Improving the emulsifying properties of canola meal protein isolate by enzymatic modification

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ABSTRACT

Solubility in aqueous solution is a major prerequisite for protein functionality; the poor solubility of canola protein isolate (CPI) has thus limited its use in food processing. In this study, food grade enzymes: alcalase, trypsin, pepsin and chymotrypsin, were employed to hydrolyse CPI in order to improve its application as a food ingredient. The methods used to measure the extent of hydrolysis were weakly correlated, but there was an increase in the number of peptide bonds cleaved with time. The free amino acid also increased with time of hydrolysis, but the extent of hydrolysis was limited to 5% with all the enzymes under the conditions employed in this study. This limited hydrolysis, however improved emulsion stability generally among the hydrolysates employed. The improved functionality indicates that canola protein hydrolysate has potential for use as a food ingredient.

Key words: canola protein – hydrolysates – degree of hydrolysis – functionality – emulsion stability

INTRODUCTION

Global canola production has grown rapidly over the past 40 years, rising from the sixth largest oil crop to the second largest. Significantly, canola oil, obtained from crushing canola seed, was the third most produced vegetable oil globally in 2008/09 and canola meal is the second largest feed meal after soybean meal (USDA, 2010). Although the oil is highly valued, oil extraction operations depend on revenues from the meal to maintain profitable operations (Howard, 1993).

Canola meal has a good amino acid profile with a well balanced amino acid composition although it has found only marginal use in the food industry, in part due to the presence of major antinutritional factors (Pastuszewska, et al. 2000; Sosulski,1979) and also because the vast majority of canola protein isolate is prepared by alkaline extraction followed by isoelectric precipitation (Tzeng, et al.1990).Although this extraction method generates high nitrogen yield, isolates prepared using this method have been shown to have very poor solubility and digestibility (Bos et al. 2007; Paulson and Tung, 1988). This is most probably due to the nature of the proteins that constitute the alkali-soluble fraction of canola meal as well as possible denaturation of these proteins during the extraction. Isolates thus possess generally unacceptable food-functional properties including poor emulsification, foaming, water-holding, gelling and oil-binding affinity. As a result, many studies have been devoted to modification of the isolates e.g., by succinylation (Paulson & Tung, 1989), acetylation, (Gruener et al. 1997) and enzymatic hydrolysis (Cumby, et al. 2008; Vioque, et al. 1999). Enzymatic modification is, however, preferred because it is milder than chemical modification. With the ongoing emphasis on the development of functional foods, including the possible generation of bioactive peptides, efforts to develop protein hydrolysates by enzymatic hydrolysis of protein isolates for both technological functionality and nutritional improvement have become popular (Panyam & Kilara 1996). Research have also suggested that canola protein hydrolysates obtained from defatted canola meal can be used for the fortification of foods because of the functional and nutritional properties they possess (Cumby et al. 2008, Vioque et al. 1999). This study therefore presents the effect of different food grade enzymes on the characterization and emulsifying functionality of canola protein hydrolysates.

MATERIALS AND METHODS

Materials

Industrial pre-toast meal was supplied by Cargill Oilseeds (Footscray, VIC Australia). Reagents, chemicals and enzymes (chymotrypsin, pepsin and trypsin) and chemicals were supplied by sigma Aldrich (Castle Hill, NSW, Australia) unless otherwise stated. Alcalase® 2.4 L FG was a gift from Novozyme (North Rocks, NSW, Australia) and canola oil was purchased from the local Woolworths store.

Extraction of CPI

Defatted canola meal (20g) was suspended in 200ml of 0.1M NaOH pH 12.0 and extracted by stirring for 1 hour and then centrifuged at 3,000*g* for 10 mins. Two additional extractions of the residue from the centrifugation process were carried out with the same volume of 0.1M NaOH. The pH of supernatant was adjusted to the isoelectric point (pH 4.0) and the precipitate formed was recovered by centrifugation as described above. The precipitate was washed with distilled water and freeze-dried for further analysis.

Enzymatic hydrolysis of CPI

CPI was hydrolysed in batches using four food grade enzymes (alcalase, chymotrypsin, pepsin and trypsin) at an enzyme substrate ratio of 1:20 singly and in combination, to obtain canola protein hydrolysates (CPH) at 1 and 24 hours. The following optimum conditions were used for each enzyme: alcalase: pH 8.0, 50ºC; pepsin: pH 3.0, 37ºC; chymotrypsin and trypsin: pH 8.0, 37ºC. The combined enzyme hydrolysis was carried out sequentially with the optimum conditions for each enzyme as mentioned above. The pH was maintained for each hydrolysis process accordingly using either 1M NaOH or 1M HCl. Hydrolysis was stopped by heating at 85ºC for 10 mins. The hydrolysates were lyophilized and stored at -20ºC for further analysis.

Characterisation of CPI hydrolysates

Degree of hydrolysis (DH), defined as the percentage of peptide bonds cleaved, was carried out using the o-pthaldialdehyde (OPA) method as described by Adler-Nissen (1979). Free amino acid was determined using the method described by Sharma, et al. (1990). Nitrogen soluble index (solubility) was determined by the method described by Lee et al. (1992) using the Leco CNS 2000 analyser for nitrogen analysis (Leco Corp., St Joseph, MI USA). Surface Hydrophobicity (H_o) was determined by hydrophobic fluorescence probes of 1-anilino-8-naphthalene sulfonate (ANS) using the method described by Church et.al. (1993).

Emulsion formulation and particle size determination

Oil in water emulsions were prepared according to the method described by Agboola, et al. (2005) using 1% (w/v) CPH and 20% (v/v) canola oil in 10mM Sodium Phosphate buffer (pH 7.0) containing 0.5%w/v sodium azide. Coarse pre emulsion were formed using an ultra turax® (T25 basic, IKA Larbortech, UK) homogeniser at speed 3 for 2 minutes before passing it through a high pressure homogeniser (EmulsiFlex™-C5, Ottawa, ON, Canada) at pressures between 100,000-150,000KPa. The emulsions were evenly distributed into centrifuge tubes and stored at 4 and 25 ºC for 7 days. The mean particles size (d_{43}) was measured with a Mastersizer E (Malvern Instruments, Malvern, UK) using Milli-Q water as dispersant, from day 0 to day 7 and the emulsions were observed physically for stability, creaming and oiling-off.

Statistical analysis

Each analysis was carried out at least in triplicates (except for the emulsions which were carried out in duplicates) and means (standard deviation) were reported. Post-hoc comparison of the data for significant difference (p<0.05) was carried out using analysis of variance on the Excel™ spread sheet.

RESULTS AND DISCUSSION

The protein isolate used for hydrolysis had a protein content of 76 % (Nx6.25) and the results for the characterisation of CPI are presented in Table 1. Degree of hydrolysis was limited to 5% for hydrolysis up to 24 hours; the rate of hydrolysis was highest within the first 30 minutes for all the enzymes used and the range between the one- and 24-hour hydrolysates was not more

than 1.5%. This shows that the enzyme activity was highest within the first hour and did not increase significantly after that. The methods used to measure the degree of hydrolysis were weakly correlated (0.45) with the FAA analysis which is another parameter that can be used to measure the extent of hydrolysis. The FAA, however, had a better representation of the peptide bond cleavage because the results obtained, when compared to the control (0.5mg/g), ranged between 4.7– 8.9 mg/g for the one- hour hydrolysates and 6.8 - 11.5 mg/g for the 24-hour hydrolysates.

Table 1. Characterization of canola protein hydrolysates.

Hydrolysates	Degree of hydrolysis (%)		Free amino acid (mg/g)		Nitrogen solubility index (%)		Surface hydrophobicity	
	1 hr	24 hrs	1 hr	24 hrs	1 hr	24 hrs	1 hr	24 hrs
Alcalase (AH)	4.10	5.01	6.58	9.47	12.3 4	11.96	86.00	40.67
Chymotrypsin (CH)	3.24	4.29	4.77	7.57	11.64	12.38	203.50	129.50
Pepsin (PH)	3.20	3.93	5.35	6.78	11.74	12.34	203.67	174.33
Trypsin (TH)	2.93	3.49	4.67	7.20	11.36	12.25	195.33	87.00
Alcalase & Chymotrypsin (ACH)	3.61	4.64	8.94	10.29	12.04	11.87	64.67	58.67
Alcalase & Pepsin (APH)	3.85	5.13	8.73	10.32	11.22	10.99	85.00	41.00
Alcalase & Trypsin (ATH)	3.94	4.91	7.58	11.47	11.63	11.51	82.33	50.67
Pepsin &Chymotrypsin (PCH)	2.61	3.58	8.27	9.66	11.72	12.21	126.33	75.00
Pepsin & Trypsin (PTH)	3.79	4.86	7.69	10.29	11.41	11.16	116.67	67.00
Trypsin & Chymotrypsin (TCH)	3.45	4.78	8.12	9.48	12.32	12.26	137.67	63.00
Control	1.90		0.47		13.51		425.50	

The solubility of all the hydrolysates was not different from the control irrespective of the time of hydrolysis and type of enzyme, indicating that both CPI and CPH were only slightly soluble at pH 7.The control also had a H_o value of 425.50 and all hydrolysates had H_o's that were much lower. Generally, H_o decreased with time of hydrolysis, and varied with enzyme type (Fig. 1). Chymotrypsin and pepsin had the highest values of 203.50 and 203.67 for the single enzyme hydrolysates respectively, while 126.33 was the highest value obtained for the hydrolysates of enzyme combinations chymotrypsin and pepsin. The gradual reduction in H_o with time of hydrolysis agrees with the FAA data and the results obtained for enzymatically modified rice endosperm proteins (Paraman et al. 2007). The peptide size of the resulting hydrolysates may also affect the affinity of the ANS probe towards the protein, lowereing H_o with time of hydrolysis (Damodaran, 1989). Philip & Beuchat (1981) postulated that breaking of peptide bonds as a result of proteolytic activity may cause a dual modification in the protein structure such that an increase in polar groups ($-NH_4^+$ and $-CO_2^-$) would increase the hydrophilicity of proteins, while an alteration in the molecular configuration would expose interior hydrophobic or lipophilic groups to an aqueous phase.It appears that the limited hydrolysis in a poorly soluble protein such as CPI did not lead to much exposure of interior hydrophobic groups of CPI. It is also probable that the CPI was already denatured by the extraction process such that hydrophobic moieties were already exposed before hydrolysis, a situation that would partly explain its inherent in-solubility as described in this study.

As shown in Fig. 2, the d_{43} average particle size measurement of the emulsions shows that after 1 hour of hydrolysis, tryptic hydrolysates formed smaller emulsion droplets than the control (CPI). The 1:1 combination of enzymes containing trypsin and chymotrypsin also generated

hydrolysates which had fairly more stable particle sizes over the 7 day storage period. While there was a clear difference between the d_{43} particle size of the emulsions formed using the control (CPI), and those obtained using hydrolysates from the single enzyme hydrolysis, there was no significant difference in particle size between the control emulsions and those formed using combined enzyme hydrolysates. Time of hydrolysis also had an effect on the d_{43} particle size of the emulsions from chymotrypsin, trypsin, 1:1 combinations of alcalase and pepsin, and pepsin and trypsin.

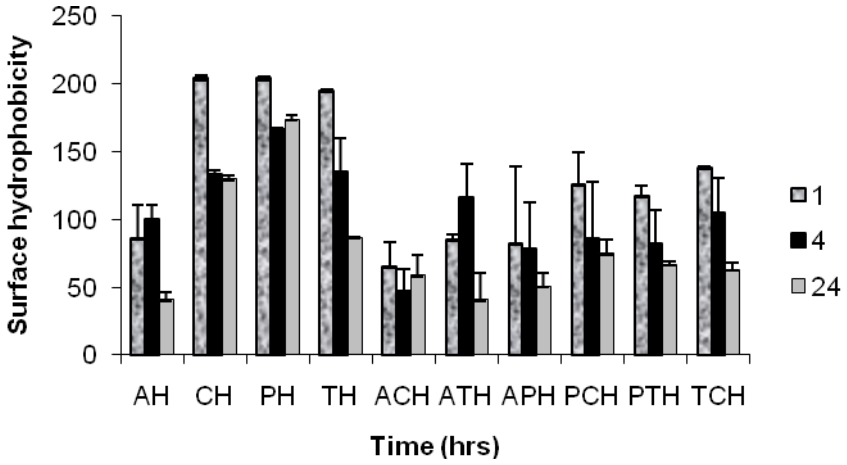

Fig.1. Surface hydrophobicity of CPH at 1, 4 and 24 hours.

In conjunction with the low average size, tryptic hydrolysates also formed emulsions with visually thicker consistency than others, a situation that normally leads to better emulsion stability (Agboola et al 2005) All the hydrolysates observed, however, formed stable emulsions with slight creaming separation after 7 days of storage at 4ºC and 25ºC, except for peptic hydrolysates which formed the thinnest emulsion consistency and showed a higher breakage level of clear serum separation than even CPI (Table 2). It would appear that the very limited hydrolysis in the majority of the hydrolysates were good enough to improve their emulsion formation and stabilisation functionality.

Table 2. Visual characterisation of emulsion stability at day seven.

	Creaming		Breakage		% Emulsion layer	
	1hr	24 hrs	1hr	24 hrs	1hr	24 hrs
Alcalase (AH)	*	*	-	-	65.0	78.0
Chymotrypsin (CH)	*	*	-	-	70.0	76.0
Pepsin (PH)	*	*	*	*	25.0	26.0
Trypsin (TH)	*	*	-	-	66.0	71.0
Alcalase & Chymotrypsin (ACH)	*	*	-	-	66.0	77.0
Alcalase & Pepsin (APH)	*	*	-	-	72.5	76.5
Alcalase & Trypsin (ATH)	*	*	-	-	65.5	76.0
Pepsin &Chymotrypsin (PCH)	*	*	-	-	70.0	71.0
Pepsin & Trypsin (PTH)	*	*	-	-	70.0	77.5
Trypsin & Chymotrypsin (TCH)	*	*	-	-	73.5	81.0
Control	*		*		68.50	

Key: *Yes; - No

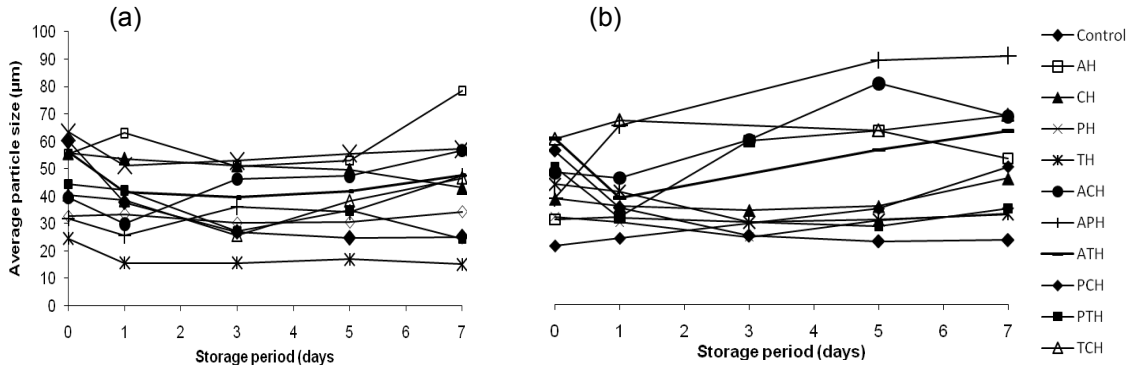

Fig. 2. $D_{(4, 3)}$ average particle size of 1% (w/v) emulsions (20%v/v oil) over 7 days at 25ºC for (a) 1 hour and (b) 24 hours hydrolysates. Legend abbreviations of figures are as in Table 1.

CONCLUSION

The type of enzyme did not significantly affect the characterisation of CPH when compared to CPI, especially by monitoring their degrees of hydrolysis as all enzymes and their combinations showed limited hydrolysis probably owing to the low solubility of CPI. However, low particle sized and more stable emulsions were formed by enzymatically modified CPI (especially those involving trypsin), except for those modified by pepsin only. More research is underway to determine other functional properties of CPH which could potentially improve the utilisation of canola meal proteins in food processing.

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