Thermal and shear processing of pea protein concentrate: Effects on secondary structure and protein hydrolysis

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Introduction

Processing of ingredients for feed manufacturing involves the use of heat and shear. Heat can induce changes in the proteins with positive (e.g. denaturation and random coil formation) or negative (e.g. protein aggregation and Maillard reactions) effects on protein digestibility (Gerrard et al., 2012). Shear is the dissipation of mechanical energy through friction of particles. Effects of shear on protein nutritional value can be similar to those of heat (Lei et al., 2007).
The disruption of the secondary or tertiary structure of the proteins due to heat or shear can facilitate the access of the enzymes for protein hydrolysis. Nevertheless, only limited effects of increasing screw speeds during extrusion of soybean meal were reported (Marsman et al., 1993).
The aim of the present study was to test the effects of thermal and shear processing on the secondary structure and hydrolysis of the proteins in a pea protein concentrate (PPC).

Materials and methods

The experiment consisted of 3 treatments: native PPC, heat-processed PPC (H) and heat and shear-processed PPC (HS). Shear processing was performed at 90 °C for 20 min in the shear cell (Laboratory of Food Processing Engineering, Wageningen University, The Netherlands) with (HS) or without (H) the input of mechanical energy. Before processing the PPC was mixed with water in a 1:3 (w/w) ratio. The shear cell is capable of simulating the conditions used during extrusion (Draganovic et al., 2014). This device consists of a stationary cone and a rotating plate, which have a grooved surface in order to avoid slippage. Temperature in the jacketed cone and plate can be controlled through an oil bath. Temperature and torque values during processing can be monitored online (Thermo drive unit, Thermo Scientific, Staffordshire, UK). Processing in the shear cell was performed in duplicate. Following processing, the samples were freeze-dried and ground through a 1 mm sieve (ZM200, Retsch, Haan, Germany).
Hydrolysis was performed using the pH-STAT method after the addition of trypsin. Briefly, 10 mL of a protein suspension in water containing 1 mg N/mL were equilibrated to pH 8 using a 0.1 M NaOH solution. After equilibration, 1 mL of a trypsin solution (1.6 mg trypsin/ml water, T8253, Sigma-Aldrich, St. Louis, MO, USA) was added. Hydrolysis was allowed to proceed for 60 min at 39 °C. Degree of hydrolysis (DH) and fractional rate of hydrolysis (k) were calculated according to the equations described by Butré et al. (2012). The PROC MODEL procedure from SAS (SAS Institute, 2011) was used to model k.
Samples were re-ground with a ball mill (MM2000, Retsch) for 3 min for secondary structure measurement. Amide I region (1600 – 1700 cm^{-1}) was measured using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Tensor 27, Bruker, MA, USA). The spectra was deconvoluted according to the procedure described by Hu et al. (2006) and analysed using the OPUS software Version 7.2 (Bruker). Identification of the resulting peaks was performed according to literature (Carbonaro et al., 2012).
Statistical analysis was performed using the PROC GLM procedure from SAS with treatment as fixed factor. *Post-hoc* testing was performed using the Bonferroni adjustment.

Results and discussion

Heat and heat-shear processing increased the formation of intermolecular □-sheet hydrogen-bonded aggregates (A2) and random coils (Fig. 1a). The proportion of random coils was higher in the H compared to HS processed PPC. In contrast, formation of A2 was higher in the HS processed compared to H processing only. Whilst random coil formation facilitates the access of enzymes for cleaving of peptide bonds, the formation of aggregates can reduce it. Although the formation of A2 and random coils originated from the more stable conformations in the secondary structure (e.g. □-sheets, □-helices and T2-turns), there was no difference ($P>0.05$) in the proportion of these structures between the processing treatments.

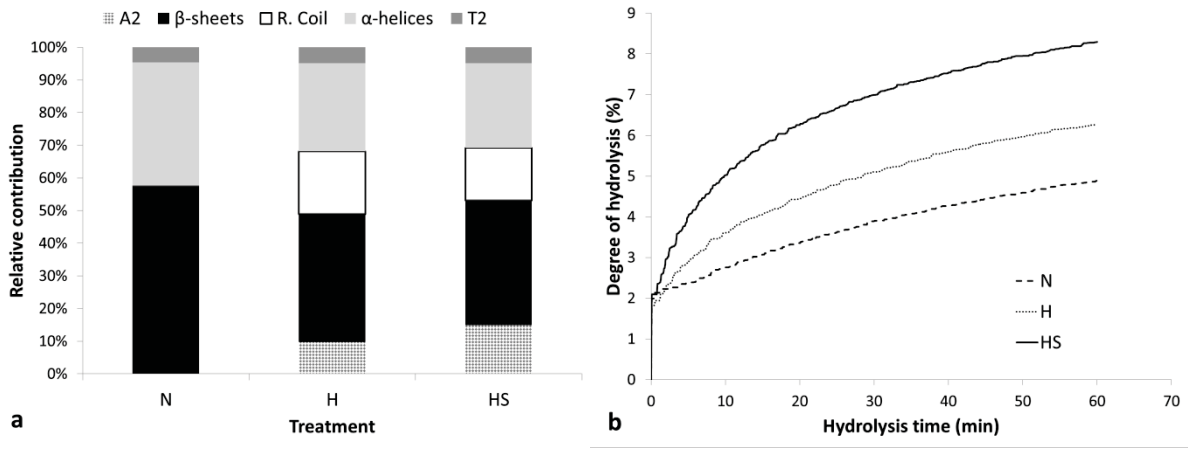

Figure 1. Proportion of secondary structure (a) and degree of hydrolysis (b) of native (N), heat (H), and heat and shear (HS) processed pea protein concentrate.

The DH was higher in the HS processed PPC compared to H processing, and higher in both of these treatments compared to the native PPC (Fig. 2b). The DH of H processed PPC was 28% higher than the native PPC, whilst that of HS processed PPC was 69% higher than native PPC. A higher DH probably indicates that the proteins are more digestible. In contrast, the *k* was higher ($P<0.05$) in the native PPC (0.100 s^{-1}) compared to either processing treatments (0.057 and 0.060 s^{-1} for H and HS treatments, respectively). The rate of hydrolysis in the processed PPC could be reduced due to the formation of aggregates (A2) in the secondary structure. However, changes in the secondary structure do not completely explain the observed changes in enzymatic hydrolysis. It could be possible that the tertiary structure of the proteins was more affected by HS processing than H processing only. This could facilitate the access of trypsin for enzymatic hydrolysis.
In conclusion, heat and heat-shear processing of PPC induces the formation of aggregates and random coils in the secondary structure of pea proteins and increases the degree of enzymatic hydrolysis.

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