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MMP11

EFFECT OF HYDROLYSIS CONDITIONS ON PROPERTIES OF WHEY HYDROLYSATES

ผลของสภาวะในการไฮโดรไลซิสที่มีต่อคุณสมบัติของเวย์ไฮโดรไลเซต

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ABSTRACT

The objective of this study was to investigate the effect of hydrolysis conditions on degree of hydrolysis (DH) and protein pattern of whey hydrolysates. Hydrolysis of whey protein isolate (WPI) was performed using trypsin, chymotrypsin and papain under different enzyme to substrate ratio (E/S) and hydrolysis time. The effect of E/S and hydrolysis time on DH and protein pattern was concentration-dependent and time-dependent, respectively. DH and the protein pattern depended on type of enzymes. Trypsin, chymotrypsin and papain effectively digested most of WPI components excluding β-lactoglobulin and α-lactalbumin which were partially hydrolyzed by trypsin and chymotrypsin but were effectively digested by papain. Selection of hydrolysis conditions relies on desired properties of protein and DH.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์ในการศึกษาผลของสภาวะในการไฮโดรไลซิสที่มีต่อค่าการไฮโดรไลซิส และแบบแผนโปรตีนของเวย์ไฮโดรไลเซต โดยใช้เอนไซม์ทริปซิน ไคโมทริปซินและปาเปน ในอัตราส่วนเอนไซม์ต่อสารตั้งต้นและเวลาในการไฮโดรไลซิสที่ต่างกัน พบว่าการเพิ่มปริมาณเอนไซม์และระยะเวลาในการไฮโดรไลซิสส่งผลให้เกิดการไฮโดรไลซิสเวย์มากขึ้น ค่าการไฮโดรไลซิสและแบบแผนโปรตีนของเวย์ไฮโดรไลเซตที่ได้ขึ้นกับชนิดของเอนไซม์ที่ใช้ เอนไซม์ทั้งสามชนิดสามารถย่อยโปรตีนที่เป็นส่วนประกอบหลักของเวย์ได้ดี ยกเว้นโปรตีน β-lactoglobulin และ α-lactalbumin ซึ่งถูกย่อยบางส่วนโดยเอนไซม์ทริปซินและไคโมทริปซิน แต่ถูกย่อยอย่างสมบูรณ์โดยเอนไซม์ปาเปน การเลือกใช้สภาวะในการไฮโดรไลซิสควรพิจารณาจากคุณสมบัติของโปรตีนที่ต้องการและค่าการไฮโดรไลซิสของเวย์ไฮโดรไลเซต

Key Words : Whey, Whey hydrolysates
คำสำคัญ : เวย์ เวย์ไฮโดรไลเซต

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1. Introduction

Whey is a complex proteins from milk. It contains proteins, sugars, minerals and lipids. Membrane-based separation technologies include ultrafiltration and diafiltration is used to improve whey quality by removing lactose, minerals and lipids with concentrating proteins. The obtained whey is called whey protein isolate (WPI). WPI composes of β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulins, bovine serum albumin, lactoperoxidase, lactoferrin and glycomacropeptide. These compounds play excellent biological properties as muscle anabolism, immunomodulation, anticancer and antioxidant (Marshall 2004, Smithers 2008). Several methods have been suggested to improve biological activities of whey. Enzymatic hydrolysis is commonly used method to enhance the activity of whey by producing several small bioactive peptides (Amiot, et al 2004, Mercier, et al 2004, Ven et al 2002). The important bioactive peptides are produced during enzymatic hydrolysis process is cysteine-containing peptides. It is reported that cysteine is glutathione precursor which enhances glutathione synthesis leads to increase intracellular antioxidant (Marshall 2004). We proposed enzymatic hydrolysis produced small bioactive peptides. These peptides may be easily transport into cell and act as precursor of glutathione to enhance antioxidant activity of cell. Antioxidant activity relies on properties of whey hydolysates such as protein pattern and degree of hydrolysis (DH). The properties are depending on hydrolysis conditions: specificity of enzyme, enzyme to substrate ratio (E/S) and hydrolysis time (Pena-Ramos and Xiong 2001). However information of hydrolysis conditions effects on properties of whey hydrolysates has limited. The objective of this study is to investigate the effect of hydrolysis conditions on the properties of whey protein isolate (WPI) hydrolysates. The knowledge of this study is used as preliminary information to evaluate antioxidant activity of WPI hydrolysates.

2. Materials and methods

2.1 Materials

WPI was purchased from IMMUNOTHAI Co., Ltd. (Thailand). Trypsin was purchased from Invitrogen Corporation (Canada). Papain, chymotrypsin, leucine and picrylsulfonic acid solution (TNBS) were purchased from Sigma-Aldrich (USA). Hydrochloric acid was purchased from Labscan Asia., Ltd., (Thailand). Sodium dodecyl sulphate (SDS) was purchased from Ajax Finechem (New Zealand). Disodium hydrogen phosphate was purchased from Ajax Finechem (Australia). Sodium dihydrogen phosphate was purchased from Merck (Germany). Tris, acrylamide/bis solution, glycine, bromophenol blue, β-mercaptoethanol, SDS, ammonium persulfate and N, N, N', N'-tetra-methyl-ethylenediamine (TEMED) and Bio-Safe Coomassie were purchased from Bio-Rad (USA). PageRuler prestained protein ladder was purchased from Fermentas (USA).

2.2 Methods

2.2.1 Preparation of whey hydrolysates

Hydrolysis of WPI was performed using trypsin, chymotrypsin and papain under E/S 1/1000, 1/200 and 1/100 at various hydrolysis time 1, 3 and 5 hr.

A 2% (w/v) WPI solution was preheated at 90°C for 5 min. The solution was hydrolyzed with the

enzymes in 0.01 M sodium phosphate buffer (pH 8.0) at 37°C (Pena-Ramos and Xiong 2001). The hydrolyzed solution was heated at 85°C for 10 min to inactivate the enzymes. The obtained hydrolysates were stored at 4°C.

2.2.2 Characterizations of whey hydrolysates

2.2.2.1 Degree of hydrolysis (DH)

DH was measured spectrophotometrically according to the method of Adler-Nissen (Adler-Nissen 1979) with modification. A leucine concentration range 0.8-2.4 mM was used as standard to calculate the amount of amino nitrogen. All samples and standard solutions were prepared in 1% (w/v) SDS, 1% (w/v) SDS was used as blank. 0.125 ml of samples or standard solutions was mixed with 1 ml of 0.2125 M sodium phosphate buffer (pH 8.2). Then added 2 ml of 0.1% (w/v) TNBS solution, followed by incubation at 50°C for 60 min in a covered water bath. After incubation, 2 ml of 0.1 N HCl was added to terminate the reaction and the mixture was allowed to cool at room temperature for 30 min before measured absorbance at 340 nm. DH values were calculated using the following equation:

$$DH = \frac{(AN_2 - AN_1) * 100}{Npb}$$

where AN_1 and AN_2 are the amino nitrogen content of the protein before and after hydrolysis (mg/g protein) respectively. Npb is the nitrogen content of the peptide bonds in whey, 123.3 mg/g protein (Spellman, et al 2003).

2.2.2.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of WPI hydrolysates were obtained from SDS-PAGE technique as described by Laemmli (Laemmli 1970) with some modification using a 15% acrylamide resolving gel and a 5%

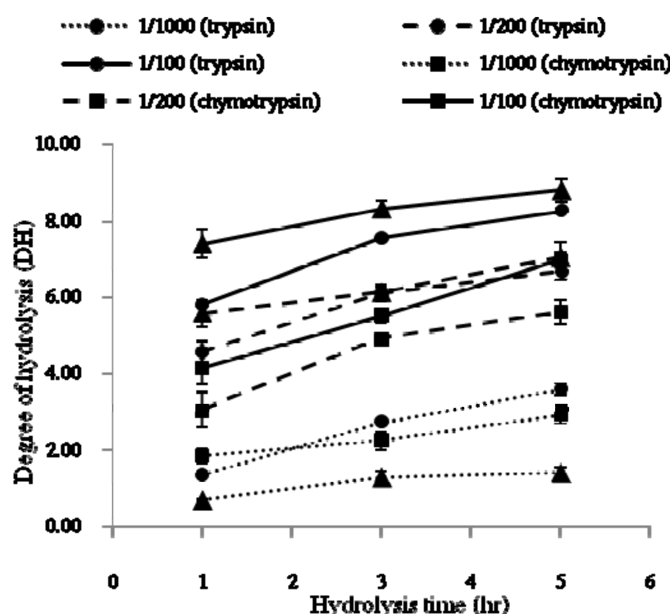

Figure 1 DH of WPI hydrolysates

acrylamide stacking gel. The SDS-PAGE was conducted in a mini-gel apparatus equipped with PowerPac Basic (Bio-Rad) run for 2 hr at 85 V (Pena-Ramos and Xiong 2001). The protein ladder performed 10, 15, 25, 35, 40, 55, 70 and 100 kDa bands.

3. Results and discussion

Properties of WPI hydrolysates depend on hydrolysis temperature, pH, type of enzyme, E/S and hydrolysis time. Hydrolysis temperature and pH should be considered for optimum conditions of selected enzyme. This study purposed to investigate the effect of type of enzyme, E/S and hydrolysis time on DH and protein pattern. Trypsin, chymotrypsin and papain were used in this study. These enzymes have different specificity to hydrolyze peptide bonds. Trypsin is specific to C-terminal side of lysine and arginine. Chymotrypsin cleaves at C-terminal side of aromatic and hydrophobic amino acid. (Amiot, et al 2004). Papain exhibits broad specificity to basic amino acids, leucine and glycine including esters and amide.

DH

As shown in Figure 1, The DH of WPI hydrolysates depended on types of enzyme, E/S and hydrolysis time. Increasing E/S produced higher DH. As expected, the effect of hydrolysis time on DH was time-dependent, increasing hydrolysis time enhanced DH. However, DH profile of WPI hydrolysates obtained from papain was little influenced by hydrolysis time. For 5 hr of hydrolysis, most of WPI hydrolysates were nearly complete hydrolysis. WPIs treated with papain in each E/S showed the highest DH compared with other enzymes except for E/S 1/1000.

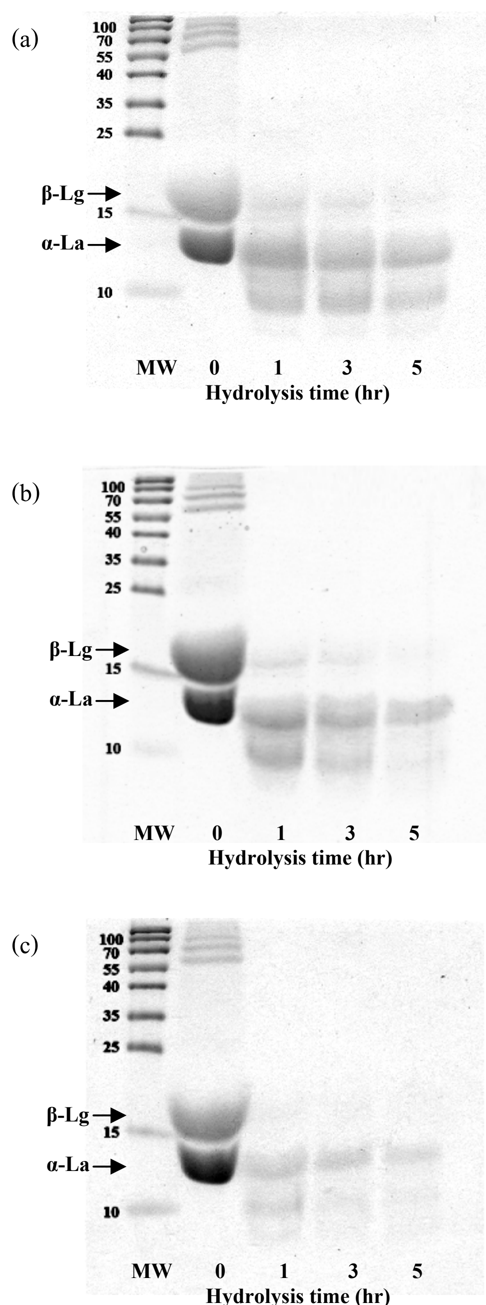

Figure 2 SDS-PAGE of WPIs treated with trypsin at various E/S 1/1000 (a), 1/200 (b) and 1/100 (c)

SDS-PAGE

WPIs treated with trypsin

The protein patterns of WPI hydrolysates treated with various hydrolysis conditions were presented in

Figure 2-4. Preheat treatment facilitated susceptibility of WPI to enzyme digestion (Pena-Ramos and Xiong 2001). Most of major components of WPI such as bovine serum albumin (66 kDa), lactoperoxidase (70 kDa), lactoferrin (80 kDa) were susceptible to trypsin and completely hydrolyzed within 1 hr at low E/S (Figure 2a). A similar result was obtained by Pena-Ramos and Xiong (2001). β-Lg and α-La account for 50% and 20% of whey, they are rich in cysteine, glutathione precursor (Marshall 2004). These proteins were partially digested by trypsin. Increasing hydrolysis time resulted in more digestion correlated with DH values (Figure 1). Besides, β-Lg appeared to be more susceptible to trypsin than α-La.

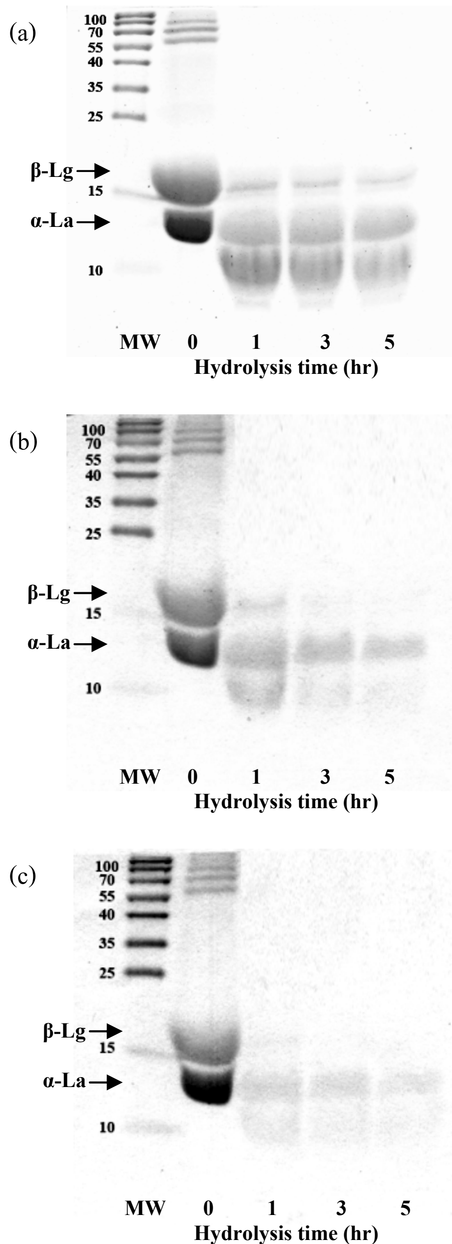

Figure 3 SDS-PAGE of WPIs treated with chymotrypsin at various E/S 1/1000 (a), 1/200 (b) and 1/100 (c)

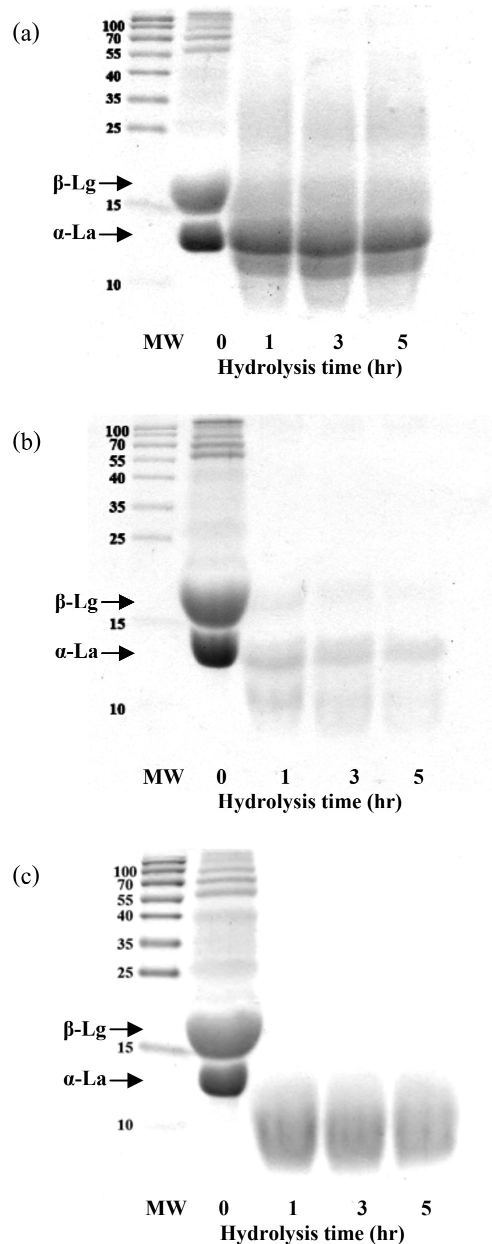

Figure 4 SDS-PAGE of WPIs treated with papain at various E/S 1/1000 (a), 1/200 (b) and 1/100 (c)

WPIs treated with chymotrypsin

Similar to WPIs treated with trypsin, most of WPI components were degraded by chymotrypsin within 1 hr of hydrolysis (Figure 3). Some components of WPI were degraded to around 10 kDa fragments. The degradation of β-Lg and α-La were obviously influenced by hydrolysis time. For WPI treated with 1/1000 chymotrypsin, the protein pattern of 3 hr of hydrolysis was slightly different from 1 hr of hydrolysis. The result related to their DH values that was little increase (Figure 1). Moreover, β-Lg was more sensitive to chymotrypsin than α-La and almost completely hydrolyzed after 5 hr of hydrolysis.

WPIs treated with papain

As presented in Figure 4, the degradation of WPI major components obtained from papain hydrolysis was not different from trypsin and chymotrypsin hydrolysis which was completely digested within 1 hr of hydrolysis. However, β-Lg and α-La were the most susceptible to papain compared with trypsin and chymotrypsin. Increasing E/S rendered more digestion of β-Lg and α-La. These components were completely hydrolyzed within 1 hr of hydrolysis for WPI treated with 1/100 papain. Hydrolysis time showed slightly effect to the protein patterns of WPIs treated with papain related to DH profile (Figure 1). Some of degradation products obtained from 1/100 papain hydrolysis were approximately 10 kDa fragments. These components possess source of cysteine, glutathione precursor. The cysteine-containing peptides obtained from papain hydrolysis may be more accessible into cell than WPI and play antioxidant effect via stimulation of glutathione synthesis. We are currently investigating the antioxidant activity of WPI hydrolysates.

4. Conclusions

The DH and protein patterns of whey hydrolysates were depending on type of enzyme, E/S and hydrolysis time. Papain was effective to hydrolyze major components of WPI including β-Lg and α-La. Whereas trypsin and chymotrypsin were less effective than papain. The effect of E/S and hydrolysis time was concentration-dependent and time-dependent, respectively. Therefore, selection of hydrolysis conditions relies on desired properties of protein and DH.

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