Evaluation of Bitterness in Enzymatic Hydrolysates of Soy Protein Isolate by Taste Dilution Analysis

W.H. SEO, H.G. LEE, AND H.H. BAEK

ABSTRACT: Although enzymatic hydrolysates of soy protein isolate (SPI) have physiological functionality, partially hydrolyzed SPI exhibits bitter taste depending on proteases and degree of hydrolysis (DH). To determine proteolysis conditions for SPI, it is important to evaluate bitterness during enzymatic hydrolysis. Taste dilution analysis (TDA) has been developed for the screening technique of taste-active compounds in foods. The objectives of the present study were to evaluate bitterness of enzyme-hydrolyzed SPI by TDA and to compare bitterness of SPI hydrolysates with respect to kinds of proteases and DH. SPI was hydrolyzed at 50 °C and pH 6.8 to 7.1 to obtain various DH with commercial proteases (flavourzyme, alcalase, neutrase, protamex, papain, and bromelain) at E/S ratios of 0.5%, 1%, and 2%. The DH of enzymatic hydrolysates was measured by trinitrobenzenesulfonic acid method. The bitterness of enzymatic hydrolysates was evaluated by TDA, which is based on threshold detection in serially diluted samples. Taste dilution (TD) factor was defined as the dilution at which a taste difference between the diluted sample and 2 blanks could be detected. As DH increased, the bitterness increased for all proteases evaluated. Alcalase showed the highest TD factor at the same DH, followed by neutrase. Flavourzyme showed the lowest TD factor at the entire DH ranges. At the DH of 10%, TD factor of hydrolysate by flavourzyme was 0 whereas those by protamex and alcalase were 4 and 16, respectively. These results suggest that TDA could be applied for the alternative of bitterness evaluation to the hedonic scale sensory evaluation.

Keywords: bitter taste, degree of hydrolysis, enzymatic hydrolysis, soy protein isolate, taste dilution analysis

Introduction

Soy protein isolate (SPI) is widely used in many foods as functional and nutritional ingredients and has been applied in the food industry (Sung and others 2006). Many studies have demonstrated that the enzymatic hydrolysis of SPI improved its functional properties, including solubility and foaming as well as emulsifying and gelation properties (Kang 1984; Jung and others 2005; Tsumura and others 2005). Also, enzyme-hydrolyzed vegetable proteins including SPI were reported to have biologically active properties. It has been reported that an SPI diet brings various health benefits such as a cholesterol-lowering effect (Bakhit and others 1994), reduction of plasma triglyceride levels (Iritani and others 1996), antiobesity effect (Aoyama and others 2000), and anticarcinogenesis effect (Messina and others 1994).

However, some proteins, especially whey, casein, and soy protein, produce bitter taste by hydrolysis process. Partially hydrolyzed SPI results in bitter taste due to the formation of low molecular weight peptides composed of hydrophobic amino acids (Matoba and Hata 1972). They also reported that bitter taste was related to the average hydrophobicity of protein and the position of hydrophobic amino acid in the peptide. Ney (1971) suggested that the degree of hydrophobicity was the most important indicator of bitterness of peptide and established the Q rule. Kim and others (1992) studied that different types of enzymes were tested for their bitterness formation during controlled partial hydrolysis of soy protein. The bitterness of hydrolyzates was varied with the type of enzyme used. Alcalase resulted in the highest bitterness, whereas neutrase and trypsin gave a relatively low level of bitterness. Cho and others (2004) fractionated bitter peptides from commercially available soy protein hydrolysates and reported that the bitterness was related to molecular mass.

Bitterness measurement is usually based on hedonic scale sensory evaluation. Sensory panels were trained to evaluate the taste intensity with standard solution such as caffeine and quinine HCl. Lim (1996) estimated the bitterness from *Ixeris dentate* Nkai by 9-point hedonic scale sensory evaluation. Johnson and Vickers (2004) evaluated the bitterness of caffeine in cream cheese by a 150-mm unstructured line scale. The bitterness intensities of β-casein and soy protein hydrolysates were evaluated by hedonic measurement (Kukman and others 1996; Singh and others 2005).

Recently, taste dilution analysis (TDA) was developed for a novel screening procedure of taste-active compounds (Frank and others 2001). This technique is based on the determination of the relative taste thresholds of compounds in serial dilutions of sample. The concept of dilution analysis was originally proposed by Tilgner (1962, 1965), who described a technique called flavor dilution profile for characterizing flavor sensations of a product. Dilution index, which is percent dilution at recognition threshold, was established to express the overall intensity of flavor of foods and beverages. This idea has been elaborated to TDA by Frank and others (2001). The TDA method was one of the dilution analysis techniques such as aroma extract dilution analysis (AEDA) (Grosch 1993) and color dilution analysis (Hofmann 1998). This method has been applied for the identification of the most

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intense taste compounds and taste impact compounds in foods. The most intense bitter taste of Maillard mixture has been identified as 3-(2-furyl)-8-[(2-furyl)methyl]-4-hydroxymethyl-1-oxo-1*H*,4*H*-quinolizinium-7-olate by TDA (Frank and others 2001). Ottinger and others (2003) discovered Maillard derived sweetness enhancer by TDA and determined its structure. Ottinger and others (2001) identified natural "cooling" compounds from glucose and L-proline in dark malt by TDA. Sequential application of solvent extraction, gel permeation chromatography, and HPLC in combination with TDA revealed that a multiplicity of bitter tastants contribute to the bitter off-taste of cold-stored carrots and commercial carrot puree (Czepa and Hofmann 2003). Recently, Lopez and others (2007) applied TDA to characterize wine taste.

The objectives of the present study were to evaluate bitterness of enzyme-hydrolyzed SPI by TDA and to compare bitterness of SPI hydrolysates with respect to kinds of proteases and degree of hydrolysis.

Materials and Methods

Materials

A commercial SPI was purchased from a local supplier and used as protein substrates. Of the 6 commercial proteases used for hydrolysis of SPI, flavourzyme, alcalase, neutrase, and protamex were obtained from Novo Nordisk Co. (Bagsvaerd, Denmark), and bromelain and papain were from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Trinitrobenzenesulfonic acid (TNBS), L-tyrosine, L-leucine, and casein were purchased from Sigma Chemical Co.

Protease activity

For measurement of protease activity, 2% casein solution was used as substrate. The casein solution was placed in a 10-mL test tube and mixed with protease solution (10 mg/mL). These solutions were allowed to stand at room temperature for 20 min and immediately transferred into test tubes containing 2 mL of 0.3 M trichloroacetic acid solution. These solutions were filtered through Whatman Nr 40 filter paper. One milliliter of filtrate was mixed with 5 mL of 0.5 N NaOH solution and 1 mL of 1 N Folin–Ciocalteu's phenol reagent (Sigma Chemical Co.). The solution was mixed immediately and incubated at 30 °C for 15 min. The solution absorbance was measured at 578 nm by spectrophotometer (Shimadzu UV-1201, Tokyo, Japan). L-tyrosine solutions (10, 20, 30, 50, 100 μg/mL) were used for standard curve.

Enzymatic hydrolysis of SPI

SPI was hydrolyzed by commercial proteases. Two hundred milliliters of SPI solutions (10% [w/v]) were placed in a 250 mL jacketed reaction vessel and preincubated at 50 °C for 30 min without pH adjustment before addition of proteases (pH 6.8 to 7.1). The enzyme to substrate ratio (E/S) was 0.5%, 1.0%, and 2.0%. Proteases were added to the vessel, and the mixture was vigorously stirred during reaction using a magnetic stirrer. Hydrolysates were removed at time intervals of 5, 10, 20, 30, 45, 60, 90, 120, and 180 min. After the hydrolysis, the enzymes were inactivated by heat treatment at 100 °C for 10 min.

Determination of degree of hydrolysis (DH)

Degree of hydrolysis (DH) was determined using the TNBS method according to Adler-Nissen (1986) and defined as follows:

$$\mathrm{DH} = (H/H_{\mathrm{tot}}) \times 100$$

H is hydrolysis equivalent, defined as concentration in milliequivalents/g of protein of α-amino group formed during hydrolysis, and H_{tot} is hydrolysis equivalent at complete hydrolysis to amino acid, calculated by summing the contents of the individual amino acids in 1 g of protein. H_{tot} value of 7.8 milliequivalents/g was used according to Adler-Nissen (1986). The TNBS method was carried out as follows: 0.25 mL of protein hydrolysate was mixed with 2 mL of sodium phosphate buffer (pH 8.0) and 0.1% TNBS, and then the solution was incubated at 50 °C for 60 min. The reaction was inactivated by adding 4 mL of 0.1 N HCl. The absorbance was measured at 340 nm by UV spectrophotometer (Shimadzu UV-1201, Tokyo, Japan). Six leucine solutions of 0.15 to 1.5 mM were used for standard curve.

Taste dilution analysis (TDA)

The bitterness of enzymatic hydrolysates was evaluated by the TDA method (Frank and others 2001). The TDA method was based on the determination of the relative taste thresholds of compounds in serial dilutions of samples. The enzymatic hydrolysates were diluted stepwise 1:1 with deodorized distilled water. Taste dilution (TD) factor was defined as the dilution at which a taste difference between the diluted sample and blanks could just be detected.

Sensory analyses

A sensory panel, which consisted of 10 graduate students, had been screened for bitterness sensitivity using a triangle test. The assessors were trained to evaluate the bitter taste of caffeine standard solution (1 mmol/L) by using a triangle test. The detection thresholds of bitterness were determined in a triangle test using 10% SPI solution as blank. The samples were presented in the order of high concentrations (serial 1:1 dilution) and evaluated for bitterness by a triangle test (1 dilute and 2 blanks [10% SPI solution]). Random 6 serving orders (AAB, ABA, BAA, ABB, BAB, and BBA) were presented for a triangle test. Triplicate analyses were performed on a triangle test by each trained panel.

Results and Discussion

Reaction progress curves

Table 1 shows protease activities used in enzymatic hydrolysis of SPI. Alcalase had the highest protease activity, followed by papain, bromelain, neutrase, flavourzyme, and protamex.

Figure 1 shows reaction progress curves for the 6 proteases used. All reaction progress curves exhibited an initial fast reaction rate followed by a slowing. The shape of these progress curves was similar to that reported for enzymatic hydrolysis of other protein sources: soy protein (Constantinides and Adu-Amankwa 1980; Netto and Galeazzi 1998); tuna waste (Guerad and others 2002); crayfish (Baek and Cadwallader 1995); veal bone (Linder and others 1996); and casein (Mannheim and Cheryan 1990). DH was dependent on the enzyme concentration, but addition of E/S 1 and 2% showed similar DH.

Table 1 — Protease activities using casein substrate.

Protease	Activity[a]
Alcalase	63.1
Papain	55.0
Bromelain	43.0
Neutrase	42.6
Flavourzyme	42.4
Protamex	41.0

[a]One unit was defined as the amount of L-tyrosine produced by 1 mg protease.

Protamex exhibited the highest DH, followed by alcalase, neutrase, bromelain, and papain. Flavourzyme showed the lowest DH. A rapid increase in DH took place during the initial 30 min of the reaction. The result was similar to previous reports (Kim and others 1997; Cho and Ahn 2002). Alcalase showed a very high rate of hydrolysis, DH increased by 15% to 18% at 60 min of reaction time, and a final DH up to 20% to 23% was achieved in 3 h of reaction time. Flavourzyme presented the lowest initial hydrolysis rate and a gradual increase in DH during 60 min. A final DH was 14% to 23% at 3 h. Cho and Ahn (2002) reported that flavourzyme showed a gradual increase in DH, while the initial rate was slow. Protamex presented the highest DH and reaction rate among other proteases. It showed the highest initial rate of hydrolysis, DH increased by 18% to 22% at 60 min, and 22% to 28% DH was achieved in the final reaction time. Neutrase, papain, and bromelain showed a similar hydrolysis tendency. They showed a rapid increase in DH during initial 30 min of the reaction, and final DH was 18% to 22%. In the case of neutrase, papain, and bromelain, Kim and others (1992, 1997) concluded that the hydrolysis reaction occurred rapidly during the first 30 min of the reaction, but there was not much difference in the DH between 1 and 3 h of reaction time. SPI with various DH could be obtained by controlling hydrolysis parameters such as reaction time and E/S.

Constantinides and Adu-Amankwa (1980) mentioned that the decrease of reaction rate might be due to a decrease in specific peptide bonds available for enzyme reaction, product inhibition, and enzyme inactivation. Also, Adler-Nissen (1986) explained that the shape of the progress curve was a result of competition between the original substrate and the peptides being constantly formed during hydrolysis. Archer and others (1973) suggested that enzymes adsorbed onto an insoluble protein in the initial reaction, degraded polypeptide chains loosely bound to the surface, and reacted on the more compact protein more slowly.

Evaluation of bitterness by TDA

Many studies in the past have focused on the intensity and removal of bitterness of enzyme-hydrolyzed proteins such as SPI, whey, casein, fish, and meat by hedonic scale sensory evaluation. The intensities of bitterness of β-casein and soy protein hydrolysates were measured by hedonic test (Kukman and others 1996; Singh and others 2005). Normally, bitterness measurement is based on hedonic scale sensory evaluation. The sensory panels

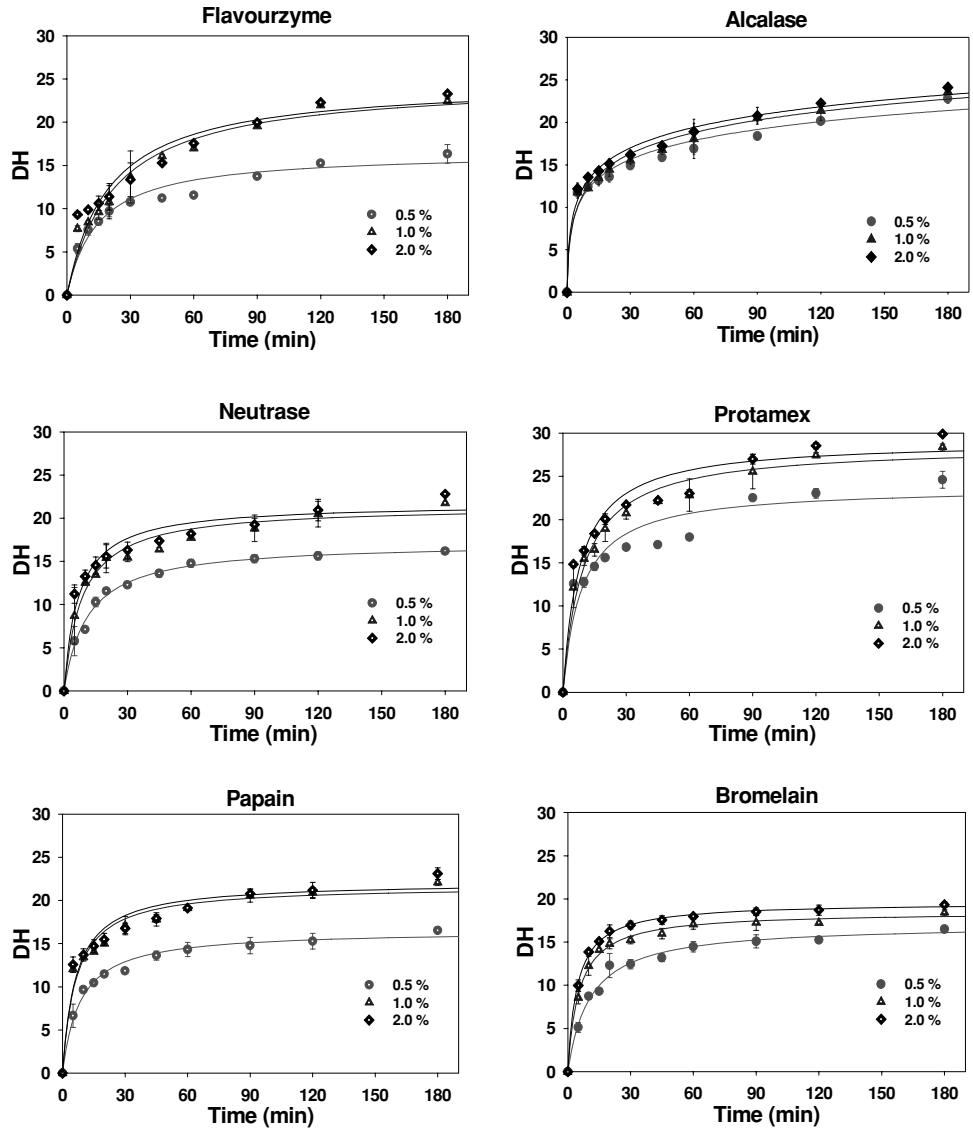

Figure 1—Reaction progress curves for enzymatic hydrolysis of SPI by proteases.

were trained to evaluate the taste intensity with standard solution such as caffeine and quinine HCl.

Recently, TDA based on sample dilution analysis has been developed for a novel screening procedure of taste-active compounds (Frank and others 2001). The TDA method was one of the dilution analysis techniques such as aroma extract dilution analysis (AEDA) (Grosch 1993) and color dilution analysis (Hofmann 1998). TDA had been developed for the screening technique of taste-active compounds and evaluation of bitterness, sweetness, and off-taste in foods (Frank and others 2001; Ottinger and others 2001, 2003; Czepa and Hofmann 2003). Therefore, TDA could be used to evaluate bitterness of enzyme-hydrolyzed SPI and to compare bitterness of SPI hydrolysates with respect to kinds of protease and DH.

Effect of kinds of protease on bitterness of SPI hydrolysates

The bitterness of enzyme-hydrolyzed SPI was evaluated by the TDA method. Figure 2 shows changes in TD factors during proteolysis with 0.5% E/S. TD factor was expressed as bitter intensity and defined as the dilution at which a bitterness difference between the diluted sample and blanks could just be detected.

As DH increased, the bitterness increased for all proteases except for flavourzyme. Alcalase showed the highest TD factor at the same DH, followed by neutrase, protamex, papain, and bromelain. Flavourzyme had the lowest. It was believed that bitterness of protein hydrolysates was controlled by kinds of protease and DH.

The bitterness of SPI hydrolysate by alcalase increased rapidly during hydrolysis. Even at the initial stage, alcalase produced hydrolysates with a high TD factor (= 16), and the final TD factor of hydrolysate was 64, which was the highest TD factor among the SPI hydrolysates in this study.

Flavourzyme showed the lowest TD factor at the entire DH ranges. Bitter taste was not detected from hydrolysates up to 12% DH. In spite of the increase in DH, the TD factor was 4 after 3 h of reaction time, which was the lowest TD factor among the hydrolysates with the same DH. Flavourzyme is a mixture of endo- and exoprotease, while the other proteases were endoproteases. Endoproteases with a broad specificity have a tendency of hydrolyzing at hydrophobic amino acid residues, leaving a nonpolar amino acid residue at the C-terminus of the peptide formed and leading to relatively high bitterness (Adler-Nissen 1986; Saha and Hayashi 2001). Bitterness of the peptides was reduced by removal of these amino acids. Exoproteases that selectively release N-terminal amino acid residues from protein can be used successfully to debitter protein hydrolysates (Saha and Hayashi 2001).

Neutrase, bromelain, papain, and protamex exhibited similar bitterness tendency. TD factor was increased to 16 at 60 min of

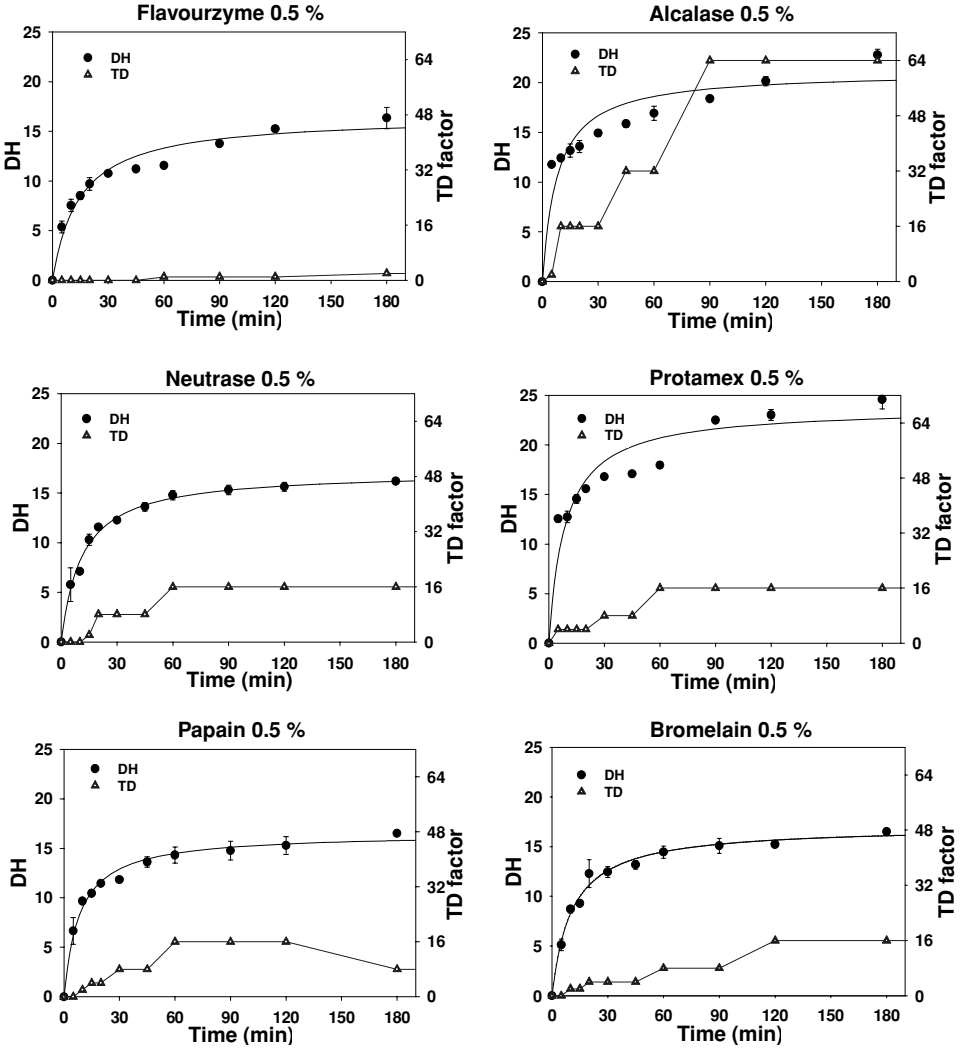

Figure 2—Changes in TD factors during proteolysis with 0.5% E/S.

reaction time and there was no much difference in TD factors after 60 min. However, papain decreased TD factor after 120 min. Protamex showed lower TD factor compared with relatively higher DH. TD factor of initial hydrolysate by protamex was 4 at 10% DH and final TD factor was 16 at 20% DH, whereas TD factor by alcalase was 64 at 20% DH.

Cho and others (2004) suggested that the bitterness of the soy peptides was predominantly associated with the medium molecular mass range peptides at 1000 to 4000 Da. Also, the bitterness intensity of the peptides depends on DH of their proteins. Ney (1971) suggested that the degree of hydrophobicity as number of hydrophobic amino acid residues (proline, leucine, and isoleucine) was the most important indicator of bitterness of peptide.

Effect of DH on bitterness of SPI hydrolysates

Figure 3 and 4 show TD factors of SPI hydrolysates with 10% and 15% DH by different proteases, respectively. At 10% DH, TD factor of SPI hydrolysate by flavourzyme was 0, whereas alcalase, protamex, papain, and bromelain were 16, 4, 4, and 4, respectively (Figure 3). Kim and others (1992) reported that alcalase gave the highest bitterness intensity at the same value of 10% DH followed by bromelain, papain, and neutrase. At 15% DH, TD factor of hydrolysate by flavourzyme was 2, whereas alcalase and bromelain were 64 and 16, respectively (Figure 4). It is suggested that kinds of protease and DH can alter the composition of amino acids and peptides during enzymatic hydrolysis, which results in different bitterness of hydrolysates. Kim and others (1997) reported that alcalase produced the highest content of hydrophobic amino acid in the peptide followed by bromelain, neutrase, and papain. Kukman and others (1996) suggested that the main reason for bitterness of soy protein hydrolysates prepared by alcalase was hydrophobic bitter peptides of relative molecular mass less than 1000.

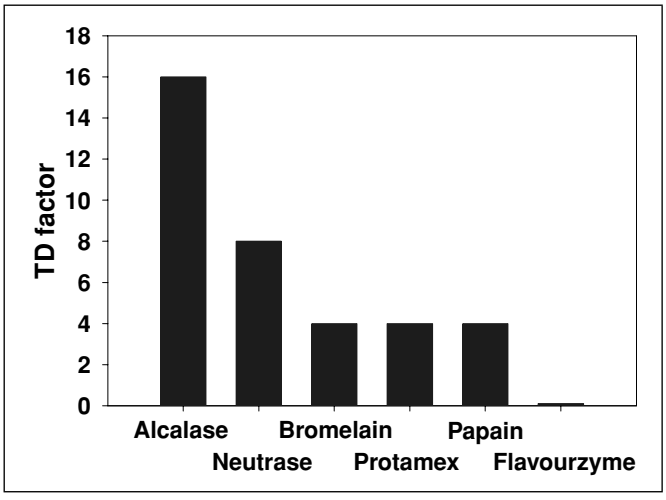

Figure 3—TD factors of SPI hydrolysates with 10% DH.

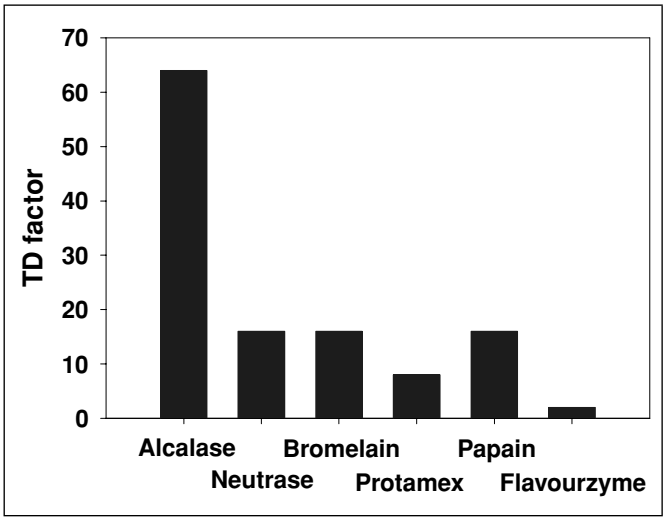

Figure 4—TD factors of SPI hydrolysates with 15% DH.

Conclusions

It was demonstrated that the TDA method could be applied for the simple and accurate method for evaluation of bitterness, which may be used as the alternative to hedonic scale sensory evaluation. This could be applied for the determination of DH with higher biological activity and less bitterness. Degree of hydrolysis was controlled by the parameters such as kinds of proteases, reaction time, and E/S. Flavourzyme showed the lowest TD factor at the entire DH range. Protamex showed a lower TD factor compared with a relatively higher DH.

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References

Adler-Nissen J. 1986. Enzymatic hydrolysis of food proteins. New York: Elsevier Applied Science Publishers.
Aoyama T, Fukui K, Takamatsu K, Hashimoto Y, Yamamoto T. 2000. Soy protein isolate and its hydrolysate reduce body fat of dietary obese rats and genetically obese mice (Yellow KK). Nutrition 16:349–54.
Archer MC, Ragnarsson JO, Tannenaum SR, Wang DIC. 1973. Enzymatic solubilization of an insoluble substrate, fish protein concentrate: process kinetic considerations. Biotechnol Bioeng 15:181–96.
Baek HH, Cadwallader KR. 1995. Enzymatic hydrolysis of crayfish processing byproducts. J Food Sci 60:929–35.
Bakhit RM, Klein BP, Essex-Sorlie D, Ham JO, Erdman JW, Potter SM. 1994. Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. J Nutr 124:213–22.
Cho HY, Ahn CB. 2002. Production and characteristics of protein hydrolysate from sharp toothed eel (*Muraenesox cinereus*). J Korean Fish Soc 35:97–104.
Cho MJ, Unklebay N, Hsieh FH, Clarke AD. 2004. Hydrophobicity of bitter peptides from soy protein hydrolysates. J Agric Food Chem 52:5895–901.
Constantinides A, Adu-Amankwa B. 1980. Enzymatic modification of vegetable protein: mechanisms, kinetics, and production of soluble and partially soluble protein in batch reactor. Biotechnol Bioeng 23:1543–65.
Czepa A, Hofmann T. 2003. Structural and sensory characterization of compounds contributing to the bitter off-taste of carrots (*Daucus carota* L.) and carrot puree. J Agric Food Chem 51:3865–78.
Frank O, Ottinger H, Hofmann T. 2001. Characterization of an intense bitter-tasting 1H,4H-quinolizinium-7-olate by application of the taste dilution analysis, a novel bioassay for the screening and identification of taste-active compounds in foods. J Agric Food Chem 49:231–8.
Grosch W. 1993. Detection of potent odorants in foods by aroma extract dilution analysis. Trends Food Sci Technol 4:68–73.
Guerad F, Guimas L, Binet A. 2002. Production of tuna waste hydrolysates by commercial neutral protease preparation. J Mol Catal B Enzymol 19:489–98.
Hofmann T. 1998. Characterization of the most intense colored compounds from Maillard reactions of pentoses by application of color dilution analysis. Carbohydr Res 313:203–13.
Iritani N, Hosomi H, Fukuda H, Tada K, Ikeda H. 1996. Soybean protein suppresses hepatic lipogenic enzyme gene expression in Wistar fatty rats. J Nutr 126:380–8.
Johnson EA, Vickers Z. 2004. The effectiveness of palate cleansing strategies for evaluating the bitterness of caffeine in cream cheese. Food Qual Prefer 15:311–6.
Jung S, Murphy PA, Johnson LA. 2005. Physicochemical and functional properties of soy protein substrates modified by low levels of protease hydrolysis. J Food Sci 70:180–7.
Kang YJ. 1984. Enzymatic modification of soy proteins: effects of functional properties of soy isolate upon proteolytic hydrolysis. Korean J Food Sci Technol 16:211–7.
Kim CH, Kim MR, Lee CH. 1992. Effect of type of enzyme on the bitterness of partial hydrolyzates of soybean protein. Food Biotech 1:79–84.
Kim CH, Kim MR, Lee CH. 1997. The bitterness of the enzymatic hydrolysate of soybean protein and the amino acid composition of the UF filtrate. Food Biotech 6:244–9.
Kukman IL, Zelenik M, Abram V. 1996. Bitterness intensity of soybean protein hydrolyzates. Z Lebensm Unters Forsch 203:272–6.
Lim SJ. 1996. A sensory evaluation of the bitter compounds from *Ixeris dentate* Nakai. Korean J Soc Food Sci 12:115–21.
Linder M, Fanni J, Parmentier M. 1996. Functional properties of veal bone hydrolysates. J Food Sci 61:712–20.

Lopez R, Mateo-Vivaracho L, Cacho J, Ferreira V. 2007. Optimization and validation of a taste dilution analysis to characterize wine taste. J Food Sci 72:345–51.
Mannheim A, Cheryan M. 1990. Continuous hydrolysis of milk protein in a membrane reactor. J Food Sci 55:381–5.
Matoba T, Hata H. 1972. Relationship between bitterness of peptides and their chemical structures. Agric Biol Chem 36:1423–31.
Messina MJ, Persky V, Setchell KD, Barnes S. 1994. Soy intake and cancer risk. Nutr Cancer 21:113–31.
Netto FM, Galeazzi MAM. 1998. Production and characterization of enzymatic hydrolysate from soy protein isolate. Lebensm Wiss Technol 31:624–31.
Ney KH. 1971. Prediction of bitterness of peptides from their amino acid composition. Z Lebensm Unters Forsch 147:64–8.
Ottinger H, Bareth A, Hofmann T. 2001. Characterization of natural "cooling" compounds formed from glucose and L-proline in dark malt by application of taste dilution analysis. J Agric Food Chem 49:1336–44.
Ottinger H, Soldo T, Hofmann T. 2003. Discovery and structure determination of a novel Maillard-derived sweetness enhancer by application of the comparative taste dilution analysis (cTDA). J Agric Food Chem 51:1035–41.
Saha BC, Hayashi K. 2001. Debittering of protein hydrolyzates. Biotech Adv 19:355–70.
Singh TK, Young ND, Drake M, Cadwallader KR. 2005. Production and sensory characterization of bitter peptide from β-casein. J Agric Food Chem 53: 1185–9.
Sung MJ, Park YS, Chang, HG. 2006. Quality characteristics of sponge cake supplemented with soy protein concentrate. Food Sci Biotechnol 15:860–5.
Tilgner DJ. 1962. Dilution tests for odor and flavor analysis. Food Technol 16:26–9.
Tilgner DJ. 1965. Flavor dilution profilograms. Food Technol 19:25–9.
Tsumura K, Saito T, Tsuge K, Ashida H, Kugimiya W, Inouye K. 2005. Functional properties of soy protein hydrolysates obtained by selective proteolysis. Lebensm Wiss Technol 38:255–61.