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## **Evaluation the Degree of Whey Protein Concentrate 80 Hydrolysis on the Health Benefits Amino Acids Content Need of the Human Body**

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**Abstract:** Whey protein concentrate (WPC 80) was modified by partial hydrolysis with Protamex to 5, 10, 15 and 20% degree of hydrolysis (DH). Whey protein concentrate 80 and its hydrolysates were analyzed, compared to FAO/WHO/UNU amino acid requirement pattern based on amino acid requirements of preschool-age child. The enzymatic hydrolysis of WPC 80 leads to numerous alterations in protein functional characteristics, like changes in solubility. The pH-protein solubility profiles of WPC 80 and its hydrolysates were used to determine protein solubility. A modified method of AOAC was used to determine Cysteine, Branched-Chain Amino Acids (BCAA) and Tryptophan at various degree of hydrolysis. Results showed that modified whey protein concentrate by partial hydrolysis degree at 15% were highly solubility and the global amino acids determined in an optimal content that makes them appropriate for food formulations as a source of health benefits amino acids.

**Key words:** Whey protein concentrate, hydrolysis, amino acid, solubility

### **INTRODUCTION**

Whey protein concentrate standardized to 80% protein content (WPC 80) is the ideal protein used in a wide range of food applications has a health benefits (Farthing, 2001; Low *et al.*, 2003; Kent *et al.*, 2003; Hazen, 2003). Kawase *et al.* (2000) found that supplement fermented milk with an added whey protein concentrate would affect serum lipids and blood pressure.

Today, whey protein is often described as a nutritionally perfect protein in the sense that it contains all the essential and non-essential amino acids required by the human body. Wheys amino acid profile is closely related to the optimal physiological needs of the human body, including an abundance of sulfur-containing amino acids, all in a highly bioavailable form also features the highest percentages of Branched-Chain Amino Acids (BCAA) (Walzem and Dilland, 2002; Pacheco and Sgarbieri, 2005).

Sometimes whey protein concentrate is modified by partial hydrolysis with enzymatic treatment (Kleber *et al.*, 2006; Bernasconi *et al.*, 2006) that transfer part of the protein into peptides to improve some properties with enrich of product supplemented (Konrad *et al.*, 2005). Partial hydrolysis can be done to the whey proteins product e.g., cheese using different protease and obtained product containing bioactive peptides that are free of any bitterness (Mann, 2000; Nelson *et al.*, 2002).

In recent years, a lot of scientific interest has been focused on physiologically active peptides deriving from milk upon hydrolysis. Partial hydrolysis of whey proteins can either change or evidence the functional properties of the peptides in this industrial residue,

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thereby increasing their applications. The hydrolysates obtained by treatment Whey Protein Concentrate (WPC) with pancreatin and protamex are good sources of peptides with activity to stimulate glutathione synthesis (Pacheco and Sagarbieri, 2005). The degree of hydrolysis is important to point out that competition for the active site between the original substrate and the peptides.

This process can be performed to different degrees; that is, vastly different size peptides can be produced having different functional characteristics. Furthermore, hydrolysis can yield a variety of new peptides that may offer many physiological benefits for humans (Otte *et al.*, 1997).

The aim of this investigate was to evaluate the meaningful health benefits of this modified whey protein at different degree of WPC 80 hydrolysis (DH) through the amino acid pattern ( $\text{mg g}^{-1}$  protein) and identify the ideal degree of hydrolyzation have unique a health benefits amino acids content.

## MATERIALS AND METHODS

Whey protein concentrate (WPC 80, 80% protein based on dry weight) was obtained from Davisco Foods International, Inc., U.S. Protamex, a commercial *Bacillus* proteinase complex, was obtained from Novo Nordisk's Enzyme Business (Wuxi, China). It is an endopeptidase with a broad specificity to hydrophobic amino acids.

Prior to enzymatic treatment the aqueous solution of WPC 80 was allowed to hydrate for 1 h at room temperature with gentle mixing, adjusted to pH 4.6 with  $2 \text{ mol L}^{-1}$  HCl and heated to  $85^\circ\text{C}$  for 30 min to denature WPC 80. The protein solution was then equilibrated at  $50^\circ\text{C}$  and the pH for Protamex hydrolysis was adjusted to 8.0 with  $1 \text{ mol L}^{-1}$  NaOH before addition of enzyme.

### Preparation of Hydrolysates and Degree of Hydrolysis

#### Preparation of Hydrolysates

Whey Protein Concentrate (WPC 80) was reconstituted at  $50^\circ\text{C}$  in distilled water to give a starting protein concentration of 5% (w/v). Prior to enzymatic treatment the aqueous solution of WPC 80 was allowed to hydrate for 1 h at room temperature with gentle mixing, adjusted to pH 4.6 with  $2 \text{ mol L}^{-1}$  HCl and heated to  $85^\circ\text{C}$  for 30 min to denature WPC 80. The WPC solution was then equilibrated at  $50^\circ\text{C}$  and initially the pH adjusted to 8 using  $0.5 \text{ N}$  NaOH to avoid any coagulation during enzymatic hydrolysis of protein to improve digestibility (Sindayikengera and Xia, 2006). Controlled enzymatic hydrolysis whey protein concentrate process by heating the reaction mixture at  $90^\circ\text{C}$  for 10 min.

Proteolytic enzyme ( $1.5 \text{ AU g}^{-1}$ ) was added at a rate of  $0.40 \text{ AU}$  per  $1 \text{ g}$  of WPC 80. Partial hydrolysis of WPC solution was carried out at  $50^\circ\text{C}$  under appropriate conditions. The degree of hydrolysis (DH) was monitored using the pH-stat technique Adler-Nissen (1986). During hydrolysis, samples were withdrawn after different times (min) and the enzymes were inactivated by heating the reaction mixture for 10 min at  $90^\circ\text{C}$ . The supernatants were taken as WPC hydrolysates and the precipitates were discarded. The WPC hydrolysates were stored at  $-20^\circ\text{C}$  for subsequent estimation of degree of hydrolysis (DH).

#### Calculation of the Degree of Hydrolysis (DH) Using the Ph-Stat Technique

The hydrolysis was carried out using the pH-stat method described by Adler-Nissen (1986) and the DH (%) was calculated from the volume and the normality of alkali used to maintain constant pH 8.

Sodium hydroxide (0.5 N) was utilized to monitor the consumption of a titrating, necessary to control the system pH during batch hydrolysis assays carried out. It calculated the number of peptide bonds hydrolyzed by the enzyme.

$$\text{DH (\%)} = B \times N_b \times \frac{1}{\alpha} \times \frac{1}{\text{MP}} \times 100\%$$

Where:

DH = Degree of hydrolysis

B = The base consumption (mL)

$N_b$  = Base normality

$\alpha$  = The average degree of dissociation of  $\alpha$ -NH groups (the values presented by Adler-Nissen (1986))

MP = The mass of protein (g)

The modified whey protein at 5, 10, 15 and 20% DH were under investigation.

#### **Determination Amino Acid Composition**

A modified method of AOAC 982.30a (AOAC, 1990) was used to determine Met-Cys and Branched-Chain Amino Acids (BCAA), except tryptophan. Sixty milligrams of freeze-dried sample were hydrolyzed with 8 mL of 6 mol L<sup>-1</sup> HCl under vacuum at 110°C for 24 h. After cooling, the hydrolysate was washed with distilled water, filtered (Whatman No. 2) and dried at 60°C (also under vacuum) in a rotary evaporator. The dried sample was then dissolved in 0.01 mol L<sup>-1</sup> HCl. The amino acids in the hydrolysate were separated and quantified by injecting 50  $\mu$ L into a Hitachi 835-50 amino acid analyzer equipped with a 2.6  $\times$ 150 mm<sup>2</sup> ion exchange column coated with resin 2619#. The column temperature was 53°C. Sodium citrate buffers (pH 3.3, 4.3 and 6.3) were used as eluents with a flow rate of 0.225 mL min<sup>-1</sup>. The light absorbance of the amino acids was detected with a 166 Detector (Beckman Instruments) at 570 nm and the amino acids were quantified by comparing them with amino acid profiles from external amino acid standard.

#### **Determination of Tryptophan**

Tryptophan was estimated by the ninhydrin method of Pintér-Szakács and Molnán-Perl (1990). One gram of sample was introduced into a 25 mL polypropylene test tube with caps and then 10 mL of 0.075 mol L<sup>-1</sup> NaOH was added and mixed until there were no lumps. The dispersion was shaken for 30 min and centrifuged at 5000 r min<sup>-1</sup> for 10 min and the supernate was transferred to a clean test tube. To 0.5 mL of supernate, 5 mL of ninhydrin reagent (1.0 g of ninhydrin in 100 mL mixture of 37% HCl and 96% HCOOH at a ratio of 2:3) was added and then solution was incubated at 35°C for 2 h and then cooled to room temperature after which the volume was made up to 10 mL with diethyl ether, thoroughly mixed with a Vortex mixer, filtrated and the clear filtrate was read at 380 nm. A standard tryptophan curve was prepared using 0~100  $\mu$ g tryptophan. From the standard graph, the concentration of tryptophan was calculated and expressed as g/100 g protein. Measurement the pH-protein solubility profiles of WPC 80 and its hydrolysates (Protein solubility) (PS).

Protein Solubility (PS) was determined in duplicate by the method of Bera and Mukherjee (1989). Two hundred milligrams of proteins were dispersed in 10 mL of deionized water. The pH of suspensions was adjusted to different levels (2.0 to 8.0) by using 1 mol L<sup>-1</sup> HCl or

1 mol L<sup>-1</sup> NaOH. The suspensions were stirred at room temperature for 30 min and then centrifuged at 10000x g for 30 min (Kika Ultra Turrax T18 basic, Germany). Protein contents in supernates were determined by Kjeldahl method (Ceirwyn, 1995). The percentage of protein solubility in each suspension was calculated by the ratio of protein in the supernate to protein in 200 mg sample.

## RESULTS

Enzymatic protein hydrolysis is the degradation of proteins into peptides and/or amino acids by proteolytic enzymes. During protein hydrolysis amide bonds are cleaved and, after addition of a water molecule, peptides and/or free amino acids are released. The most commonly used parameter describing the result of a hydrolysis process is the degree of hydrolysis (DH), used as an indicator of the extent of hydrolysis.

### Solubility

The whey protein solubility was measured in the pH range of 2 to 8 (the pH-protein solubility profiles of WPC 80 and its hydrolysates; (Fig. 1). Results showed that WPC 80 and its hydrolysate had minimum solubility at pH 4.0-5.0. Solubility in the pI range increased from 75.0 to 77.2, 79.0, 81.1 and 86.1% for WPC 80 and its hydrolysates at 5, 10, 15 and 20% DH, respectively.

At pH away isoelectric point (pI), the number of ionizable groups increased specially in which related to the peptides formed from the hydrolysate that improved the solubility. The WPC 80 and its hydrolysates had the highest solubility values at the both acidity pH range between 2.0 and 3.0 and alkaline pH range between 7.0 and 8.0. The results showed also that changes in solubility with DH were small at 5 and 10% DH and became more noticeable up to 15 and 20% DH for WPC 80.

### The Degree of Hydrolysis and the Health Benefits Amino Acid Content

Results showed that WPC 80 and its hydrolysates were the high content of sulfur-containing amino acids (Met and Cys), so WPC 80 had 79.0 mg g<sup>-1</sup> protein and

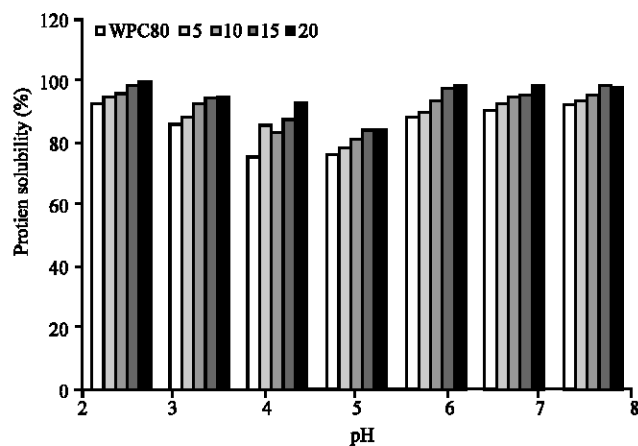


Fig. 1: Protein solubility of WPC 80 and its hydrolysate

at 5, 10, 15 and 20% DH had 40.2, 43.1, 41.4 and 42.0 mg g<sup>-1</sup> protein, respectively as shown in (Fig. 2) whereas FAO/WHO/UNU recommended requirements of preschool-age child were 25.0 mg g<sup>-1</sup> protein.

The three Branched-Chain Amino Acids (BCAA) are Leucine, Isoleucine and Valine. Leucine in WPC 80 had 106.0 mg g<sup>-1</sup> protein and at 5, 10, 15 and 20% DH had 104.0, 109.0, 107.4 and 105.2 mg g<sup>-1</sup> protein respectively whereas the optimal physiological needs of the human body are 66.0 mg g<sup>-1</sup> protein as recommended by FAO/WHO/UNU (Fig. 3-5). It is obvious that Leucine less sensitive to dehydrolyzation. Isoleucine in WPC 80 had 48.6 mg g<sup>-1</sup> protein and at 5, 10, 15 and 20% DH had 53.0, 52.0, 52.5 and 48.2 mg g<sup>-1</sup> protein, respectively whereas the optimal physiological needs of the human body are 28.0 mg g<sup>-1</sup> protein. Valine in WPC 80 had 17.5 mg g<sup>-1</sup> protein and at 5, 10, 15 and 20% DH had 52.5, 50.0, 53.4 and 48.2 mg g<sup>-1</sup> protein, respectively whereas the optimal physiological needs of the human body are 35.0 mg g<sup>-1</sup> protein.

The nutritional quality of the protein used in the formulation could be extrapolated to the expected tryptophan content. Tryptophan content in WPC 80 was 17.0 mg g<sup>-1</sup> protein and the requirement pattern is 11 mg g<sup>-1</sup> protein. With increasing the DH 5, 10, 15 and 20%

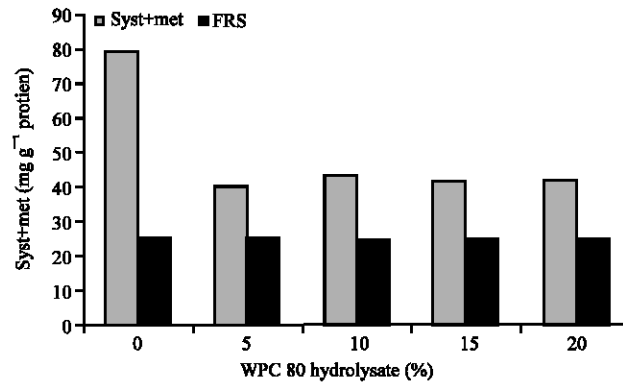


Fig. 2: Met and Syst content in WPC 80 and its hydrolysate compared to FAO/WHO/UNU reference standard

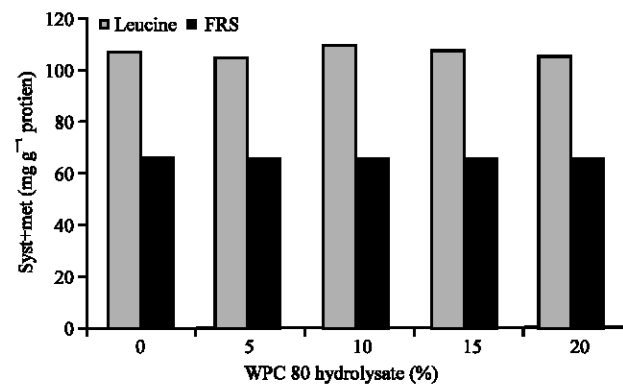


Fig. 3: Leucine content in WPC 80 and its hydrolysate compared to FAO/WHO/UNU reference standard

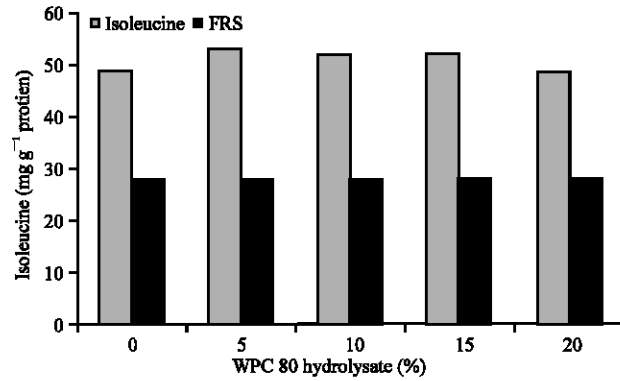


Fig. 4: Isoleucine content in WPC 80 and its hydrolysate compared to FAO/WHO/UNU reference standard

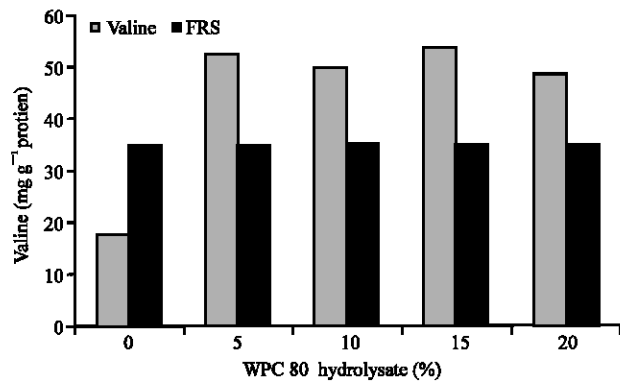


Fig. 5: Valine content in WPC 80 and its hydrolysate compared to FAO/WHO/UNU reference standard

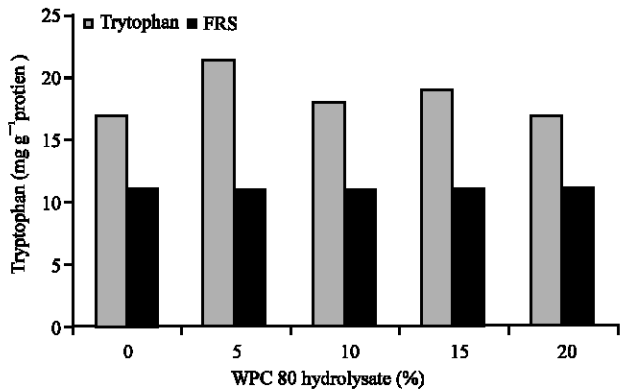


Fig. 6: Tryptophane content in WPC 80 and its hydrolysate compared to FAO/WHO/UNU reference standard

in WPC 80, Trp. content increased or kept the around the same concentrate as in non-hydrolyzed WPC 80. As gradually increase DH, Trp. content was 21.3, 18.0, 19.0 and 17.0 mg g<sup>-1</sup> protein, respectively (Fig. 6).

## **DISCUSSION**

In whey proteins, most peptide bonds are located in the interior of the protein and are not accessible for the enzyme. For these globular proteins it was postulated by Linderström-Lang that reversible denaturation of the protein is needed for protein breakdown, as after denaturation more peptide bonds are exposed and at this stage the unfolded molecules are susceptible to degradation by proteolytic enzymes.

Generally, any protein has a solubility minimum at its isoelectric pH. The solubility at the isoelectric point (pI) of whey proteins concentrate 80 increases with hydrolysis, which is mainly the result of reduction in molecular weight and the increase in the number of polar groups (Chobert *et al.*, 1988; Nielsen, 1997). Increasing the number of ionizable groups  $\text{NH}_4^+$ ,  $\text{COO}^-$  in the aqueous environment was increased in hydrophilicity of the residues that follow by increasing the solubility. A high solubility of the concentrates produces a high significant biological activity (Bounous and Gold, 1991). This indicates that the proteins present are essentially in undenatured form. This functional parameter is important for application of hydrolysates in food products.

Although, Met and Cys decreased with increasing the hydrolysis, the content of these amino acids but it still above the requirements as FAO/WHO/UNU recommended. Cystine is considered to be key factors for synthesis of the glutathione (GSH), one of the major detoxifiers and antioxidants of the body. Glutathione is essential in supporting the immune system, including natural killer cells (Droege and Holm, 1997) and in the maintenance of T-lymphocytes (Gutman and Schattini, 1998). In higher concentrations compared to the recommended dose immune function is enhanced through intracellular conversion to glutathione that supporting natural killer cells (Droege and Holm, 1997) and in the maintenance of T-lymphocytes (Gutman, 1998).

Isoleucine and Valine concentration were increased by increasing the partial hydrolysis compared to its concentration in WPC 80. Increasing partial hydrolysis of WPC 80 is useful for tissue growth and repair, in protein metabolism during the translation-initiation pathway of protein synthesis (Anthony *et al.*, 2001) and synthesizing new proteins (Walzem and Dilland, 2002; Bos *et al.*, 2000).

Tryptophan is important for the production of serotonin. Serotonin is one of the key brain chemicals involved in regulating mood. A number of studies indicate that normal mood depends in large part on adequate brain serotonin stores (Booij *et al.*, 2005; Ruhe *et al.*, 2007; Kaye *et al.*, 2000). Dietary intake of L-Tryptophan directly influences the amount of serotonin in the plasma, brain and throughout the entire body.

## **CONCLUSIONS**

In this study, it is apparent that WPC 80 enzymatic hydrolysis affected amino acid availability as indicated by some nutritional parameters such as the amino acid composition. This enzymatic hydrolysis improved the solubility and WPC 80 and its hydrolysates. The enhanced solubility of WPC hydrolysate is expecting increasing the biological activity. The undenatured conformation of whey protein was rich in cystine and with increasing DH kept cystine above the optimal physiological needs of the human body that is benefit for enhancing glutathione synthesis. Generally modified WPC 80 by partial hydrolysis was lead to increasing the concentration of Branched-Chain Amino Acids (BCAAs) that will be most useful for tissue growth and growth muscles. Tryptophan also was increased with increasing DH.



Whey protein concentrate standardized to 80% protein content (WPC 80) is the ideal protein used in a wide range of food applications. This study obvious that the optimal modified WPC 80 by partial hydrolysis was at DH 15% that is a good and makes them appropriate for food formulations or as nutritional supplements.

According to the results obtained, the content of beneficial health amino acids produced from WPC 80 hydrolysis depend on DH. It is prefer to recognize in generally the protein DH to get the optimal amino acid content benefit both this protein used as supplementation or was inside the product. Other health benefits can get from partial whey protein hydrolysates. Recent studies have described the antioxidant activity of milk protein hydrolysates and individual peptides released after hydrolysis (Pena Ramos and Xiong, 2001). The antioxidant activity has been attributed to certain amino acid sequences (Suetsuna *et al.*, 2000).

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