrophobicity of sunflower 11S globulin and conferred the better oil binding capacity of its hydrolysates.

CONCLUSION

Alcalase could be used to modify sunflower 11S globulin by hydrolysis to generate a new protein hydrolysate with the modified structural and functional properties. The hydrolysis influenced the properties of sunflower 11S globulin significantly. The hydrolysis decreased its molecular weight and surface hydrophobicity. Hydrolysates with shorter hydrolysis time showed relatively higher foaming and emulsifying properties and oil binding capacity compared to the original 11S globulin. The modified protein showed its potential as an ingredient for the food industry.

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doi: 10.17221/154/2015-CJFS

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Received: 2015–03–29
Accepted after corrections: 2015–08–14

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