Selective thermal denaturation of *Pisum sativum L.* proteins using salt

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Introduction

Globular proteins from various sources play important roles in many foodstuffs; their ability to develop very interesting technological functionalities is of great interest. Protein isolates from pea (*Pisum sativum L.*) are characterized by a high content of two mains globulins: legumin and vicilin. Both globulins exhibit different characteristics so that their ratio value as an impact on isolates properties. In terms of amino acids composition, legumin contains more sulfur-containing amino acids and arginine, whereas vicilin is higher in isoleucine, leucine, phenylalanine and lysine. So increasing the legumin/vicilin ratio should improve the nutritional quality of pea protein isolates. On the other hand, vicilin content is the main factor influencing functional properties such as emulsifying properties, foaming properties and heat induced gelation.

Full exploitation of pea protein isolates potentialities and development of strategies for rational modifications of those properties make it necessary to understand the relations between processing conditions and functional properties. Thermal treatment is one of the most common parts of industrial processes and heating has a strong incidence on functional properties of protein isolates as described for various sources such as whey, egg or vegetal as rice and this incidence is closely linked to physicochemical conditions of proteins environment as pH and ionic strength. Observed changes are linked to structural changes at the molecular level in studied proteins. Such treatment will have a significant impact on functional properties and nutritional qualities of pea protein isolates.

Methodology

Preparation of protein extract

The protein dispersion used in this investigation was prepared from Pisum sativum L. seeds (c.v. Baccara). Fine flour was milled from complete pea seeds (M-20, IKA). Salt-soluble proteins were then extracted into a 100 mM phosphate buffer containing potassium sulfate, pH 7.2 with a flour-to-buffer ratio of 1:10. Protein extract was then dialyzed against distilled water and freeze-dried.

Preparative-scale protein separation

Legumin and vicilin were separated using a gel filtration chromatography followed by an affinity chromatography, both using FPLC system (AMERSHAM BIOSCIENCES).

Analytical-scale protein separation

Legumin and vicilin were separated by gel filtration chromatography using 3 combined columns: Hydroguard, Ultrahydrogel 1000 and Ultrahydrogel 250 (WATERS) characterized by decreasing molecular weight ranges. Identification of each peak was obtained by injecting dispersions of purified globulins previously prepared.

Legumin/vicilin ratio estimation

Response factors of legumin and vicilin in chromatographic UV detector (λ=280 nm) were calculated by injecting increasing known amount of available purified globulins. Chromatographic peak areas and response factors were then used to estimate the legumin/vicilin ratio.

Thermal treatment using thin tubes

Heating of total protein extracts dispersions was carried out by batch treatment in stainless-steel coiled tube (5 mm internal diameter, 50 ml capacity). Tube filling was performed under a positive pressure at piston flow to prevent air bubble development. Heating was performed by immersing the entire coil in a oil bath.

Results

1. Legumin/vicilin ratio estimation

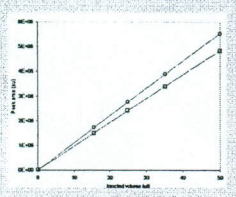

Figure 1. Peak area of increasing injected amount of globulins.
O legumin, □ vicilin.

Accurate evaluation of legumin/vicilin ratio first needed the determination of response factors characterizing both globulins. Injections of increasing amounts of purified globulins (Figure 1) allowed the calculation of the ratio between legumin and vicilin response factors (l=280 nm). The calculated absorbance ratio was 1.6.

Samples characterized by known ratio obtained from purified globulins were then injected to check the efficiency of the chromatographic method. Figure 2 shows the existing deviation between injected and calculated ratios. Equation defining the relation between injected and measured ratios and corresponding correlation coefficient are showed. On the basis of that equation, estimated ratio should be corrected by a multiplicative factor equal to 1.2. So both UV-absorbance ratio (1.6) and correction factor (1.2) should be used for legumin/vicilin ratio estimation.

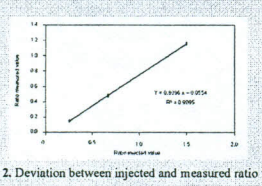

Figure 2. Deviation between injected and measured ratio values.

2. Calorimetric measurements

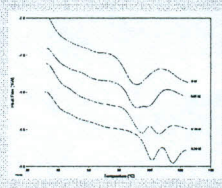

Figure.3. Influence of K2SO4 at pH 7.2 on thermal denaturation of pea protein dispersions. (DSC 2110 (TA Intruments) over a temperature range of 25-125°C at a heating rate of 10°C min-1)

Figure 3 shows DSC thermal curves of total proteins extract dispersed in increasing ionic strength phosphate buffer (0 - 0.5M K2SO4). Proteins seem to be thermally stabilized by salt addition. K2SO4 induces a global increase of both *T*0 and *T*max values. Thermal curves are characterized by two endotherms and the separation between endotherms increases with increasing salt concentration. This indicates a higher stabilizing effect of the salt on the high temperature endotherm.

Figure 4 a and b shows DSC thermal curves of purified pea globulins in the presence of 0.5M of K2SO4. Those curves allowed the determination of *T*0 and *T*max for legumin (107.6, 114.0) and vicilin (100.7, 104.9) and the identification of both DSC endotherms previously described.

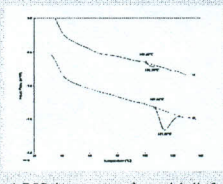

Figure 4. DSC-thermograms of pure globulin fractions.
(a) vicilin, (b) legumin.

3. Thermal treatment using thin tubes

Table 1. Composition of soluble and insoluble fractions as determined by reduced SDS-PAGE.

Temperature	Insoluble fraction			Soluble fraction		
(°C)	Albumins	vicilin	legumin	Albumins	vicilin	legumin
80	+	+	-	+	++	++
110	+	++	++	+	+	++
125	+	++	++	+	+	++

The aim of this experiment was to evaluate the ability of a controlled thermal denaturation to induce the formation of specifically enriched fractions. On the basis of *T*0 and *T*max values, three temperatures were selected for thermal treatment: 80, 110 and 125°C. Dispersions of total protein extract in phosphate buffer (pH 7.2, 0.5M K_2SO_4) were processed at selected temperature. First observation was the formation within the treated dispersion of an insoluble deposit that could be recovered by centrifugation. Proteic profile of soluble and insoluble fractions was determined by reduced SDS-PAGE (Table 1) and protein content was measured and legumin/vicilin ratio was estimated by the chromatographic method in the soluble fraction (Table 2).

A limited thermal treatment (80°C, 5 min) leads to an insoluble fraction free from legumin and a soluble fraction containing both globulins with very close concentrations (estimated ratio close to 2). Higher tested temperatures induce partial precipitation of legumin and nearly total precipitation of vicilin so that the soluble fraction is characterized by high legumin concentration (ratio above 10). Estimated legumin/vicilin ratio for pea c.v. Baccara is 0.94.

Table 2. Protein content (percentage of thermally treated proteins) of soluble and insoluble fractions and legumin/vicilin ratio of soluble fractions.

Temperature	Insoluble fraction	Soluble fraction	
(°C)	Proteins (%)	Proteins (%)	Ratio
80	14	85	2
110	31	69	12
125	45	54	17

Conclusions

Thermal denaturation of the two major storage proteins of field pea varied with concentration of the salt environment. The increase in stability was different for legumin and vicilin and can lead to a nearly complete analytical separation of both globulins. Limited scaling-up consisted in processing of pea proteins dispersions within thin tubes. Thermal treatment at 80°C for 5 min produced limited amount of water insoluble deposit of vicilin free of legumin. Use of higher temperatures (110 and 125°C) eliminated most of the vicilin by thermal precipitation and so provided a soluble fraction characterized by a high legumin/vicilin ratio (respectively 12 and 17).

Transposition of such adapted thermal treatments to an industrial scale could be considered as a way to produce selectively enriched pea protein isolates. Such isolates could show particular functional properties or increased nutritional value as a function of most represented globulins family and denaturation level. Indeed selective thermal denaturation of the most sensitive globulins – vicilins previously described as functionality promoting – could result in interesting functional properties development while the legumins native state should preserve their nutritional value.