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Flavor characteristics of soy products modified by proteases and alpha-galactosidase

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Dedicated to Dr.Cheryll A. Reitmeier and Dr. Patricia A. Murphy,

for guidance and friendship through a wonderful journey

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ABSTRACT

Improved flavor characteristics of soy protein hydrolysates are needed so that soy protein ingredients with valuable properties can be used in food applications. The primary objective of this research was to evaluate the aroma and flavor alteration of soy protein hydrolysates using descriptive analysis (DA). Data were analyzed using analysis of variance and differences were determined ($p<0.05$). Sensory panelists (12) described differences in extruded-expelled soy flour (EESF) hydrolysates, particularly fishy, nutty, toasted, sweet, grassy beany, raw beany, bitter, and astringent attributes. Greater fishy aroma was detected in jet-cooked treatments of EESF compared to uncooked treatments. Control and Multifect-Neutral (MN)-modified EESF samples were more fishy than bromelain and Experimental Exopeptidase-C (EEC) treatments. For flavor attributes, bromelain hydrolysate was no different than the control. EEC and MN treatments had less sweetness and more intense grassy-beany flavor, astringency and bitterness than the control. MN-modified EESF was the most bitter and had the least nutty and toasted flavors among samples. A trained panel (10) evaluated sweetness and bitterness of EESF treated with MN and α-galactosidase (α-GAL). The non-digestible sugar in EESF hydrolyzed by α-galactosidase (α-GAL) increased sweetness and decreased bitterness of MN-modified EESF. α-GAL modification could be used to mask the bitterness of soy protein hydrolysates. Eleven trained panelists evaluated 3 protease-modified soy protein isolates (SPI) samples and un-hydrolyzed control with high- and low-protein dispersibility indices (PDI). High- and low-PDI SPIs were similar in most of the sensory attributes, but low-PDI SPI had more raw beany and bitter flavors than high-PDI SPI. SPI and soy flour hydrolyzed by MN were the most bitter and astringent and lowest in sweetness than other enzyme treatments. FPC-modified SPI had higher cooked beany flavor,

less bitter flavor and less astringency than MN-modified SPI and the control. MN+FPC treatment had the sensory attributes between the single enzyme treatments. “Cooked beany” was a single attribute defined by sensory panelists, but is likely a mixture of aromas and flavors attributed to by-products of nonenzymatic browning and fatty acid oxidation, volatile carbonyls from lipid oxidation and furans. Proteolysis modification reduced chalkiness and grittiness of soy protein, which would be a favorable attribute for beverages. Bitterness and astringency are the primary flavor problems of protein hydrolysates. Bromelain and FPC proteases produced soy protein hydrolysates with little bitterness and may be potential hydrolysates for food applications. The use of MN + α-GAL and the MN + FPC combinations produced less bitter soy hydrolysates than the MN treatment alone.

CHAPTER 1. GENERAL INTRODUCTION

INTRODUCTION

Sales of soy foods increased from $300 million to $3.9 billion from 1992 to 2004 due to consumers' interest in the health benefits of soy, according to Soyfoods Association of North America (SANA 2007). Soymilk sales increased from $652 million to $742 million (14%) from 2003 to 2004 and new soy food categories such as soy-based drinks, drinkable cultured soy, non-dairy frozen desserts, soy entrees, pastas and snack foods grew steadily in the market. A total of 479 new soy products were introduced in 2006 alone (SANA 2007). Despite the growth in soy foods popularity, soy flavor was recognized as an "off-flavor" by consumers in the United States and foods labeled as "containing soy" were perceived negatively for flavor (Wansink and Park 2002).

The soy food industry continues to be concerned about these undesirable flavors. Bitterness is a major problem of soy protein modified by enzymes to improve functional characteristics. Current knowledge of flavor alteration of enzymatic-hydrolyzed soy proteins is limited. Therefore, methods to improve the flavor characteristics of soy ingredients need to be investigated before soy foods will be widely accepted. In this thesis, flavor alteration of extruded-expelled soy flour and soy protein isolates with protease modifications, cooking methods and α-galactosidase treatment will be reviewed.

RESEARCH OBJECTIVES

The objectives of present studies were : 1) to describe the aroma and flavor attributes of unhydrolyzed extruded-expelled soy flour (EESF), EESF treated with an exopeptidase and two endopeptidases (bromelain and neutral protease) processed with and without jet-cooking; 2) to determine the sweetness and bitterness of neutral protease-modified EESF,

unhydrolyzed EESF and both of them treated with α-galactosidase; and 3) to characterize the sensory attributes of soy protein isolate and 3 soy protein isolate hydrolysates with high or low protein dispersibility indices (PDI).

THESIS ORGANIZATION

The thesis consists of 4 chapters and an Appendix. Chapter 1 provides a general introduction of the thesis, the research objectives, and a general literature review. The literature review includes topics related to nutritional benefits of soy, soy products, current trends of soy foods in market, sensory attributes of soy, and enzyme modification of soy. Chapter 2 presents the first study: Flavor attributes of extruded-expelled soy flour modified by proteases and α-galactosidase hydrolysis. Chapter 3 discusses the sensory attributes of protease-modified soy protein isolate with different protein dispersibility index. Both papers of Chapters 2 and 3 will be submitted to the Journal of Food Science. The overall conclusions of chapter 2 and 3, and possible direction for future research will be covered in Chapter 4.

Tables, figures and references are at the end of each chapter. Data obtained in this research but not reported in the previous chapters are presented in the Appendix.

LITERATURE REVIEW

Health benefits and nutritional claims

Whole soybeans contain 35-40% protein, 15-20% fat, 30% carbohydrates, 10-13% moisture, and 5% minerals and ash. Composition varies with variety and growing conditions. The carbohydrates include dietary fiber and about 10% sugars (stachyose, raffinose, and sucrose). Soybeans contain minerals such as potassium, sodium, calcium, magnesium, sulfur and phosphorus. The water-soluble vitamins in soybeans include thiamin, riboflavin, niacin,

pantothenic acid, biotin, folic acid, inositol and choline and the fat-soluble vitamins are vitamins E (Soyatech 2007). Fifty percent of the fat in soybeans is linoleic acid (a polyunsaturated fatty acid and an essential nutrient) and 8% of soybean oil is α-linolenic acid, an omega-3 fatty acid that may help reduce cholesterol and the risk of heart disease compared to saturated fats in the diet. (Soyatech 2007).

Today, the United States is the world's largest soybean producer, followed by China and Brazil (Li 2006). Soybean oil is the predominant vegetable oil source and contributes 80% of the edible oil in the United States (United Soybean Board 2007). The use of soy oil such as frying oil, ingredients in salad dressing and other foods contributes 12% of kcal to the diets of United States consumers (Li 2006).

In October 1999, the U.S. Food and Drug Administration (FDA) approved a health claim for products high in soy protein – "soy protein (25 g or more per day) may reduce the risk of heart disease in diets that are low in saturated fat and cholesterol" (Federal Register 1999). The food product must contain at least 6.25 g of soy protein per reference amount customarily consumed.

Soy protein is high in the essential amino acids isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, typrosine, threonine, tryptophan, valine and histadine (Zarkadas and others 1993). The World Health Organization (WHO) and the FDA adopted the protein digestibility corrected amino acid score (PDCAAS) method as the official assay for evaluating protein quality. Soybean proteins have a PDCAAS score of 1.0 and are comparable to animal protein sources such as milk (PDCAAS=1.0) and beef (PDCAAS=0.92) (Henley and Kuster 1994). Soy protein has been included as a protein source in enteral formulas for malnourished children who have absorption problems and

allergic reactions to cow's milk proteins. De Regil and de la Barca (2004) suggested that enzymatically modified soy protein could be considered to be an alternative to milk proteins for enteral formulas.

Khalil and others (2005) reported that soy is a rich source of isoflavones, a group of compounds classified as natural selective estrogen receptor modulators, and may be a potential therapy for age-related decline of bone mass and osteoporosis. There was a significant dose-dependent effect of soy isoflavones on attenuating bone loss at the spine and femoral neck in a study of the bone-sparing effect of isoflavones in Chinese postmenopausal women (Ye and others2006). A daily intake of two glasses of soymilk containing 76 mg total isoflavones prevented lumbar spine bone loss in postmenopausal women (Lydeking-Olsen and others 2004).

These positive effects of soy protein and isoflavones on bone health may be in part due to improved intestinal calcium absorption, similar to estrogen but without exerting a uterotrophic effect (Arjmandi and others 2002). In contrast, Lees and coworkers (1998) reported that a soy protein isolate diet alone did not prevent increased cortical bone turnover in ovariectomized macaques, but it increased bone turnover when compared to casein or lactalbumin-fed monkeys. Nagata and others (2002) reported that bone mineral density (BMD) in a study of 87 postmenopausal Japanese women was associated with endogenous estrogen levels but was not associated with soy or isoflavone intake and serum isoflavonoid levels.

Soy isoflavones may have anti-cancer properties. Stoll (1997) reported that soy proteins, as a major dietary component, were responsible for the incidence of breast cancer in Japanese and Chinese women being one-fifth that of Western women. Mukhopadhyay and

coworkers (2006) demonstrated by histological grading that soy protein protects against dimethylbenz[a]anthracene (DMBA) breast tumors in female rats. There is also evidence suggesting that men who consume a high amount of soy milk are at reduced risk for prostate cancer (Jacobsen and others 1998). In contrast, there was no association between soy food intake and breast cancer survival for 1459 breast cancer Chinese patients who commonly ate soy foods, and there was no evidence to indicate that soy foods were unsafe for breast cancer patients (Boyapati and others 2005). Aoyama and others (2000) reported that soy protein isolate (SPI) and its hydrolysate (SPI-H) reduced the body fat of dietary obese rats and genetically obese mice, and may be suitable protein sources in energy-restricted diets for the obesity treatment.

Soy products

In East Asia, soy is traditionally incorporated into entrées, desserts, snacks, beverages and a large variety of other foods. Imitation meat products made of soy have been important in the Chinese vegetarian cuisine for several hundred years. According to Fukushima (1999), the soy products that are traditional in East Asia are classified as unfermented and fermented soy products. The unfermented products include soymilk, tofu, tofu-related products such as yuba, toasted soybean and soybean powder, boiled green soybeans, and soy sprouts. The fermented products are soy sauce, miso, natto, tempeh, and sufu.

Tofu, soy sauce, soy milk and textured soy protein products have been incorporated into many cuisines around the world (Li 2006). Soy beverages such as fruit-flavored drinks, flavored soymilk, smoothies, and coffee drinks are widely available. Textured soy protein products are commonly used to make imitation meat products, such as vegetarian sausage,

meatballs, burgers, and chili. Soy products available in U.S. supermarkets are shown in Table 1 (SANA 2007).

As food ingredients, soy protein products fall into three major groups based on protein content: soy flours and grits, soy protein concentrates (SPC), and soy protein isolates (SPI). Soy flours and grits are made by grinding and screening soybean flakes, and the protein content is in the range of 40 to 54%. There are three types of soy flours: full-fat flours, partially defatted extruded-expelled flours and defatted soy flours. SPC is processed by aqueous treatment, heat treatment and water extraction process to remove carbohydrates and obtain at least 65% protein. SPI is defatted soy flour from which sugars, cotyledon fibers and water-soluble materials have been removed, and are the most highly refined soy protein products commercially available, containing more than 90% protein (Endres 2001).

Soy flour is often used in baking applications for color and texture improvement and some manufacturers use it for soymilk production. Textured SPC as fibers and chunks is used to make the imitation meat, pork, poultry, seafood products. Because of its good nutrition and high functionality, SPI is used in infant formula, baked goods, and meat and dairy products (Endres 2001).

Consumer perceptions and trends

Since ancient times, soybeans have been a significant source of nutrition and the chief source of protein in the Orient. In Western countries, soy protein products have been used as nutritional and functional food ingredients in foods since the 1960s. Soy protein products are an ideal source of nutrition to complement cereal proteins, which are inadequate for satisfactory growth due to an imbalance in essential amino acids (Fukushima 1999). Soy,

with its high quality protein, is an ideal vegetarian food and is less expensive than meat protein (Skarra 2004).

After the FDA approved the health claim for soy protein associated with heart health, over 2,500 new soy products were introduced in the U.S market from 2000 to 2006, (SANA 2007). According to United Soybean Board (USB), one-quarter of Americans consumed soy products once a week or more in 2007, more than in 2006. Consumers (85%) viewed soy protein as a healthy choice and 37% specifically looked for soy products (United Soybean Board 2007).

Sensory attributes of soy foods

Flavor and aroma characteristics of soy products are a major issue related to consumer acceptability of soy products. Soy products with improved flavor are expected by consumers who desire soy foods for health benefits. To satisfy consumer expectations, the food industry requires low-cost soy ingredients with high functional properties and desirable flavor profiles for applications in many products (Li 2006).

Acceptance of soy flavor may be associated with early feeding experiences of humans. Mennella and coworkers (2004) showed that children who were fed soy infant formulas were more likely to prefer bitter-flavored apple juice at a later age compared to children who were fed commercial milk-based or hydrolysate formulas. This demonstrated that early flavor experiences influence subsequent flavor preferences (Mennella and others 2004).

Chambers and others (2006) identified 29 sensory attributes in fresh soymilk, commercial soy powder and ready-to-drink plain soy milk and developed a descriptive sensory lexicon for soymilk evaluation based on these commercial products and previous

literature. The attributes included almond, astringent, banana, beany, brown, butter rum, cardboard, fruity, green/peapod, milky, molasses, nona-lactone, nutty, oats, oil-like, overall grain, wheat, processed, rancid, raw, salty, starch-like, sour, sweet, sweet aromatics, tooth-etch and vanilla/vanillin. A combination of this lexicon with a review of sample attributes may be used for evaluating other commercial soymilk or new products.

Most scientific literature refers to soy flavor as "beany", an undesirable attribute or off-flavor. However, the term "beany" has different meanings in different cultures (Torres-Penaranda and Reitmeier 2001). The use of a single standard that has various components in a matrix may represent the combination of different "beany" characteristics and may include desirable and detrimental attributes. To counter this problem, the sources of "beany" flavor corresponding to more specific terms should be established and investigated for proper description of soy flavor. Previous studies have separated beaniness into several terms to better describe the different beany characteristics including "raw as hexanal", "grassy", "sweet as green floral", "raw beany" and "cooked beany" (Torres-Penaranda and Reitmeier 2001, Torres-Penaranda and others 1998, Vara-Ubol and others 2004). Specific terms for separate beany characteristics should only be used with trained sensory panelists since it may be difficult for the untrained panelists to detect differences.

The undesirable flavor of soy products have been characterized as beany, green, grassy, painty, chalky, astringent, and bitter (Rackis and others 1979). Flavor components in raw soybean have been studied and identified by researchers. Kinsella and Damodaran (1980) summarized the compounds contributing to off-flavor, including furans (green beany), aldehydes (green beany, grassy, stale, cardboard), alcohols (oxidized, grassy/beany), trihydroxy fatty acids (bitter), fatty acid dimers (bitter), phenolics (sweet-nauseating,

astringent), furfurals (cerealy), browning products (roasted), oxidized phosphatidylcholine (bitter) and volatile amines (fishy). Volatile fatty acids and volatile amines were identified using gas and paper chromatography by Arai and coworkers (1966) (Table 2). Aldin and coworkers (2006) found that malonyl-B-glucoside isoflavones and the DDMP-conjugated saponins may contribute to bitterness and off-flavor more than do other saponins and isoflavones in defatted soy flakes, soy protein isolate, and soy germ extracts.

Bott and Chambers (2006) reported that hexanal did not contribute a beany odor individually but did in combination with other non-beany or beany compounds. These combinations produced an overall beany odor that was neither present nor similar in individual compounds. Addition of hexanal to other compounds maintained or increased the beany aroma, and adding hexanal to 1-octen-3-one had the highest overall beany intensity. The combination of some two beany chemicals changed the overall beany intensity of the samples (Bott and Chambers 2006).

A total amount of 256 and 736 ppm phenolic compounds were isolated from soybean flakes (Maga and Lorenz 1974) and soybean flour (Dabrowski and Sosulski 1984), respectively. Phenolic acids (syringic, vanillic, ferulic, gentisic, salicylic, *p*-coumaric and *p*-hydroxybenzoic acids), which have sour, bitter and astringent flavors, were identified in defatted soybean flour (Arai and others 1966). Astringency is a puckering or drying sensation caused by the interaction of polyphenols and proline-rich proteins in saliva (Kallithraka and others 1998). Huang and Zayas (1991) reported that the recognition threshold of p-coumaric acid (48 ppm) taste was bitter, astringent and unpleasant), ferulic acid (90 ppm) taste was sour and the combination of them (20 ppm) tasted sour and bitter. A lower taste threshold for

a combination of phenolics was due to a synergistic effect; free phenolic acids are the flavors in food (Huang and Zayas 1991).

Boatright and Lei (1999) reported the major odor components (dimethyl trisulfide, trans, trans-2,4-decadienal, an unidentified burnt soy sauce-like odor, 2-pentyl pyridine, trans,trans-2-4-nonadienal, hexanal, an unidentified charred sweaty feet-like odor, acetophenone and 1-octen-3-one) in soy protein isolate. Solina and others (2005) identified the volatile aroma components of commercial soy protein isolate (SPI) and acid-hydrolyzed vegetable protein (aHVP) by gas chromatography-olfactometry (GCO) and gas chromatography mass spectrometry (GC-MS). SPI contained mainly aliphatic aldehydes and ketones while aHVP had pyrazines and sulphur containing compounds. A total of 95 volatile compounds were identified in SPI (33) and aHVP (78) in aqueous suspension. Some of the volatile components identified were derived from lipid, sugar, amino acid and unknown components. Ames and Macleod (1984) identified 146 aroma volatiles with 26 odor descriptors in unflavored textured soy protein (TSP). Possible compounds with corresponding odor descriptors in SPI are summarized in Appendix A (Ames and Macleod 1984, Boatright and Crum 1997, Boatright and Lei 1999, Solina and others 2005).

Generally, unprocessed soybeans have a mild and plain flavor. Detrimental flavors are often generated through enzymatic lipid oxidation, autoxidation, proteolytic modification or flavor-binding alterations during processing. Soybeans are rich in lipoxygenases (L-1, L-2 and L-3) and unsaturated fatty acids (linoleic and linolenic). During soybean processing, lipoxygenases catalyze unsaturated oxidation of lipids. The hydroperoxidized lipids are unstable and further catalyzed by hydroperoxide lyase. Wang and others (1998) reported that lipoxygenase and hydroperoxide lyase catalyzed the polyunsaturated fatty acids and created

"beany" flavor in soybeans. To prevent the formation of detrimental flavor from lipoxygenase, high processing temperature (Wilkens and others 1967, Omura and Takechi 1991), pH adjustment (Kon and others 1970, Che Man and others 1989), oxygen atmosphere control (Yuan and Chang 2007), or genetically modified soybeans (Kobayashi and others 1995) have been used for enzyme inactivation or activity control.

Soy foods made from lipoxygenase-free soybeans have a lower intensity in beany characteristics including “hexanal, grassy”, “sweet as green floral”, “raw beany”, “cooked beany aroma” and “cooked beany flavor (Torres-Penaranda and others 1998, Torres-Penaranda and Reitmeier 2001). Yuan and Chang (2007) reported that beany odor content (hexanal, hexanol, 1-octen-3-ol, trans-2, trans-4-decadienal) was correlated with lipoxygenase activity, linoleic acid and protein content in traditional process soymilk. Cooking significantly reduced these components and beany aroma (Yuan and Chang 2007).

Volatile components of aerobic and anaerobic processed soymilk to control the enzymatic lipid oxidation of lipoxygenase were analyzed using GCO and GC-MS method (Feng and others 2001). The odor spectra were different for both aerobic and anaerobic processing methods (Table 3). Controlling lipid oxidation with anaerobic processing may enhance soymilk aroma by reducing the lipid oxidation products. Cheman and others (1989) reported that irreversible inactivation of lipoxygenase occurred at pH $\leq$3.

Several methods, such as supercritical carbon dioxide extraction, enzyme treatments, and organic solvent extraction, have been investigated to prevent, reduce or remove off-flavors in soy products. Maheshwari and others (1995) reported that supercritical carbon dioxide removed off-flavors in soy protein isolate with no detrimental influence on functionality. Enzyme treatment with aspergillopeptidase removed the off-flavor of partially

hydrolyzed tofu and soybean flour (Naguchi and others 1970). Maheshwari and others reported (1997) that treatment with 0.5% porcine liver aldehyde oxidases reduced the off-flavors (acetalaldehyde, n-propanal, n-butanal, n-pentanal and n-heptanal) in soy protein.

Bitterness was the primary flavor problem in proteolytic-modified soy products (Kunst 2003, Cho and others 2004, Kodera and others 2006). The formation of bitter peptides may be associated with the hydrophobicity of the peptides following the *Q* rules (Ney 1979, Adler-Nissen 1986), proteolytic treatment exposed the hydrophobic amino acids in the interior of protein structure to interact with the taste buds and increase bitterness (Kurst 2003), degree of hydrolysis of the parent soy protein and the molecular weight of the peptides (Cho and others 2004), and selection of enzyme and its substrate specificity (Kodera and others 2006). Based on the Ney's *Q* rules, bitter peptides were correlated to the hydrophobicity of the peptides, which was estimated by the average free energy fro the transfer of the amino acid side chains from ethanol to water (Ney 1979). Cho and others (2004) identified the peptides of medium molecular mass range (1 to 4 K Dalton) as the primary bitter soy peptides in commercial soy protein hydrolysates. A protease (novel protease D3) derived from germinated soybean cotyledons tended to cleave the C-terminus of a hydrophilic amino acid adjacent to the C-terminus of hydrophobic amino acids and produced soy protein hydrolysate with low bitterness (Kodera and others 2006).

Interaction of flavor compounds in a food matrix also affects flavor. Therefore, knowledge of protein-flavor-binding interactions is critical to improve the flavor of soy products. Crowther and others (1980) studied the heat adsorption and adsorption coefficients of several aliphatic alcohols, aldehydes, and ketones with soy protein heated at different temperatures and moisture contents. Heat-treated soy protein isolate had lower binding

affinity than unheated protein due to protein denaturation (Crowther and others 1980). Chung and Villota (1989) suggested the interactions of alcohols with SPI may involve hydrophobic associations and hydrogen bonding. Denaturation of the heated soy protein may reduce the formation of hydrogen bonds with alcohols and therefore decrease the binding capacity for alcohols.

Previous studies suggested that the binding affinity of soy protein with carbonyls increased with chain length and hydrophobic interactions of the protein (Arai and others 1970, Damodaran and Kinsella 1981). However, O'Keefe and coworkers (1991) proposed that covalent binding may have occurred in carbonyl protein interactions. Soy glycinin had a higher binding affinity to carbonyl compounds than β-conglycinin in an aqueous model system using a headspace GC technique (O'Keefe and others 1991).

Siebert and others (1996) proposed that hydrophobic bonding may be stronger than hydrogen bonding in phenolic-protein interactions. Hofmann and others (2006) suggested that the astringency response was due to the hydrophobic interaction of tannin with soluble proteins. Previous studies reported that phenolic-protein interactions were affected by pH, temperature, phenolic concentration and molecular weight, salt concentration, ethanol concentration, and type of protein (Saeed and Cheryan 1989, Charlton and others 2002, Serafini and others1997, Oh and Hoff 1987). Hagerman and Butler (1981) reported that phenolic-protein interactions were affected by protein characteristics including isoelectric point, secondary/tertiary structure, and amino acid composition, and that tannins had a high relative binding affinity with proline-rich proteins.

Masking undesirable flavor with sugars, flavors or gums is an option to improve soy flavor if prevention treatments are not effective. Drake and others (2001) found that

additional sweeteners (sucrose, fructose, and sucrose/fructose) and fruit flavors (lemon, strawberry) decreased soy aromas, soy flavors, and astringency in soy fortified dairy yogurts with increasing concentration. Chocolate, almond or vanilla flavorings in soymilk improved the aroma profiles and added gum partially masked the beany off-flavor of soymilk (Wang and others 2001).

Proteases modified soy protein

Various proteases have been investigated to modify the functionality of soy proteins. Soy protein generally has good functionality but heat treatment, pH, inonic strength, and other factors may affect funtionality (Panyam and Kilara 1996). Limited hydrolysis of proteolytic-modified soy protein improved protein solubility, emulsification stability, and foaming capacity in soy products (Jung and others 2005, Lamsal and others 2006, Chaing and others 1999).

Proteolytic hydrolysis involves a decrease in molecular weight, an increase of ionizable groups and exposure of hydrophobic groups in the protein secondary and tertiary structures (Panyam and Kilara 1996). Amino acid composition and sequence, the specific endopeptidase or expeptidase, the substrate specificity for the enzyme, pH, temperature, enzyme to substrate ratio, reaction time, enzyme inactivation, product inhibition and substrate availability in the reaction conditions influenced the flavor profiles, nutritional value and functional properties of protein hydrolysates (Kunst 2003). The pH-stat method to calculate the degree of hydrolysis (DH) is commonly used to monitor enzyme hydrolysis (Adler-Nissen 1984). A constant pH is maintained during hydrolysis by adding base.

Hydrolysis of non-digestible oligosaccharides in soy

Soybeans are generally high in non-digestible α-galacto-oligosaccharides (NDO),

mainly stachyose and raffinose (Leske and others 1993). Consumption of soy products has been hampered by the presence of NDO because mammals lack pancreatic α-galactosidase (α-GAL), which is necessary for hydrolysis. Soy products may cause excessive intestinal gas or gastrointestinal disorders, which results from colonic bacterial fermentation of the indigestible oligosaccharides raffinose and stachyose in sensitive individuals. The structures of stachyose, raffinose and the enzyme cleavage are shown in Figure 1 (LeBlanc and others 2004a).

Cicek and others (2006) reported *Glycine max* (V71-370) soybeans contained approximately 77 g sucrose, 5 g raffinose and 39 g of stachyose per kg. Soy flour derived from conventional soybeans (3.33% stachyose, 0.51% raffinose and 6.37% sucrose) had higher sugar content than soy flour processed from low-oligosaccharide soybeans (0.46% stachyose, 0.16% raffinose and 6.52% sucrose) (Suarez and others 1999).

Several methods including genetic modified low-oligosaccharide soybeans, enzyme treatment and engineered lactic acid bacteria have been investigated to eliminate the gastrointestinal disorders derived from NDO consumption. Suarez and coworkers (1999) found that soy flour derived from low-oligosaccharide soybeans resulted in less gas production than that derived from conventional soybeans. Smiricky and others (2002) reported that soy oligosaccharides reduced nutrient digestibility, but the reductions were small, ranging from approximately 1.1 to 7.4%.

LeBlanc and coworkers (2004b) reported that soymilk fermentation by *Lactobacillus fermentum CRL 722* resulted in the reduction of NDO concentrations in soymilk, thus eliminating these undesirable physiological effects. Connes and others (2004) also demonstrated that lactic acid bacteria had the potential for degradation of α-galactosides of

soy, both in soy milk fermentation and in the small intestine when they were administrated orally to animals as probiotic preparations. Ghazi and others (2003) reported that commercial soybean meal treated with protease and α-galactosidase had improved nutritive value when feed to broiler cockerels and broiler chicks.

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Table 1. Variety of soy foods available in the supermarkets

Section	Products
Produce	Tofu, soy nuts (near the croutons), soy veggie dogs, soy burgers, soy patties, soy crumbles, soy cheese, soy deli meat
Freezer	soy macaroni & cheese, sweet soybeans, edamame, soy burgers, soy patties, soy nuggets, soy tenders, soy veggie dogs, soy crumbles (ground meat alternative), miso, tempeh
Refrigerated Milk	various flavors of soymilk
Refrigerated Dairy	soy cheese, soy yogurt, soy nondairy-cream cheese, soy "buttery"spread
Baking	various flavors of shelf-stable soymilk, soy flour, soy protein, soy oil (vegetable oil), soy brownie and biscuit mixes
Snack and Cracker Aisle	roasted soy nuts, soy chips, soy crisps, soy bars, soy trail mix
Ice Cream	soy nondairy frozen desserts, soy frozen yogurt
Jellies and Spreads	soy nut butter
Coffee	soy coffee, soy creamer
Breakfast and Cereal	soy bars, soy cereals and flakes, soy pancake mixes, soy shakes
Breakfast Freezer	soy sausage links, soy flax waffles, soy breakfast burritos
Canned Food /Pasta	canned soy beans/soy pasta
Healthy and Organic Foods	soy flour, shelf-stable soymilk, soy protein bars, soy meal replacement, soy crisps, soy chips, soy protein powder, soy bars, soy cereals and flakes, soy pancake mixes, soy shakes
International/Asian Food	soy sauces, shelf-stable soymilk, miso, tempeh, seitan

Source: The Soyfoods Association of North America 2007

Table 2. Volatile fatty acids and volatile amines identified from raw soybeans

Identity	Flavor characteristic	Identity	Flavor characteristic
Fatty acid		Amines	
acetic	acetic acid like	ammonia	
propionic	-	monomethylamine	
isovaleric	-	dimethylamine	dried-fish product like
n-valeric	-	piperidine	dried-fish product like
isocaproic	green bean like	cadaverine	dried-fish product like
n-caproic	green bean like		
n-caprylic	green bean like		
n-nonanoic	waxy		
n-capric	Waxy or butter like		

Source: modified from Arai and others 1966

Table 3. Aroma components from aerobic and anaerobic processed soymilk by gas chromatography-olfactometry method

Odor Spectra	Compound	Process Method
Grassy	Hexanal	aerobic
Wheaty	Unknown	aerobic/anerobic
Rancid	Methional	aerobic/anerobic
Beany	2-Heptanone	aerobic/anerobic
Sugar-sweet	Unknown	aerobic
Smoky	Unknown	aerobic
Syrup sweet	Maltol	aerobic/anerobic
Cucumber	(E,Z)-2,6-Nonadienal	aerobic/anerobic
Chalky	(E)-2-Nonenal	aerobic/anerobic
Off-oil	(E,E)2,4- Nonadienal	aerobic/anerobic
Off-oil	(E,Z)2,4- Decadienal	aerobic
Off-oil	(E,E)2,4- Nonadienal	aerobic/anerobic
Off-oil	2,4- Nonadienal	anerobic
Metallic	(E)-4,5-Epoxy-(E)-2-decenal	aerobic/anerobic
Cream	Vanillin	aerobic/anerobic
Flower-sweet	B-damascenone	aerobic/anerobic
Mushroom	1-Octen-3-one	anerobic
Fatty-rancid	Unknown	anerobic
Band-aid	4-Vinyl-2-methoxyphenol	anerobic

Source: modified from Feng and others 2001

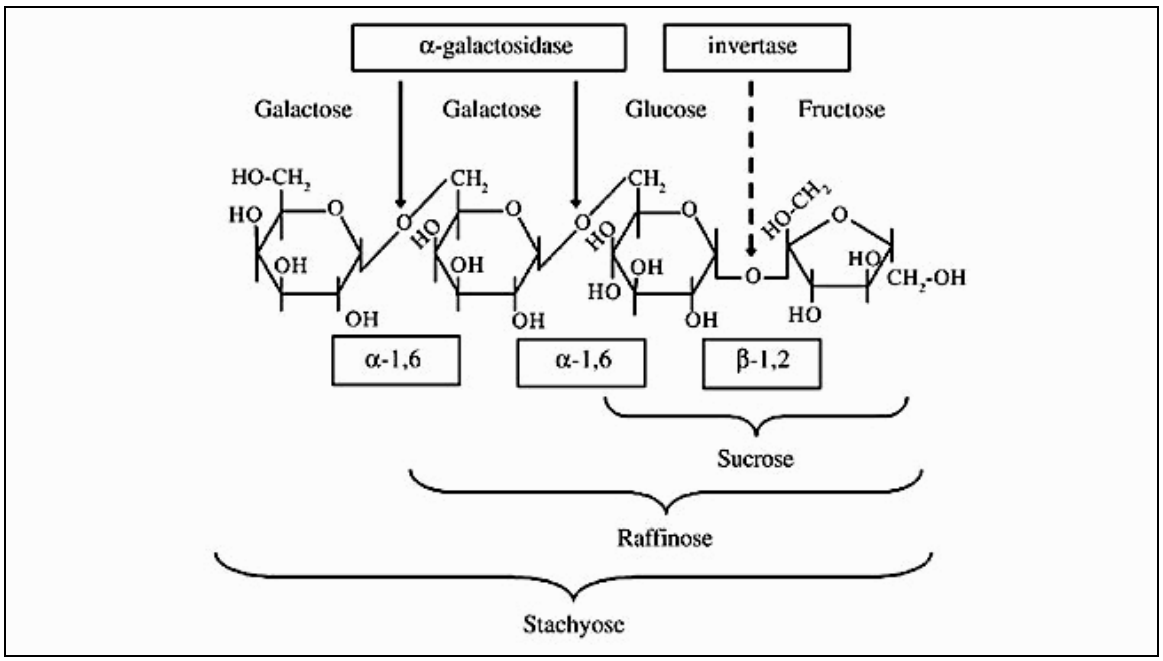

Figure 1. The structures of stachyose, raffinose and the cleavage of α-galactosidase and inverstase.

Source: LeBlanc and others 2004a

CHAPTER 2. FLAVOR ATTRIBUTES OF EXTRUDED-EXPELLED SOY FLOUR MODIFIED BY PROTEASES AND α-GALACTOSIDASE HYDROLYSIS

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ABSTRACT

The objectives of these studies were to describe the aroma and flavor of EESF treated with an exopeptidase and two endopeptidases (bromelain and neutral protease) processed with or without jet-cooking; and to determine the sweetness and bitterness of EESF modified by neutral protease-modified and α-GAL. Twelve trained panelists evaluated 3 protease-modified extruded-expelled soy flours (EESF) at 4% degree of hydrolysis (DH) and un-hydrolyzed control with or without jet-cooking using descriptive analysis (DA). A separate panel of 10 trained panelists evaluated α-GAL-treated un-hydrolyzed and neutral protease-modified EESF using DA. Data were analyzed using analysis of variance and differences were determined (p<0.05). Greater fishy aroma was detected in jet-cooked treatments of EESF compared to uncooked treatments. Control and neutral protease-modified EESF samples were more fishy than bromelain and exopeptidase treatments. For flavor attributes, bromelain hydrolysate was no different than the control. Exopeptidase and neutral protease-modified EESF had less sweetness and more intense grassy-beany flavor, astringency and bitterness than the control. Neutral protease-modified EESF was the most bitter and had the least nutty and toasted flavors among samples. The non-digestible sugar in EESF associated

with abdominal discomfort can be hydrolyzed by α-galactosidase (α-GAL). After hydrolysis, D-Galactose increased 3.2-3.6% by wet weight compared to unhydrolyzed EESF. α-GAL hydrolysis increased sweetness and decreased bitterness of protease-modified EESF. Enzymatic modification optimizes soy protein functionalities but concomitantly increases the bitterness. Bromelain hydrolysis may be the most suitable proteolytic modification for soy food applications. α -GAL modification could be used to increase sweetness and mask the bitterness of soy protein hydrolysates.

Keywords: soy protein hydrolysis, α -galactosidase, flavor

INTRODUCTION

Proteolytic modification of soy protein has been investigated to modify the functionality of soy protein. Enzyme hydrolysis improved some functional characteristics of soy products (Jung and others 2005, Drewnnowski and Gomez-Carmeros 2000). Lamsal and others (2006) reported that low protein dispersibility index (PDI) extruded-expelled soy flour (EESF) treated with endopeptidase or exopeptidase improved protein solubility, emulsification stability, and foaming capacity of soy protein at 4% degree of hydrolysis (DH), but altered the flavor.

Bitterness has been the primary flavor problem in proteolytic-modified soy products (Kunst 2003, Cho and others 2004, Kodera and others 2006). The formation of bitter peptides is associated with hydrophobicity of the peptides following the *Q* rules (Ney 1979), degree of hydrolysis of the parent soy protein and the molecular weights of the peptides produced (Cho and others 2004), and the enzyme and its substrate specificity (Kodera and others 2006). As the degree of hydrolysis (DH) increases, lower molecular weight peptides are produced, resulting in more hydrophobic amino acids accessible for interaction with taste

buds and increased bitterness (Kunst 2003).

Cho and others (2004) determined that the primary bitter peptides in commercial soy protein hydrolysates had a molecular mass range of 1 to 4K Dalton. A protease (novel protease D3) derived from germinated soybean cotyledons cleaved the C-terminus of hydrophilic amino acids adjacent to the C-terminus of hydrophobic amino acids and produced soy protein hydrolysate with low bitterness (Kodera and others 2006).

Soy beans are generally high in non-digestible α-galacto-oligosaccharides (NDO), mainly stachyose and raffinose (Leske and others 1993). Humans lack α-galactosidase (α-GAL) to hydrolyze the NDO and soy consumption can induce flatulence in sensitive individuals. A-GAL treatment hydrolyzes raffinose and stachyose into galactose and sucrose. Moreover, the similarities in structures of bitterness and sweetness molecules allow the sweet molecules to fit into the bitter receptors and decrease bitterness (Lindsay 1996). In addition to potentially masking the bitterness of protease-modified EESF, α-GAL modification may reduce the incidence of flatulence after soy consumption (Suarez and other 1999).

Bitterness is a critical factor in the acceptance or rejection of a food. Methods to reduce the bitterness or to produce non-bitter soy hydrolysates are necessary for utilization of soy proteins in food applications. Current knowledge of the bitterness characteristics of soy hydrolysates and other potential off-flavors is limited and development of techniques to improve soy flavor is needed.

The purposes of this study were to characterize the aroma and flavor attributes of unhydrolyzed EESF and 3 soy hydrolysates processed with or without jet-cooking, and to determine the sweetness and bitterness of EESF modified by neutral protease-modified and α-GAL compared to unhydrolyzed EESF.

MATERIALS AND METHODS

Material Preparation

Proteolytic modified EESF

Extruded-expelled soy flour (EESF) and EESF treated with 3 protease enzymes, with or without a jet-cook treatment, were provided by the Center for Crops Utilization Research (CCUR), Iowa State University, Ames, IA. The EESF, purchased from Nutriant Co. (Cedar Falls, IA) contained 7.3% sugar, 7.0% residual oil and 4.2% fiber on dry basis weight. The average moisture and protein (dry weight basis) contents of EESF were 2.62% and 50.9%, respectively (Lamsal and others 2006).

Bromelain^{TM}(BR), an endopeptidase proteolytic enzyme derived from pineapple, was obtained from Bio-Cat Inc. (Trop, VA) while Experimental Exopeptidase C^{TM} (EEC) and Multifect Neutral^{TM}(MN) exopeptidase were provided by Genencor International Inc. (Rochester, NY). EEC (EC 3.4.4.23.18) was an exopeptidase produced by *Aspergillus oryzae*, also known as Aspergillopepsin I. MN has been renamed to "Protex 7L". MN had mainly metalloendopeptidase produced by *Bacillus amyloliquefaciens* (EC 3.4.24.28) and low level of subtilisin-like protease (EC 3.4.21.62). The activity of Multifect® Neutral is expressed as azo units/g (AU) based on hydrolysis of Azo-casein substrate at pH 7.5 for 5 min at 30 ^{o}C. The activity of this protease is >1600 AU, effective at pH 6.0-8.0 and 40-60 ^{o}C, with optimum performance at pH 6.5.

The average degrees of hydrolysis (DH) for BR, EEC and MN were 4.2 ± 0.2, 4.0 ± 0.2 and 4.6 ± 0.4%. An unhydrolyzed control and the three soy flour hydrolysates were treated with or without jet cooking at 104^{o}C for 19 s to ensure microbial safety. The samples

were spray-dried at 165°C (Anhydro compact dryer, APV Crepaco Inc., Attleboro Falls, MA) and stored at 4°C (Lamsal and others 2006).

α-GAL hydrolyzed EESF

Unhydrolyzed EESF and MN-treated EESF were further hydrolyzed with alpha-galactosidase (α-GAL).Experimental α-GAL (Genencor International Inc., Rochester, NY) produced by *Aspergillus niger* with enzyme activity of 110,000 units/gram via Food Chemicals Codex V method was used.

A sample (90 g) of EESF was dissolved in distilled water and adjusted to pH 5.0 with 3*N* citric acid (final volume 700 mL). The sample was stirred at 500 rpm in a 60°C water bath. After 1 hr of stirring, 0.1 g of enzyme was added to the mixture for 3 hr of hydrolysis at 60°C. The pH was adjusted to 7.0 with 2*N* NaOH to terminate the reaction. Samples were freeze-dried for 72 hr. Freeze-dried materials were stored at 4°C until sensory evaluation. All samples were processed in a food-grade facility.

A 2-mL mixture of soy and water was analyzed for galactose production using a Megazyme Lactose and D-Galactose (rapid) assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) (modification of AOAC method 984.15; lactose in milk) before and after 3 hr of hydrolysis.

Selection of panelists

Two sensory evaluation studies were conducted with different panelists at different dates. Twelve panelists were selected to characterize the flavor profiles of unhydrolyzed EESF and three protease-modified EESF processed with and without jet-cooking. A second set of 13 panelists was selected to evaluate the sweetness and bitterness of α-GAL modification of neutral protease-modified EESF compared to unhydrolyzed EESF.

Sensory evaluation candidates were recruited from Iowa State University students and staff via e-mail messages. Subjects were selected based on interest, availability, and willingness to taste soy flour. Procedures for this study were approved by ISU Institutional Review Board (IRB) and each panelist consented to participate in the research study. Panelists' abilities were tested using pre-screening questionnaires and intensity ranking tests of four basic tastes (Meilgaard and others 1991).

Panelists were required to distinguish solutions of 1.5% sucrose in water as sweet, 0.2% sodium chloride in water as salty, 0.1% citric acid in water as sour and 0.004% quinine sulfate in water as bitter, and to correctly rank 1.0, 1.5 and 2.0% sucrose in water for sweetness; 0.1, 0.2 and 0.5% sodium chloride in water for saltiness; 0.05, 0.10 and 0.15% citric acid in water for sourness; and 0.002, 0.004 and 0.006% quinine sulfate in water for bitterness.

Sensory testing environment

The training and evaluation sessions were conducted in the sensory facilities of the Center for Designing Foods to Improve Nutrition (CDFIN), Iowa State University. During training, panelists were seated around a circular table in a room with white fluorescence lights. The last training session and all evaluation sessions were conducted in partitioned booths with minimal noise and sensory interference under white fluorescent lights.

Training of panelists

Proteolytic hydrolysate panel

Selected panelists (12) were trained during a 5-wk period in two 1-hr sessions per week (10 hr total). The panelists were presented a variety of commercial soy flours and soy milks to become familiar with the major attributes of soy products. Unsalted crackers,

napkins, water, a rinse of 0.55% microcrystalline cellulose (MCC) and an expectorant cup were provided. Judges were instructed to rinse their mouths with MCC rinse and water between samples and to expectorate samples. None of the panelists were excluded from the panel after initial selection.

The panelists generated a list of terms for aroma, appearance, flavor and texture of commercial soy flour samples. The initial list of terms was narrowed to 4 aroma attributes (fishy, roasted, nutty and beany) and 7 flavor attributes (sweet, toasted, nutty, raw-beany, grassy-beany, bitter and astringent) by consensus. The panelists evaluated EESF as dry flour for aroma attributes and as 7.5% (w/v) EESF/water dispersion for flavor attributes.

A 15-cm intensity line scale anchored from "none" to "intense" for each attribute was developed by the panelists and panel leader. Panelists defined a reference for each attribute and reached consensus for each standard and descriptor. Different concentrations of standards were used to identify the maximum intensity of the standards (15 = intense) (Table 1). The concentration of the sample solutions and serving size were determined by the panelists. Light snacks were provided to the panelists at the end of each session. Monetary rewards were provided to panelists after completion of the study.

At the tenth training session, panelists participated in a simulated sensory test to become familiar with evaluation procedures and the computer program (CompuSense Five™, version 4.4.8, Guelph, Ontario, Canada).

α-GAL hydrolysate panel

Thirteen panelists were trained 6 hr during a 3-wk period (2 hr per week). The objective was to determine the effect of *α –GAL* treatment on EESF and EESF hydrolysate. Judges were presented a variety of commercial soy flours to become familiar with sweetness

and bitterness of soy products. Two attributes, sweetness and bitterness, were evaluated and references (15 = intense) for each attribute were defined by the panelists. A 5% sucrose solution and a 0.002% quinine sulfate solution were defined as the references for sweetness and bitterness, respectively. Both attributes were determined by evaluation of 6% (w/v) EESF in water dispersion.

Sample preparation for sensory evaluation

Samples were prepared in the Center for Designing Foods to Improve Nutrition, Human Nutritional Sciences building, Iowa State University. For the proteolytic hydrolysate panel, EESF or EESF hydrolysate (37.5 g) was diluted to 500 mL volume with tap water and stirred at 700 rpm for 5 min for uniform dispersion. Sample solutions were poured into paper cups (40 mL per serving) and stored at 4 ^{o}C for 2 hr. Sample solutions were kept at room temperature for 30 min before serving. Flour samples (3 g per serving) were capped in 1-oz white plastic cups with lids and set at room temperature 2 hr before serving. All samples were coded with randomly selected 3-digit numbers.

For the α-GAL hydrolysate panel, EESF or EESF hydrolysate (30.0g) was diluted to 500 mL volume with tap water, and served as for proteolystic hydrolysate samples.

Descriptive Analysis

Proteolytic hydrolysate panel

Trained panelists evaluated 8 soy flour samples using descriptive analysis (DA) methods in two sessions. After the training, panelists received 4 non-jet-cooked samples (control EESF, EESF modified with BR, EEC, or MN protease). In the second session, 4 jet-

cooked samples (control EESF, EESF modified with BR, EEC, or MN protease) were presented.

Judges were instructed to evaluate aroma attributes by sniffing the samples and the corresponding standards. Panelists were instructed to wait 15 s between each aroma evaluation to determine intensity of each attribute. To evaluate the flavor attributes, panelists were instructed to hold a sample or standard in their mouths, move it around their tongues 5 s and expectorate it. They then waited for 5 s before evaluating another sample.

Panelists determined the level of intensity for each attribute compared to the standard, and marked a response on the line scale using computer software. Panelists were allowed to access standards or samples any time during the test, and change of response.

α –GAL hydrolysate panel

Trained panelists evaluated 4 soy flour samples using DA methods in 2 sessions on 2 different days. They received 4 jet-cooked samples (control EESF, α-GAL modified EESF, MN-treated EESF, and α-GAL modified MN-treated EESF). Judges were instructed to evaluate the sweetness and bitterness of the samples with the corresponding standards, and to mark the responses on a paper score sheet. The panelists followed procedures similar to the proteolytic hydrolysate panel for evaluating all standards and samples.

Statistical design and analysis

Proteolytic hydrolysate panel

A randomized complete block (RCB) factorial design (2 cooking methods and 4 treatments) was used for proteolytic hydrolysate evaluation due to a limited amount of samples. The panelists were treated as randomized blocks and 12 replications were

generated. Using a restricted randomized sequence, panelists received the control as the first sample at each session, followed by the three hydrolyzed samples in randomized order.

Analysis of variance (ANOVA) was constructed with mixed model procedure (PROC MIXED) using SAS statistical program, version 9.1 (SAS Institute, Inc., NC, 2003) to test the effect of cooking method and enzyme treatment. Main effect means were reported because there was no interaction between cooking method and enzyme hydrolysis treatment. Differences of least squares means were obtained from a multiple comparison procedure using the Tukey's Honestly Significant test *(p*<0.05). No data was excluded from the statistical analysis.

α –GAL hydrolysate panel

A complete factorial design with panelist (10) and treatment (4) as the main effects was used for α –GAL hydrolysate panel. Panelists (13) evaluated 4 samples at 2 sessions for 2 replications. Data from 3 panelists were excluded from analysis because discrepancies were observed in training and duplicate sample evaluations. A general linear procedure (PROC GLM) was used to construct the ANOVA structure for data analysis using SAS 9.1. Main effect means were reported. Mean separations were analyzed using Tukey's Honestly Significant Test (*p*<0.05).

RESULTS AND DISCUSSION

Proteolytic hydrolysate panel

Aroma Attributes

The aroma profiles of EESF with or without a cooking treatment are presented in Table 2. Greater fishy aroma was detected in jet-cooked treatments compared to non-jet-cooked treatments. There is evidence that heating could increase the production of volatile

compounds (Ames and Macleod 1984) may increase the fishy aroma in jet-cooked EESF. Possible volatile compounds associated with the unpleasant fishy odor, such as ammonia, monomethylamine, dimethylamine (DMA), piperidine and cadaverine, have been isolated from raw soybeans (Arai and others 1966b). The thresholds of DMA and ammonia are 30 ppm and 110 ppm, respectively (O'Keefe 2000).

The control and MN-modified EESF had more fishy aroma compared to BR- and EEC-modified EESF (Table 3). A previous study conducted by Lamsal and coworkers (2006) indicated that β-conglycinin was almost completely hydrolyzed by BR, EEC or MN based on the SDS-PAGE profiles, although glycinin was hydrolyzed differently by the 3 enzymes (Appendix B). BR and EEC partially hydrolyzed the acidic subunits of glycinin based on SDS-PAGE profiles, but MN did not (Lamsal and others 2006).

Roasted, nutty and beany aromas were not different for uncooked and jet-cooked EESF (Table 2) or for enzyme treatments (Table 3). Heating, enzyme treatments or the combination of both treatments did not alter the roasted, nutty or beany aromas of EESF compared to the control.

Flavor Attributes

Heating generally denatures soy protein and alters flavor-components interactions but there were no difference in sweetness, toasted, nutty, grassy-beany, raw-beany, bitter and astringent flavors in uncooked- and jet-cooked EESF (Table 4). Protein denaturation of EESF samples mainly occurred during extruding-expelling, resulting in no sensory difference in cooking treatment.

EEC-and MN-modified EESF were less sweet and more bitter than the control and BR-treated EESF (Table 5). Low sweetness may have been caused by the similarities of

bitterness and sweetness in structure, which allow bitter molecules to fit into the receptors for sweetness (Lindsay 1996). Sweetness was inversely related to the bitterness of EESF.

MN-modified EESF had the least toasted and nutty flavor among all enzyme treatments. Toasted and nutty flavors are mainly related to the products (alkyl pyrazines) of Streaker degradation and Maillard browning reactions, which occurred during extruding-expelling. The high temperature of extruding-expelling causes browning reactions between reducing sugars and free amino acids in the soy flour to occur. This heating process denatures the protein, reduces functionality and enhances the toasted and nutty flavors through browning reactions or deamidation (Kinsella and Damodaran 1980, Zhang and others 1993).

Solina (2005) identified pyrazine volatile components derived from Maillard browning reactions in acid-hydrolyzed soy hydrolysate. The SDS-PAGE profile (Lamsal and others 2006) showed that MN hydrolyzed the soy protein differently than BR and EEC hydrolysis, resulting in the difference in toasted and nutty flavors of EESF.

Stronger grassy-beany flavor was detected in the EEC- and MN-modified EESF compared to the control EESF (Table 5). BR treatment was not different in grassy-beany flavor compared to others. According to Kinsella and Damodaran (1980), grassy-beany flavor was caused by the volatile carbonyls derived from lipid oxidation such as n-hexanal, cis-hex-3-enal, n-alkanols (C5, C6, C7), n-2-pentyl furan and ethyl vinyl ketone. Feng and coworkers (2001) also identified volatile aldehydes and ketones derived from lipid oxidation that were associated with undesirable beany flavors in soymilk. Bott and Chambers (2006) reported that hexanal alone did not contribute beany odor but hexenal combined with other non-beany or beany compounds did have (different) beany odor. In contrast, proteolytic modification of EESF did not influence raw-beany flavor.

Most literature refers to “beany” as an undesirable attribute or off-flavor.However, the term “beany” has various aspects and different meanings in different cultures (Torres-Penaranda and Reitmeier 2001). The use of a single standard with various components in a matrix may represent different “beany” characteristics and may include desirable and detrimental attributes. To counter this problem, the actual sources of “beany” flavor corresponding to more specific terms should be established and investigated for proper description of soy flavor. Previous studies have separated beaniness into several terms to better describe the different beany characteristics such as “raw as hexanal”, “grassy”, “sweet as green floral”, “raw beany” and “cooked beany” (Torres-Penaranda and Reitmeier 2001, Torres-Penaranda and others 1998, Vara-Ubol and others 2004).

The bitterness of MN-modified EESF was highest, followed by EEC-modified EESF compared to control and BR-modified EESF (Table 5) even though the DH of the hydrolysates were similar (4.0-4.6%) (Lamsal and others 2005). Ney (1979) hypothesized that the bitterness of peptides was dependent on the hydrophobicity of the peptides and established the *Q* rules. According to Adler-Nissen (1986), proteolytic treatment exposed the hydrophobic amino acids in the interior of protein structure to interact with the taste buds and increase bitterness. However, Cho and coworkers (2004) proposed that the bitterness level of peptides was associated with DH of the parent soy protein and the MW of the peptides. Exopeptidase-treated hydrolysates produced less bitterness by producing less hydrophobic peptides than endopeptidase-treated hydrolysates (Kunst 2003). However, our data indicated that the MN-modified EESF, an endopeptidase-treated hydrolysate, was more bitter than the EEC-modified EESF, an exopeptidase-treated hydrolysate. Recently, Kodera and coworkers (2006) concluded that bitter peptide formation was dependent on the selected enzyme and its

substrate specificity. The MN and EEC proteases may tend to produce hydrophobic peptides than the BR due to its substrate specificity (Appendix C and D), resulting in more intense bitterness than the control and BR treatment.

The EEC and MN treatments had higher scores for astringency than control and BR treatments (Table 5). Phenolic compounds isolated from soy products (Maga and Lorenz 1974, Dabrowski and Sosulski 1984) were bitter and astringent (Arai and others 1966a). Enzymatic modification of protein increases the exposure of hydrophobic groups in the secondary and tertiary structures, and caused a change of hydrophobic interactions between amino acid residues within the same molecule or between molecules (Panyam and Kilara 1996). The exposure of peptides with amino acids that have phenolic groups may increase the astringency of hydrolysates. Both MN- and EEC-modified proteins may have structural changes that are unfavorable for phenolic-protein binding, resulting in more free phenolic acids and more astringency than the EESF control and BR-treated EESF.

α –GAL hydrolysate panel

Control and MN-treated EESF had the same level of sweetness prior to α-GAL hydrolysis; sweetness increased in each after α-GAL treatment (Table 6). The α-GAL hydrolyzed the NDO, mainly stachyose and raffinose into galactose and sucrose, After hydrolysis, D-Galactose increased 3.2-3.6% by wet weight compared to unhydrolyzed EESF. As a result, α-GAL-modified samples increased the sweetness of EESF. MN-treated EESF had the highest bitterness among samples but it decreased to the same level as control and α-GAL treated EESF. The increase of galactose and sucrose reduced the bitterness of MN-modified hydrolysate, probably by fitting into the bitter receptors (Lindsay 1996).

CONCLUSIONS

Improved functional properties and acceptable flavor are necessary before soy products will be consumed. EESF treated with MN protease and EEC had more intense bitterness and astringency than the control and BR-treated EESF. This indicated that endopeptidase or exopeptidase treatment is not the only factor affecting the bitterness of the same substrate hydrolyzed to the same DH. The flavor profiles of soy hydrolysates are due to amino acid composition, sequence of the peptides and the specificity of the enzymes. α-GAL modification could be used to increase sweetness and mask the bitterness of soy protein hydrolysates. BR-modified EESF had similar flavor attributes as control EESF and may be the most suitable hydrolysate in food applications. However, methods to reduce the bitterness and other off-flavors of soy hydrolysate, and development of proteases that produce non-bitter peptides, should be investigated in the future.

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Table 1- Sensory descriptors and standards for proteolytic hydrolysate panel

Term	Standard	Concentration for "intense" (15 on linescale)
Aroma		
Fishy	Dried fish snack	-
Roasted	All-purpose flour and sucrose	10:1 Baked at 350°F for 50 min
Beany	Green alfalfa sprouts blended in water	10:1
Nutty	'Planters' roasted peanut	-
Flavor		
Sweet	Sucrose in water	5%
Toasted	All-purpose flour	Baked at 350°F for 35 min
Nutty	'Planters' roasted peanut	-
Grassy-beany	Fresh green alfalfa sprouts	-
Raw-beany	Raw soaked soy beans	-
Bitter	Quinine sulfate in water	0.003%
Astringent	Alum in water	0.1%

Table 2 - Aroma attributes of jet-cooked and uncooked EESF

	Aroma Attributes			
Treatments	Fishy	Roasted	Nutty	Beany
Soy flour, uncooked	5.15[a]	3.36	2.51	1.84
Soy flour, jet-cooked	6.54[b]	3.41	2.65	3.26
		NS	NS	NS

Main effect means are responses of 4 enzymatic and non-enzymatic treatments and 12 panelists, 0 = none to 15 = intense. Means in the same column with different letters are significantly different at $p < 0.05$. NS = not significant.

Table 3 - Aroma attributes of EESF and EESF hydrolysates

	Aroma Attributes			
Enzyme Treatments	Fishy	Roasted	Nutty	Beany
Control, no enzyme	6.86[a]	3.69	2.43	2.90
Bromelain (BR)	5.10[b]	3.59	2.75	2.34
Experimental Exopeptidase C (EEC)	4.77[b]	3.19	2.59	2.32
Multifect Neutral (MN)	6.67[a]	3.06	2.55	2.63
		NS	NS	NS

Main effect means are responses of 2 cooking methods and 12 panelists, 0 = none to 15 = intense. Means in the same column with different letters are significantly different at $p < 0.05$. NS = not significant.

Table 4 - Flavor attributes of jet-cooked and uncooked EESF

	Flavor Attributes						
Treatments	Sweet	Toasted	Nutty	Grassy-Beany	Raw-Beany	Bitter	Astringent
Non-jet-cooked	2.27	3.77	3.92	2.11	4.48	5.92	5.92
Jet-cooked	2.25	3.72	3.62	2.56	5.53	5.17	5.27
	NS	NS	NS	NS	NS	NS	NS

Main effect means are responses of 4 enzymatic and non-enzymatic treatments and 12 panelists, 0 = none to 15 = intense. NS = not significant at $p<0.05$.

Table 5 - Flavor attributes of EESF and EESF hydrolysates

	Flavor Attributes						
Enzyme Treatments	Sweet	Toasted	Nutty	Grassy-Beany	Raw-Beany	Bitter	Astringent
Control	3.11[a]	4.46[a]	4.42[a]	1.55[a]	4.40	2.13[a]	4.18[a]
Bromelain (BR)	2.72[a]	3.98[a]	4.10[a]	2.27[ab]	5.02	2.98[a]	4.42[a]
Experimental Exopeptidase C (EEC)	1.81[b]	3.84[a]	4.05[a]	2.61[b]	4.66	7.03[b]	6.35[b]
Multifect Neutral (MN)	1.41[b]	2.73[b]	2.51[b]	2.90[b]	5.96	10.05[c]	7.42[b]
					NS		

Main effect means are responses of 2 cooking methods and 12 panelists, 0 = none to 15 = intense. Means in the same column with different letters are significantly different at $p<0.05$. NS = not significant.

Table 6 – Effect of α –GAL treatment on EESF and EESF hydrolysate

	Flavor Attributes	
Treatments	Sweet	Bitter
Control, no enzyme	1.8[a]	2.6[a]
α –GAL	3.8[b]	1.3[a]
Multifect Neutral (MN)	1.5[a]	8.7[b]
Multifect Neutral (MN) + α –GAL	4.3[b]	2.8[a]

Main effect means are responses of 10 panelists, 0 = none to 15 = intense. Means in the same column with different letters are significantly different at $p<0.05$.

CHAPTER 3. SENSORY CHARACTERISTICS OF HIGH- AND LOW-PDI SOY PROTEIN ISOLATES MODIFIED BY PROTEASE HYDROLYSIS

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ABSTRACT

The objectives of these studies were to characterize the aroma and flavor attributes of two commercial SPIs (high and low PDI) treated with Fungal Protease Concentrate (FPC) with primarily exopeptidase activity, Multifect® Neutral (MN) with endopeptidase activity, and a combination of MN+FPC at 4% degree of hydrolysis (DH). Eleven trained panelists evaluated 3 protease-modified soy protein isolates (SPI) samples and un-hydrolyzed control with high (66.2) and low (3.3) protein dispersibility indices (PDI) using descriptive analysis (DA). Data were analyzed using analysis of variance and differences were determined with Tukey's Honestly Significant test ($p<0.05$). Greater raw beany and bitterness flavors were detected in low-PDI samples than high-PDI samples. The PDI of protein had influence on the raw beany and bitter flavors of soy hydrolysates with different enzyme treatments. The bitterness of MN-modified SPI was highest, followed by the MN+FPC-modified SPI, and FPC-modified SPI, then the control. MN treatment had more intense astringency than FPC treatment. MN+FPC treatment was not different in astringency than FPC and MN treatment alone. In contrast, FPC-modified SPI had higher cooked beany flavor and was less bitter and astringent compared to MN-modified SPI and the control. Differences in the flavor profiles

of soy hydrolysates from the same substrate (same composition and sequence of amino acids) and at the same DH are due the specificity of selected enzymes. Proteolysis modification reduced chalkiness and grittiness of soy protein, which would be a favorable attribute for beverages. FPC protease provided soy protein hydrolysate with low bitterness and may be a potential hydrolysate in food applications.

Keywords: enzymatic hydrolysis, soy protein isolate, sensory

INTRODUCTION

Sensory attributes of soy products continues to be a concern in the soy food industry. In October 1999, the U.S. Food and Drug Administration (FDA) approved a health claim for products high in soy protein – "soy protein (25 g or more per day) may reduce the risk of heart disease in diets that are low in saturated fat and cholesterol" (Federal Register 1999). Sales of soy foods increased from $300 million to $3.9 billion from 1992 to 2004 due to consumers' interest in the health benefits of soy (SANA 2007). Despite the growth in soy foods popularity, soy flavor was recognized as an "off-flavor" by U.S. consumers and foods labeled as "containing soy" were perceived negatively for flavor (Wansink and Park 2002).

Enzyme hydrolysis improved some functional characteristics, such as protein solubility, emulsification stability, and foaming capacity of soy products (Jung and others 2004, Jung and others 2005, Drewnowski and Gomez-Carmeros 2000, Chaing and others 1999). Bitterness was reported as the primary flavor problem in proteolytic-modified soy products (Kunst 2003, Cho and others 2004, Kodera and others 2006). Bitterness of hydrolysate may be associated with hydrophobicity of the peptides (Ney 1979, Alder-Nissen 1986), degree of hydrolysis of the parent soy protein and the molecular weight of the peptide

bonds (Cho and others 2004) and the substrate specificity of enzyme (Kodera and others 2006).

Low protein dispersibility index (PDI) extruded-expelled soy flour (EESF) treated with endopeptidase or exopeptidase improved protein solubility, emulsification stability, and foaming capacity of soy protein at 4% DH (Lamsal and others 2006). Bromelain treatment produced hydrolysate with lower bitterness and astringency scores than MN treatment while MN-modified EESF had less sweetness and more intense grassy-beany flavor, astringency and bitterness than the control (Lock 2007).

The sensory attributes of enzymatic-modified EESF has been addressed (Lock 2007). The composition of soy flour is more complex than SPI. Therefore, using SPI will have less interference on flavor component interactions than soy flour. The production of SPI includes production of defatted soy flour, alkali extraction, okara removal, acid precipitation, neutralization and spray drying. SPI contains a minimum of 90%. The degree of protein denaturation in a soy product is measured by PDI. High PDI value is associated with low level of protein denaturation and high solubility. SPI with high and a low PDI levels have different degrees of protein denaturation, and may influence the sensory profiles of the hydrolysates.

The purpose of this study was to characterize the aroma and flavor attributes of two commercial SPIs (high and low PDI levels) treated with Fungal Protease Concentrate (FPC, primary exopeptidase), Multifect® Neutral (MN, an endopeptidase), and a combination of MN+FPC at 4%DH.

MATERIALS AND METHODS

Materials

Two commercial SPIs (Profam 825 and Profam 955) were provided by Archer Daniels Midland Co. (ADM, Decatur, IL). The proximate moisture content, dry-basis protein content and pH of Profam 825 and Profam 955 were 3.9 and 92.4% at pH 7.0, 3.6 and 95.1% at pH 5.4, respectively, with < 4% fat content. The protein dispersibility indices (PDI) of Profam 825 and Profam 955 were 66.2 and 3.3%, respectively, determined by Eurofins Scientific Inc. (Des Moines, IA). Profam 825 was the high PDI SPI and Profam 955 was the low PDI sample in this study. Profam 825, with high solubility and emulsification, is typically used for making beverages, nutrition bars, and extruded cereal pieces (information provided by ADM). Profam 955 has high dispersibility and is used in fresh cheese applications (information provided by ADM).

Fungal Protease Concentrate (FPC) and Multifect® Neutra l(MN) were provided by Genencor International Inc. (Rochester, NY). Fungal Protease Concentrate has been renamed to "Protex 51FP" (EC 3.4.23.18). FPC is an exopeptidase-endopeptidase complex derived from a selected strain of *Aspergillus oryzae* var. The activity of FPC is expressed in Hemoglobin Units (HU). The activity of one HU liberates 0.0447 mg of non-protein nitrogen in 30 min at pH 4.7 and 40 ^{o}C, as determined by the supplier. FPC has >400.000 HU/g activity, effective at pH 6.0-9.0 and 25-60 ^{o}C with optimum performance at 50 ^{o}C and pH 7.5.

Multifect® Neutral has been renamed to "Protex 7L". MN is a bacterial neutral enzyme prepared by controlled fermentation of a non-genetically modified strain of *Bacillus amyloliquefaciens* with mainly metalloendopeptidase (EC 3.4.24.28) and very low level of a subtilisin-like protease (EC 3.4.21.62). The activity of Multifect® Neutral is expressed as azo

units/g (AU) based on hydrolysis of Azo-casein substrate at pH 7.5 for 5 min at 30 °C. The activity of this protease is >1600 AU, effective at pH 6.0-8.0 and 40-60 °C, with optimum performance at pH 7.5.

Proteolytic hydrolysis

Two SPI hydrolysates were prepared by 10% (w/w) suspensions (SPI/distilled water) at 50 °C for 2 replications, one day before sensory evaluation. Samples were adjusted to pH 7.0 with 2*N* NaOH with stirring. Enzymes were added to the SPI at 15 min after pH stabilization to solubilize the protein. The use of different enzyme ratios to SPI for the 3 protease treatments to reach 4% DH was determined (Table 1). The enzyme/substrate ratio (E/S) was defined as the percentage of enzyme (g) added per 100 g of soy protein. Fungal Protease Concentrate (FPC) was added after Multifect® Neutral (MN) protease reached 1.5% DH in the combination of two enzymes treatments. It is difficult to inactivate the proteases. To reduce the enzyme activity, hydrolysates were adjusted to pH 5.8 with 3*N* citric acid at the end of 4% DH, then chilled in an ice water bath for 20 min before storing at 4°C. No proteolytic activity was observed at pH 5.8 at room temperature.

The pH-stat (718 STAT Titrino; Metrohm, Brinkmann Instruments Inc., Westbury, NY) was used to monitor and control the hydrolysis. DH was calculated based on the formula: $DH = [(V_{NaOH} \times N_{NaOH}) / (\alpha \times MP \times h_{tot})] \times 100\%$ (Alder-Nissen 1986). The α value was the average degree of dissociation of the α-amino groups, which was 0.440 for the reaction at 50 °C and pH 7.0, according to Adler-Nissen (1986). MP was the mass of protein (g) and the h_{tot} was the total number of peptide bonds in the protein substrate (meqv/g protein). Hydrolysis was performed in a food-grade laboratory.

Selection of panelists

Twelve panelists were selected to characterize the flavor profiles of unhydrolyzed SPI and 3 protease-modified SPI at 2 different PDI. Sensory evaluation candidates were recruited from Iowa State University students and staff via e-mail messages. Subjects were selected based on interest, availability, and willingness to taste soy protein. Procedures for this study were approved by ISU Institutional Review Board (IRB) and each panelist consented to participate in the research study. Pre-screening questionnaires and intensity ranking tests of four basic tastes (Meilgaard and others 1991) was conducted to test the panelists' sensory abilities.

The panelists were instructed to distinguish solutions of 1.5% sucrose in water as sweet, 0.2% sodium chloride in water as salty, 0.1% citric acid in water as sour and 0.004% quinine sulfate in water as bitter, and to correctly rank 1.0, 1.5 and 2.0% sucrose in water for sweetness, 0.1, 0.2 and 0.5% sodium chloride in water for saltiness, 0.05, 0.10 and 0.15% citric acid in water for sourness and 0.0004, 0.002 and 0.004% quinine sulfate in water for bitterness.

Sensory testing environment

The training and evaluation sessions were conducted in the sensory facilities of the Center for Designing Foods to Improve Nutrition (CDFIN), Human Nutritional Sciences Building (HNSB), at Iowa State University. The panelists were seated around a circular table in a room with white fluorescence lights for all training sessions. The evaluation sessions were conducted in partitioned booths with minimal sensory and noise contaminants under white fluorescent lights.

Training of panelists

Selected panelists were trained during a 4-wk period in 1-hr sessions for 3 days a week (12 hr total). Judges received a variety of commercial soy flours, soy protein isolates and soymilks to become familiar with the major attributes of soy products. Unsalted crackers, napkins, water, and a expectorate cup were provided. The panelists were instructed to rinse their mouths with water between samples and to expectorate samples.

The panelists generated a list of terms for aroma, appearance, flavor and texture of commercial SPI. The initial list of terms was narrowed to 3 aroma attributes (raw beany, cooked beany, and wheat flour), 5 flavor attributes (sweet, raw beany, cooked beany, wheat flour and bitter), and 3 mouthfeel attributes (chalky, gritty and astringent) by consensus.

A 15-cm intensity line scale anchored from "none" to "intense" for each attribute was developed by the panelists and panel leader. Panelists defined a reference for each attribute and reached consensus for each standard and descriptor. All selected standards were used to identify the maximum intensity of each attribute at intense = 15 on the line scale (Table 2). Panelists received light snacks as compensation at the end of each session and monetary rewards after completion of the study.

Sample preparation for sensory evaluation

Samples were prepared in the Center for Designing Foods to Improve Nutrition (CDFIN), Human Nutritional Sciences Building (HNSB), Iowa State University. SPI hydrolysate samples stored at 4^{o}C were diluted to 1000 mL with tap water and stirred at 700 rpm (5 min) for uniform dispersion. Control SPI (50g) was diluted to 1000 mL with tap water and stirred. Sample solutions (40 mL) were served in 2-oz white plastic cups with lids, after

30 min at room temperature. All samples were coded with randomly selected 3-digit numbers.

Descriptive Analysis

The panelists evaluated 8 SPIs using descriptive analysis (DA). The panelists evaluated the samples as 5% (w/v) SPI/water dispersions. The panelists were instructed to evaluate aroma and flavor attributes of samples and the corresponding standards, and mark the responses on a paper score sheet. The panelists were instructed to wait 15 s between each aroma evaluation and 5 s between each flavor evaluation. The panelists were allowed to re-evaluate samples and to change response during testing.

Statistical Design and Analysis

A complete factorial design with 11 panelists, 4 enzyme treatments and 2 soy isolates (high and low PDI) as the main effects was used. Panelists evaluated 4 samples (control and 3 hydrolysates) at the same PDI level, 2 replications of each. A total of 4 separate sessions were conducted on 3 consecutive days. Data from one panelist were excluded from analysis as discrepancies were observed in training and duplicate sample evaluations.

Analysis of variance (ANOVA) was constructed with general linear procedure (PROC GLM) using SAS statistical program, version 9.1 (SAS Institute, Inc., NC, 2003) to analyze the data. Main effect means were reported because there were no interactions between enzyme treatment and PDI levels of SPI. Differences in least squares means were obtained from a multiple comparison procedure using the Tukey's Honestly Significant Test *(*$p<0.05$).

RESULTS AND DISCUSSION

No differences in aroma attributes, sweet, cooked beany, and wheat flour flavors, and

chalky, gritty and astringent mouthfeel attributes were observed between SPIs with low- and high-PDI values (Table 3). More intense raw beany and bitter flavors were detected in low-PDI soy isolate compared to high-PDI SPI (Table 3). By-products of oxidized fatty acids may responsible for the beany flavor. As both SPI samples had <4% fat, similar beany flavor scores were expected. Low-PDI SPI may have more denatured protein then high-PDI SPI but the exact processing methods was not provided by the suppliers. Bitterness has been detected in commercial SPI (Russell and others 2006). Low-PDI isolate may cause more hydrophobic amino acids to interact with the taste buds and increase bitterness compared to high-PDI SPI.

More intense raw beany aroma was detected in the control SPI compared to SPI with enzyme treatments (Table 4). Cooked beany aroma among all treatments was not different. FPC- and MN+FPC-modified SPI had lower wheat flour aroma than the control. Raw beany and wheat flour aromas are often caused by the volatile aldehydes and ketones (Feng and others 2001, Kinsella and Damodaran 1980, Aaslyng and others 1999, Bott and Chambers 2006). Crowther and others (1980) studied the heat adsorption and adsorption coefficients of several aliphatic alcohols, aldehydes, and ketones with soy protein heated at different temperatures and moisture contents. Heat-treated SPI decreased the binding affinity of SPI due to protein denaturation (Crowther and others 1980). It is likely that volatile components that contribute raw beany and wheat flour aromas were lost due to the proteolytic treatment. MN-modified SPI resulted in the least sweetness and the most bitterness in flavor among all treatments (Table 4). Low sweetness may have been caused by the similarities of bitterness and sweetness in structure, which allow the bitter molecules to fit into the receptors for sweetness (Lindsay 1996). The bitterness in FPC- and MN+FPC-modified samples was not intense enough to reduce the sweetness.

Proteolytic modification of SPI did not influence raw beany and wheat flour flavors (Table 4). Wheat flour flavor was detected as "flour paste" flavor in commercial SPI (Russell and others 2006). The most intense cooked beany flavor was detected in FPC-modified SPI than the rest of the treatments. Volatile components, such as pyrazine derived from Maillard browning reactions (Solina 2005), volatile carbonyls derived from lipid oxidation, by products of oxidized fatty acids and furan (Kinsella and Damodaran 1980, Feng and others 2001, Boatright and Lei 1999) and protein may contribute to cooked beany flavor.

The bitterness of MN-modified SPI was highest, followed by the MN+FPC-modified SPI, FPC-modified SPI, and the control (Table 4). Hydrophobicity of the peptides and free amino acids (Ney 1979) and substrate specificity of enzyme (Kodera and others 2006) were responsible for the bitterness of hydrolysates. FPC protease had mainly endodeoxyribon-nucleases producing 5'phosphomonoesters (EC 3.1.21.14) and metalloexopeptidase, thermolysin (EC 3.4.24.4). MN protease had the activity of serine endopeptidase (EC 3.4.21.62) and metalloendopeptidase (3.4.24.38). Both FPC and MN proteases (Appendix C and D) cleaved at different bonds of the substrate and yielded different products. The MN proteases may tend to produce more hydrophobic peptides, resulting in more intense bitterness than FPC protease. In previous work (Lock 2007), MN-treated EESF had a bitterness score of 10.05 (15 = intense) which was similar to the score for MN-treated SPI (10.8).

Chalky and gritty mouthfeel were more intense in all enzyme treatments than in the control (Table 4). Chalkiness was detected as a texture/mouthfeel in commercial SPI (Russell and others 2006). Jung and others (2004) reported that MN and FPC hydrolysates of soy flour improved protein solubility. Enzyme modification decreases the molecular weight of

the soy protein and reduces the size of soy proteins, which could explain the reason enzyme-treated SPI samples were less gritty than the control SPI.

MN treatment had more intense astringency than FPC treatment. MN+FPC treatment was not different in astringency than FPC and MN treatment alone (Table 4). Astringency in soy products was associated with phenolic compounds (Maga and Lorenz 1974, Dabrowski and Sosulski 1984, Arai and others 1966). Proteolytic modification increased the exposure of hydrophobic groups in the secondary and tertiary structures of protein (Panyam and Kilara 1996). MN treatment may produce peptides that have more exposure of aromatic amino acids with phenolic groups than FPC treatment, which may increase astringency in hydrolysates than the control. The astringency score of MN-treated EESF (7.42, 15 = intense) was similar to the astringency score of MN-treated SPI (7.5)(Lock 2007).

CONCLUSIONS

Improved flavor characteristics of soy protein hydrolysates are needed for food applications. The PDI levels of protein influenced the raw beany and bitter flavors of soy hydrolysates with enzyme treatments. MN-treated SPI was more bitter and astringent and lower in sweetness than control. In contrast, FPC-modified SPI had more intense cooked beany flavor and was less bitter with similar astringency compared to MN-modified SPI. MN+FPC treatment had sensory attributes between both single enzyme treatments. Differences in the flavor profiles of soy hydrolysates from the same substrate (same amino acids composition and sequence) and at the same DH are due the specificity of selected enzymes. The endopeptidase or exopeptidase treatment is not the only factor affecting the bitterness of hydrolysate. Proteolysis modification reduced the chalkiness and grittiness of soy protein and maybe favorable for beverage applications. FPC protease provided soy

protein hydrolysate with low bitterness and may be a potential hydrolysate in food applications.

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Table 1- Enzyme/Substrate (E/S) ratios used to prepare SPI hydrolysates

Enzymatic Treatments	E/S (%)
Control, no enzyme	-
Multifect® Neutral	0.42
Fungal Protease Concentrate	0.70
Multifect® Neutral followed by Fungal Protease Concentrate	0.21+0.35[a]

[a] Fungal Protease Concentrate was added after 1.5% DH by Multifect® Neutral protease

Table 2 - Sensory descriptors and standards for SPI and SPI hydrolysates

Term	Standard	Concentration for "intense" (15 on linescale)
Aroma		
Raw beany	Raw soaked soy beans	40 g dried soybeans in 200 mL water
Cooked beany	Organic canned soybean	-
Wheat flour	All-purpose flour in water	25 g flour:75 mL water
Flavor		
Sweet	Sucrose in water	3%
Raw beany	Raw soaked soy beans	40 g dried soybeans in 200 mL water
Cooked beany	Organic canned soybean	-
Wheat flour	All-purpose flour in water	25 g flour:75 mL water
Bitter	Quinine sulfate in water	0.003%
Mouthfeel		
Chalky	Corn starch	-
Gritty	Cornmeal	-
Astringent	Alum in water	0.1%

Table 3 – Effect of protein dispersibility indices (PDI) on sensory attributes of soy protein isolates (SPI)

	Soy Protein Isolates		
	Profam 825	Profam 955	
Sensory Attribute	PDI=66.2	PDI= 3.3	
Aroma			
Raw beany	4.3	4.1	NS
Cooked beany	4.0	4.1	NS
Wheat flour	4.9	4.6	NS
Flavor			
Sweet	2.5	2.7	NS
Raw beany	5.5[a]	6.6[b]	
Cooked beany	5.1	4.9	NS
Wheat flour	5.1	5.0	NS
Bitter	6.7[a]	7.8[b]	
Mouthfeel			
Chalky	3.9	4.0	NS
Gritty	2.1	1.8	NS
Astringent	5.7	6.2	NS

Main effect means are responses of SPI and 3 SPI hydrolysates, and 11 panelists for 2 replications, 0 = none to 15 = intense. Means in the same column with different letters are significantly different at $p < 0.05$. NS = not significant.

Table 4 - Sensory attributes of SPI and protease modified SPI

Sensory Attribute	Treatments				
	Control	Multifect ® Neutral	Fungal Protease Concentrate	Multifect®Neutral + Fungal Protease Concentrate	
Aroma					
Raw beany	5.7[a]	3.7[b]	3.2[b]	4.0[b]	
Cooked beany	4.9	3.7	3.8	3.9	NS
Wheat flour	6.0[a]	4.7[ab]	4.1[b]	4.2[b]	
Flavor					
Sweet	3.1[a]	1.4[b]	3.0[a]	2.4[a]	
Raw beany	5.6	6.9	5.6	6.0	NS
Cooked beany	4.6[a]	3.9[a]	6.6[b]	5.0[a]	
Wheat flour	5.4	4.3	5.7	4.9	NS
Bitter	4.3[a]	10.8[b]	6.0[c]	7.8[d]	
Mouthfeel					
Chalky	5.9[a]	2.7[b]	3.7[b]	3.4[b]	
Gritty	4.2[a]	1.1[b]	1.3[b]	1.2[b]	
Astringent	3.6[a]	7.5[b]	6.1[c]	6.7[bc]	

Main effect means are responses of 2 SPI with high and low PDI levels, 11 panelists for 2 replications, 0 = none to 15 = intense. Means in the same column with different letters are significantly different at $p < 0.05$. NS = not significant.

CHAPTER 4. GENERAL CONCLUSIONS

The primary objective of this research was to evaluate the aroma and flavor alteration of soy protein hydrolysates. Improved flavor characteristics of soy protein hydrolysates are needed so that soy protein ingredients with valuable properties can be used in food applications. Aroma and flavor perceptions in food systems are complex. Aromas arise from many components and aroma intensity varies with concentration and complexity of the food matrix. Past studies have attempted to match specific aromas of SPI with specific chemical compounds using gas chromatography-olfactory and sensory evaluation techniques. The most prominent compounds, proposed sources and odor descriptors are summarized in Appendix A.

Soy protein hydrolysates have different aroma and flavor profiles based on 1) composition of the enzymes in commercial proteases, 2) the specificity of these enzymes, 3) the substrate matrix, 4) the composition and sequence of amino acids, 5) the molecular weight of peptides produced, 6) hydrophobicity of the amino acids and peptides, and 7) degree of protein hydrolysis. Enzyme specificity of the protease enzymes used in this research is described in Appendix C and D. Bitterness and astringency are the primary flavor problems of protein hydrolysates. The formation of bitter peptides depends on DH, amino acid composition and sequence, molecular weight of the peptides and enzyme specificity. The use of endopeptidase or exopeptidase is not the only factor affecting the sensory profiles of hydrolsates.

Substrates of different composition affect flavor perception. Water, proteins, lipids, carbohydrates, sugars, and salts interact differently with flavor components. The composition of EESF is more complex than that of SPI. This resulted in different aroma and sensory

profiles for EESF and SPI. The composition of EESF and SPI and the sensory descriptors for each soy product are noted in Appendix E.

Sensory panelists described differences in soy flour hydrolysates, particularly fishy, nutty, toasted, sweet, grassy beany, raw beany, bitter, and astringent attributes. Greater fishy aroma was detected in jet-cooked treatments of EESF compared to uncooked treatments. Control and MN-modified EESF samples were more fishy than bromelain and Experimental Exopeptidase-C (EEC) treatments. For flavor attributes, bromelain hydrolysate was no different than the control. EEC and MN treatments had less sweetness and more intense grassy-beany flavor, astringency and bitterness than the control. MN-modified EESF was the most bitter and had the least nutty and toasted flavors among samples. The non-digestible sugar in EESF hydrolyzed by α-galactosidase (α-GAL) increased sweetness and decreased bitterness of MN-modified EESF. α-GAL modification could be used to mask the bitterness of soy protein hydrolysates.

SPIs with different degrees of protein denaturation (based on PDI) were similar in most of the sensory attributes, but SPI with low PDI had more raw beany and bitter flavors than SPI with high PDI. SPI and soy flour hydrolyzed by MN were the most bitter and astringent and lowest in sweetness compared to other enzyme treatments. FPC-modified SPI had higher cooked beany flavor, less bitter flavor and less astringency than MN-modified SPI and the control. MN+FPC treatment had the sensory attributes between the single enzyme treatments. “Cooked beany” was a single attribute defined by sensory panelists, but is likely a mixture of aromas and flavors attributed to by-products of nonenzymatic browning and fatty acid oxidation, volatile carbonyls from lipid oxidation and furans.

The use of protease + α-GAL modification and the MN + FPC modification decreased the bitterness of soy hydrolysates than the MN treatment alone. However, it may be costly to apply the 2 enzyme treatments. Protease + α-GAL modification can be only used on soy ingredients with non-digestible sugar and not SPI. Improved functionality of soy flour with acceptable flavor profiles may increase the value of soy flour.

Bromelain and FPC proteases produced little bitterness in soy flour and SPI, respectively, and may be the most suitable hydrolysate for food applications. Flavor profiles should be considered as important as functionality and cost in the development of soy hydrolysates.

APPENDIX A. POSSIBLE COMPOUNDS ASSOCIATED WITH "BEANY" ODOR IN SOY PROTEIN ISOLATES

Compound	Derived source	Aroma descriptor
Butyric acid [1]	-	Sweaty feet [1]
2-methyl butyric acid methyl ester [1]	-	Fruity [1]
2-pentyl pyridine [1,2]	-	Penetrating grassy [1,2]
Hexanal [1]	Lipid oxidation/degradation [3,4]	Oxidized/nutty [2], green, bitey, very sweet, dripping-like on dilution [4]
Octanal [4]	Lipid oxidation/degradation [3]	Stink bug [4], oily, nut oil [3]
Pentanal [2]	Lipid oxidation/degradation [3]	Oxidized/nutty[2], nutty, sweet and green on dilution [4], grassy [3]
Dimethyl disulfide [2]	Heated sugars and /or amino acids [3]	Sulfur [2], unpleasant, stink bug, sulphyry, crushed ant on dilution [4]
Dimethyl trisulfide [2]	-	Sulfurous.green onion [2], must, sweet, fruity [4]
Pyrazines	Heated sugars and /or amino acids [3]	-
1-octen-3-one [2]	-	Mushroom [2]
1-octen-3-ol [4]	-	Medicinal, mushroom-like on dilution [4]
2,3-butadione [2]	-	Buttery [2]
2,3-pentadione [2]	Heated sugars and /or amino acids [3]	Buttery [2]
2-pentyl furan [2]		Grassy 2, green [4]
Benzaldehyde [2]	Heated sugars and /or amino acids [3]	Almonds [2], metallic and hearbaceous [4], almond, nutty [3]
Acetophenone [2]	Heated sugars and /or amino acids [3]	Penetrating green [2],slight meaty [3]
Trans-2,4-nonadienal [2]	Lipid-derived [4]	Oxidized/fatty [2]
Trans-2,4-decadienal [2]	Lipid-derived [4]	Oxidized/fatty [2]

Source : [1] Boatright and Crum 1997.
[2] Boatright and Lei 1999.
[3] Ames and Macleod 1984.
[4] Solina and others 2005.

APPENDIX B. SDS-PAGE PROFILES

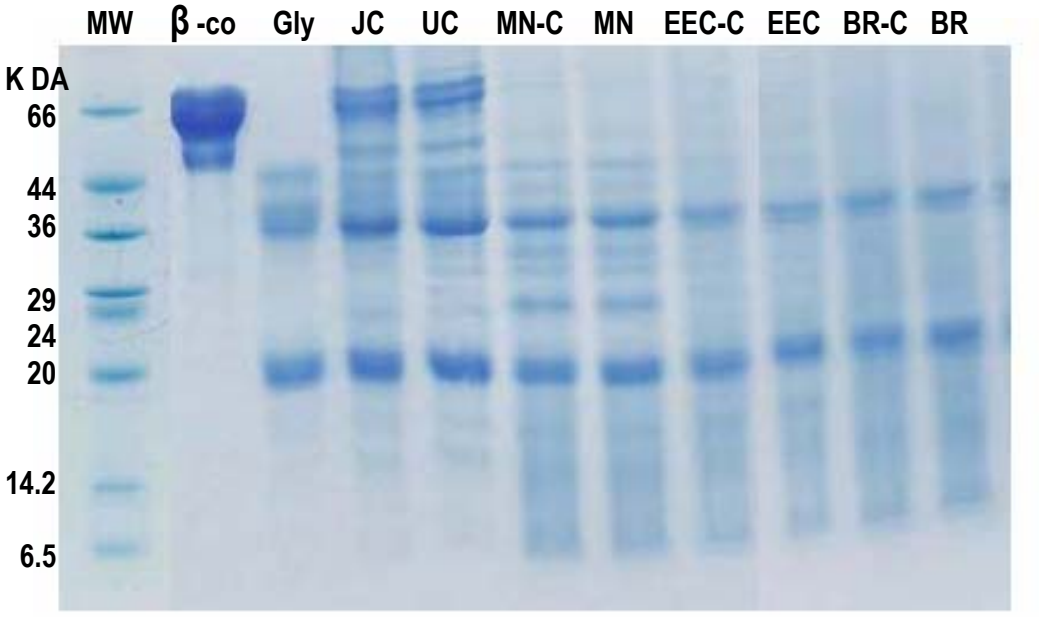


Figure 1- Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of EESF hydrolysates at marker: MW=molecular weight, β-co= β -conglycinin,Gly=glycinin, JC=Jet-cooked control, UC=Uncooked control, MN-C= MN hydrolyzed and Jet-cooked, MN=MN hydrolyzed and uncooked, EEC-C= EEC hydrolyzed and Jet-cooked, EEC=EEC hydrolyzed and uncooked, BR-C= BR hydrolyzed and Jet-cooked, BR=BR hydrolyzed and uncooked; 1 µg protein/lane (Lamsal and others 2006).

APPENDIX C. ENZYME NOMENCLATURE

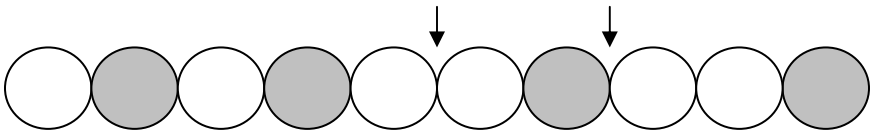
Commercial Enzyme	EC reported by supplier	Reaction Catalyzed
Bromelain		Acting on peptide bonds. Possible to be EC 3.4.22.33 - cysteine endopeptidase. Hydrolysis of proteins with broad specificity for peptide bonds.
Experimental Exopeptidase C	EC3.4.23.18	Acting on peptide bonds. Aspartic protease. Hydrolysis of proteins with broad specificity. Generally favors hydrophobic residues in P1 and P1', also accept Lys in P1, which leads to activation of trypsinogen.
Fungal Protease Concentrate (Renamed to Protex 51FP)	EC 3.4.23.18	Acting on peptide bonds. Aspartic protease. Hydrolysis of proteins with broad specificity. Generally favors hydrophobic residues in P1 and P1', also accept Lys in P1, which leads to activation of trypsinogen.
Multifect® Neutral (Renamed to Protex 7L)	EC3.4.24.28	Acting on peptide bonds. Similar but not identical to thermolysin. Thermolysis prefer cleavage at Xaa-|-Leu > Xaa-|-Phe
Multifect® Neutral (Renamed to Protex 7L)	EC3.4.21.62	Acting on peptide bonds. EC 3.4.21.62 – Subtilisin is a serine endopeptidases. Hydrolysis of proteins with broad specificity for peptide bonds, and a preference for a large uncharged residue in P1. Hydrolyzes peptide amides.

Source: ExPASy. 2007. NiceZyme view of enzyme. Available from (www.expasy.org). Accessed Jul 16, 2007.

APPENDIX D. EXPECTED REACTION OF ENZYMES

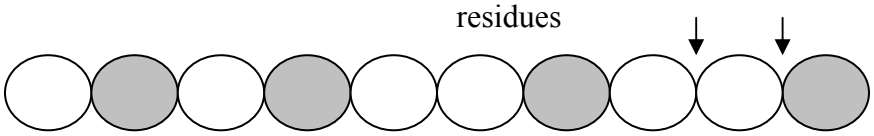
Bromelain

cleaves the middle of the polypeptide chain with broad specificity



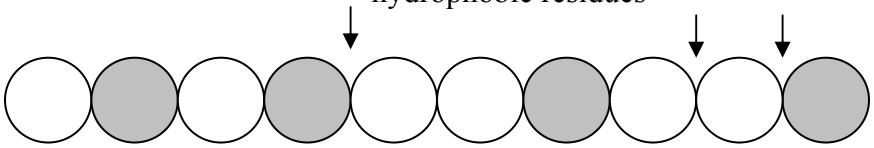
Experimental Exopeptidase C

cleaves the end of the polypeptide chain with broad specificity but favor hydrophobic residues



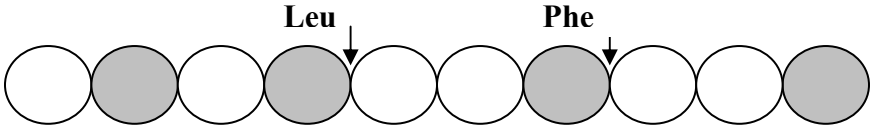
Fungal Protease Concentrate (Renamed to Protex 51FP)

cleaves the middle and the end of the polypeptide chain with broad specificity but favor hydrophobic residues

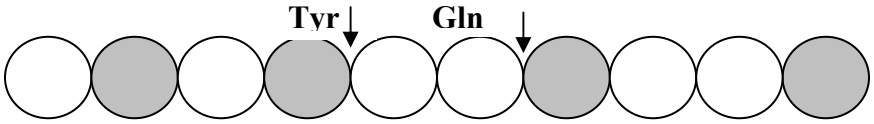


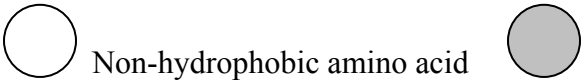
Multifect® Neutral (Renamed to Protex 7L)

EC3.4.24.28- cleaves the middle of the polypeptide chain, similar to thermolysin that prefer cleavage at Xaa- | -Leu > Xaa- | -Phe



EC3.4.21.62- cleaves the middle of the polypeptide with broad specificity but a large uncharged residue in P1





APPENDIX E. COMPOSITION AND SENSORY ATTRIBUTES OF EXTRUDED-EXPELLED SOY FLOUR AND SOY PROTEIN ISOLATE

Extruded-Expelled Soy Flour (EESF)	Soy Protein Isolate (SPI)
Composition (% in dry basis)	
Protein 50.9±0.12 [a] (PDI=21 [c])	Protein >90 [b] (PDI=66.2 and 3.3 [c])
Sugar 7.3 [a]	Fat <4 [b]
Oil 7 [a]	Ash <5 [b]
Fiber 4.2 [a]	
Moisture content 2.62±0.01 [a]	**Moisture content <6 [b]**
Sensory Profiles	
Aroma	**Aroma**
Fishy	Raw beany
Roasted	Cooked beany
Beany	Wheat flour
Nutty	
Flavor	**Flavor**
Sweet	Sweet
Toasted	Raw beany
Nutty	Cooked beany
Grassy-beany	Wheat flour
Raw-beany	Bitter
Bitter	
Astringent	**Mouthfeel**
	Chalky
	Gritty
	Astringent

Source: [a] Lamsal and others 2006.
[b] Archer Daniels Midland Co 2007.
[c] Eurofins Scientific Inc 2007.

APPENDIX F. INFORMED CONSENT DOCUMENT

Enzyme Modification to Enhance Soy Protein Ingredients in Food
November 06 to May, 07

This research is being conducted by Sheue-lei Lock and Dr. Cheryll Reitmeier, Department of Food Science and Human Nutrition, Iowa State University. The training involves the sensory evaluation of standard tastes and soy protein isolate products. The soy samples will be evaluated in water solutions or in food products.

You must be 18 years of age or above to participate in the sessions. Panelists are not allowed to participate if allergic to soy foods. You will be participating in descriptive analysis training and evaluation during spring 2007. The sensory evaluation will be conducted in CDFIN sensory facility, Human Nutritional Sciences Bldg., ISU.

Responses to the sensory evaluation will be used only in coded statistical analysis without reference to the respondent. There is no risk associated with the evaluation of soy flour in water or food. Benefits include a reward of food at each session, a gift of $50.00, a significant contribution to soybean research and no direct benefit to the participant. Dr. Reitmeier (294-4325, creitmei@iastate.edu) will be available throughout the study to answer questions associated with the evaluation.

If you have any questions about the rights of research subjects or research-related injury, please contact the IRB Administrator, (515) 294-4566, IRB@iastate.edu, or Director, (515) 294-3115, Office of Research Assurances, Iowa State University, Ames, Iowa 50011.

***** I understand the research being conducted and agree to evaluate soy protein products. I understand that I should be present for evaluation sessions but I can withdraw at anytime. I will notify the investigator if I can no longer participate in this research. *****

NAME : _____

DATE : _____

APPENDIX G. PANEL QUESTIONNAIRE-TRAINING

History

Name: Gender:

E-mail: Phone number:

Health

1. Do you take any medications which affect your senses, especially taste and smell?

_____

2. Do you have any allergies to soy foods?

_____

3. Do you have any health problem that causes you not able to take certain type of food?

_____

Daily Living Habits

1. What are your favorite foods?_____
2. What are the foods you dislike to eat?_____
3. What are the foods you can not eat? _____
4. What is your favorite snack? _____

Flavor Quiz

1. How would you describe the difference between flavor and aroma?

_____

2. How would you describe the difference between flavor and texture?

_____

3. Describe some of the noticeable flavors in chocolate.

_____

Please refrain from eating food; chewing gum and drinking juice or soda 1 hour prior to this session. Try not to wear heavy smelling perfumes, Thanks!

-Thank you for your participation-

APPENDIX H. SCORE CARD FOR BASIC TASTES RECOGNITION

TEST-TRAINING

Name: _____
Date: _____

Please taste the 4 samples labeled with sweet, sour, salty and bitter. Rinse your mouth with distilled water, then place the solution in your mouth and move it around your tongue. Expectorate the sample and rinse your mouth with water, wait for 5 seconds before tasting the second sample.

Use the sample procedure as above and taste the set of coded samples. Record the codes and marked an X on the corresponding line.

Sample Code	Sweet	Sour	Salty	Bitter
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

Comments:

-Thank you for your participation-

APPENDIX I. SCORE SHEET FOR INTENTSITY RANKING TEST-TRAINING

Name: _____
Date: _____

Please rank each coded solution in ascending order of intensity/strength of the solution. Rinse your mouth with distilled water, then place the solution in your mouth and move it around your tongue. Expectorate the sample and rinse your mouth with water, wait for 5 seconds before tasting the second sample.

	Sample Code
Least sweet	_____
	_____
Most sweet	_____

	Sample Code
Least sour	_____
	_____
Most sour	_____

	Sample Code
Least salty	_____
	_____
Most salty	_____

	Sample Code
Least bitter	_____
	_____
Most bitter	_____

-Thank you for your participation-

APPENDIX J. SCORE SHEET FOR SOY FLOUR HYDROLYSATE EVALUATION

-PROTEOLYTIC HYDROLYSATE PANEL

Name/Code: _____
Date: _____

Instruction: Quick sniff the sample several times and try to identify the best sample to match the odor of the soy flour solution. After the discussion, an appropriate value will be assigned based on the intensity of the sensory attribute. You may re-sniff if you wish, but allow some time (15 sec) to elapse before you try it again.
Please mark the relative intensity of the aroma on the scale

AROMA

1. Fishy

_____
None Intense

2. Roasted

_____
None Intense

3. Beany

_____
None Intense

4. Nutty

_____
None Intense

Please taste the sample and find the best sample to match the flavor of soy flour solution. Rinse your mouth with special rinse between samples. Hold the sample in your mouth and move it around your tongue. You may expectorate the sample, rinse your mouth and wait for 5 seconds before you re-taste it.

FLAVOR

5. Sweet

_____
None Intense

6. Toasted

None Intense

7. Nutty

None Intense

8. Grassy-beany

None Intense

9. Raw-beany

None Intense

10. Bitter

None Intense

11. Astringent

None Intense

Comments:

-Thank you for your participation-

APPENDIX K. SCORE SHEET FOR ENZYMATIC MODIFIED-SOY FLOUR

EVALUATION- ALPHA-GALACTOSIDASE PANEL

Number code: _____
Date: _____

Instructions:

1. Taste the samples provided as Standards. Determine the suitability of the Standards for the flavors identified.
2. Taste the soy flour solutions and mark the intensity on the linescale for each attribute, compared to the appropriate Standard.

Rinse your mouth with water between samples. Hold the sample in your mouth and move it around your tongue. You may expectorate the sample, rinse your mouth and wait 5 seconds before re-tasting.

Sample code:_____
Sweetness

None Intense

Sample code:_____
Sweetness

None Intense

Sample code:_____
Sweetness

None Intense

Sample code:_____
Sweetness

None Intense

Instructions:

1. Taste the samples provided as Standards. Determine the suitability of the Standards for the flavors identified.
2. Taste the soy flour solutions and mark the intensity on the linescale for each attribute, compared to the appropriate Standard.

Rinse your mouth with water between samples. Hold the sample in your mouth and move it around your tongue. You may expectorate the sample, rinse your mouth and wait 5 seconds before re-tasting.

Sample code:_____
Bitterness

None Intense

Sample code:_____
Bitterness

None Intense

Sample code:_____
Bitterness

None Intense

Sample code:_____
Bitterness

None Intense

-Thank you for your participation-

APPENDIX L. SCORE SHEET FOR SOY PROTEIN ISOLATE EVALUATION

Code Number:
Name: _____

Instruction: Please taste each sample and evaluate the sample for intensity of aroma flavor, mouthfeel by marking a line on the intensity scale. Rinse your mouth with water before you taste the sample. Hold the sample in your mouth and move it around your tongue. You may expectorate the sample, rinse your mouth and wait for 5 seconds before tasting the next sample. Thank you.

Soy Protein Isolate Attributes:

Aroma

1. Raw Beany

None Intense

2. cooked beany

None Intense

3. Wheat flour

None Intense

Flavor

4. Sweet

None Intense

5. Raw beany

None Intense

6. Cooked beany

None Intense

7. Wheat flour

None Intense

8. Bitter

None Intense

Mouthfeel

9. Chalky

None Intense

10. Gritty

None Intense

11. Astringent

None Intense

Comments:

-Thank you for your participation-

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