Production of Seafood Flavor from Red Hake (*Urophycis chuss*) by Enzymatic Hydrolysis

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Protein hydrolysates were prepared as a natural flavor stock from the red hake (*Urophycis chuss*) headed−gutted (H&G) mince and frame mince using commercial enzymes, Flavourzyme and Savorase, at the natural pH of fish (6.8) and the water/fish ratio of 2:5. The addition of 1.5% NaCl and 0.4% STPP improved the flavor quality of the hydrolysate by masking bitterness and off-flavor. A 6 h hydrolysis of H&G mince with Flavourzyme yielded a hydrolysate of the highest acceptability. Hydrolysis increased the concentration of most free amino acids except Arg and His. Leu, Lys, and Arg were predominant free amino acids in the hydrolysates, whereas Leu and Arg were major ones in the cooking juice. The concentration of Glu responsible for umami taste was increased by 6−9 times upon hydrolysis. Hydrolysates contained higher percentages of free amino acids giving both umami and sweet tastes than did cooking juice.

Keywords: *Red hake; mince; Flavourzyme; hydrolysate; flavor*

INTRODUCTION

As part of our ongoing utilization effort, red hake (*Urophycis chuss*) was selected for production of white fish flavor because it is an underutilized fish species in northeastern and mid-Atlantic coasts and has a uniquely mild pleasant flavor with low fat (0.8%, wet basis) (Regenstein et al., 1980). Red hake has not been widely used for value-added products because of the undesirable development of rubbery texture during frozen storage (Gendron, 1980). The production of seafood flavor from red hake could broaden red hake utilization, and the use of filleting waste (frame mince) would potentiate a commercial prospect of flavor manufacturing from red hake.

The production of seafood flavors from the underutilized fish species through protein hydrolysis is somewhat challenging due to the difficulty of ensuring high organoleptic quality. The hydrolysis of protein often accompanies flavor defects such as bitterness and off-flavor along with the formation of desirable flavor (Kilara, 1985).

Flavor quality of hydrolysate depends on several parameters. The quality of raw material plays a critical role in determining flavor quality. Fatty fish species are not desirable because of their high susceptibility to lipid oxidation and the high cost of removing excess fat (Ritchie and Mackie, 1982). The extent of hydrolysis determines sensory quality and is dependent upon the specificity of protease, level of enzyme, water-to-substrate ratio, pH, and temperature.

Currently, several commercial proteases are available for the production of protein hydrolysates, and their optimum processing conditions are generally suggested by manufacturers. However, the selection of the suitable hydrolytic enzymes and the extent of hydrolysis need to be refined according to the nature of applications.

This study was conducted to determine optimum processing conditions for natural flavor extraction from red hake headed and gutted (H&G) mince and frame mince and to examine their flavor quality.

MATERIALS AND METHODS

Materials. Fresh red hake (*Urophycis chuss*) was obtained from the local seafood company (Narragansett, RI), headed and gutted, and deboned to prepare mince using a deboning machine (model 694, Baader Machineries, Germany). Frame mince, on the other hand, was prepared by running frames free of viscera through the same deboning machine. Prepared H&G mince and frame mince were vacuum packed in 100 g portions and stored in the freezer (−20 °C) until hydrolysis.

Enzymes. The two most effective commercial enzymes were selected on the basis of the degree of hydrolysis (DH) and the quality of flavor generated. They were Flavourzyme MG (a declared activity of 1000 LAPU/g, Novo Nordisk Bioindustrials, Franklinton, NC) and Savorase M (a declared activity of 300 LAPU, Imperial Biotechnology U.S., St. Louis, MO). Both were of food grade and contained endo-and exopeptidases.

Chemicals. Food grade salt (Morton International Inc., Chicago, IL) and sodium tripolyphosphate (STPP) (Monsanto Co., St. Louis, MO) were used. *o*-Phthaldialdehyde (OPA) and an internal standard for amino acid analysis were obtained from Sigma Chemical Co. (St. Louis, MO). Sample diluent and eluents A and B for Pico Tag amino acid analysis were purchased from Waters Millipore Co. (Bedford, MA). All other chemicals used were of analytical or HPLC grade and obtained from Fisher Scientific (Pittsburgh, PA).

Preparation of Fish Mince Hydrolysates and Flavor Control. Frozen fish mince (100 g) was thawed overnight in the refrigerator (4 °C), transferred into a 600 mL digestion vessel, and brought to 50 °C prior to addition of enzyme at 0.3% (w/w) on a mince weight basis. This level was obtained on the basis of the manufacturer's recommendation, namely, 2% (w/w) on a sample protein weight basis. The enzyme was slowly added to the mince with gentle agitation at various amounts of added water. The pH was not adjusted for hydrolysis because the initial pH of the mince (6.8) was in the optimum pH range for both enzymes (Flavourzyme, 5−7; Savorase, 6−8). To one of the batches were added 1.5% NaCl and 0.4% STPP 30 min after the addition of the enzyme. This

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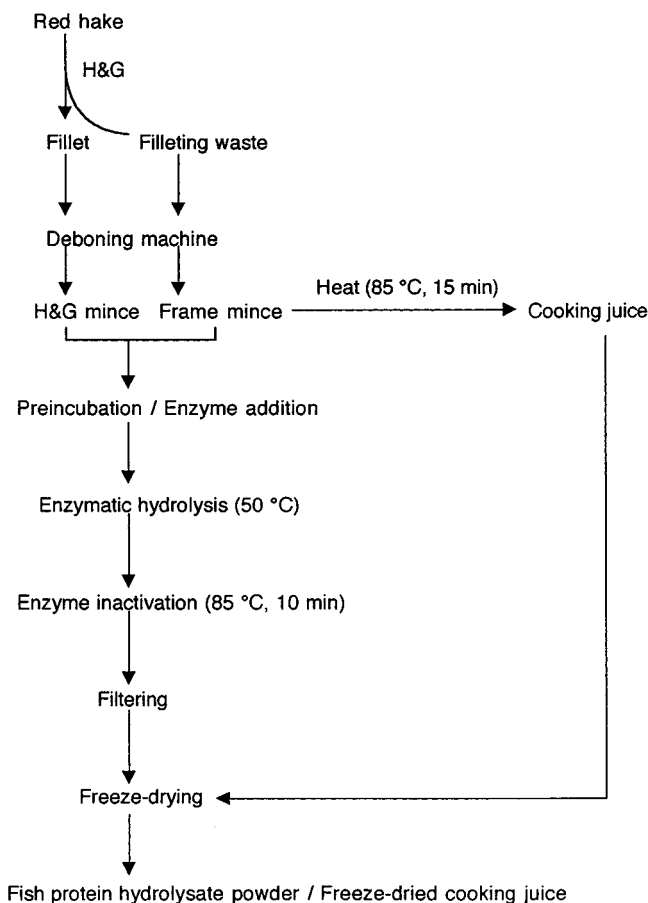
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Red hake

H&G

Fillet   Filleting waste

Deboning machine

H&G mince   Frame mince —— Heat (85 °C, 15 min) ——▶ Cooking juice

Preincubation / Enzyme addition

Enzymatic hydrolysis (50 °C)

Enzyme inactivation (85 °C, 10 min)

Filtering

Freeze-drying ◀——

Fish protein hydrolysate powder / Freeze-dried cooking juice

Figure 1. Preparation of protein hydrolysate from red hake H&G mince and frame mince.

1.5% salt level was chosen for its optimum sensory saltiness, which was determined by our preliminary trial; 0.4% STPP was used to reduce off-flavor and prevent oxidation during storage as suggested by Matlock et al. (1984). After hydrolysis with moderate agitation (~100 rpm) for a specified time using a propeller (7.6 cm diameter) attached to the heavy-duty stirrer (Cole-Parmer Instrument Co., Chicago, IL), the enzyme was inactivated by raising the temperature from 50 to 85 °C and holding for 10 min with constant stirring. The hydrolysate was filtered through two layers of cheesecloth (grade 50, Fisher Scientific) and freeze-dried. The fraction of hydrolysate that passed through the cheesecloth is referred to as "hydrolysate" in the following sections. Two control samples were prepared by freeze-drying cooking juice collected upon cooking H&G and frame mince at 85 °C for 15 min, respectively. The schematic flow diagram of the process is shown in Figure 1.

For the measurement of pH change during hydrolysis, the mince was dispersed in the equal amount of deionized water. After the temperature of sample was equilibrated at 50 °C, the initial pH was measured. The pH change was monitored every hour using an Accument pH meter (Fisher Scientific).

Effect of Endogenous Protease on DH. To determine the role of endogenous protease on protein hydrolysis, fish mince was subjected to the same hydrolysis condition without enzyme added. Therefore, any changes in the amount of hydrolysate protein content or DH would have been a result of endogenous enzyme activity. To verify the presence of endogenous protease activity, one batch of mince was heated to 85 °C and held for 10 min prior to hydrolysis while the other batch was not heated before determination of hydrolysate protein and DH.

Effect of Water/Mince Ratio on DH and Yield. The effect of different amounts of water addition on DH and yield of hydrolysates was examined using Savorase, as it is highly hydrolytic. Water was added to the mince before hydrolysis at 20, 40, 60, 80, and 100% (w/w) of the mince weight,

respectively. DH at 1, 3, and 6 h and yield at 6 h of hydrolysis were determined.

DH. DH is defined as the percent ratio of the number of peptide bonds broken to the total number of bonds per unit weight. An OPA method described by Petersen et al. (1995) was used to determine DH.

Proximate Composition and Yields of Hydrolysates. Moisture, ash, protein, and lipid contents in H&G mince, frame mince, and freeze-dried hydrolysates were determined using the AOAC (1984) methods with some modifications. For the crude protein ($N \times 6.25$) analysis, an ammonium ion electrode (Orion model 290A, Fisher Scientific) was used for the determination of N after digestion (Pivarnik et al., 1998).

The yield of freeze-dried hydrolysate was determined as follows:

$$\text{yield (\%)} = (\text{weight of hydrolysate solids/} \atop \text{weight of fish mince solids}) \times 100$$

Free Amino Acid Composition. Sample preparation for free amino acids followed the method of Sekiwa et al. (1997) with some modifications. Free amino acids were analyzed by HPLC (Perkin-Elmer, Norwalk, CT) using an amino acid analyzer column (Waters Pico Tag, 3.9 mm × 15 cm), and the analytical procedure followed the manual provided by Waters (1986). Aliquots of liquid hydrolysates and cooking juice (control) were subjected to 7.5% (w/w) TCA precipitation, followed by centrifugation at 30000g for 15 min. The supernatants were filtered through a 0.2 μm syringe filter (Whatman Inc., Clifton, NJ) before injection.

Sensory Evaluation. Sensory evaluation of hydrolysates was conducted by the eight-member panel from a pool of graduate students and faculty members in the Department of Food Science and Nutrition. The panel was composed of four female and four male individuals. The panelists were instructed to detect basic flavor attributes such as bitterness and umami taste, and individuals who were unfamiliar with or lack the ability to discern those flavor notes were excluded during the course of three trial sessions. The panelists also had an experience in evaluation of similar products such as lobster flavor and other seafood products on a regular basis. The 15 point line scale with 5 word anchors was used. "poor"−"very good" was used for the degree of liking, whereas "not at all"−"very strong" was for the determination of intensity. Hydrolysate solutions (3%, w/w) were prepared by dissolving freeze-dried hydrolysate in water (20 °C) and served to panelists. Four different attributes including liking as fish chowder flavor, off-flavor (muddy, cardboardy, or painty flavor), bitterness, and umami taste (meaty or MSG-like taste) were evaluated. Overall liking was also evaluated using a 9 point hedonic scale.

For the evaluation, two hydrolysates prepared by 6 h Flavourzyme hydrolysis and 3 h Savorase hydrolysis were used. The selected hydrolysis times were found to show the best flavor quality among those tested ranging from 3 to 8 h. Three samples consisting of control and two different hydrolysates were evaluated at a time and served in random order. For the same level of saltiness, the same amount of salt and STPP were added to the control and hydrolysate samples.

Statistical Analysis. The sensory data were analyzed by one-way ANOVA using SYSTAT v. 5.2 (SYSTAT, 1992). Paired t test was used to examine the difference in proximate compositions between H&G mince and frame mince and between hydrolysates. All hydrolysis experiments and composition analyses were repeated at least once. The significance of difference was set at $p < 0.05$.

RESULTS AND DISCUSSION

pH Change during Hydrolysis. When the pH change in the mince as a function of hydrolysis time was monitored, the initial pH was around 6.80 and dropped to 6.27 and 6.12 for Flavourzyme and Savorase hydrolysates, respectively, after 6 h of hydrolysis (Figure 2).
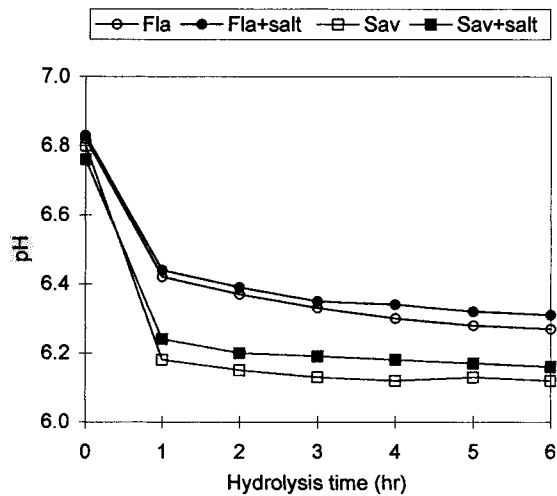
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Figure 2. Changes in pH during hydrolysis in two different enzyme systems. H&G mince was dispersed in the equal amount of water, and enzyme was added at 2% (w/w, protein weight). Salt mixture (1.5% NaCl + 0.4% STPP) was added after 30 min of hydrolysis based on the initial weight of the mince. Fla, Flavourzyme; Fla+salt, Flavourzyme containing salt mixture; Sav, Savorase; Sav+salt, Savorase containing salt mixture.

Major pH drops occurred within 1 h in both enzyme systems, followed by slight decreases with further hydrolysis. Peterson (1981) reported that dissociation of the α-amino group increased when hydrolysis was done at pH >6.5. The increase in the number of protonated α-amino groups by the cleavage of peptide bond results in decreased pH because carboxyl groups are not readily protonated at this pH range.

Pommer (1995) found that an enzyme complex of fungal origin such as Flavourzyme has a better hydrolysis efficiency in natural drifting pH than controlled pH condition for hydrolyzing soy protein isolate. Therefore, an enzyme system such as Flavourzyme would allow optimal hydrolysis of fish mince without pH adjustment. Furthermore, the natural pH system is more practical in a large scale process. Rebeca et al. (1991) reported that hydrolysis of mullet (*Mugil cephalus*) using bacterial protease with pH control resulted in faster fish protein hydrolysis in the first 2 h, but there was no difference in the soluble nitrogen content between uncontrolled and controlled pH systems at 3 h of hydrolysis.

When the salt mixture was added, pH remained slightly higher in both enzyme systems, but the pattern of pH change was similar to that without a salt mixture.

Effect of Endogenous Protease on DH of Mince. Mince without enzyme did not liquefy even after 6 h of incubation at 50 °C, whereas complete liquefaction occurred within 30 min with Flavourzyme or Savorase at a 2% (w/w) level. The protein content in the filtered hydrolysate with endogenous enzymes was slightly higher for unheated H&G mince or frame mince than heated ones (Figure 3), but it was still less than half of that in the hydrolysate prepared with added exogenous enzymes (data not shown). At an early stage of hydrolysis, protein content in the hydrolysate mainly reflected the protein content of water soluble proteins. An increase in the protein content with time was due to further solubilization of hydrolyzed proteins by endogenous enzymes. The lower protein content of heated mince or frame mince might be partly due to the limited amount of water soluble protein content released into
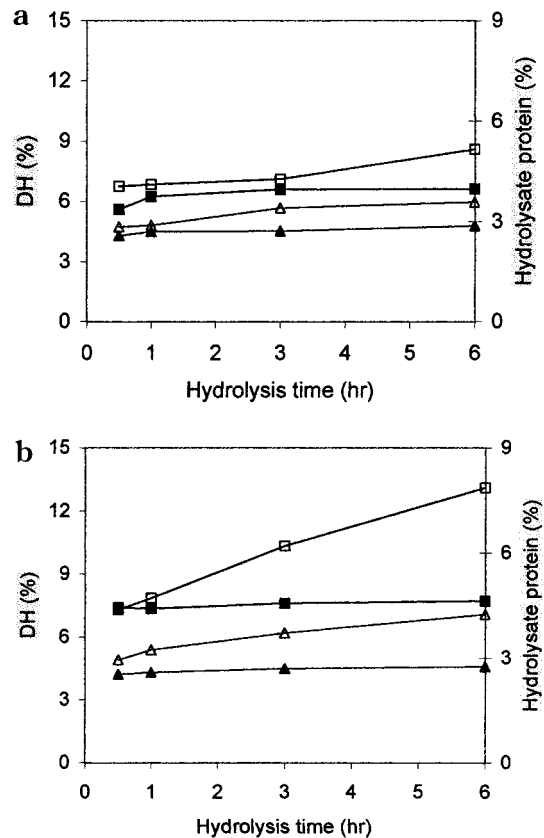

Figure 3. (a) Changes in DH and hydrolysate protein contents by endogenous enzyme in H&G mince: (□) DH of unheated H&G mince; (■) DH of heated H&G mince; (△) hydrolysate protein content of unheated H&G mince; (▲) hydrolysate protein content of heated H&G mince. H&G mince was dispersed in the equal amount of water. Heat treatment of the mince was done at 85 °C for 10 min prior to addition of an equal amount of water. (b) Changes in DH and hydrolysate protein contents by endogenous enzyme in frame mince: (□) DH of unheated frame mince; (■) DH of heated frame mince; (△) hydrolysate protein content of unheated frame mince; (▲) hydrolysate protein content of heated frame mince. Frame mince was dispersed in the equal amount of water. Heat treatment of the mince was done at 85 °C for 10 min prior to addition of an equal amount of water.

solution. Mohr (1980) reported that a considerable proportion of water soluble proteins in fish was not easily solubilized by heating. The extent of hydrolysis by endogenous protease appeared to be insignificant in H&G mince until 3 h, but a slightly higher DH was observed with extended incubation time. The difference in DH between unheated and heated frame mince increased with time, and the DH of unheated frame mince was 2 times higher than that of heated frame mince after 6 h of incubation. This result suggests that frame mince contains higher endogenous protease activity than H&G mince, although its endogenous protease activity was not enough to liquefy the sample alone. Shahidi et al. (1995) suggested that predigestion of fish mince prior to the addition of exogenous enzyme may increase the yield of hydrolysate.

Effect of Water/Fish Ratio on DH and Yield. The optimum amount of water for stirring needs to be determined. Typically, an equal amount of water is added to samples (Yu and Tan, 1990; Martin and Porter, 1995), but a low water/mince ratio can be more desirable because a lower drying cost is required. As shown in Figures 4 and 5, DH was not substantially changed by the ratio, whereas higher hydrolysate protein content
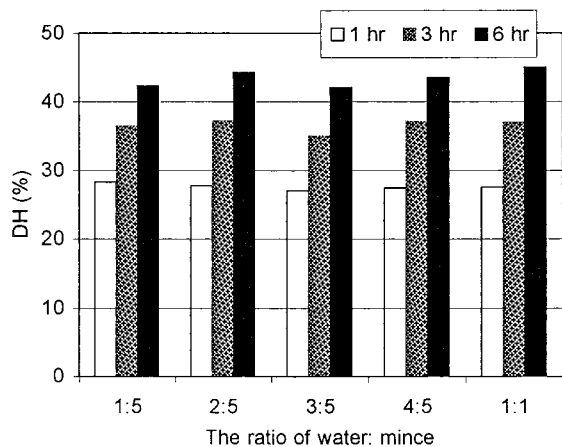
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Figure 4. Effect of water/H&G mince ratio on DH. Water was added to the H&G mince at the ratios (water/H&G mince, w/w) of 1:5, 2:5, 3:5, 4:5, and 1:1. Savorase was added at 2% (w/w, protein weight).

Figure 5. Effect of water/H&G mince ratio on hydrolysate protein content. Water was added to the H&G mince at the ratios (water/H&G mince, w/w) of 1:5, 2:5, 3:5, 4:5, and 1:1. Savorase was added at 2% (w/w, protein weight).
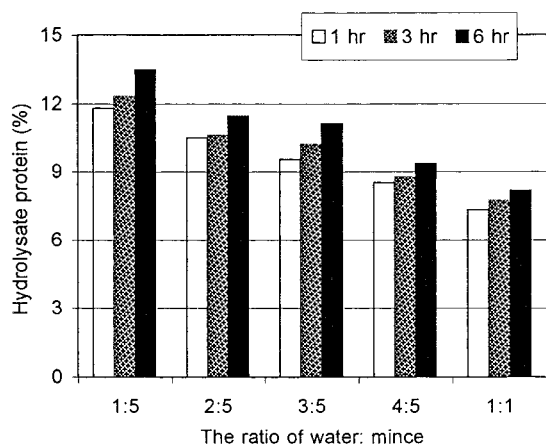
was found at lower water/mince ratios. A lower protein content at the higher water/mince ratio was probably caused by the dilution of substrate. Surowka and Fik (1994) suggested that a greater amount of water could have a beneficial effect by increasing enzyme distribution and reducing localized concentration of hydrolysis products, but no obvious DH change with different water/mince ratios was observed under the tested conditions. The ratio of water/mince did not much influence the extent of hydrolysis, but the amount of water to be removed is of practical importance as reported by Raghunath (1993).

At the 1:5 water/mince ratio, the liquid hydrolysate presented a problem with filtering due to the high viscosity. This difficulty was also reflected in the yield by showing a realtively lower yield compared to other ratios. The yield (solid recovery after hydrolysis and cheesecloth filtration) tended to increase with increasing water level (Figure 6). This could be a result of increased water soluble proteins and filtration facilitated by water because there was no increase in DH with higher ratios. The yield of hydrolysate after 6 h of hydrolysis was in the range from 70 to 79% on a solid weight basis (13.5−15.2% based on the initial mince weight). This range was close to the typical yield of fish protein hydrolysate (10−15%) reported by Quaglia and Orban (1990) and Rebeca et al. (1991). Among ratios studied, 2:5 (water/
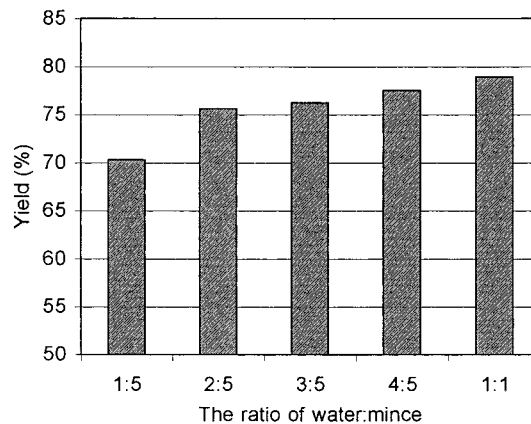

Figure 6. Effect of water/H&G mince ratio on yield of hydrolysates. Water was added to the H&G mince at the ratios (water/H&G mince, w/w) of 1:5, 2:5, 3:5, 4:5, and 1:1. Savorase was added at 2% (w/w, protein weight). After 6 h of hydrolysis, the yield was calculated by using the following equation: yield (%) = (weight of hydrolysate solids/weight of fish mince solids) × 100.
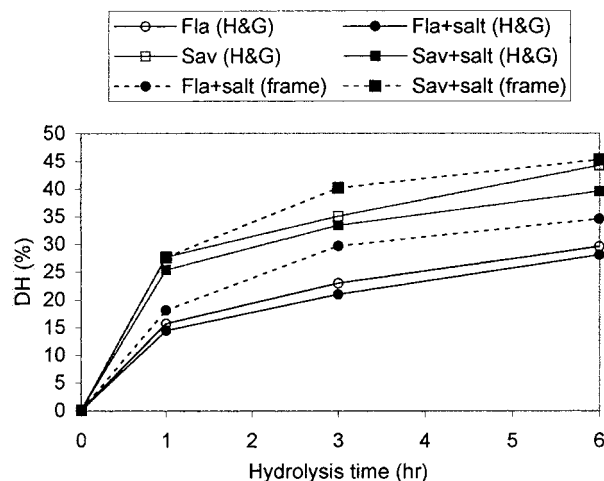

Figure 7. Changes in DH for H&G mince and frame mince treated with Flavourzyme or Savorase. Water/mince ratio of 2:5 and 2% (w/w, protein weight) of each enzyme were used for hydrolysis. Salt mixture (1.5% NaCl + 0.4% STPP) was added after 30 min of hydrolysis.

mince) was chosen and used for further testing because this ratio might be cost-effective in the dehydration process without an appreciable yield loss.

Changes in DH by Flavourzyme and Savorase Enzyme Systems. When the hydrolytic abilities of the two chosen enzymes were compared at the same levels, Savorase yielded a higher DH than Flavourzyme (Figure 7). The DH curve as a function of time appears to be similar to those of other fish protein hydrolysate preparations (Diniz and Martin, 1996; Shahidi et al., 1995). Within 1 h, DH reached more than half the level of the 6 h hydrolysis. Flavourzyme showed high tolerance to salt without a decrease in DH, whereas Savorase showed ~10% decrease in DH in the presence of salt.

Proximate Composition. The proximate compositions of H&G mince, frame mince, and corresponding freeze-dried hydrolysates are shown in Table 1. There was a significant difference in protein content between H&G mince and frame mince on a wet weight basis but not on a solid weight basis. This was due to the difference in moisture content, not to a difference in tissue protein content. Except for ash content, no

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Table 1. Proximate Composition of H&G Mince, Frame Mince of Red Hake, and Corresponding Hydrolysates Prepared by Two Different Enzymes[a]

| | | | freeze-dried hydrolysate | | | |
| | | | H&G mince | | frame mince | |
composition[a] (%)	H&G mince	frame mince	Fla[b]	Sav[c]	Fla	Sav
moisture	83.02 ± 0.30[d]	84.39 ± 0.37[d]	1.71 ± 0.22[e]	1.87 ± 0.07[e]	5.92 ± 0.31[f]	5.33 ± 0.35[f]
protein	15.54 ± 0.12[d]	14.18 ± 0.05[e]	77.18 ± 0.54[f]	78.47 ± 0.61[f]	72.06 ± 0.51[g]	72.26 ± 0.07[g]
lipid	1.44 ± 0.16[d]	1.38 ± 0.02[d]	6.10 ± 0.28[e]	6.44 ± 0.04[e]	5.08 ± 0.44[f]	5.20 ± 0.24[f]
ash	1.09 ± 0.10[d]	1.00 ± 0.06[d]	13.61 ± 0.05[e]	12.43 ± 0.29[f]	15.75 ± 0.02[g]	15.27 ± 0.20[g]

[a] Wet weight basis. [b] Freeze-dried hydrolysate made with Flavourzyme for 6 h hydrolysis. [c] Freeze-dried hydrolysate made with Savorase for 3 h hydrolysis. [d–g] Values are means and standard deviations of triplicate measurements. Means with different letters in a row are significantly different ($p < 0.05$).

Table 2. Free Amino Acids Composition of H&G Mince, Frame Mince of Red Hake, and Corresponding Hydrolysates Prepared by Two Different Enzymes

			free amino acids[a] (mg/100 mL)			
			hydrolysate			
			H&G mince		frame mince	
amino acid	H&G mince control[b]	frame mince control	Fla[c]	Sav[d]	Fla	Sav
alanine	6.09 (3.17)	7.19 (3.19)	20.95 (3.63)	19.89 (3.00)	27.43 (3.37)	24.39 (3.08)
arginine	58.32 (30.38)	66.51 (29.53)	54.56 (9.45)	59.93 (9.04)	52.49 (6.46)	58.07 (7.34)
aspartic acid	3.24 (1.69)	1.66 (0.74)	15.50 (2.68)	8.54 (1.29)	24.98 (3.07)	15.57 (1.97)
cystine	0.08 (0.04)	0.09 (0.04)	2.64 (0.46)	5.39 (0.81)	2.51 (0.31)	5.21 (0.66)
glutamic acid	3.24 (1.69)	5.40 (2.40)	29.25 (5.06)	20.98 (3.16)	47.64 (5.86)	34.04 (4.30)
glycine	3.12 (1.63)	4.43 (1.97)	17.34 (3.00)	9.82 (1.48)	27.20 (3.35)	13.87 (1.75)
histidine	15.21 (7.92)	12.13 (5.39)	14.88 (2.58)	15.82 (2.39)	9.20 (1.13)	14.07 (1.78)
isoleucine	0.86 (0.45)	1.57 (0.70)	20.46 (3.54)	20.34 (3.07)	36.64 (4.51)	30.74 (3.88)
leucine	53.76 (28.00)	71.71 (31.84)	134.35 (23.26)	141.40 (21.32)	185.88 (22.87)	186.20 (23.53)
lysine	15.02 (7.82)	15.77 (7.00)	66.82 (11.57)	118.85 (17.92)	98.66 (12.14)	110.29 (13.94)
methionine	2.15 (1.12)	1.38 (0.61)	28.89 (5.00)	33.86 (5.10)	36.10 (4.44)	38.52 (4.87)
phenylalanine	15.24 (7.94)	17.94 (7.96)	24.25 (4.20)	45.63 (6.88)	45.54 (5.60)	55.89 (7.06)
proline	6.74 (3.51)	8.15 (3.62)	37.54 (6.50)	22.03 (3.32)	61.23 (7.53)	32.60 (4.12)
serine	2.54 (1.32)	3.29 (1.46)	22.93 (3.97)	10.71 (1.61)	35.31 (4.34)	15.01 (1.90)
threonine	3.37 (1.76)	3.87 (1.72)	32.29 (5.59)	64.50 (9.72)	43.95 (5.41)	74.26 (9.38)
tyrosine	2.03 (1.06)	2.87 (1.27)	20.68 (3.58)	37.63 (5.67)	31.78 (3.91)	42.47 (5.37)
valine	0.99 (0.52)	1.28 (0.57)	34.30 (5.94)	27.97 (4.22)	46.34 (5.70)	40.26 (5.09)

[a] Results are means of quadruplicate measurements. Free amino acids were analyzed from the supernatant of control and hydrolysates after 7.5% TCA precipitation followed by centrifugation at 30000g for 15 min; percent distribution in parentheses. [b] Cooking juice. [c] Hydrolysate made with Flavourzyme for 6 h hydrolysis. [d] Hydrolysate made with Savorase for 3 h hydrolysis.

differences were found in the compositions of hydrolysates prepared from the same raw material by the two enzymes.

The ash contents of hydrolysates were higher than those of typical fish protein hydrolysates (6–7%, solid weight basis) made from nonfatty species (Mackie, 1982). The higher ash content was due to the addition of salt mixture during hydrolysate preparation.

Composition of Free Amino Acids. The amino acid composition was hardly changed by hydrolysis except for some loss of sulfur-containing amino acids such as Cys and Met depending on the hydrolysis conditions (Mackie, 1982). However, after hydrolysis, the composition of free amino acids will change because the enzyme cleaves peptide bonds and liberates free amino acids and small peptides. Free amino acid composition is more meaningful than the composition of muscle tissue amino acids because free amino acids are active primary flavor components in the hydrolysate. Thus, the distribution and relative amounts of active taste components affect the flavor quality (Fuke, 1994).

No marked difference was found in the free amino acid composition between H&G mince and frame mince controls (cooking juice) (Table 2). Leu and Arg were predominant free amino acids in H&G mince and frame mince controls. The concentrations of most free amino acids were increased upon hydrolysis except for those of Arg and His, which barely changed. Overall, frame

mince released more free amino acids than H&G mince except for Arg, Cys, and His. The concentration of umami taste giving Glu was found to be 6–9 times higher in hydrolysates than in the unhydrolyzed control. According to Fuke (1994), primary taste active free amino acids responsible for umami and sweetness are Glu, Met, Ser, and Ala and Thr, Gly, Ser, and Pro, respectively. On the basis of the percent distribution, hydrolysates contained higher concentrations of free amino acids giving both umami and sweet tastes than did cooking juice. Hydrolysates scored higher in the umami taste intensity without salt mixture added. However, when salt mixture was added, no difference in umami taste was detected between control and hydrolysates (Tables 3 and 4).

Although free amino acids can impart more than one basic taste, individual amino acids are usually represented by one predominant basic taste. Among these, hydrophobic L-amino acids such as Arg, Leu, Phe, and Val mainly contribute to bitterness (Fuke, 1994). The greater concentrations of Leu, Phe, and Val were found in frame mince hydrolysates. Kato et al. (1989) reported that threshold values of bitterness for Arg, Leu, Phe, and Val were 50, 190, 90, and 40 mg/100 mL, respectively. These threshold values were close to the concentrations of each amino acid present in frame mince hydrolysate. A relatively higher sensory bitterness score (Table 2) found in frame mince hydrolysates might be

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Table 3. Sensory Scores (Given by Eight Panelists; Means with Standard Deviations in Parentheses) of Hydrolysates Prepared from H&G and Frame Mince of Red Hake by Two Different Enzymes

| | H&G mince | | | | | | frame mince | | |
| | unsalted | | | salt mixture added[a] | | | salt mixture added | | |
attribute	Con[b]	Fla[c]	Sav[d]	Con[e]	Fla	Sav	Con	Fla	Sav
fish chowder flavor[f]	7.35^{gh}	9.30^{h}	6.16^{g}	10.36^{g}	11.01^{g}	9.76^{g}	10.29^{g}	9.24^{g}	7.09^{h}
	(1.39)	(1.83)	(2.36)	(2.64)	(1.90)	(1.70)	(2.66)	(2.85)	(2.45)
off-flavor	5.00^{g}	2.43^{g}	4.07^{g}	2.36^{g}	1.65^{g}	2.46^{g}	2.10^{g}	2.40^{g}	3.11^{g}
	(4.20)	(1.07)	(3.66)	(1.72)	(1.42)	(2.09)	(1.55)	(1.63)	(2.14)
bitterness	2.40^{g}	2.39^{g}	3.95^{g}	1.34^{g}	1.44^{g}	2.45^{g}	1.15^{g}	2.13^{g}	4.93^{h}
	(2.43)	(2.05)	(3.12)	(1.17)	(1.26)	(1.42)	(1.48)	(2.22)	(3.23)
umami	5.30^{g}	6.46^{g}	6.60^{g}	7.45^{g}	7.34^{g}	6.38^{g}	5.83^{g}	5.96^{g}	5.03^{g}
	(2.99)	(2.60)	(2.83)	(3.54)	(2.96)	(1.96)	(2.59)	(2.30)	(1.88)
overall liking	4.75^{g}	6.38^{h}	4.63^{g}	7.00^{g}	6.75^{g}	6.25^{g}	6.75^{g}	6.38^{g}	5.13^{g}
	(1.28)	(0.92)	(2.26)	(0.76)	(1.16)	(1.58)	(1.16)	(0.92)	(1.64)

[a] Salt (1.5%) and STPP (0.4%) mixture was added after 30 min of hydrolysis with either enzyme. [b] Freeze-dried cooking juice. [c] Freeze-dried hydrolysate prepared with Flavourzyme for 6 h hydrolysis. [d] Freeze-dried hydrolysate prepared with Savorase for 3 h hydrolysis. [e] Same level of salt mixture was added to freeze-dried cooking juice. [f] Liking as fish chowder flavor. [g,h] Means with different letters in a row within the same group are significantly different ($p < 0.05$).

Table 4. Sensory Scores (Given by Eight Panelists; Means with Standard Deviations in Parentheses) of Hydrolysate and Unhydrolyzed Frame Mince

attribute	hydrolysate[a]	unhydrolyzed frame mince[b]
fish chowder flavor[c]	10.90 (2.02)[d]	6.78 (2.29)[f]
off-flavor	1.76 (1.53)[d]	3.03 (3.35)[d]
bitterness	3.14 (3.31)[d]	2.80 (3.51)[d]
umami	8.73 (3.15)[d]	3.73 (2.74)[f]
overall liking	7.13 (0.64)[d]	4.25 (1.16)[f]

[a] Frame mince hydrolysate prepared with Flavourzyme 6 h hydrolysis. Salt (1.5%) was added after 30 min of hydrolysis. [b] Unhydrolyzed frame mince was prepared according to the same procedure as hydrolysate preparation except enzyme addition. [c] Liking as fish chowder flavor. [d–f] Means with different letters in a row are significantly different ($p < 0.05$).

related to the higher concentration of hydrophobic free amino acids. Flavourzyme hydrolysates contained more Pro and Ser, but less Lys and Thr, than Savorase hydrolysates.

Sensory Evaluation of Hydrolysates. Without salt, the Flavourzyme hydrolysate had a significantly higher overall liking score than the control or the Savorase hydrolysate (Table 3). With respect to fishy flavor, the Savorase hydrolysate had a higher intensity than the Flavourzyme hydrolysate, and this might be a reason for higher off-flavor and lower overall liking scores for the Savorase hydrolysate. Small molecular weight nonprotein compounds present in fish mince could be responsible for typical off-flavors. Enzymatic hydrolysis of soy protein was reported to liberate off-flavors (Petersen, 1981).

Gillete (1985) reported that the addition of sodium chloride enhanced fullness and balance of perception, whereas it decreased bitterness and off-flavor note. Phosphate ions might contribute to the perception of umami taste and saltiness (Hayashi et al., 1981).

When salt mixture was added, the unhydrolyzed H&G mince control (cooking juice) did not show significant difference in overall liking. As for the frame mince, the Savorase hydrolysate had significantly lower scores in liking as fish chowder flavor and bitterness than the control and the Flavourzyme hydrolysate.

The Savorase frame mince hydrolysate, which showed a relatively higher bitterness score, had a higher DH (40.7%) compared to the Flavourzyme frame mince hydrolysate (35.8%) (Figure 7). Barzana and Garcia-Garibay (1994) suggested that the intensity of bitterness depends on DH and protease specificity because hydro-

phobic amino acids responsible for bitterness can be liberated by endopeptidase. Therefore, with an increase in DH, more hydrophobic amino acid could be generated from interior peptide chains, resulting in increased bitterness as in the case of Savorase.

All hydrolysate solutions had a slightly chalky mouth-feel, which may have affected the overall liking. However, this will not be a problem in commercial products because the chalky mouthfeel can be easily masked by added ingredients.

The effect of enzymatic hydrolysis on flavor quality was examined by comparing sensory scores for frame mince hydrolysate and unhydrolyzed frame mine. As shown in Table 4, the hydrolysate received significantly higher scores in overall liking than the unhydrolyzed frame mince. The hydrolysate had a much higher umami taste score than unhydrolyzed frame mince, but there were no significant differences in off-flavor and bitterness between them. Therefore, desirable flavor quality and fullness could be obtained by controlled enzymatic hydrolysis.

Conclusions. Protein hydrolysate prepared from red hake has a great potential as a fish flavorant and can be used as a flavor supplement and sauce for various seafood products such as fish chowder. The hydrolysate prepared from frame mince had a flavor quality comparable to that of H&G mince hydrolysate, suggesting that a good quality fish flavor can be produced from unutilized frame waste. The sensory quality of hydrolysates was improved by the addition of salt and STPP.

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