Subcritical water hydrolysis of pea protein concentrate and its mixture with citrus pectin

H.P.H. Vo, M.D.A. Saldaña*

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada
Corresponding author: marleny.saldana@ualberta.ca

1. Introduction: Pea, *Pisum sativum*, is one of the pulse crops that can provide a considerable amount of protein. Canada is the world largest pea producer with a production yield of 4.2 million tons in 2019, followed by Russia, China, and the USA. The major pea protein is globulin which accounts for 70-80%, and a minor portion of albumin that has 10-20% of total protein. Globulin is a salt-soluble globular protein, which is made up of legumin (11S, ~360 kDa, hexamer), vicilin (7S, ~180 kDa, trimer), and convicilin (7S, ~290±40 kDa, tetramer)[1].

Several studies have demonstrated the health promoting and antioxidative effects of pea peptides obtained from enzymatic hydrolysis of pea protein concentrate[2]. Enzymatic digestion is a well-known approach to hydrolyze protein; however, it has several disadvantages: high-cost enzymes, time consuming, complex protocol, and strictly controlled parameters. A green alternative technology is the use of subcritical water to hydrolyze pea protein concentrate, as well as investigate the behavior of pea protein under high temperature and pressure conditions. Concurrently, to enhance the protein degree of hydrolysis, pectin was used as a catalyst for the hydrolysis reaction

2. Materials and Methods: Pea protein concentrate (51% protein) was mixed with citrus pectin at a mass ratio of 1:1 (w/w). The mixture was then dissolved in 0.2M phosphate buffer at pH 8. After that, the suspension was stirred overnight under constant speed at 4°C to hydrate the protein. The hydrolysis was conducted using a Parr 4590 system at different temperatures and times under constant pressure of 50 bar. The hydrolysates were characterized by the degree of hydrolysis (OPA assay), amino acid profile (HPLC, Supercosil LC-18 column, and fluorescence detector) and peptide size distribution (HPLC, Superdex 75 Increase 10/300 GL column, and UV detector).

3. Results and discussion

3.1. *Degree of hydrolysis (DH)*: The degree of hydrolysis indicates the amount of free amino groups in the hydrolysates. As shown in **Fig. 1A**, with increasing time, the DH increased and reached the maximum value of 50.5% at 40 min. Afterwards, the DH remained unchanged even with longer times of 50 and 60 min. This phenomenon could be attributed to the dissociation constant (K_w) of water, which depends on temperature and pressure of the media[3]. Hence, the DH could not further increase after the proteins were hydrolyzed by the available ionic species in the sCW. From 10 to 40 min, the concentration of H_3O^+ and OH^- ions stay unchanged under constant pressure and temperature condition, and the DH was significantly enhanced due to better interactions between H_3O^+ or OH^- ions and protein. **Fig. 1B** shows the effect of temperature on the DH. From 160°C to 200°C, the DH increased slightly. However, the DH was significantly improved and peaked at 220°C with the highest value of 64.8% (**Fig. 1B**). The K_w value of water at 220°C is approximately 700 times higher than the one at ambient conditions. Hence, the proteins underwent a more severe hydrolysis due to higher concentration of H_3O^+ and OH^- ions which could potentially attack the peptide bonds; eventually increasing the amount of free amino groups in the hydrolysates. The samples with protein alone also showed the highest DH value at 40 min and 220°C, however, compared to the hydrolysates of protein + pectin, they had significantly lower DH. Pectin, a polymer of D-galacturonic acids (GalA), can be hydrolyzed into its monomer due to the susceptibility

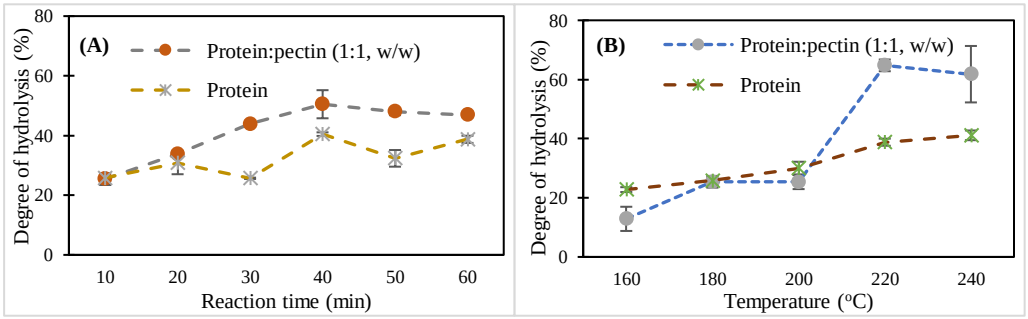

Figure 1. Effect of reaction times (A) and temperatures (B) on the degree of hydrolysis of pea protein concentrate and its mixture with pectin

of glycosidic bonds in sCW conditions. The released GalA could then act as a catalyst for the hydrolysis reaction of protein.

3.2. Amino acid profile: **Table 1** shows the amino acid profile of pea protein hydrolysates. The hydrophobic amino acids had increasing trends at higher sCW temperatures up to 220ºC. The results suggested that the hydrophobic characteristic of the hydrolysates was enhanced by the high temperatures of the sCW, resulting in the exposure of hydrophobic amino acids from the interior of proteins to the surface. Dielectric constant of sCW decreases at increasing temperature which favors the dissolution of non-polar molecules. Hydrophobic amino acids normally possess non-polar side chains, which solubility is considerably enhanced at high temperatures of sCW[4]. There was a slight increase in hydrophobic amino acids content from 35.93 to 37.63g/100g protein at 10 and 60 min of reaction time, respectively. Also, the contents of hydrophilic amino acids were the highest compared to hydrophobic and uncharged polar ones. Hydrophilic amino acids increased with increasing temperatures and reached the maximum value of 50.39g/100g protein at 240ºC. The reaction times did not considerably affect the changes in amino acids profile of pea protein concentrate.

Table 1. Amino acid composition of pea protein + pectin hydrolysates.

Amino acid (g/100g protein)	Protein:pectin 1:1 (w/w), 10 min				Protein:pectin 1:1 (w/w), 180ºC		
	160ºC	180ºC	220ºC	240ºC	10min	40min	60min
Uncharged polar	20.48	15.77	15.31	14.63	15.77	15.43	15.9
Hydrophobic	33.08	35.93	42.08	34.99	35.93	37.41	37.63
Hydrophilic	46.45	48.29	42.59	50.39	48.29	47.17	46.46

3.3. Peptide size distribution: Size exclusion chromatography was used to monitor the changes in molar mass of peptides. **Fig. 2A** shows the effect of reaction times. With longer time, the intensity of 4.3 kDa peak considerably increased. Also, the peak area of 4.3 kDa at 20 min and 40 min were 1.8 and 5.5 times higher than the one at 10 min, respectively. This observation agreed with the degree of hydrolysis (**Fig. 1A**) where the highest DH was obtained at 40 min, which is likely due to the high content of peptides in the hydrolysate. Compared to the reaction time, temperature had more pronounced effect in reducing the peptides molecular mass (**Fig. 2B**). With increasing temperature, better hydrolysis and smaller peptides were therefore produced. The peptides found at 180ºC had molecular mass between 4.5-4.9 kDa with relatively low intensity of 53-55 mAu. However, at a high temperature of 220ºC, the peaks eluted at 30 and 31 min disappeared, instead, there was a prominent peak detected at 32 min with a considerably higher intensity of 153 mAu. The molar mass of the identified peptides was 4.1 kDa. In **Fig. 2B**, the area of 4.1 kDa peptides at 220ºC were 6.5 times higher than the one at 180ºC, suggesting that the proteolytic activity of the media was enhanced at higher temperature. This result also explained the increment of the degree of hydrolysis at 220ºC in **Fig. 1B**.

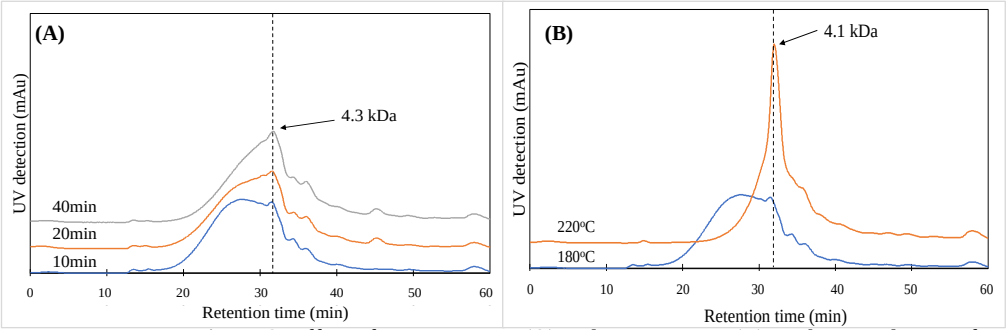

Figure 2. Effect of reaction times (A) and temperatures (B) on the peptide size of pea protein concentrate + pectin hydrolysates.

4. Conclusions: For the first time, sCW was employed to hydrolyze pea protein concentrate. The effect of temperature on the DH was more pronounced than the reaction time. The highest DH value obtained was 64.76% at 220ºC/10min. The hydrophilic amino acids were the dominant type in the hydrolysates. The most distinguished peptide had molar weight (MW) of 4.1-4.3 kDa. sCW showed to be a promising technology to effectively produce small MW peptides and amino acids from the mixture of pea protein concentrate and pectin in a short time using non-hazardous chemicals.

References: [1] Boukid, F., Rosell, C. M., & Castellari, M. (2021) *Trends Food Sci Technol. 110*, 729-742. [2] López-Barrios, L., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2014) *J. Food Sci. 79*(3), 273-283. [3] Martinez-Monteagudo, S. I., & Saldaña, M. D. A (2014). *Food Eng. Rev. 6*(4), 105-127. [4] Ziero, H. D. D., Ampese, L. C., Sganzerla, W. G., Torres-Mayanga, P. C., Timko, M. T., Mussatto, S. I., & Forster-Carneiro, T. (2022) *J. Supercrit. Fluids. 181*, 105492.