Protein Hydrolysates from Pacific Whiting Solid Wastes

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Alcalase and Neutrase showed optimum activity against Pacific whiting solid wastes (PWSW) at pH 9.5, 60 °C and pH 7.0, 55 °C, respectively. Alcalase had a higher proteolytic activity than Neutrase. Enzyme concentration, reaction time, and waste/buffer ratio significantly affected the hydrolysis and nitrogen recovery (NR) ($p < 0.05$). Optimum conditions for PWSW hydrolysis were 20 AU Alcalase/kg, 1 h reaction time, waste/buffer ratio of 1:1 (w/v). Correlation between the degree of hydrolysis (DH) and NR ($R^2 = 0.970-0.978$) was high. Freeze-dried hydrolysate was brownish yellow in color ($L^* = 54.59$, $a^* = 6.70$, $b^* = 27.89$) and contained 2.77% moisture, 79.97% protein, 13.44% ash, and 3.83% lipid. Amino acid composition of freeze-dried hydrolysate was similar to that of PWSW and Pacific whiting muscle but tryptophan was reduced to 21.50% and 14.74%, respectively.

Keywords: *Waste; Pacific whiting; hydrolysate; Alcalase; Neutrase*

INTRODUCTION

Due to the abundance of Pacific whiting (*Merluccius productus*) off the west coast of the United States, it has been exploited as a raw material for surimi production (Morrissey et al., 1996). During processing, solid wastes including eviscera, head, skin, bone, and some muscle tissue are generated and can be as high as 70% of the original raw material. Normally, these wastes have been used as fish meal or fertilizer. Novel means of processing are required to convert the underutilized wastes into more marketable and acceptable forms. To upgrade protein byproducts, proteases from plant, animal, and microbial origin have been applied to convert seafood processing wastes and underutilized species into protein concentrate (Onodenalore and Shahidi, 1996; Shahidi et al., 1994; 1995; Beak and Cadwallader, 1995; Hoyle and Merritt, 1994; Rebeca et al., 1991; Quaglia and Orban, 1987; Cheftel et al., 1971). Commercial enzymes were also used for protein hydrolysate production from chicken heads (Surowka and Fik, 1992, 1994) and veal bone (Linder et al., 1995; 1996). Autolysis caused by endogenous enzymes can contribute to the protein hydrolysis, however, it is difficult to control the rate of hydrolysis due to several factors including the fish species and seasonality as well as the type and amount of enzymes (Sikorski and Naczk, 1981). Consequently, application of exogenous enzymes is more common, particularly for protein hydrolysate production since the hydrolysis and properties of resultant product can be manipulated. Alcalase and Neutrase are endopeptidases produced from *Bacillus licheniformis* and *Bacillus amyloliquefaciens*, respectively. Both enzymes have been reported as the potent proteinases for hydrolyzing the muscle proteins as well as agricultural wastes (Quaglia and Orban, 1987; Hoyle and Merritt, 1994; Beak and Cadwallader, 1995; Shahidi et al., 1995). The objective of this investigation was to study the production and composition of hydrolysate from Pacific whiting solid waste (PWSW) using Alcalase and Neutrase.

MATERIALS AND METHODS

Reagents. 2,4,6-Trinitrobenzenesulfonic acid (TNBS), sodium sulfite, and L-leucine were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium dodecyl sulfate (SDS) was purchased from Bio-Rad Laboratories, Hercules, CA. Boric acid was obtained from Mallinckrodt, Inc., St. Louis, MO. Alcalase 2.4 L (a declared activity of 2.4 AU/g and a density of 1.18 g/mL) and Neutrase 0.5 L (a declared activity of 0.5 AU/g and a density of 1.25 g/mL) were provided by Novo Nordisk Biochem North America, Inc. (Franklinton, NC).

Materials. Ground PWSW including head, skin, bone, eviscera, and muscle tissue was obtained from Point Adams Packing Co. (Hammond, OR). Pacific whiting was caught and processed within 16−24 h of capture. Solid processing wastes were ground finely and mixed thoroughly at room temperature within 2 h of processing. The samples were transported to OSU Seafood Laboratory, vacuum-packed in polyethylene bags, and kept at −20 °C until used.

Enzymic Hydrolysis of PWSW by Alcalase and Neutrase. *pH and Temperature Profile for Alcalase and Neutrase on PWSW.* Optimum pH of Alcalase and Neutrase against PWSW was studied at 60 and 50 °C, respectively. PWSW (5 g) was added with 0.2 M McIlvaine buffer (pH 4.5−8.0) or 0.2 M borate buffer (pH 8.5−11.5) at the ratio of 1:2 (w/v) and pH of mixture was rechecked and adjusted with 6 N NaOH or 6 N HCl. The mixtures were incubated and well-shaken at reaction temperature for 10 min before the reaction was initiated by adding 20 μL of enzymes. After 10 min, a 500 μL aliquot was mixed with 2.0 mL of 1% hot SDS solution (85 °C) and placed in a water bath at 85 °C for 15 min.

The studies of optimum temperature for Alcalase and Neutrase on PWSW were carried out under optimum pH for each enzyme. The rest of the conditions were the same as those in the pH study described above.

α-Amino acid released was measured and expressed as L-leucine. Increased amount of α-amino acid was determined by subtracting the α-amino acid at 0 min from that of hydrolyzed PWSW at 10 min.

Effect of Enzyme Concentration. Two levels of enzyme concentration (5 and 10 AU/kg) were used to compare the hydrolytic activity between Alcalase and Neutrase. The reactions were held at optimum conditions, pH 9.5, 60 °C for Alcalase and pH 7.0, 55 °C for Neutrase. The rest of the conditions were the same as before. Increased α-amino acid concentration was analyzed at different reaction times.

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To study the effect of enzyme concentration on degree of hydrolysis (DH) and nitrogen recovery (NR), different amounts of enzyme were added to the suspension of PWSW in buffer (1:2 ratio, w/v) and the reaction was carried out under optimum conditions. After 30 min, reaction was stopped by heating at 90 °C for 5 min. The supernatant was obtained by centrifuging at 3000*g* for 10 min.

Effect of PWSW and Buffer Ratio. Effect of PWSW and buffer ratio on α-amino acid and NR was investigated. PWSW was mixed with buffer at a ratio of 1:0.5, 1:1, 1:2, 1:3, 1:5, and 1:8 (w/v). Enzyme (20 AU/Kg) was added and reaction was maintained for 30 min under optimum conditions. α-Amino acid and NR were determined as described below.

Determination of Optimum Condition for PWSW Hydrolysate Production Using Alcalase. Three parameters were studied to optimize the hydrolysate process as follows: enzyme concentration (10, 20, 40 AU/kg), PWSW:0.2 M borate buffer ratio (1: 0.25, 1: 0.5, 1:1, 1:3, w/v), and reaction time (30, 60, 120 min). The hydrolysate obtained from different conditions was determined for NR. The liquid hydrolysate produced under optimum conditions was freeze-dried at −60 °C for 12−24 h (Labconco Corp., Kansas City, MO).

Determination of α-Amino Acid and Degree of Hydrolysis (DH). Modified methods of Adler-Nissen (1979) and Crowell et al. (1985) were used to determine α-amino acid content. Properly diluted samples (125 μL) were mixed thoroughly with 2.0 mL of 0.2125 M phosphate buffer, pH 8.2, followed by the addition of 1.0 mL of 0.01% TNBS solution. The mixtures were then placed in a water bath at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 mL of 0.1 M sodium sulfite. The mixtures were cooled down at ambient temperature for 15 min. The absorbance was measured at 420 nm and α-amino acid was expressed in terms of L-leucine. The DH was determined using the modified method of Beak and Cadwallader (1995) and defined as follows:

$$\mathrm{DH} = [(L_t - L_0)/(L_{\max} - L_0)] \times 100$$

where L_t corresponded to the amount of α-amino acid released at time t. L_0 was the amount of α-amino acid in original PWSW. $L_{\max}$ was the maximum amount of α-amino acid in PWSW obtained after acid hydrolysis. PWSW suspension (500 μL) was mixed with 4.5 mL of 6 N HCl. The tube with sample mixtures was flashed with nitrogen gas and sealed tightly with screw-cap. The hydrolysis was run at 100 °C for 24 h (Beak and Cadwallader, 1995). The acid-hydrolyzed sample was filtered through Whatman paper no. 1 to remove the unhydrolyzed debris. The supernatant was neutralized with 6 N NaOH before α-amino acid determination.

Determination of NR. After the hydrolysis reaction, the supernatant was obtained by centrifuging at 3000*g* for 10 min. The dense lipid layer was skimmed using two-layers of cheese cloth. The volume of soluble fraction was recorded and total nitrogen in supernatant was determined using Kjeldahl method (AOAC, 1984). NR was calculated using the following equation: NR (%) = [total nitrogen in supernatant (mg)/total nitrogen in substrate (mg)] × 100.

Electrophoresis. Alcalase or Neutrase (10 AU/kg) was added to the mixture of PWSW and buffer (ratio 1:2, w/v). The reaction was carried out under optimum conditions at different times (5, 10, 30, and 60 min). After the exact reaction time, SDS was added to obtain the final concentration of 5% (w/v) and the mixture was heated at 85 °C for 1.5 h. The undissolved debris was removed by centrifuging at 3500*g* for 5 min.

SDS−polyacrylamide gel electrophoresis (SDS-PAGE) was run according to the method of Laemmli (1970) using 4% stacking gel and 15% separating gel. Sixty micrograms of protein determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard was applied to the gel. Proteins were stained in 0.125% Coomassie brilliant blue R-250 and destained in 25% ethanol and 10% acetic acid. M_r of protein bands was estimated using protein standards. High molecular weight standards (Sigma Chemical Co., St. Louis, MO) included rabbit muscle myosin (205 000), *E. coli* β-galactosidase (116 000), rabbit muscle phosphorylase *b* (97 000), bovine serum albumin (66 000), ovalbumin (45 000), and bovine erythrocytes carbonic anhydrase (29 000). Low molecular weight standards (Phamacia Biotech, Inc., Piscataway, NJ) contained phosphorylase *b* (94 000), albumin (67 000), ovalbumin (43 000), carbonic anhydrous (30 000), trypsin inhibitor (20 100), and α-lactalbumin (14 400).

Composition Analyses. Chemical compositions of samples including freeze-dried hydrolysate, PWSW, and Pacific whiting muscle were determined as described below. Moisture content was measured by oven drying at 105 °C until a constant weight (AOAC, method 24.002, 1984). Protein and ash were determined according to method 24.027 (Kjeldahl method) and 18.025, respectively (AOAC, 1984). Lipid was assayed using a modification of method 18.043 (AOAC, 1984). For lipid analysis, the centrifuge tube with Teflon-lined screw cap was used instead of Majonnier fat extraction flask. The upper layer of solvent containing fat was transferred by pipet.

Amino acid composition was determined after hydrolysis at 115 °C for 20 h in 6 N HCl with 0.05% mercaptoethanol and 0.02% phenol. The amino analysis was performed by post-column derivatization with ninhydrin using a Beckman System 6300 amino acid analyzer (Beckman Instruments, Inc., Fullerton, CA). Cysteine was determined as cysteic acid. Performic acid oxidation was run at 50 °C for 15 min prior to acid hydrolysis (Hirs, 1967). Tryptophan was measured by alkaline hydrolysis at 135 °C for 48 h (Hugli and Moore, 1972).

Color Measurement. L^* (lightness), a^* (redness, + or greenness, −), and b^* (yellowness, + or blueness, −) of samples including freeze-dried hydrolysate, PWSW, and Pacific whiting muscle were measured using a Minolta Chroma meter CR-310 (Minolta Corp., Ramsey, NJ). A Minolta calibration plate ($Y_{CIE} = 94.5$, $x_{CIE} = 0.3160$, $y_{CIE} = 0.330$) and a Hunter Lab standard plate ($L^* = 82.13$, $a^* = -5.24$, $b^* = -0.55$) were used to standardize the instrument with D65 illuminant and 2° observer.

Statistical Analyses. Data were analyzed using the analysis of variance procedure. Mean difference was determined using the least significant difference (LSD) multiple range test (Statgraphics Version 6.0, Manugistics Inc., Rockville, MD). Significance of difference was established at $p < 0.05$. All hydrolysis experiments were carried out in duplicate and composition analysis was performed in triplicate.

RESULTS AND DISCUSSION

pH and Temperature Profile of Alcalase and Neutrase on PWSW. The pH activity curves of Alcalase and Neutrase are shown in Figure 1. The optimum pH values for Alcalase and Neutrase on PWSW were 9.5 and 7.0, respectively. Alcalase showed a broad activity in alkaline pH range. However, a sharp decrease in activity was observed at pH 11.5. Adler-Nissen (1986) reported that Alcalase was more active at alkaline pH and remained active to pH 6.0. For Neutrase, the activity reached the maximum at pH 7.0. The activity was high in pH range of 6.5−8.5 but showed considerable loss of activity at pH 10.5. A change in pH affects both the substrate and enzyme by changing the charge distribution and conformation of the molecules (Adler-Nissen, 1986). Most enzymes undergo irreversible denaturation in a very acid and alkaline solution, causing the loss of stability (Whitaker, 1994). The pH also affects the ionization of prototrophic groups which are involved in maintaining proper conformation of the active site of enzyme, binding of substrate to enzyme, and transforming substrate to product (Whitaker, 1994).

The effect of temperature on enzyme activity is shown in Figure 2. Alcalase showed a high activity in the high-temperature range (55−70 °C) with an optimum at 60 °C. However, an appreciable decrease in enzyme activity was observed above 70 °C, due to thermal denaturation. At 20 °C, the activity was approximately 4-fold lower than that obtained at 60 °C. Adler-Nissen (1986)

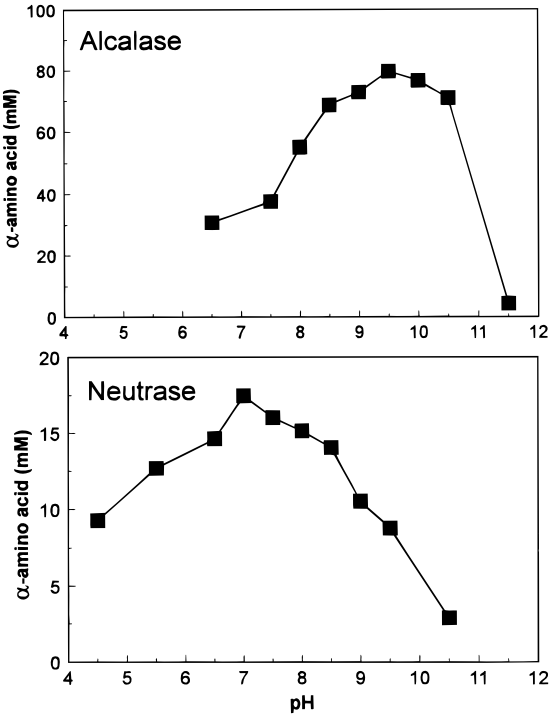

Figure 1. pH profile of Alcalase and Neutrase on PWSW. The hydrolytic reaction was run for 10 min at 60 and 50 °C for Alcalase and Neutrase, respectively. The hydrolysis product was expressed as α-amino acid (mM).

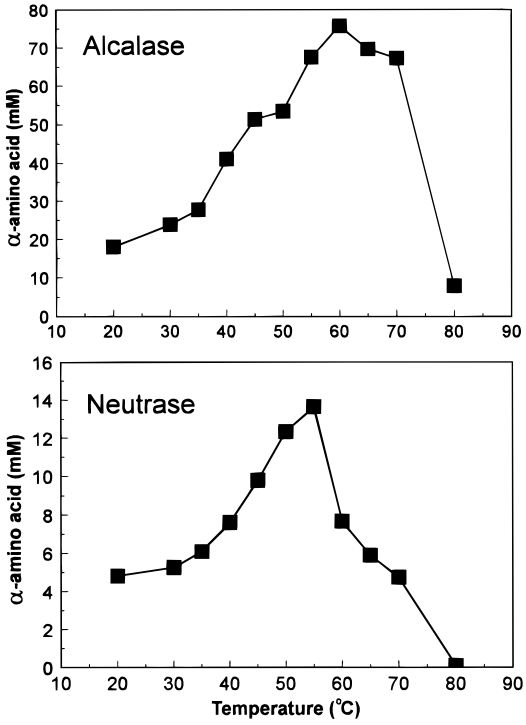

Figure 2. Temperature profile of Alcalase and Neutrase on PWSW. The hydrolytic reaction was run for 10 min at pH 9.5 and 7.0 for Alcalase and Neutrase, respectively. The hydrolysis product was expressed as α-amino acid (mM).

reported that the activity of Alcalase doubled for every temperature rise of 12 °C. Beak and Cadwallader (1995) found that the optimum temperature of Alcalase on crayfish processing by-products was 70 °C. This difference was probably due to the different substrate and reaction conditions. For Neutrase, the activity increased to 55 °C followed by a sharp decrease in activity. No activity was found at 80 °C. At high

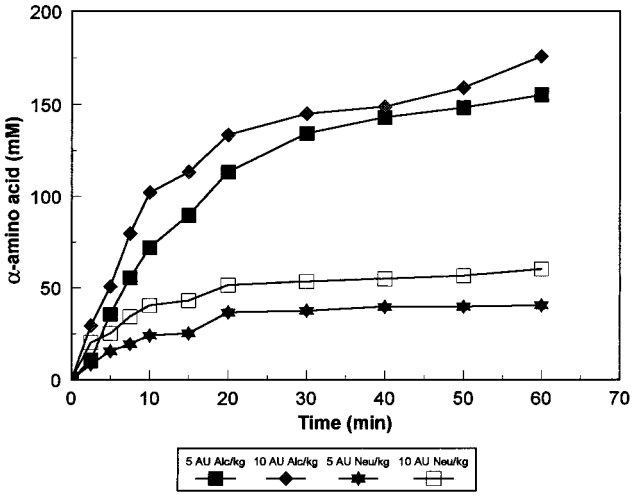

Figure 3. Proteolytic activity of Alcalase and Neutrase on PWSW. The hydrolytic reaction was run at pH 9.5, 60 °C and pH 7.0, 55 °C for Alcalase and Neutrase, respectively. The hydrolysis product was expressed as α-amino acid (mM).

temperatures, most enzymes are irreversibly denatured. The temperature activity curves indicate that Alcalase was more heat stable and active at a higher temperature for PWSW hydrolysis.

Enzymatic Hydrolysis of PWSW. Hydrolytic curves of PWSW by Alcalase and Neutrase were compared (Figure 3). For each enzyme, α-amino acid in PWSW treated with 10 AU/kg was slightly higher than that treated with 5 AU/kg. At the same level of enzyme, Alcalase showed a higher hydrolytic activity than Neutrase. A rapid reaction rate was obtained in the first 10 min, then the rate of hydrolysis subsequently decreased. A constant rate was observed with PWSW treated with Neutrase after 20 min of reaction time, suggesting that the enzymatic reaction reached the steady-state phase. A similar curve was reported for the enzymatic hydrolysis of crayfish byproduct (Beak and Cadwallader, 1995), capelin (Shahidi et al., 1995), sardine (Quaglia and Orban, 1987), and veal bone (Linder et al., 1996). Generally, the enzyme absorbs rapidly onto the insoluble protein particles, then the polypeptide chains that are loosely bound to the surface are cleaved. The more compacted core proteins are hydrolyzed more slowly. The rate of enzymic cleavage of peptide bond controls the overall rate of hydrolysis (Archer et al., 1973). However, available substrate decreases as time of reaction increases.

Hydrolysis Pattern on SDS-PAGE. SDS-PAGE of control and enzyme-treated PWSW is shown (Figure 4). In the control sample (C), protein bands with M_r ranging from 82 000 to 111 700 were observed. These bands were postulated to be the native proteins or the degradation products from myosin due to autolysis during handling or waste generation process. Actin with M_r 45 000 was prominent in PWSW. Benjakul et al. (1997) reported that 45% of Pacific whiting myosin heavy chain (MHC) was degraded within 8 days iced storage, but no noticeable difference was observed in actin. A broad range of low M_r bands was also noted. After treatment of PWSW with Alcalase, high M_r bands were totally removed and the low M_r bands were observed. No obvious difference was found with different reaction times. Different hydrolytic patterns of PWSW proteins were noted after treatment with Neutrase. After Neutrase treatment, the bands ranging from M_r 82 000 to 117 000 entirely disappeared while an increase of bands

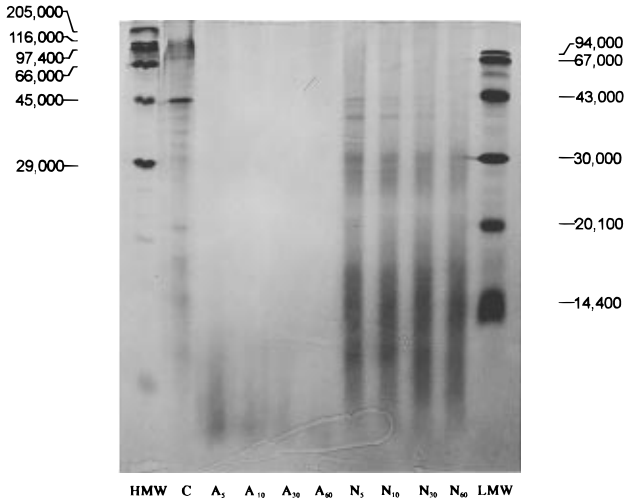

Figure 4. SDS-PAGE patterns of PWSW proteins during hydrolysis by Alcalase (A) and Neutrase (N). Numbers designate the reaction time (min). HMW, LMW, and C stand for high molecular weight standards, low molecular weight standards, and control (PWSW in the absence of enzyme).

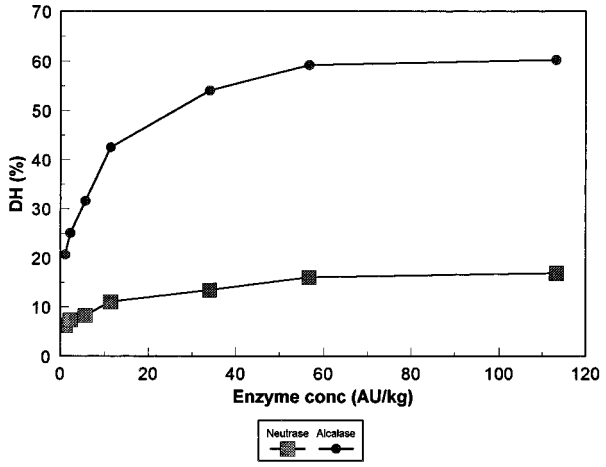

Figure 5. Effect of Alcalase and Neutrase concentration on DH of PWSW. Different amounts of enzyme were added to the suspension of PWSW in buffer (1:2 ratio, w/v). The reaction was run for 30 min at pH 9.5, 60 °C and pH 7.0, 55 °C for Alcalase and Neutrase, respectively.

at M_r 30 000 and M_r lower than 17 000 were observed. At 5 min (N_5), a decreasing intensity of band with M_r 45 000 was found and band intensity was decreased as time increased. This band totally disappeared after 60 min (N_{60}). From SDS-PAGE results, it can be inferred that Alcalase showed a considerably higher hydrolytic activity on PWSW when compared with Neutrase. The result coincided with a higher amount of α-amino acid in hydrolysate obtained after treatment of PWSW with Alcalase (Figure 3).

Effect of Enzyme Concentration on DH and NR. When the enzyme concentration was increased, DH and NR of PWSW treated with both Alcalase and Neutrase increased (Figures 5 and 6). Significant changes in DH and NR occurred with the enzyme treatment at concentrations ranging from 0 to 34 AU/kg ($p < 0.05$). However, no significant increases for both DH and NR were found with treatment of enzyme at concentration above 57 AU/kg ($p > 0.05$). PWSW hydrolysate treated with Alcalase showed appreciably higher DH and NR than that treated with Neutrase. DH has been used as an indicator for the cleavage of peptide bond whereas NR reflects the yield that can be recovered from the

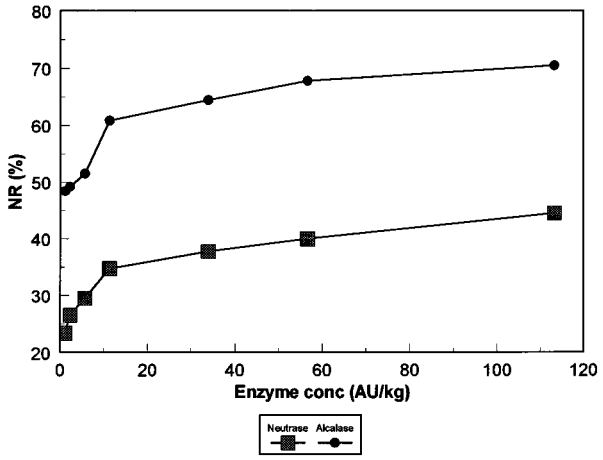

Figure 6. Effect of Alcalase and Neutrase concentration on NR of PWSW. Different amounts of enzyme were added to the suspension of PWSW in buffer (1:2 ratio, w/v). The reaction was run for 30 min at pH 9.5, 60 °C and pH 7.0, 55 °C for Alcalase and Neutrase, respectively.

hydrolysis process. The value of DH in this study was similar to that obtained in crayfish processing byproduct hydrolysate treated with Alcalase (Beak and Cadwallader, 1995). Early work by Cheftel et al. (1971) on fish protein concentrate showed that an increase in enzyme concentration has a positive effect on overall proteolysis with subsequent increases in solubilization of protein.

When $\log_{10}$(enzyme concentration) vs DH was plotted, a linear relationship was observed (Figure 7). The correlation coefficients of $R^2 = 0.978$ and 0.972 were obtained for Alcalase and Neutrase, respectively. This result was in agreement with Beak and Cadwallader (1995) who reported that the relationship between $\log_{10}$(protease amount) and DH for enzymatic hydrolysis of crayfish processing byproducts was linear. From this relationship, the exact concentration of enzyme required to hydrolyze PWSW to a required DH in 30 min under optimum conditions can be calculated. Hale (1969) found that a logarithmic plot of digestion ratio vs enzyme concentration for enzymatic hydrolysis of fish protein substrate powder was linear.

Our results showed that NR was directly proportional to DH (Figures 8 and 9). The correlation coefficients of $R^2 = 0.978$ and 0.970 were obtained for Alcalase and Neutrase, respectively, indicating that a close relationship existed between DH and NR. Shahidi et al. (1995) reported that considerable soluble protein was released during initial phase and no increase in soluble hydrolysate was observed when additional enzyme was added during the stationary phase of hydrolysis. The rate of hydrolysis and NR was reduced with a high concentration of soluble peptides in reaction mixtures. In our study, the increase in enzyme concentration caused an increase in DH and soluble peptides, leading to a higher NR. However, a reduction in rate of DH and NR production with increasing enzyme concentration was observed (Figure 5 and 6).

Effect of Substrate/Buffer Ratio on Hydrolysis and NR. The effect of substrate/buffer ratio on hydrolysis and NR of PWSW by Alcalase and Neutrase is shown (Figures 10 and 11). In general, an increase in waste/buffer ratio resulted in an increase in α-amino acid concentration as well as NR. For Alcalase, the increase in ratio up to 1:3 significantly increased both α-amino acid concentration and NR ($p < 0.05$). However, no significant changes were observed at the ratio

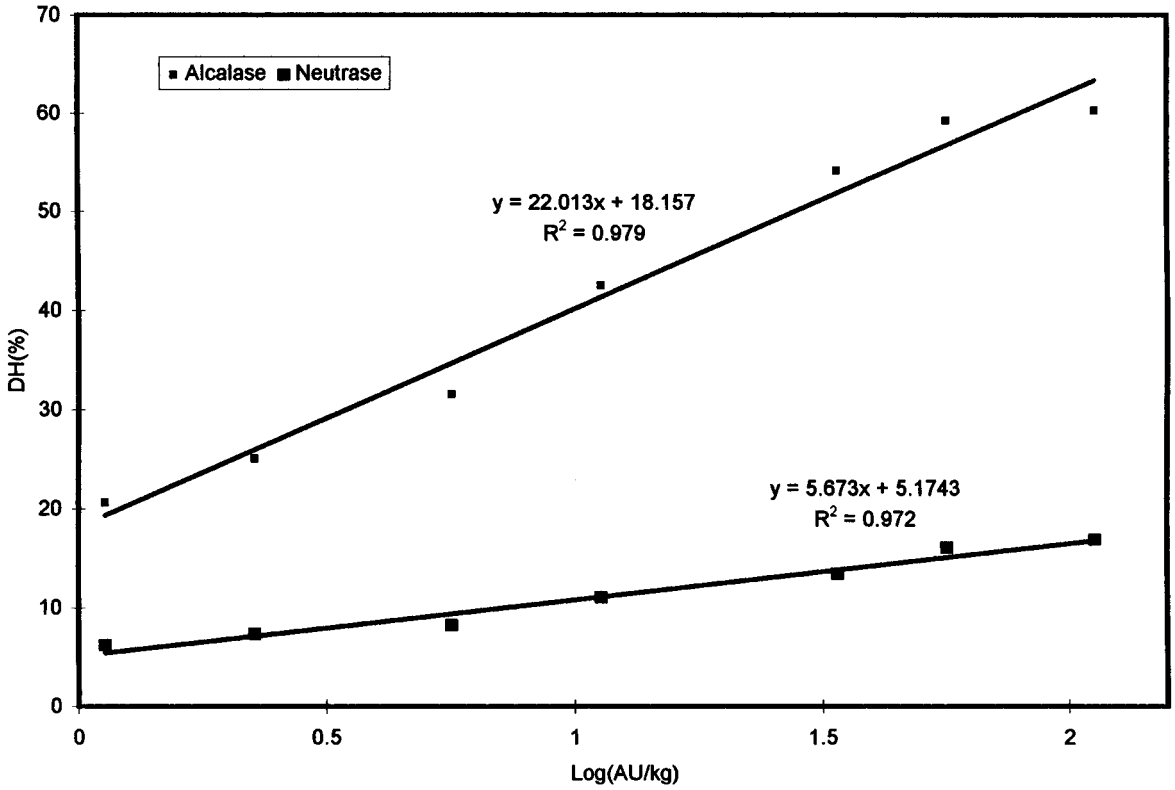

Figure 7. Relation between $\log_{10}$(enzyme concentration) and DH for PWSW treated with Alcalase or Neutrase. Different amounts of enzyme were added to the suspension of PWSW in buffer (1:2 ratio, w/v). The reaction was run for 30 min at pH 9.5, 60 °C and pH 7.0, 55 °C for Alcalase and Neutrase, respectively.

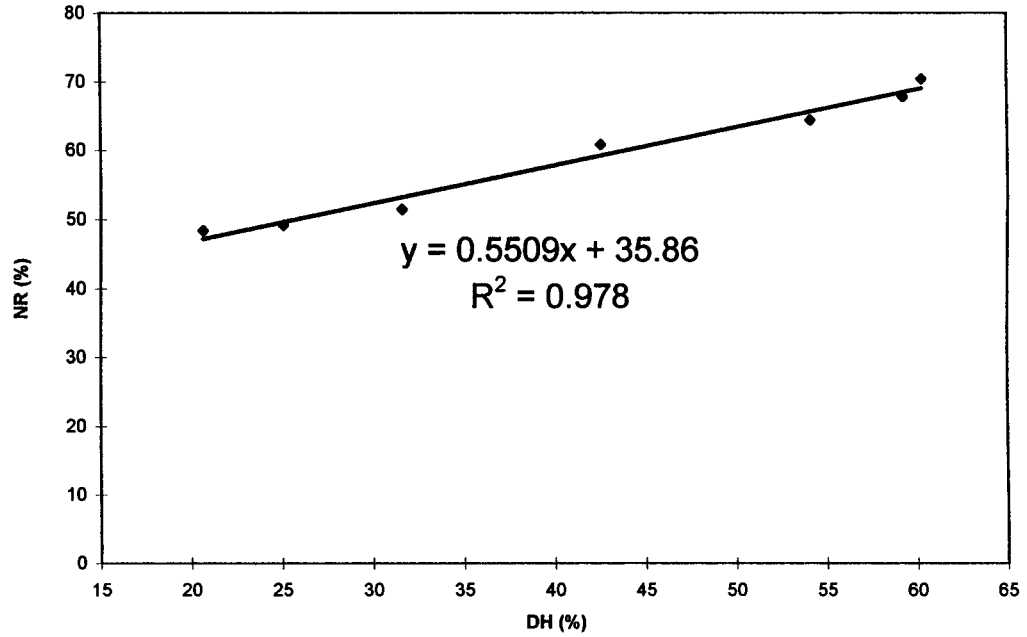

Figure 8. Relation between DH and NR for PWSW treated with Alcalase. The hydrolytic reaction was run for 30 min at pH 9.5, 60 °C with PWSW:buffer ratio of 1:2 (w/v).

higher than 1:3 ($p > 0.05$). For Neutrase, the ratio above 1:1 did not cause a significant increase in NR ($p > 0.05$). Sufficient buffer provided buffering capacity for the reaction, worked as media for enzyme dispersion and was considered as an important factor for PWSW hydrolysis. From these results, waste/buffer ratio of 1:2 and 1:3 (w/v) was sufficient for enzymatic reaction. Increased water added to substrate enhanced enzyme homogeneity, promoted tissue swelling, and reduced the localized concentration of hydrolysis products (Surowka and Fik, 1994). This result was in accordance with Surowka and Fik (1992, 1994), who applied pepsin and neutrase to recover the proteinaceous substances from chicken heads and found that an increase in the ratio of added water resulted in an increase in nonprotein nitrogen.

Optimum Condition for PWSW Hydrolysis by Alcalase. Due to the high activity of Alcalase in PWSW hydrolysis, it was chosen for hydrolysate optimization tests. In these tests, yield is represented by NR instead of α-amino acid concentration. All three parameters including enzyme concentration, waste/buffer ratio, and

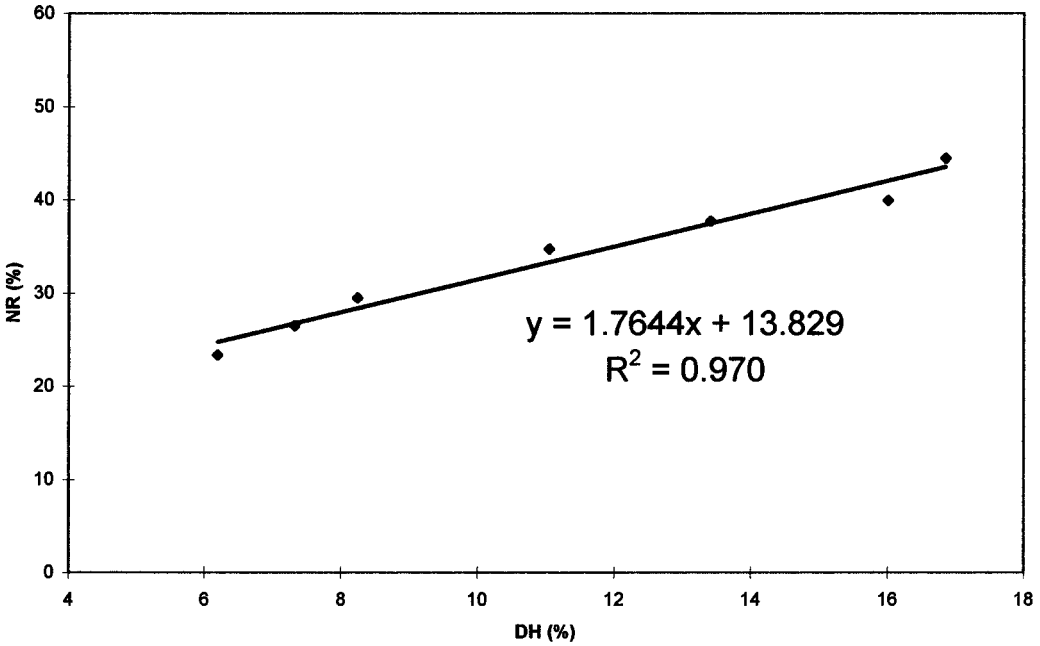

Figure 9. Relation between DH and NR for PWSW treated with Neutrase. The hydrolytic reaction was run for 30 min at pH 7.0, 55 °C with PWSW:buffer ratio of 1:2 (w/v).

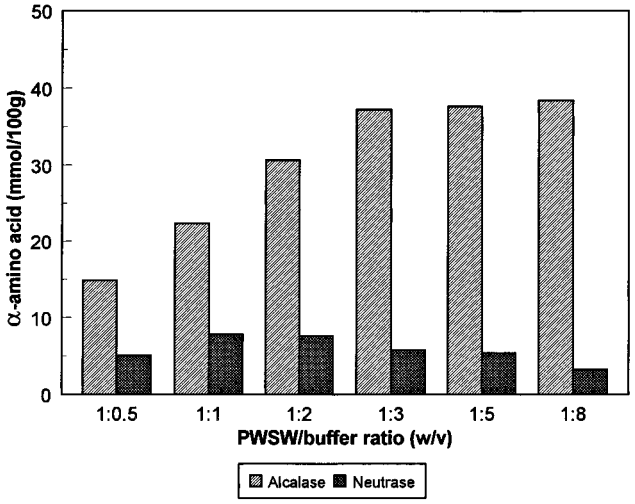

Figure 10. Effect of waste:buffer ratio on hydrolysis of PWSW treated with Alcalase or Neutrase. PWSW was mixed with buffer at different ratios. Enzyme (20AU/kg) was added and reaction was run for 30 min at pH 9.5, 60 °C and pH 7.0, 55 °C for Alcalase and Neutrase, respectively. The hydrolysis rate was expressed as α-amino acid (mmol/100 g sample).

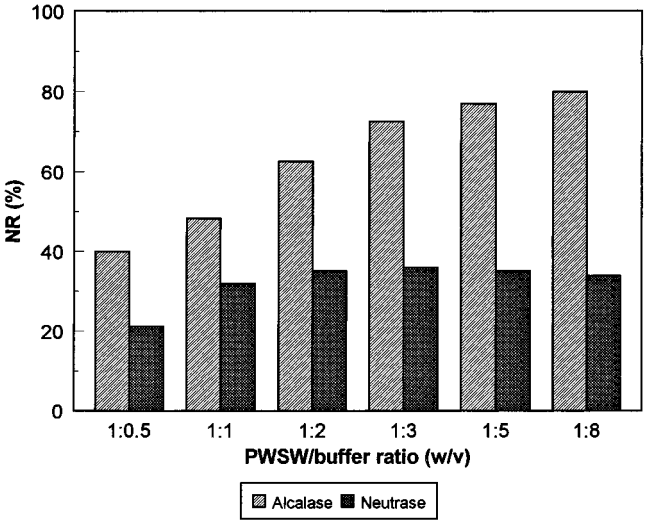

Figure 11. Effect of waste:buffer ratio on NR for PWSW treated with Alcalase or Neutrase. PWSW was mixed with buffer at different ratios. Enzyme (20AU/kg) was added and the reaction was run for 30 min at pH 9.5, 60 °C and pH 7.0, 55 °C for Alcalase and Neutrase, respectively.

reaction time significantly affected NR ($p < 0.05$) (Figure 12). Enzyme concentration showed an interaction with waste/buffer ratio and time ($p < 0.05$). To minimize the cost of enzyme and energy for water removal to produce dry hydrolysate as well as to reduce the reaction time, the conditions for enzymic hydrolysis of PWSW with Alcalase 2.4L were selected as pH 9.5, 60 °C, 1 h reaction time, waste:0.2 M borate buffer ratio of 1:1 (w/v), and 20 AU/kg waste.

Compositions of PWSW Hydrolysate. The freeze-dried hydrolysate, PWSW, and whole Pacific whiting muscle contained 2.77, 81.10, and 84.62% moisture, respectively. On dry weight basis, freeze-dried hydrolysate contained higher protein content but lower ash and lipid content than PWSW (Table 1). The high protein content was a result of the solubilization of protein during hydrolysis, the removal of insoluble undigested non-protein substances, and partial removal of lipid after hydrolysis. The inorganic substances reported in the hydrolysate were possibly due to the addition of borate buffer during the enzymatic reaction. However, inorganic compounds were lower than PWSW. The main source of minerals in PWSW was the bone and scale which were ground together with the whole waste. Removal of the fat layer after hydrolysis caused a low lipid content in hydrolysate. During the hydrolysis process, the muscle cell membranes tend to round up and form insoluble vesicles, leading to the removal of membrane structured lipids (Shahidi et al., 1995). Reduced lipid content was reported in harp seal (Shahidi et al., 1994), capelin (Shahidi et al., 1995), and shark hydrolysates (Onodenalore and Shahidi, 1996). Hoyle and Merritt (1994) reported hydrolysate preparation with a low lipid content by using the ethanol-extracted herring. For Pacific whiting muscle, a considerably high protein content with a low ash and lipid content were observed compared with PWSW and freeze-dried hydrolysate.

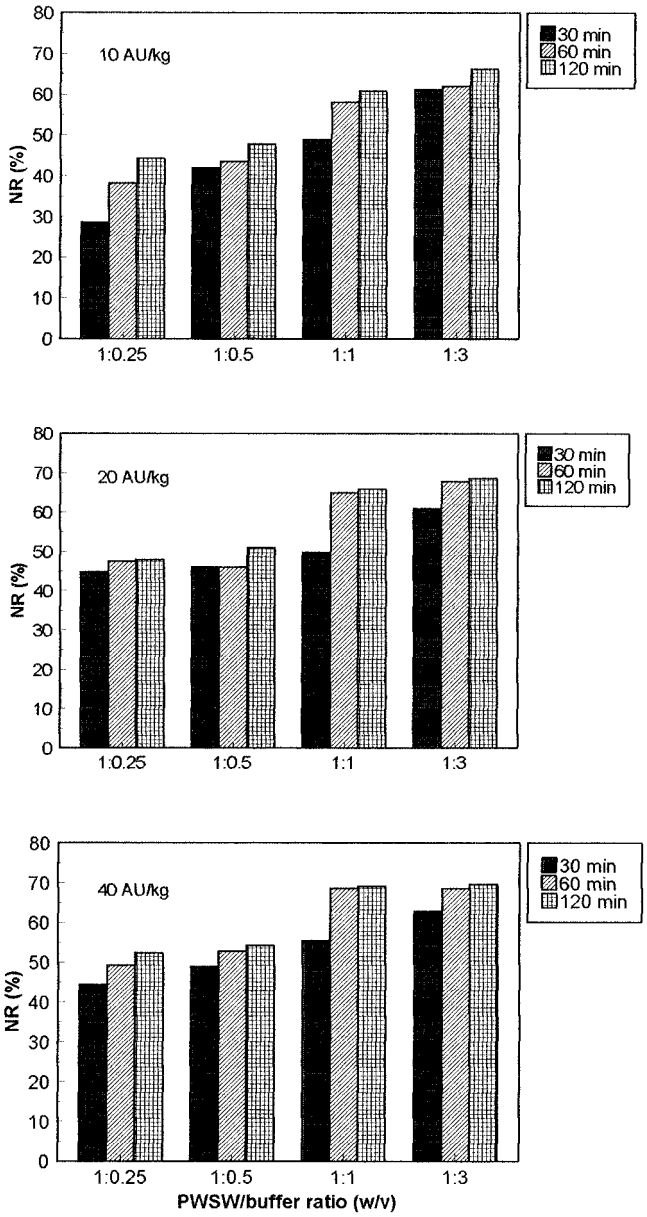

Figure 12. Effect of enzyme concentration, reaction time, and waste/buffer ratio on NR for PWSW treated with Alcalase. The hydrolytic reaction was run at pH 9.5, 60 °C under various conditions.

Table 1. Chemical Composition of Freeze-Dried Hydrolysate, PWSW, and Pacific Whiting Muscle[a]

compositions (% dry weight basis)	freeze-dried hydrolysate	PWSW	Pacific whiting muscle
protein	82.25 ± 0.05	69.36 ± 3.28	92.05 ± 3.97
lipid	3.94 ± 0.13	20.31 ± 0.74	2.53 ± 0.75
ash	13.82 ± 0.08	20.74 ± 4.34	6.50 ± 0.65

[a] Freeze-dried hydrolysate, PWSW, and Pacific whiting muscle contained 2.77 ± 0.12, 81.10 ± 0.29, and 84.62 ± 0.28% moisture, respectively.

Freeze-dried hydrolysate was brownish yellow in color (L^* 54.59, a^* 6.70, b^* 27.89) and lighter than PWSW, the dark color of which was probably due to the oxidation of myoglobin and the melanin pigment in the skin (L^* 45.94).

The amino acid compositions of freeze-dried hydrolysate were similar to those of PWSW and Pacific whiting muscle (Table 2). Freeze-dried hydrolysate contained a lower amount of glutamic acid and tryptophan than PWSW and the muscle. Tryptophan in hydrolysate was reduced to 14.74−21.5% of that in PWSW and muscle, respectively. Shahidi et al. (1995) reported that the tryptophan in capelin hydrolysate was reduced by approximately 60% when compared with the original muscle. Freeze-dried hydrolysate and PWSW had a similar amount of glycine which was approximately 2-fold higher than the muscle. Some differences in amino acid profile among PWSW, hydrolysate, and muscle were possibly due to the differences in protein composition. PWSW contained skin and other connective tissues, leading to the differences in amino acid composition from the muscle. From this result, freeze-dried hydrolysate contained a comparable amount of amino acids when compared with Pacific whiting muscle. In future studies, the functional properties of hydrolysate will be tested.

Table 2. Amino Acid Compositions of Freeze-Dried Hydrolysate, PWSW, and Pacific Whiting Muscle

amino acid compositions	% of total protein: freeze-dried hydrolysate	PWSW	Pacific whiting muscle
alanine	6.53	6.45	5.51
arginine	7.29	7.71	7.28
aspartic acid	10.10	9.72	10.50
cysteine	0.92	0.82	1.13
glutamic acid	13.80	18.50	15.00
glycine	7.88	8.09	3.87
histidine	2.10	2.19	2.35
isoleucine	4.30	4.28	4.97
leucine	7.16	7.08	8.05
lysine	8.33	8.19	10.20
methionine	3.02	2.96	3.20
phenylalanine	3.80	3.87	4.06
proline	6.00	6.25	4.04
serine	5.33	5.35	4.80
threonine	5.12	4.86	4.86
tryptophan	0.14	0.65	0.95
tyrosine	3.50	3.38	4.18
valine	4.72	4.61	4.94

Conclusion. Alcalase showed an efficient hydrolysis toward PWSW at pH 9.5 and 60 °C. Optimum conditions for PWSW hydrolysis were 20 AU/kg, 1 h reaction time, and waste:buffer ratio of 1:1 (w/v). The hydrolysate had a high protein content and an amino acid composition comparable to fish muscle.

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