ABSTRACT

NISHKU, SARA. Development of a Lexicon for Plant Protein Powders. (Under the direction of Dr. MaryAnne Drake).

Plant proteins are increasing as a protein alternative. As such, there is a need to identify a sensory language to provide a standardized tool to identify flavor attributes of plant proteins and protein specific flavor changes following heat treatment. A total of 66 samples of plant proteins, including pea, chia, soybean, rice, wheat, faba bean, hemp, sacha inchi, mung bean, pumpkin, and potato were collected. Proteins were rehydrated at 10 % solids (w/v) in deionized water and a highly trained descriptive panel identified, defined and referenced a lexicon with 24 aromatics, 4 basic tastes and 2 mouthfeel attributes. Selected representative plant protein solutions (n=13) were incorporated into model beverages, ultra-pasteurized at 140°C for 3 s, and evaluated. Key differentiating attributes for plant proteins included basic tastes (umami, bitter) and aromatics (green/grassy, cardboard, doughy, fruity, fecal/indole, cereal/grain) ($p<0.05$). Flavor intensities and astringency changed with heat treatment ($p<0.05$) and only a few new flavors specific to heat treatment were documented. The developed lexicon provides a tool for characterization and differentiation of specific flavors contributed by plant proteins that can be used by product developers for development and characterization of protein-rich foods.

Development of a Lexicon for Plant Protein Powders

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DEDICATION

First and foremost, to my parents and brother who have supported me with constant love and encouragement. To my friends across the country, for making me laugh during the times I needed it most. To Austin, for being a shoulder to lean on. To the kittens, for the snuggles. Of course, all of this wouldn't be possible without the guidance of Dr. Drake and the rest of the MAD lab, who I have truly considered to be a second family during this time.

BIOGRAPHY

Sara Nishku was born on May 12, 1995 and grew up in Palm Harbor, FL. Following high school, Sara attended University of Florida, where she graduated in 2017 with a Bachelor's degree in Food Science. Her journey continued to North Carolina State University in Raleigh in January 2018, where she pursued her Master's degree in Food Science under the direction of Dr. MaryAnne Drake. Sara graduated in June 2020. While not at work, Sara spends her time joining volleyball leagues and run clubs, hiking, cooking, and traveling across the globe.

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Introduction

Plant proteins are being used in new product development as dairy alternative proteins (Mintel, 2018; Sethi et al., 2016; McKinsey and Company, 2019). Plant protein sources are more sustainable and ethical to produce than animal-based protein sources in the minds of many consumers (Pimentel and Pimentel, 2003; Aiking, 2011; Food Navigator-USA, 2018). The nutritional and functional properties of plant proteins should also be assessed. Plant proteins do not contain all essential amino acids which make them incomplete proteins and not as nutritional as dairy proteins (Pasiakos, 2015; Gilbert et al., 2011). Plant proteins also do not perform functionally as well as dairy proteins in many food applications (Webb et al., 2002; Zayas, 2012). Since plant proteins are used in beverage formulations, there needs to be more research into the characterization of differences regarding sensory changes after heat treatment for plant proteins. The understanding of sensory and functional properties of proteins from different plant sources can give insight to manufacturers of high protein products. This literature review will address sensory techniques to characterize the flavors of different plant proteins and how certain factors, like heat treatment and protein interaction, can affect the overall sensory profile.

Plant Proteins as an Alternative Protein Source

Proteins are essentially important to the human body and they are widely used by the food industry for nutritional and functional purposes in foods. The global high protein-based food market size is expected to reach USD 91.1 billion by 2021, with an expected 11% increase in size annually (Business Wire, 2017). Animal based proteins, such as whey or milk, have generally dominated this industry, however in the past five years, 54% of protein products that have launched using the “high protein” label have been vegan or plant based (Mintel, 2013). Plant protein sources can be categorized as legume, nut, grain, or vegetable. Soy protein has

dominated the plant protein market, however other proteins, such as pea, are becoming more popular (Grand View Research, 2020). Soy protein experienced criticism because it is considered a genetically modified organism (GMO), making it a controversial topic for many consumers (McKinsey & Co, 2019). Soy protein has also been documented to have allergenic tendencies and hormonal estrogenic effects (Harvard, 2019; Friedman and Brandon, 2001).

There are a variety of plant based high protein product applications such as bars, ready-to-mix (RTM) powders, and ready-to-drink (RTD) beverages. Overall, there is an increasing demand among consumers to create products with dairy alternative proteins (Henchion et al., 2017). Many consumers view plant proteins as environmentally sustainable and more ethical in terms of animal welfare over dairy-based proteins (Aiking, 2011; McCarthy et al., 2016; Mintel, 2018). Plant proteins have shown increasing potential of being used as an alternative high protein source, but there are still many challenges in terms of flavor and functionality. There has been much research conducted to refine dairy proteins in terms of protein functionality and flavor that has yet to be performed for plant proteins. Flavor is a key for consumer acceptance regarding the use of plant proteins in products (Natdathur et al., 2017; Mintel, 2018). More than 40 y ago, Rackis et al. (1979) documented that vegetable protein sources had undesirable off-flavors such as beany, grassy and high intensities of bitter taste and astringency. Since that time, sensory properties of soy products, including soy proteins, have been addressed in many studies, but sensory properties of plant proteins remain undefined as demand for plant-based foods continues to increase.

Nutritional Profile of Plant Proteins

Proteins are a key part to any diet and are needed for growth and development and to keep maintenance of the body. The recommended daily allowance (RDA) is 0.8 g protein per kg of body weight for healthy adults 19 years and older (Institute of Medicine, 2005). The recommended dietary allowance for adults ages 19-70 y is about 46 g and 56 g for women and men, respectively (FDA, 2011). Proteins can be found in both plant and animal sources. The protein quality, or the nutritive value of a protein, depends on the amino acid composition, and physiological utilization of specific amino acids after digestion, absorption, and minimal obligatory rates of oxidation (Friedman, 1996). There are twenty amino acids that are found in nature that are the building blocks of all proteins. A "complete" protein is one that contains all nine essential amino acids (EAA). EAA must be obtained from the diet because they cannot be synthesized by the body. Proteins allows the body to perform essential actions like regulation of enzymes in biological pathways, binding of complex molecules, amplify and transduce signals, cause motion, and control genetic form. In comparison to animal proteins, all plant proteins lack lysine and leucine and have an imbalanced ratio of cysteine to methionine (van Vilet et al., 2015; Massey, 2003). Animal based proteins are viewed as higher quality since they contain all EAA that are needed for muscle protein synthesis, have higher digestibility and have higher absorption of EAA relative to plant protein sources (Pasiakos, 2015; Gilbert et al., 2011).

When it comes to plant proteins, there is a wide range of protein quality. Protein quality can be measured using protein efficiency ratio (PER), digestible indispensable amino acid score (DIAAS), and protein digestibility corrected amino acid score (PDCAAS). PER is the oldest method, developed in the early 1900s, and is based on the weight gain of a test subject divided by its intake of a protein during the test period. This approach has been replaced by newer

methods. DIAAS is the ratio of the digestible amino acid content in the food (mg/g of protein) to the same amino acid in a reference pattern taken from age-specific amino acid requirements. DIAAS is the newest method, developed in 2013, that considers the ileal digestibility coefficients of each amino acid instead of true fecal digestibility that is used for PDCAAS (Marinangeli and House, 2017). DIAAS is still not yet implemented by industry and PDCAAS remains the recommended score by FDA and is widely used.

The PDCAAS is determined by expressing the content of the first limiting amino acid of the test protein as a percentage of the content of the same amino acid content in a reference pattern of essential amino acids (Hoffman and Falvo, 2005). The PDCAAS reference score is based on the amino acid profile to the needs of a two-to-five-year-old – the most critical requirement of any age group except infants (Endres, 2001). The digestibility is based on the proportion of dietary protein derived amino acids that is effectively digested and absorbed to be used in body protein synthesis reactions (van Vilet et al., 2015). Based on the PDCAAS score, isolated soy protein meets the same efficiency as animal protein, whereas wheat protein may be less than 50% useable compared to animal protein (American Dietetic Association, 2003). PDCAAS has limitations such as the reference pattern is based on minimum amino acid requirements and does not reflect optimum intake. Mathai et al. (2020) compared the DIAAS and PDCAAS scores for dairy and plant proteins and determined, that regardless of the method, dairy proteins had greater digestibility than plant proteins taken from soy, pea and wheat. Other studies have also determined that some plant proteins, like isolated soy protein, provide the same nutritive quality as milk protein (Steinke, 1980; Young et al., 1994). While soy has the same protein quality as animal protein by some studies, there are other health factors associated with soy protein that have yet to be fully understood (Xiao, 2008; Patisaul and Jefferson, 2010). The

plant estrogen compounds in soy, called isoflavones, have been found to cause hormonal interactions in the body (Friedman and Brandon, 2001). Allergic reactions in feeding infants using soy-based formulas have also been documented and some infants may not tolerate soy-based diets (Cantani and Lucenti, 1997).

Most plant proteins lack one or more amino acids which alters the rate that the absorbed amino acids are used in our body. Plant protein sources can be combined in order to create a complete protein or have an improved amino acid composition in order to create a complete protein (Brito and Nunez, 1982; Craig and Mangels, 2009; Shiriki et al., 2015; Gorissen et al., 2018). This dietary process is called complementation. Steinke et al. (1980) determined that a blend of 40-60% isolated soy protein with both animal and vegetable proteins maintained a more balanced amino acid profile than regular isolated soy protein alone. Complementary proteins do not have to be consumed at the same time in order to ensure nitrogen retention and absorption of all EAAs (Young et al., 1994). Further research needs to be conducted to test the effectiveness and nutritional benefits of plant proteins and plant protein blends.

Processing Protein Isolate and Concentrate

There are a variety of different plant sources that are used to make commercial plant proteins. Most commonly used industrial plant protein sources include soy, pea, rice, spirulina, hemp, and pumpkin seed (Grand View Research, 2020). Generally, similar process procedures are followed in order to produce plant protein isolates and concentrates. First, the plant protein sources themselves must be screened of foreign materials and damaged products with additional steps depending on structure, i.e. seeds, leaves, hulls, etc. For example, the soybeans are cleaned by air-screen cleaning and gravity separators. There are many factors to consider when determining a processing method for a plant protein source including: species, seasonal variation,

and development stage of the product, etc. Among all plant-based proteins, the most processing research conducted has been with soy proteins because of its many uses and its long use as a cultural food staple in many Asian countries (Myers, 1988; Lusas and Riaz, 1995; Ashaolu, 2019).

Various plant protein types are used to produce protein products including flours, concentrates, and isolates. Flours contain 40-65% protein, concentrates contain 65-90% protein and isolates contain 90% protein (Swanson, 1990; Enders, 2001). Protein flours are the least refined protein product. Flours can be fatted or defatted. Fatted flours are the plant source that has been dehulled and milled into a powder. Defatted flour involves an extra step to remove oils in fatted flours. The oils are commercially extracted using solvent extraction using hexane, ethanol and water (US 201001 12187A1, 2010; Campbell et al., 2013; Endres, 2001). The remaining wet defatted material is then spray dried or evaporated into a flour. Protein concentrates are defatted flour made by removing most of the water soluble, nonprotein constituents such as sugar, water and/or alcohol (Altschul and Wilcke, 2013). Protein isolates represent the major protein fraction and have most of the non-protein contents removed, as well as fibrous material.

Plant proteins sources generally go through three major levels of refinement to attain a soluble form of the protein. The first mandatory level removes any antinutritional factors. Anti-nutritional factors, such as trypsin inhibitors, phytic acid, tannins and lectins, are often present naturally in plant food protein sources including soybean, peas and faba beans (Schaafsma, 2012). Antinutritional factors are biological compounds present in foods that reduce nutrient utilization or food intake, thereby contributing to impaired gastrointestinal and metabolic performance. Most of the antinutritional factors are inactivated by heat, but any remaining heat

stable compounds, like saponins, phytic acid, tannins, are removed using extraction or ultrafiltration from aqueous alcohol solutions (Omosaiye and Cheryan, 1978; Ghodsvali et al., 2005; Majinda, 2012). Some heat stable compounds include saponins and isoflavones. These compounds should be removed because they contribute to off-flavors, like bitter taste (Heng et al., 2006; Laszitity, 2009; Damodaran and Arora, 2013). There are some anti-nutritional factors that develop during heat processing of proteins such as Maillard reaction products, protein-bound D-amino acids, and lysinolalanine (LAL) that negatively affect protein digestibility (Gilani, 2011).

After the anti-nutritional factors are removed, the indigestible sugars are extracted. Indigestible sugars are present in most legumes and can be extracted without enzymatic manufacturing processes. Some carbohydrates found in plants are not broken down by enzymes in our body, which can cause gas production and mild to severe abdominal discomfort. The most common oligosaccharides are galacto-oligosaccharides, raffinose, stachyose, and verbascose (Karr-Lilienthal et al., 2005). Indigestible sugars are removed using ultrafiltration from aqueous alcohol solutions (Liener, 1994; Lawrence, 2011). Finally, dry fractionation into protein concentrates and isolates from insoluble starch, fibrous and cellulose material occurs through iso-electric precipitation (Schutyser et al., 2015).

Refinement of the raw plant protein source can occur through mechanical separation such as air classification or dry milling. Air classification mills grind the whole or dehulled seed into a very fine flour and the flour is then classified into a perpetual spiral stream until the starch and protein are separated (Boye et al., 2009). This technique is variable since the milling efficiency can depend on the thickness and structure of the cell wall and the degree of adhesion between the cell contents and proteinaceous material and starch granules.

The protein purity and yield are easily affected by the processing conditions and can be variable depending on the different time temperature profiles used, pH, and protein solubility of the starting material (Gonzalez-Perez and Arellano, 2009). Further separation of starch and protein components occurs based on the particle size (Tulbek et al., 2017). Protein fractions undergo a wet process where they are separated as fine particles that are then clarified by centrifugation, filtration and membrane processing in order to concentrate the protein, usually by ultrafiltration or precipitation (Gonzalez-Perez and Arellano, 2009). Most commonly used to create protein isolates is alkaline extraction followed by isoelectric precipitation (IEP). This is commonly used for legume proteins such as pea, mung bean, faba bean, etc. The proteins are kept at an alkaline pH (8-11) with a base, commonly sodium hydroxide, which solubilizes the proteins at an elevated temperature. For example, wheat gluten is adjusted to pH 10-11 at 95oF using ammonia solutions and for soy, pH is adjusted to pH 8-10 between 80-1000F using potassium hydroxide solutions (US 8,309,152 B2, 2012; WO 2009/006144 Al, 2009). The solution undergoes ultrafiltration or precipitation to remove any insoluble material and then the pH is adjusted so that the isoelectric point induces protein precipitation (Gonzalez-Perez and Arellano, 2009). The isoelectric point of a specific protein is the pH where the net charge of that specific protein is zero (Table 1.1). Individual proteins are more likely to aggregate with each other when like charges are not present (Huppertz et al., 2018). Control of the pH is a critical factor to maintain the functional properties of the isolated protein. The ionic strength is a contributing factor to the solubility of the isolate (Myers, 1988). The solution is then centrifuged to collect the protein, washed to remove salts, neutralized and dried. Generally, spray drying is the most common economical method. During this drying step, further denaturation occurs due

to air-water interaction and aggregation that can lead to protein unfolding (Haque and Adhikari, 2015).

Lipid Oxidation Effects on Plant Proteins

Lipids are hydrophobic compounds that can be added to foods or occur naturally. They can affect the perception of overall flavor, aroma, and mouthfeel of a food product. Within a food matrix, lipids are likely to undergo oxidation that leads to the degradation of quality and sensory attributes, i.e. off-flavor. Lipid oxidation can occur naturally or as a result of manufacturing or product shelf life and is increased by heat, light, chemical catalysts or non-enzymatic and enzymatic processes. These reactions in plants are induced by atmospheric oxygen (oxidation), native lipoxygenases (enzymatic oxidation), and free radicals (autooxidation) (Damodaran and Arora, 2013). The overall mechanism is described through the spontaneous reaction of lipids with atmospheric oxygen through a chain reaction of free radicals (Figure 1.3) (Cinkova et al., 2014).

Lipids can be found naturally in all plants and can affect the overall flavor in plant proteins because of heat treatment and storage conditions. Lipid oxidation in plant proteins can also cause decolorization of pigments, lipid-protein interaction, and lead to decreased nutritive quality through decreased solubility, digestibility, and availability of essential amino acids (Swanson, 1990; Damodaran and Arora, 2013). During the processing of plant protein extraction several non-protein materials, such as lipids and lipid oxidation products, can be co-extracted with the plant protein leading to off flavors (Harwood, 1997). Plant proteins contain varying amounts of total fat content, however once the fat is removed, the remaining protein flour still contains fats that are equally susceptible to oxidation reactions. The total fat content in plant protein isolates, such as soy, is usually around 2.4-5% (Fang et al., 2004). The major

phospholipids found in plant sources are polar phospholipids, like phosphatidylcholine (Sessa et al., 1976; Aldin et al., 2006; Gonzalez-Thuilier et al., 2015). Plant based protein isolates, such as soy and pea, contain unsaturated fatty acids including monounsaturated fatty acids, like oleic acid, and polyunsaturated fatty acids, like linolenic and α-linoleic acids (Messina, 1999; Brynda-Koptowska, 2018). The high polyunsaturated fatty acid content in plant fats and the phospholipids in plant proteins caus3 high susceptibility to lipid oxidation (Swanson, 1990). The enzyme lipoxygenase is found in many plants and catalyzes the oxygenation of fatty acids to produce hydroperoxide derivatives and lipid peroxidation-derived free radicals (Harwood, 1997; Sosulski, 1979; Sessa and Rackis, 1977). Inactivation of this native enzyme is critical in the initial handling and processing of plant proteins.

Beany and cardboard flavors and bitter taste have been attributed to oxidation derivatives of polyunsaturated fatty acids to aldehydes, ketones and alcohols (Swanson, 1990; Sessa and Rackis, 1977; Weder and Belitz, 2003). The catalyzed enzymatic oxidation of linoleic acid and linolenic acid by native lipoxygenases also contributes to bitter taste in plant products (MacLeod et al., 1988; Yu et al., 2017; Weder and Belitz, 2003). These sources of potential flavors and odors that naturally occur in pea protein can include 2-hydroxypalmitic acid, α-linolenic acid, octacosa-6,9,19,22-tetraen (15), and (10E,12E)-9-hydroxyoctadeca-10,12-dienoic acid (Glaser et al., 2020). For soy protein isolate, the contributing flavor and odor sources include sulfur containing amino acids like methionine as well as fatty acids which can produce a host of different volatile compounds, dimethyl trisulfide, 2-pentyl pyridine, hexanal, trans, trans-2,4-decadienal, trans, trans-2,4-nonadienal, and acetophenone (Boatwright and Lei, 1997; Boatright and Crum, 1997). Hexanal is one of the primary and secondary identifiable decomposition products of linoleic acids. Hexanal is one of many presumed compounds to be responsible for

grassy, green aromas in pea flour and beany aroma in faba beans and soy flours and isolates (Sessa and Rackis, 1977; MacLeod et al., 1988; Boatwright and Crum, 1997; Glaser et al., 2020). Other lipid oxidation compounds, like (E)-2-Octenal, have been found in soy isolates and green pea extract associated with green pea and green aroma (Siddiq and Uebersax, 2018). To further prevent lipid oxidation reactions from happening, improved processing and storage conditions can prevent some reactions from occurring.

Maillard Reactions and Effect on Plant Proteins

The Maillard reaction occurs between free amino acids and free amino acid groups on proteins, and carbonyl groups of reducing sugars. The Maillard reaction is comprised of three stages: early stage, intermediate stage, and final stage. During the early stage, the sugar-amine condenses, and the Schiff base and Amadori rearrangement products (ARPs) are formed (Hodge, 1955; Wrodnigg and Eder, 2001). The intermediate stage involves sugar dehydration, fragmentation and amino acid degradation. During this intermediate stage, one of the ARPs, the Stecker aldehydes, can react with sugar derived Maillard intermediates leading to aroma changes in food (Ruan et al., 2018). In the final stage, there are several different products formed- which can include polymeric compounds, like melanoidins, which act as brown pigments. Both color and flavors can be formed.

The Maillard reaction can be caused due to heat treatment during food processing when temperatures range from 100 to 250 ºC and/or during storage for long periods at room temperature. The negative impacts of this reaction can lead to off flavor development, discoloration, and loss of protein nutritional value (Ames, 1990). Regarding proteins, the ε-amino group of lysine residues and sometimes guanidino groups of arginine can react with reducing sugars – causing decreased nutritional and functional quality (Weder and Belitz, 2004;

Ruan et al., 2018). The temperature, time, pH, water content and types of sugars and amino acids present also affects the flavor and aroma development of the product (van Boekel, 2006; Yu et al., 2018).

The processing steps used for plant protein isolates and concentrates can hydrolyze residual sugars into glucose and fructose, which can result in Maillard reactions (Finot, 1983). Commonly occurring in plant protein sources is the formation of the Amadori compound resulting from the reaction occurring during the high drying temperatures and poor storage conditions (Wettlaufer and Leopold, 1991; Zhang et al., 2017). This reaction occurs between lysine, a commonly found amino acid in plant proteins, and a reducing sugar resulting in the loss of availability of lysine and decreased protein digestibility (Finot, 1983).

There have been many studies that have observed how the Maillard reaction can affect solubility, flavor and color during storage of dairy proteins (Anema et al, 2006; Sithole et al. 2006, Le et al., 2011; Carter and Drake, 2018). Color is altered due to nonenzymatic browning reactions that occur from the chemical reactions with sugars resulting in melanoidins, brown-colored pigments. There have been studies that have shown that whey protein isolate + glucose systems brown more readily than soy protein isolate and glucose system, probably due to the increased lysine content found in milk proteins (Laubuza and Schmidl, 1986; O'Brien and Morrisey, 1989; Davies et al., 1998). Maillard reactions also produce flavors and aromas (Zhou et al, 2002; Romero and Ho, 2007; Cerny and Davidek, 2003; Sikorski et al., 2008; van Borkel, 2006). The deamination of proteins leads to the liberation of an ammonia molecule from the conversation of asparagine or glutamine into an acid. Free ammonia is a driver of the Maillard reaction and formation of pyrazines, which contribute to roasted and baked notes (Romero and Ho, 2007; Zhang et al., 2019). Soy protein peptides that have undergone Maillard reaction

increase the intensity of meaty flavor and umami taste and decrease bitter taste (Lan et al., 2010; Song, 2013). The transformations in the protein structure leads to a decrease in bitterness in amino acids and peptides. Maillard reactions can also negatively impact protein solubility (Shih et al., 2016; Fan et al, 2018). In dairy proteins, the lactose interacts with free amino groups from amino acids which results in protein crosslinking, reducing solubility (Anema et al., 2006; Fan et al., 2018). Martins and Netto (2006) determined that Maillard reactions also led to the decline of solubility in soy protein isolate gels during storage conditions, especially with increased temperature and relative humid. Proper storage conditions and controlled processing procedures should be considered during and after processing plant proteins to limit Maillard reactions.

Flavor and proteins

Taste and aroma perception have a key role in the acceptability of any product (Cardello, 1992). Flavors can be added to foods to improve overall flavor and mask undesirable attributes, like bitterness and astringency. Proteins have their own flavor profile that carry over into the final product (McDaniel and Chan, 1988; Russell et al., 2006; Whetstine et al., 2005; Suppavorasatit and Cadwallader, 2010; Oltman et al., 2015; Childs and Drake., 2010; Keefer et al., 2020). When proteins are added into a food matrix, they can also bind flavor compounds that have been added to a food product and alter the overall taste profile of the product. The process of purifying (processing alterations) and isolating (refining, drying, storage) proteins also affects the final protein flavor (Gonzalez-Perez and Arellano, 2009; Sessa and Rackis, 1977; Carter and Drake, 2018). There are two main interactions that occur between proteins and flavor compounds. The first is reversible binding including hydrogen bonds, hydrophobic interactions, and ionic bonds (Overbosch et al., 2009). The second is irreversible binding between covalent linkages, for example amide and ester formation. However, there is no universal mechanism for

flavor binding in foods – it depends on the characteristics of the flavor compounds and proteins present in the matrix (Kuhn et al., 2006).

When proteins undergo changes in pH, temperature and high pressure, the conformation is altered. This change in conformation affects protein-flavor interactions, including the protein binding of flavor compounds. Kuhn et al. (2008) examined flavor binding with whey protein isolate and determined that increased heat and pressure had notable effects on protein-flavor binding and changes to the overall flavor profile. Not all flavor compounds have the same binding capabilities, depending on their hydrophobic nature, indicating that not all flavor compounds bind in the same intensity. This flavor binding capability for some aroma compounds is found in the hydrophobic sections of the protein, which become more available as the protein uncoils though denaturation (Grinberg et al., 2002). The alterations in protein structure and the effect on flavor binding varies between different types of proteins. For example, the binding of vanillin to soy protein isolate is a result of changes in the conformation of protein structure to allow for flavor-protein binding, while the binding of vanillin with casein and whey protein isolate is driven by the interactions of the carbonyl and hydroxyl groups of the vanillin with the protein (Price et al., 1979; Li et al., 2000). Temperature changes in plant-based protein solutions alter the protein structurally and change the binding capabilities, similarly to what happens in dairy proteins, such as whey protein (Damodaran et al., 1960; Heng, 2006).

The binding of flavor compounds leads to changes in the flavor profiles of the overall food matrix. Hansen and Heinis (1991) used trained sensory panels to determine that the increased presence of whey protein concentrate and casein (0.125-0.5% w/v) lead to a decreased perception of vanilla flavor in model solutions. Li et al. (2000) compared the binding capabilities of six concentrations of vanillin (mg/L) for 2% (w/v) solutions of dairy (whey protein isolate and

casein) and soy protein isolate using Klotz plots and thermodynamic parameters to determine that dairy proteins, especially whey protein, had a stronger binding for vanillin than soy protein in protein solutions. Houde et al. (2018) compared vanillin binding with pea protein concentrate (74.3% protein content) and whey protein isolate (94.9% protein content) using spectroscopic quantification of unbound flavor ligands in protein [2% (w/v) x vanillin (0.29 to 32.86 mmol/L)] solutions. Sensory analysis using different the different proteins (30% w/w) and vanilla (4.45-26.7% w/v) found that the perception of vanilla flavor was higher in the whey protein isolate using enriched cookies and that off-flavor intensity increased as protein content increased for both protein types. Ng et al. (1989) determined that the concentration of the flavorant and faba bean protein isolate 5% (w/v) and the conformation of the protein contributed to the extent of binding of the flavorant by the protein. More research is needed to examine the differences between how proteins bind flavor compounds and how plant protein – flavor interactions differ from dairy protein flavor interactions.

Descriptive Analysis

Descriptive analysis is a commonly used sensory technique that provides a complete and detailed sensory profile of a product (Lawless and Heymann, 2010). Descriptive analysis typically requires 6-12 participants that have been trained prior to the actual data collection for the product (Chambers et al., 2004). Descriptive analysis is a quantitative method that provides objective data –thus the panel itself is an instrument and requires calibration. Panel training usually involves many hours to provide accurate and precise data. The panel uses food or chemical references at different intensities of concentrations or intensity strengths for standardization and to provide valid and reliable data. There are also different types of scaling techniques that can be used, including category scales, line scales, and magnitude estimation

scales (Lawless and Heymann, 2010). Commonly used techniques include the Flavor Profile method, Quantitative Descriptive Analysis™ (QDA) method, Spectrum Method™, Time Intensity descriptive analysis, and Free Choice Profiling (Civille and Carr, 1999). While there are several different techniques the overall result is the same – an objective sensory description of a product or a detailed comparison or discrimination of several products using a scaled system.

Descriptive analysis can be a useful technique for quality control in order to determine off flavors, color changes, etc of products to developing flavor or texture profiles to assist product development before running consumer testing (Gacula, 1997). Descriptive analysis is commonly used to develop lexicons for a variety of products. Lexicons are scientific languages that standardize sensory protocols and allow reproducibility and facilitate communication (Drake and Civille, 2003). Lexicons are not limited to food or beverages (Krinsky et al., 2006; Seo et al., 2009; Drake et al., 2010), but can also be applied to home care, personal care and fragrance products (Civille et al., 1990; Retiveau et al., 2004). When developing a lexicon, a select panel is chosen to be used across different sessions to evaluate products in that category to generate a list of terms that accurately discriminate those group of products (Drake and Civille, 2003). Panelists evaluate a subset (5-10) of samples from the whole sample set (15-25 products) to create an initial list of terms. After the samples have been evaluated, the terms are organized, and redundant or repetitive attributes are removed - usually after statistical analysis. The final terms are then defined by the panel and references are identified. Lexicons are fluid and can be re-visited over time as the product category changes.

Sensory Properties of Protein Isolates and Concentrates

Descriptive analysis can be used to examine and characterize sensory properties of protein isolates and concentrates. Lexicon development can be used to understand the differences among

the products within this category (Lawless and Civille, 2013). These studies can serve as a platform to understand processing, storage, and formulation changes. Drake et al. (2003) determined the sensory attributes of dried milk powders and dairy ingredients and found that there was notable flavor variation among the rehydrated skim milk and whole milk powders from different suppliers.

Studies have investigated the flavors associated with dried dairy protein concentrates and isolates and have documented flavor variability (Carunchia-Whetstine et al., 2005; Drake et al., 2003; Drake et al., 2014). Flavor variability in dairy products is affected by animal feed, season, location, storage time before drying, and storage conditions (Floyd et al., 2009; Kinsella and Melachouris, 2009; Carter and Drake, 2018; Croissant et al., 2007). The liquid whey source also contributes to the flavor variability in whey protein (Carunchia-Whetstine et al., 2003; Karagul-Yuceer et al., 2003). Similarly, flavor variability can also be seen in plant sources due to the variety of plant types due to the varieties of each plant type that are being harvested and the time/temperature profiles that are being used to process the plants into isolates or concentrates (Yu et al., 2017; Khazaei et al., 2019). Further variation can be explained by the significant variation of protein distribution in the developing seed or germination caused by production conditions whole growing (Myers, 1988; Kinsella and Melachouris, 2009).

Most of the sensory research conducted for plant proteins has been primarily focused on soy proteins (Drake et al., 2006; Russell et al., 2006; Dervisoglu et al., 2005; Friedeck et al., 2003). Russell et al. (2006) compared the sensory properties of rehydrated soy and whey proteins and determined that there were sensory differences for each protein type. Sweet aromatic, cardboard and brothy flavors were associated with each protein type. Whey proteins had more metallic and soapy flavors, while soy proteins had cereal, malty, flour paste and roasted attributes. Trikusuma

(2016) determined that the highest flavor intensities in a rehydrated (10% w/v) pea protein isolate were beany, potato, pasta, and cooked green bean flavors. There are also naturally occurring reactions in the protein source that can affect the overall flavor profile as well. Carunchia-Whetstine et al. (2005) determined that lipid oxidation and heat generated compounds contributed to the flavor of whey proteins. With legume-based proteins, the fatty acid composition changes as the plant matures creating lipid oxidation compounds and impacting the overall levels of bitter taste and green/grassy flavors (Cowan et al., 1973; Kumar et al., 2006). However, very little recent work has investigated this topic.

A source of bitterness that naturally occurs in most legume seeds, such as pea, soybean, lentils and lupins, comes from phytochemicals including isoflavones, saponins, and other phenolic compounds (Heng et al., 2006; Laszitity, 2009; Damodaran and Arora, 2013). Isoflavones are classified as flavonoids which are a group of phenolic compounds that are responsible for antioxidant and anti-inflammatory properties (Yu et al., 2016). Isoflavones contribute to the activation of bitter taste receptors (Roland et al., 2011; Soares et al., 2013; Roland et al., 2013). Saponins are heat stable glycoside compounds that act as surface active agents responsible for foaming. Saponins have been found to bind to proteins and remain in the protein isolate after processing (Shimoyamada et al., 1998). However, saponins also have beneficial effects, such as inhibiting the adsorption of cholesterol from the small intestine and anti-inflammation and antioxidation effects (Shi et al., 2004). Most of the naturally occurring phenolic acids in plant sources are removed during the extraction of protein, however free phenolic acids are still a concern regarding flavor (Sosulski, 1979; Damodaran and Arora, 2013). There still needs to be more research to document the exact role of these compounds in the flavor of plant proteins.

Ready-to-Drink Protein Beverages

Today's consumers value premium products that are convenient and nutritious (Mintel, 2018). Protein-rich drinks made up 44.5% of the high protein food market, of which 25.5% make up high protein and high energy sports drinks (Business Wire, 2017). There has been a large increase in ready-to-drink protein beverages that provide nutritional value, convenience, and shelf stability. Protein beverages are functional foods that are defined as "foods and food components that provide a health benefit beyond basic nutrition" (Institute of Medicine, Food and Nutrition Board, 1994; Serafini et al., 2011). The FDA (21 CFR 101.54) states that a "high" protein label is consistent with a food that contains 20% or more of the reference daily intake (RDI) per reference amount customarily consumed. As such, there must be at least 10 g of protein per serving (FDA, 2014). These beverages can be defined by their protein, fat, carbohydrate, and fiber composition (Paulsen et al., 2005). There are various pockets in the marketplace for these ready-made beverages including: blended milk products, milk alternatives, pharmaceutical/nutritional, meal replacers/weight loss, and cream alternatives.

Most proteins that are used in ready-to-drink (RTD) protein beverage formulation include casein proteins, milk proteins and plant proteins (Oltman et al., 2015). Many plant proteins that are being developed use around 4-6.5% protein/serving. When adding a protein to a functional beverage it creates some complications to the final product. Many proteins lack heat stability, are highly viscous, lack clarity and have an unpleasant taste. Proteins that are clear, neutral in taste and can be incorporated across a wide pH and have heat stability are best suited for functional beverages (Boye et al., 2014). Protein load, or grams of protein per serving, is of chief importance to category consumers and is consistently attractive to all types of consumers (Oltman et al., 2015; Harwood and Drake, 2019). According to Harwood and Drake (2019), the

ideal protein load for a high protein product is 20-29g/serving. The other factor that is of high importance is great taste. Banovic et al. (2018) conducted focus group sessions that determined consumer preferences for plant proteins in protein products. Participants chose cereal plant proteins due to sensory profile (taste), sustainable production and perceived naturalness. Legume based proteins were beneficial due to higher protein content, however there were concerns about the naturalness of the protein and bad sensory qualities, and in terms of soy – perceived lack of healthiness. Protein products may not be evaluated solely by flavor but by functionality – however flavor acceptability is a baseline need (Gruenwald and Paquin, 2009; Oltman et al., 2015). Kinsella and Melachouris (2009) suggested that solubility of proteins is the most important factor and an indicator of their functionality. Dairy proteins have significantly higher solubility than plant proteins and have been traditionally used in food applications such as beverages (Webb et al., 2002; Zayas, 2012). Soy protein has been traditionally the most used plant protein in beverage production due to its mild flavor and high solubility. Other plant proteins generally have decreased functionality when used in beverage applications (Hermansson, 1979; Kinsella and Melachouris, 2009). Improvements are needed regarding the performance of plant proteins in beverage applications.

Processing Beverages

Beverages are typically processed through homogenization and heat treatment. There are many other considerations when making protein beverages, whether they are plant based or animal-based protein. The pH, viscosity, dispersibility of ingredients, color stability, flavor stability, and phase separation and storage stability must be taken into consideration. The pH of the beverages is generally related to the stability of the beverage. Most protein beverages are processed at a neutral pH since most proteins are not functional at lower pH levels (Zayas, 2012).

Neutral pH beverages have a pH of 4.6-7.5 and have to be thermally sterilized via retort processing (115-125 °C for 10-40 min) or ultra-high temperature treatment (135-150 °C for 2-6 s) (Etzel, 2004; U.S. Dairy Export Council, 2006). Whey protein, for example, is unstable to heat at a neutral pH, but can be applied in acidified (pH ≤ 3) protein beverages (Deeth and Lewis, 2017). Acidic beverages have a pH of 2.5-3.0 and can be heat processed to 90-95 °C to be shelf stable at lower temperatures (Etzel, 2004; Rittmanic and Burrington, 2006). The viscosity is the measured resistance against the flow of the beverages. Viscosity is a functional property that is directly related to the sensory properties regarding texture and consistency (Szczesniak, 2002; Mudgil and Barak, 2016). Beverages with low viscosity have an undesirable watery texture and those with higher viscosities reduce the liquid properties of the beverage. The solubility of the beverages during storage is a critical parameter. The dispersibility is generally adjusted using stabilizers and emulsifiers. Color is also a large factor in consumer acceptability. Colors are generally added to improve the appearance of these beverages. The color should be consistent throughout the storage period, so it should be compatible with other ingredients and pH. Similarly, it is also important for flavors to have stability to last throughout the shelf life and not interact with other ingredients in the product formulation.

Homogenization is a common practice where a liquid product is pushed at high pressure through a narrow aperture and high shear reduces the protein particle sizes until a uniform distribution is reached. Homogenization of beverages may be necessary to maintain protein stability/solubility. Beverages can be pasteurized with different heat treatments, including high temperature-short time (HTST), ultra-pasteurization (UP), and ultra-high temperature (UHT) processing. HTST parameters state that the product needs to be heated at 160-165°C for 14-30 s. Generally, beverages are processed at higher temperatures in order to maintain a longer shelf life.

"Ultra-pasteurization" is defined by the PMO as thermal processing "at or above 138°C for at least two (2) seconds, either before or after packaging, which has an extended shelf life under refrigerated conditions" (FDA, 2013). Ultra-high temperature (UHT) sterilization or aseptic processing (4-15 s at 135 – 150 ^{o}C) is ultra-pasteurization procedures followed by aseptic filling. UHT temperatures and aseptic packaging produces a shelf stable product that has at least 6 months of shelf stability (Mellema and Bot, 2009).

Conclusion and Objectives

The objectives of this project are to develop a lexicon for rehydrated plant proteins and to determine flavor changes in model protein beverages after ultra-pasteurization heat treatment.

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Table 1.1. Isoelectric points for protein isolates.

Protein Isolate	Iso-electric Point
Pea	4.5
Soy	4.5
Rice	4.5
Whey	4.5
Milk	6.6
Casein	4.6

	Pea			Lupin			Soy		
	Seed	Conc.	Isol.	Seed	Conc.	Isol.	Seed	Conc.	Isol.
Protein	26.0	48.5	89.6	38.2	60.5	94.8	38.1	62.3	85.1
Lipid	1.4	0.9	1.6	11.1	1.5	1.4	17.3	0.5	0.8
Ash	3.0	3.0	2.6	3.9	4.1	1.7	4.6	6.2	4.2
Moisture	13.0	8.6	5.3	9.0	7.3	2.4	8.5	4.5	3.9

Figure 1.1: Percent proximate composition of pea, lupin, and soy protein products [%] (taken from Tomoskozi et al., 2001)

Protein	PDCAAS
	Truncated
Whey protein isolate	100 (i), 99 (ii), 97 (ii)
Whey protein concentrate	100 (i), 100 (ii)
Milk protein concentrate	100 (i), 100 (ii)
Skim milk powder	100 (ii)
Casein	100 (iv)
Pea protein concentrate	89.3 (i), 75 (ii), 71 (ii)
Soy protein isolate	100 (i), 97.9 (i), 93 (ii), 86 (ii)
Soy flour	98 (ii), 93 (ii)
Rice protein concentrate	41.9 (i)
Wheat	50 (ii), 51 (ii)
Almonds	23 (iii)
Dehulled hemp seed	63–66 (iv)

Figure 1.2: PDCAAS scores for different protein sources. (i) Rutherfurd, Fanning, Miller, and Moughan (2015); (ii) Mathai et al., 2017; (iii) Boye et al. (2012); (iv) House, Neufeld, and Leson (2010) PDCAAS was calculated as [mg of limiting amino acid in 1 g of test protein/mg of the same amino acid in 1 of reference protein × true faecal digestibility of protein (%)] × 100 (taken from Chalupa-Krebzdak et al., 2018).

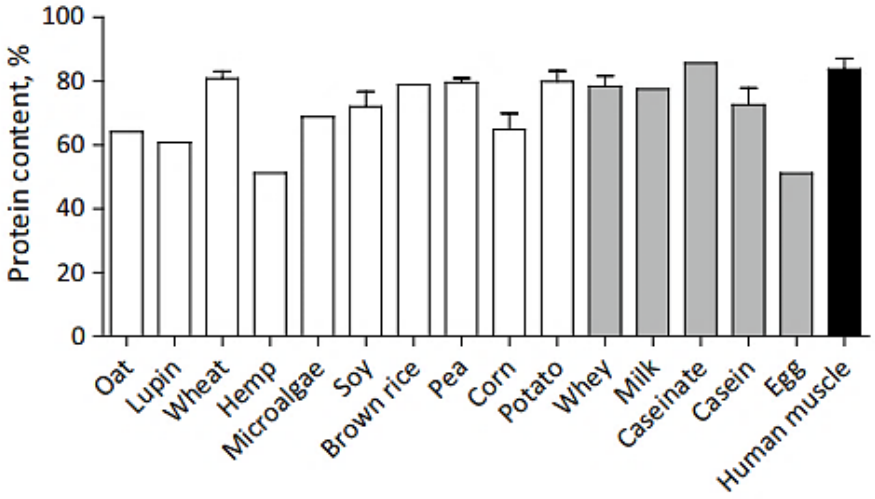

Figure 1.3: Mean (+- SEM) protein isolate content (% of raw material) of various commercially available protein sources and human skeletal muscle tissue (taken from Gorissen et al., 2018).

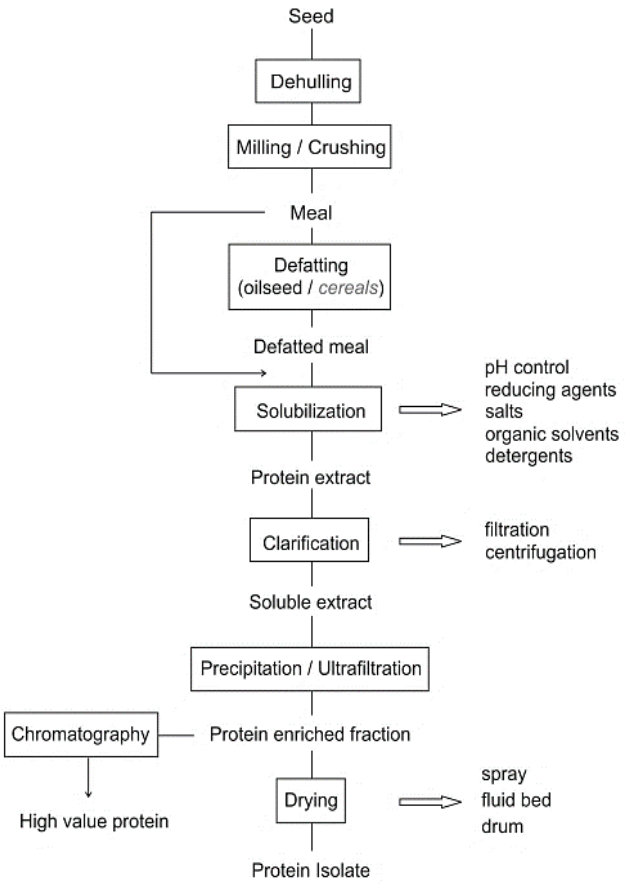

Figure 1.4. Diagram of plant processing into protein isolates (taken from Gonzalez-Perez and Arellano, 2009)

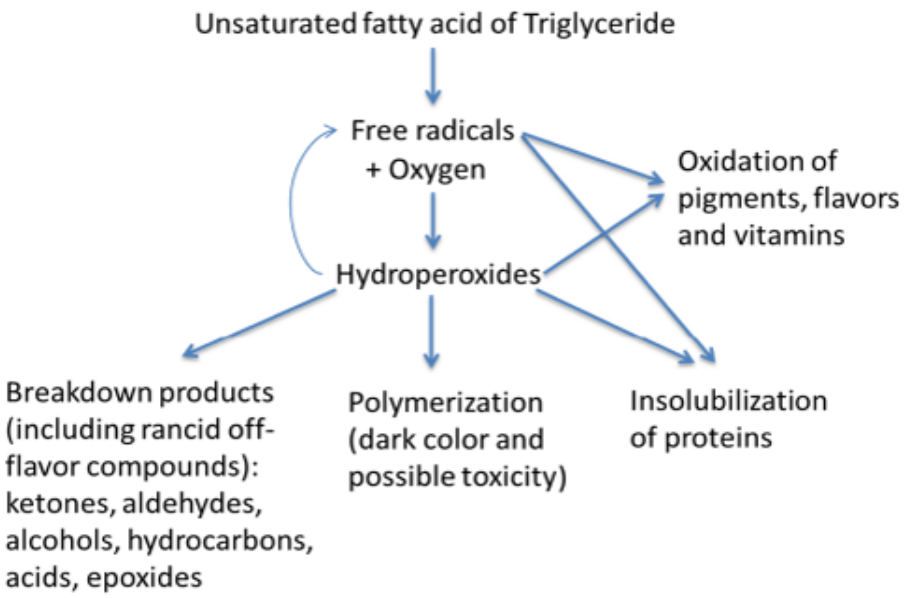

Figure 1.5. Lipid oxidation mechanism (taken from Cinkova et al., 2014).

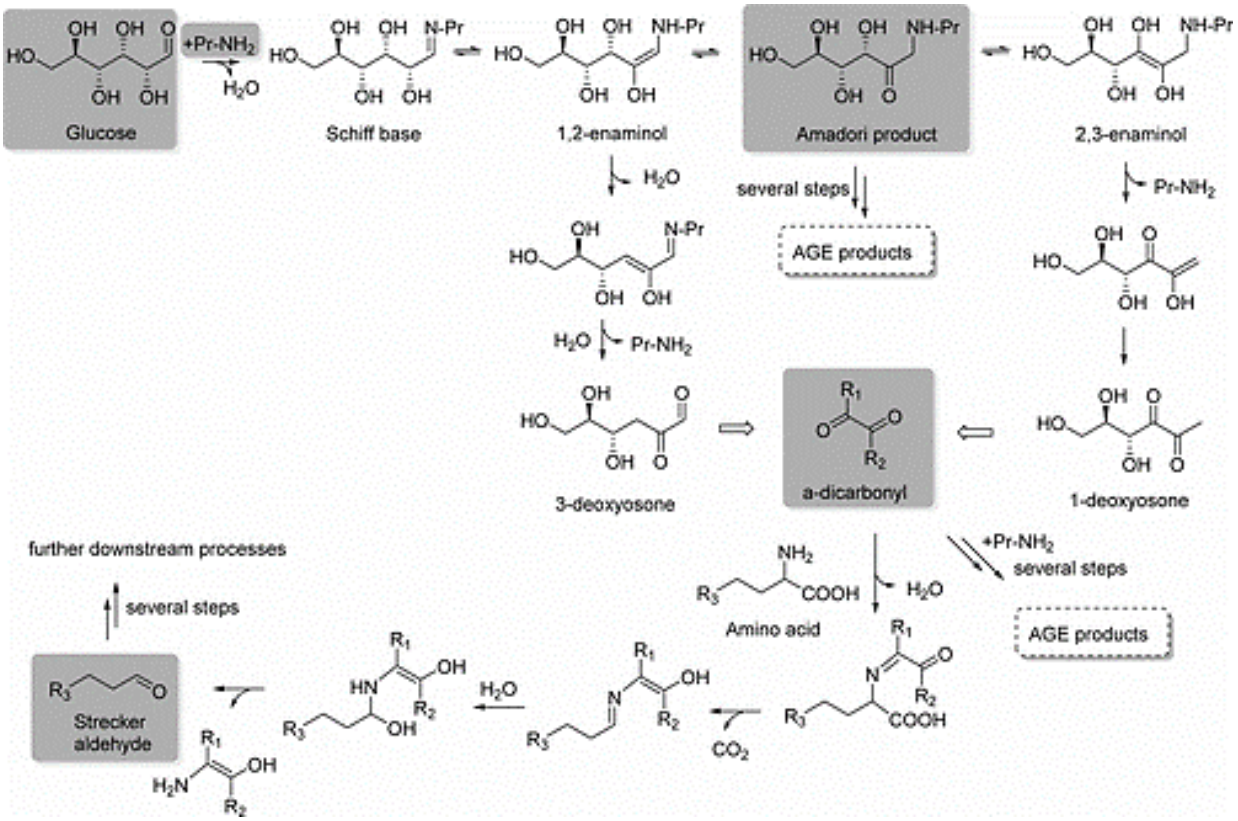

Figure 1.6: Simplified scheme of the Maillard reaction (taken from Lund et al., 2017)

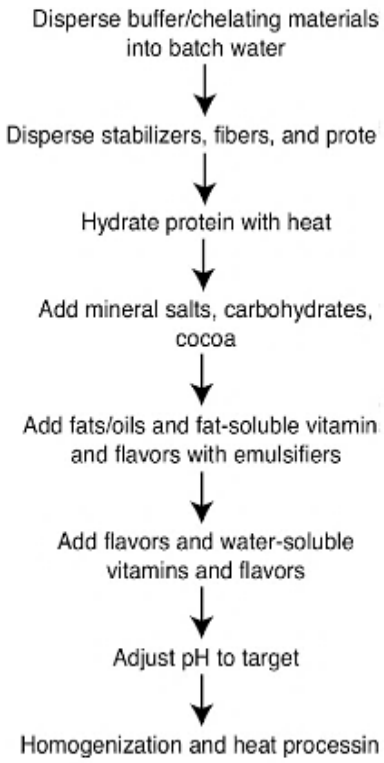

Figure 1.7. Process flow for typical ready-to-drink beverage (taken from Paulsen et al., 2005)

CHAPTER 2:

DEVELOPMENT OF A SENSORY LEXICON FOR PLANT PROTEIN POWDERS

TITLE: Development of a Sensory Lexicon for Plant Protein Powders

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Abstract

Plant proteins are increasing as a protein alternative. As such, there is a need to identify a sensory language to provide a standardized tool to identify flavor attributes of plant proteins and protein specific flavor changes following heat treatment. A total of 66 samples of plant proteins, including pea, chia, soybean, rice, wheat, faba bean, hemp, sacha inchi, mung bean, pumpkin, and potato were collected. Proteins were rehydrated at 10 % solids (w/v) in deionized water and a highly trained descriptive panel identified, defined and referenced a lexicon with 24 aromatics, 4 basic tastes and 2 mouthfeel attributes. Selected representative plant protein solutions (n=13) were incorporated into model beverages, ultrapasteurized at 140°C for 3 s, and evaluated. Key differentiating attributes for plant proteins included basic tastes (umami, bitter) and aromatics (green/grassy, cardboard, doughy, fruity, fecal/indole, cereal/grain) ($p<0.05$). Flavor intensities and astringency changed with heat treatment ($p<0.05$) and only a few new flavors specific to heat treatment were documented. The developed lexicon provides a tool for characterization and differentiation of specific flavors contributed by plant proteins that can be used by product developers for development and characterization of protein-rich foods.

Introduction

Proteins are added to food products for nutritional value or functional purposes. Consumers place a strong emphasis on high protein products that are easily accessible and ready-made. New products that had "high protein" labels on packaging increased by 157% in 2018 (Nielsen, 2018). There has been a large increase in consumption of ready-to-drink (RTD) protein beverages that provide nutritional value, convenience, and shelf stability (Ozer and Kirmaci, 2010; Corbo et al., 2014). Protein-rich RTD drinks made up 44.5% of the high protein food market, of which 25.5% make up high protein and high energy sports drinks (Business Wire, 2017). Plant-based alternatives have contributed to a large increase in the dairy alternative market, appearing as more ethical and environmentally more sustainable than dairy proteins to some consumers (Mintel, 2018; Sethi et al., 2016; McKinsey and Company, 2019). Around 14% of consumers in the U.S. regularly consume plant alternatives (NPD, 2018). Plant based proteins have started to attract many consumers who are interested in replacing or complementing animal proteins (Mintel, 2018; Food Navigator USA, 2018).

The consumer acceptance of a food product relies heavily on the sensory profile. With the rise in plant protein-based products, further investigation of the sensory properties of these protein ingredients is needed. Protein ingredients have flavors and these flavors carry through into ingredient applications (Oltman et al., 2015; Keefer et al., 2020). Previous research has addressed the sensory properties of dairy proteins, sensory lexicons have been developed for dairy proteins and dried dairy ingredients (Drake et al., 2009; Carunchia-Whetstine et al., 2005; Drake et al., 2003; Smith et al., 2016). There are various commercial plant proteins currently on the market including soy, pea, wheat, oat, lupin, hemp, potato, corn and brown rice (Gorissen, 2018; Mintel 2018). The sensory properties of these plant protein ingredients are largely

unknown. Russell et al. (2006) documented and compared sensory properties of whey and soy proteins and noted distinct flavors that were unique to each protein source. Other previous studies have documented off flavors and aromas of plant proteins (Rackis et al., 1979; Sessa and Rackis, 1976, Trikusuma, 2018), however, these papers did not provide specific sensory vocabularies for plant proteins.

There are many challenges when adding proteins into a food matrix –especially regarding the retention of the added flavorants that can be altered throughout the processing and storage of the food (Schutte et al., 1979; Gremli, 1974; Gonzalez-Perez and Arellano, 2009) as well as the flavors of the proteins themselves. Proteins have flavor binding capabilities that can negatively alter the inherent or added desirable flavors in the final product (Sessa and Rackis, 1977; Plug et al., 1994; Suppavorasatit and Cadwallader, 2010). Ng et al. (1989) determined that the concentration of vanillin and conformation of the faba bean protein in 5% w/w solutions influenced the binding and overall flavor impact of the solution. Proteins also have their own flavor profile that can carry over into the final product (McDaniel and Chan, 1988; Russell et al., 2006; Carunchia-Whetstine et al., 2005; Oltman et al., 2015; Childs et al., 2010). Keefer et al. (2020) recently compared sweeteners and protein sources (whey protein isolate, milk protein isolate, and pea protein) in protein bars and determined that the protein source affected both functionality and sensory texture properties as well as flavor properties of the protein bars. Pea protein bars were associated with yellow pea/beany, pyrazine, fecal, and cardboard flavors, while the whey protein bars had more distinct cardboard and sulfurous flavors and the milk protein bars had milky, sweet aromatic and tortilla flavors. Protein beverages are subjected to further heat processing, and additional protein specific flavors can be formed during the heat process (White et al., 2013). There is a need to understand the flavor profiles of plant-based proteins and

how they are affected by heat treatment that may be encountered in processing to create better products through product development and flavor masking techniques. The objective of this study was to characterize the sensory profiles of plant proteins and to further characterize the role of heat treatment on flavor of model plant protein beverages. A variety of plant proteins were selected including pea, soybean, rice, wheat, faba bean, hemp, sacha inchi, pumpkin, hemp, and potato protein sources based on popularity and availability in the market.

Material and Methods

Experimental Overview

Sixty-six plant proteins were collected in duplicate lots. Sensory properties of the collected rehydrated plant proteins were evaluated by a trained panel to generate a lexicon. Sixteen representative plant proteins were subsequently chosen for heat treatment using a benchtop ultra-pasteurization system, and sensory properties following heat treatment were documented. Thirteen model plant protein beverages were then processed and evaluated by the trained sensory panel.

Plant Proteins

Plant proteins were sourced in duplicate lots from the US, Denmark and China. The sixty-six different plant protein isolates and concentrates were selected based on availability and popularity in the market. These included pea, soybean, faba bean, hemp, rice, chia, sacha inchi, wheat, potato, mung bean, and pumpkin (Table 2.1). Samples were received in duplicate lots as 1 kg, 5 kg or 25 kg samples. Proteins were transported and stored in unopened packaging. Upon receipt, proteins were stored at -20 ºC.

Descriptive Analysis

All sensory testing was conducted in compliance with the NCSU Institutional Review Board for Human Subjects approval. Panelists were highly experienced (5 females and 2 males, ages 23 to 56 y) and each had greater than 500 h of descriptive sensory analysis experience using the Spectrum™ method to evaluate flavor and texture of multiple products, including previous experience with dried dairy and soy protein ingredients and plant based alternative beverages (Meilgaard et al., 1999; Drake and Civille, 2003). Attributes were scaled using the 0- to 15-point universal Spectrum™ intensity scale (Meilgaard et al., 1999). Once the initial terms were identified (Table 2.2), the panelists were further trained (an additional 30 h) to consistently identify and score intensities of flavor and mouthfeel attributes present in the rehydrated plant proteins. Analysis of variance of the data collected from the last part of training indicated that the panel and panelists could consistently use the attributes to differentiate the products.

Sensory analyses were conducted individually in a positive air pressure, odor free room dedicated to descriptive sensory analysis. There were approximately 20 sessions of 45 min each to generate descriptors for the 66 rehydrated plant proteins. Samples were presented monadically in a randomized balanced block design; 3-4 samples were evaluated per session. Plant proteins were rehydrated at 10% solids (w/v) with deionized water and allowed to hydrate overnight at 4C. The next day, samples were poured into 118 ml plastic soufflé cups (PFS Sales, Raleigh, NC) with 3-digit blinding codes and lidded. A reconstitution of 10% (w/v) solids was chosen in conjunction with previous research on dried dairy and soy ingredients (Drake et al., 2003; Russell et al., 2006; Wright et al., 2009). Products were gently mixed by hand with spring water and then mixed briefly with a KitchenAid™ hand blender to disperse lumps (KitchenAid, Benton Harbor, MI). Plant proteins were evaluated at room temperature (20ºC). A 2 min wait

time was enforced between samples; samples were expectorated. The final sensory attributes included aromatics, basic tastes and mouthfeel attributes (Table 2.3). Panelists evaluated samples individually in the same manner as for language identification and refinement. Each rehydrated protein was evaluated by each panelist in duplicate in separate sessions. Data was collected on paper ballots.

Heat Treatment of Rehydrated Plant Proteins

Sixteen representative plant proteins, which included representative samples from each plant protein type evaluated in this study, were selected for heat treatment based on examination of principal component biplots from descriptive analysis of the sixty-six rehydrated plant proteins. Proteins were rehydrated as previously described and subjected to an ultra-pasteurization (UP) treatment using a benchtop oil bath as described by Jo et al. (2019). This benchtop UP system served as a preliminary step to pilot plant processing to determine if proteins could functionally withstand heat treatment (e.g. no coagulation). A 60 ml solution was subjected to a pre-heat treatment in an oil bath at 150 ºC until the temperature reached 85 ºC and then the solution was transferred to the second oil bath at 180 ºC until the temperature reached 140 ºC and held for 3 s. The temperature was monitored consistently for each solution with a wire temperature probe to ensure each solution reached proper temperature. The bottles were cooled at room temperature for 5 min and then placed in an ice bath until 10ºC was reached. Solutions were then stored at 4ºC. The order of heat treatment was randomized between replicates to account for process order effects. The experiment was repeated in duplicate.

Descriptive analysis was conducted on the heated solutions at room temperature (20ºC) by the same sensory panel that evaluated the rehydrated plant proteins to confirm whether new attributes needed to be added to the lexicon. Samples were presented monadically in a

randomized balanced block design; no more than 6 samples were evaluated per session. Heated solutions were poured into 118 ml plastic soufflé cups (PFS Sales, Raleigh, NC) with 3-digit blinding codes and lidded. A 2 min wait time was enforced between samples; samples were expectorated.

Following evaluation of heated solutions, thirteen plant proteins and a milk protein were selected for pilot plant model beverage processing. The number of representative samples was reduced to thirteen based on performance in the oil bath benchtop system; samples were not included if sedimentation/gelling was observed. The milk protein was included as a control for which sensory properties and beverage functionality have been previously established (Smith et al., 2016). For model beverages, 765 g plant protein, 5 g of stabilizer (Colony Gums, Gellan Gum LA: Monroe, NC, 0.03% w/w) and 0.1% (w/w) potassium citrate (ADM, Decatur, IL) were mixed with deionized (DI) water for approximately 10 minutes and until no clumps were observed. Then, 5% w/w of each protein powder was added slowly and mixed gently with a hand-mixer into solution until no clumps were observed. The model beverages had an average of 8 $\pm$ 2 g of protein/240 ml serving, depending on the protein type and protein content of the plant protein powder. A Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) and clean fill station were used for beverage processing. Direct steam injection (DSI) was selected because DSI has shown to preserve protein quality and cause less denaturation of proteins (Kelleher et al., 2018; Dickow et al., 2012). Prior to processing, the system was sterilized by heating the unit to 121.1 °C recorded by the last thermocouple and held at that temperature for 20 minutes. The beverages were first were preheated to 90°C, heated to 140°C for 3 s by direct steam injection, then cooled to 85°C by vacuum cooling to remove both heat and added water. The final product was cooled to 10°C and filled in a laminar flow hood into

sanitized 500 mL bottles (Container and Packaging Store, Eagle, ID). Pasteurized plant protein beverages were stored at 4 °C. The order of processing was randomized between replicates to account for process order effects. The experiment was repeated in duplicate.

Descriptive analysis was conducted on the model beverages 24 h after heat treatment by the same panel that evaluated the rehydrated solutions. The model beverages were evaluated across 7 sessions of approx. 30 min each. Samples were presented monadically in a randomized balanced block design; 3-4 samples were evaluated per session. Model beverages were poured into 118 ml plastic soufflé cups (PFS Sales, Raleigh, NC) with 3-digit blinding codes and lidded. Model beverages were evaluated at room temperature (20^{o}C). A 2 min wait time was enforced; samples were expectorated. Data was collected on paper ballots.

Statistical Analysis

Descriptive analysis data was evaluated with analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) test ($p<0.05$) by protein type and attributes. Rehydrated proteins of a single type with six or more samples were analyzed with principal component analysis (PCA). PCA was also applied to trained panel means from the average sensory profile for each plant protein type to visualize similarities and differences among protein types. Following heat treatment, two-sample independent t-tests on individual proteins and PCA were applied to visualize differences before and after heat treatment for all proteins. Statistical analyses were conducted using XLStat (Version 2018.7, Addinsoft; Paris, France).

Results and Discussion

Lexicon Development

During initial sessions, 35 attributes were identified that included 27 aromatics, 5 basic tastes and 2 mouthfeel factors. During the development stage of the lexicon, the panel removed redundant attributes and defined attributes. The final lexicon had 24 aromatics, 4 basic tastes and 2 mouthfeel attributes (Table 2.3).

Rehydrated Plant Proteins

The plant protein lexicon was developed to assess the range of important sensory characteristics in rehydrated plant proteins and to compare sensory attributes among plant protein groups. The analysis of variance of descriptive sensory results revealed differences and similarities between plant protein groups (Table 2.4). In general, the mean attribute intensities for rehydrated proteins were relatively low (< 4 on a 0 to 15- point intensity scale). These results are consistent with previous work on protein ingredients (Smith et al., 2016; Russell et al., 2006, Trikusuma, 2019). Protein flavor attributes are not intense, but they are distinct and carry through into ingredient applications (Childs et al., 2997; Childs and Drake, 2010; Oltman et al., 2015; Zhang et al., 2020; Keefer et al., 2020). Sweet aromatic and cardboard flavors and bitterness and astringency were documented in most proteins. Cardboard flavor and astringency are established attributes in dairy and soy proteins as well and may be ubiquitous protein sensory attributes (Russell et al., 2006; Leksrisompong et al., 2012; Drake et al., 2014; Keefer et al., 2020). Plant proteins such as pumpkin, sacha inchi, chia and hemp that had lower protein concentrations also had lower levels of astringency compared to other proteins. Astringency is a complex sensory attribute and has been attributed to proteins as well as other food components including phenolic compounds which are also present in plant sources (Carter et al., 2020; Bajec

and Pickering, 2008). Cardboard flavor has been attributed to lipid oxidation products (Whitson et al., 2010). Bitter taste in legume seed sources has been attributed to naturally occurring phenolic compounds, like saponins and isoflavones (Roland et al., 2011; Soares et al., 2013; Roland et al., 2013).

Most of the plant proteins also shared variable intensities of cereal/grain, beany, fecal, and green/grassy flavors, and umami taste. Grassy/green and beany aromas in pea, faba beans and soy ingredients have been attributed to lipid oxidation derivatives including hexanal, trans-2-nonenal, and 2-pentyl furan (Sessa and Rackis, 1977; MacLeod et al., 1988; Boatwright and Crum, 1997; Glaser et al., 2020). Vara-Ubol et al. (2005) characterized beany flavor as a result of a combination of sensory attributes like musty/earthy, green/pea pod, or brown derived from multiple compounds. For example, 1-octen-3-ol, at low levels, contributed to a musty/earthy aroma, while 2-pentyl furan and hexanol contributed to a green/pea pod aspect of beany flavor.

Salty taste was present in pea, soy and wheat proteins, and sour taste was only present in wheat and potato proteins. These attributes may be due to the manufacturing procedures such as salt extraction and/or manipulation of pH for isoelectric precipitation of protein (Kinsella and Melachouris, 1976; Gonzalez-Perez and Arellano, 2009). Some attributes were only present and distinctive of certain plant proteins or individual plant proteins. Seaweed flavor was only documented in mung bean and potato proteins. Metallic flavor was only present in faba bean protein. Bader et al. (2009) determined that metallic flavor was present in lupin flour, which is the same family as faba beans. Oxidized flavor was only present in rice protein. Oxidized flavor, due to lipid oxidation, has been attributed to 2-nonenal and octanal found in rice during the breakdown of linoleic and oleic acid oxidation (Lam and Proctor, 2003). Earthy/soil flavor was only present in hemp protein. Shen et al. (2020) determined that myrcene was a source of earthy

aroma found in hemp protein isolate. Woody flavor was only documented in mung bean and pumpkin proteins. There was a high intensity (≥2) of cooked cereal/grain in almost all the wheat proteins. Sun and Zhuang (2012) attributed meaty and roasted aromas in enzyme hydrolyzed wheat protein to Maillard reactions and production of high levels of asparagine. Berends et al. (2004) also detected malty flavor and umami and salty tastes in wheat gluten hydrolysates. Pumpkin and sacha inchi had higher intensities of nutty flavor than other proteins. Jain et al. (2015) documented nutty and beany flavors in ground peanut protein isolates, which is in the same family as sacha inchi. Siegmund and Murkovic (2004) detected nutty and pyrazine aroma compounds in pumpkin seed oil.

Although viscosity/mouthfeel of the rehydrated proteins was not examined extensively, it is important to note that there were differences in viscosity/mouthfeel across all protein groups. Many of the rehydrated proteins had sedimentation and solutions were swirled gently prior sensory evaluation to account for this issue. Sandy mouthfeel was used to describe the gritty mouthfeel of proteins such as rice, chia, sacha inchi, pumpkin, hemp and potato. In comparison to dairy proteins, many plant proteins lack solubility and sedimentation is likely to occur (Webb et al., 2002; Zayas, 2012).

For all plant proteins, a cumulative of 64% of the data variability was explained by the first four principle components (Figures 2.1 and 2.2). Thirty-eight percent (38%) of the variability was explained on the first two components (Figure 2.1). PC1 was characterized by aroma intensity, sweet aromatic, cardboard, green/grassy, doughy, fruity and astringency (Table 2.5). PC2 was characterized by cereal/grain, fecal, woody, bitter, sour, and umami. PC3 and PC4 explained 24% of the variability. PC3 was characterized by green pea, nutty, beany, pyrazine, malty, salty taste, and sandy mouthfeel, and PC4 was characterized by painty and cooked cereal/grain flavors.

Within each plant protein category, there was variability, but as might be expected, different plant proteins had distinctive sensory attributes. Pea proteins were distinguished mostly by pyrazine and green pea flavors and umami taste. The rice, potato and wheat proteins had a distinctive cereal/grain flavor, but potato and wheat proteins had more sour and bitter tastes. Faba bean protein had metallic and fruity flavors. Hemp, sacha inchi and mung bean and chia proteins had distinctive earthy/soil and green/grassy flavors. Pumpkin protein was more associated with nutty and tortilla flavor. Soy protein had the lowest the lowest aroma intensity and had more mild flavor intensities compared to other plant proteins (Table 2.4). Soy proteins have been marketed for many years. Presumably, tremendous research has been documented to optimize soy varieties and processing steps to minimize flavor (MacLeod et al., 1988; Miroljub et al., 2004; Damodaran and Arora, 2013). It is important to note that there are some limitations of our results due to the number of samples collected for some protein groups (mung bean, chia, faba bean, pumpkin, sacha inchi). Some of the data in the current study may not be fully representative of those particular plant protein group. More research needs to be conducted to confirm representative attributes.

Correlation analysis was conducted on the sensory results from the entire plant protein set (results not shown) to evaluate possible redundant terms. Attribute relationships were associated with product specific relationships and did not reveal redundancies. Sour taste was positively correlated with malty, cooked cereal/grain flavors and astringency ($r = 0.66$, 0.56, and 0.59 respectively, $p<0.05$). Fruity was positively correlated with green/grassy, pyrazine and metallic ($r = 0.63$, 0.60, and 0.63 respectively, $p<0.05$). Metallic and green/grassy flavors were also positively correlated with fruity flavor ($r = 0.56$, $p<0.05$). Sweet aromatic flavor was negatively

correlated with fecal flavor ($r = -0.56$, $p<0.05$). Cooked cereal/grain was positively correlated with malty flavor ($r=0.66$, $p<0.05$).

Further analysis of each group of individual plant proteins demonstrated flavor variability within a plant protein group as well (Figures 2.3-10). The variety within a protein group can be due to the different processing methods by each manufacturer, the different varieties of plants used and/or seasonal and geographical effects (Yu et al., 2017; Khazaei et al., 2019). Flavor variability has been previously documented in soy proteins (Russell et al., 2006), and is also seen in dairy proteins due to changes in animal feed, season, location, differences in processing steps, and storage conditions (Carter and Drake, 2018; Croissant et al., 2007). Pea proteins (n=26) were differentiated by green pea and pyrazine flavors (PC1) and cereal/grain, cardboard, fecal, doughy, burnt flavors and bitter and umami taste (PC2) (Figure 2.3-4). Similar variability within a protein group was observed for rice, wheat, and soy proteins (Figures 2.5-10). Wheat proteins (N=10) were characterized by sweet aromatic, sulfur, green/grassy, beany, and malty flavors, sour taste, and sandy texture (PC1) and cereal/grain flavor and salty taste (PC2) (Figure 2.5-6). Rice protein were characterized by aroma intensity, cereal/grain, beany, painty, cooked cereal/grain, and oxidized flavors, bitter taste, and sandy and astringent mouthfeel (PC1) and cardboard, fecal, and pyrazine flavors (PC2) (Figure 2.7-8). Soy proteins were characterized by aroma intensity, sweet aromatic, cereal/grain, malty, fecal, sulfur flavors and bitter taste (PC1) and cardboard and beany flavors and salty, umami, and bitter tastes and astringency (PC2) (Figure 2.9-10). Cereal and other grain flavors, like malty, have also been previously reported for soymilks and soy proteins (Russell et al., 2006; N'Kouka et al., 2004; Lawrence et al., 2016).

Correlations between sensory attributes were also examined within pea, soy, rice and wheat proteins. Few correlations were observed among pea and soy protein sensory attributes

(results not shown). For pea protein, pyrazine was positively correlated with aroma intensity and green pea (r=0.54 and 0.71 respectively, p>0.05) and umami taste was positively correlated with doughy. For soy proteins, sweet aromatic was positively correlated with cereal/grain and, malty doughy (r=0.70, 0.71 and 0.71 respectively, p>0.05) and negatively correlated with fecal (r=-0.72, p>0.05). Similar correlations were also seen in previous studies (Russell et al., 2006; Chamba et al., 2013). Cardboard flavor was negatively correlated with aroma intensity (r=-0.67, p>0.05). More notable correlations were documented among wheat and rice protein sensory attributes (Tables 2.6, 2.7). For wheat protein, painty flavor was negatively correlated with sweet aromatic, cardboard, and malty (r=-0.70, -0.67, and -0.69 respectively, p>0.05). Sweet aromatic flavor was positively correlated with malty and sour (r=0.93 and 0.67 respectively, p>0.05). Nutty flavor was positively correlated with cereal/grain (r=0.75, p>0.05). Salty taste was negatively correlated with cardboard flavor (r=-0.68, p>0.05). Sour taste was positively correlated with green/grassy flavor (r=0.84, p>0.05). For rice proteins, cereal/grain flavor was negatively correlated with oxidized flavor and sandy and astringency (r=-0.78, -0.80, and -0.83 respectively, p>0.05). Bitter taste was positively correlated with cereal/grain flavor (r=0.78, p>0.05). Onion and sulfur flavors were correlated (r=0.98, p>0.05). Doughy and sweet aromatic flavors were correlated (r=0.89, p>0.05). Beany flavor was negatively correlated with cardboard flavor (r=-0.68, p>0.05).

Heat Treatment

Some differences were documented within selected proteins before and after heat treatment (Table 2.8, Figure 2.11-14). Flavor changes within a specific protein before and after heat treatment are summarized for each of the selected proteins (Table 2.9). Before heat treatment, thirty-seven percent variability was explained by the first two factors (Figure 2.11).

PC1 was explained by aroma intensity, sweet aromatic, beany, woody, earthy/soil, bitter, umami, and astringency and PC2 was explained by cereal/grain, cardboard, sulfur, malty, green/grassy, fruity and sour (Table 2.10). Following heat treatment, thirty-two percent variability was explained by the first two factors (Figure 2.12). PC1 was explained by aroma intensity, cardboard, green pea, pyrazine, sulfur, cooked cereal/grain, metallic, fruity and umami and bitter taste (Table 2.11). PC2 was explained by cereal/grain, doughy, green/grassy, seaweed, earthy/soil, and sandy mouthfeel.

Following heat treatment, most proteins increased or decreased in specific flavor intensities (Table 2.9). Milk protein increased in sulfur flavor ($\alpha<0.05$). This is consistent with previous research on the sensory profiles from rehydrated milk proteins and fluid milk following ultra-pasteurization temperatures (Smith et al., 2016; Jo et al., 2018). Most proteins had a loss of sweet aromatic flavor and sandy mouthfeel. Soy proteins had no detectable fruity or cardboard flavors or bitter taste following heat treatment. Soy protein flavor decreased after heat treatment for studies that have examined flavors in tofu and soymilk (Kwok and Niranjan, 1995; Groff and Gropper, 1999; Yoon and Kim, 2007). Pea protein increased in pyrazine flavor and decreased in beany, cereal/grain, and sulfur flavors and umami taste. Triksuma (2019) also concluded that after UHT treatment of pea protein there was a decrease in aroma compounds that contributed to beany and pasta aromas.

Another limitation with this study is that the formulation for the model beverages that were used for heat treatment included a relatively low protein content and no added flavors. We selected a lower protein load to accommodate as many as possible of the plant proteins since many of them had minimal solubility following heat processing Further research needs to be conducted with higher protein content (for those plant proteins that remain soluble) and more

extensive examination of the sensory and functional properties, especially to determine if higher content of plant protein would lead to higher protein flavor intensities. Other studies with dairy proteins have documented increased flavor and mouthfeel issues with increased protein content (Oltman et al., 2015). The role of plant proteins with added flavors on flavor interactions (such as binding) also need to be addressed.

Conclusion

This study demonstrated that plant proteins can be differentiated using a single sensory language. Some attributes were documented in all plant proteins while others were specific to a protein type. Furthermore, the flavor profiles of plant proteins were influenced by heat treatment. The sensory language may be further refined or expanded for specific plant proteins. This lexicon can help the industry to better define and differentiate the potential of future plant proteins.

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Table 2.1 - Plant protein type and country of origin

Plant Protein Type	Country of Origin	Number of Proteins*	Protein Content Range
Pea	USA	26	80-90%
Wheat	USA	10	90%
Rice	China, USA	9	80%
Soy	USA	7	90%
Potato	Netherlands	3	90%
Hemp	China, USA	3	45-60%
Sacha Inchi	USA	2	60%
Pumpkin	China, USA	2	50-60%
Faba Bean	USA	2	60%
Mung Bean	China	1	80%
Chia	USA	1	60%

**Duplicate lots were obtained from each supplier*

Table 2.2 - Preliminary plant protein list

Flavor	Basic Taste	Texture/Mouthfeel
Aroma Intensity	Bitter	Astringent
Sweet Aromatic	Umami Sweet	Sandy
Cereal / Grain Cooked Cereal/Grain	Salty	
Cardboard Green Pea Yellow Pea	Sour	
Beany		
Oxidized		
Burnt		
Fecal/Indole		
Nutty		
Potato		
Green/Grassy		
Seaweed		
Pyrazine		
Malty		
Doughy		
Tea/Smoky		
Sulfur		
Fruity		
Painty		
Animal/Wet Dog		
Tortilla		
Onion		
Earthy		
Woody		

Table 2.3 – Lexicon for rehydrated plant proteins

Sensory Attribute	Description	Reference
Sweet aromatic[a]	Sweet aromatic associated with cake mix or grains such as oatmeal	Quaker oatmeal, 50 g soaked in 500 mL water Vanilla cake mix
Cereal/grain[a]	Aromatics associated with cereals and grains	Cheerios, 50 g in 200 mL water
Cardboard[a]	Aromatics associated with wet cardboard and brown paper	2 cm x 2 cm piece of brown paper bag boiled in water for 30 min
Fecal/indole[c]	Aroma associated with complex protein decomposition	Indole, skatole (20 mg kg−1)
Green pea[f]	Aromatics associated with fresh, green peas	Frozen green peas
Pyrazine[c]	Aroma associated with freshly cut green peppers	Methoxy pyrazines (5 μg kg−1); freshly cut bell pepper
Sulfur[c]	Aromatics associated with sulfurous compounds	Boiled mashed egg. H2S bubbled through water; struck match
Doughy[a]	Aromatic reminiscent of biscuit dough and cooked pasta	Cooked drained pasta water
Nutty[c]	The nut-like aromatic associated with different nuts	lightly toasted unsalted nuts, wheat germ. unsalted Wheat Thins
Oxidized[c]	Aroma associated with oxidized fat	2,4-Decadienal, 20 mg kg^{-1}
Painty[g]	The aromatics associated with linseed oil or oil-based paint	Linseed oil
Cooked Cereal/grain[b]	Aroma and flavor associated with cooked cereals such as oats	Rolled oats boiled 10 min
Tortilla[g]	The aromatic associated with caramelized or browned cornmeal	Fresh white corn tortillas, Tostitos tortilla chips
Onion[c]	Aromatics associated with dried onion, sweet, brown, slightly pungent	--
Earthy/Soil[e]	Aromatic associated with dirt or soil	Fresh soil and mushrooms
Burnt[e]	Scorched dark brown aromatic that may be somewhat sharp and acrid; produced by over-heating	AFF's wheat cereal, burnt toast
Beany[d]	Aromatics characteristic of beans	Kroger canned great northern beans
Fruity[c]	The aromatic blend of different fruit identities; the aromatics associated with different fruits	Canned fruit salad (in syrup); canned fruit cocktail juice; ethyl butyrate (0.1% in PG); fresh pineapple; ethyl hexanoate (20 mg kg−1)
Woody[d]	Flat, dark dry aromatics associated with the bark of a tree or wood by-products.	Popsicle stick

Table 2.3 (continued)

Malty[c]	Sweet fermented aromatic associated with dried sprouted grains	Grape nuts cereal, 20 g in 500mL water
Potato[b]	Flavor and aftertaste of boiled potato	Unpeeled white potato cubes boiled until soft
Green/Grassy[e]	An unripe aroma characterized by cut grass and unripe or green fruit	Cis-5-hexanal [300 ppm], fresh cut grass clippings
Seaweed[d]	Aromatics associated with shell fish, fresh fish, and ocean vegetation	Dried seaweed
Bitter[c]	Basic taste elicited by various compounds including caffeine and quinine	0.5% caffeine solution
Salty[d]	Basic taste elicited by salts	2% NaCl solution
Sour[d]	Basic Taste elicited by acids	1% citric acid solution
Umami[c]	Chemical feeling factor elicited by certain peptides and nucleotides	1% monosodium glutamate in water
Astringency[c]	The complex of drying, puckering, shrinking sensations in the oral cavity causing contraction of the body tissues; a mouth-drying and harsh sensation	0.1% Alum in water
Sandy[h]	The perception of small, hard particles reminiscent of sand between the teeth.	Malt-O-Meal Original = 2.0; Jiffy Corn Bread Mix = 5.0.

Terms in this list were developed, defined, and referenced using standard materials by trained panels. [a] Russell et al., 2006; [b] N'Kouka et al., 2004; [c]Drake et al., 2017; [d]Cherdchu, and Chambers IV, 2013; [e]Oliver et al., 2019; [f]Lott and Chambers IV 2006; [g]Torres and Reitmeier, 2001; [h]Donfrancesco et al., 2012.

Table 2.4: Trained panel sensory attribute means for rehydrated protein groups averaged across all samples within each group

	Aroma Intensity	Sweet Aromatic	Cereal / Grain	Cardboard	Malty	Fecal	Green Pea	Pyrazine	Sulfur	Green / Grassy	Doughy	Woody	Nutty	Beany	Seaweed
Rice (n=9)	3.4	0.5	1.2	2.2	ND	0.7	ND	ND	ND	ND	ND	ND	0.6	ND	ND
Pea (n=26)	3.2	0.7	1.0	2.1	ND	0.6	1.8	ND	ND	ND	0.6	ND	ND	1.8	ND
Soy (n=7)	2.0	1.3	2.3	1.8	ND	ND	ND	ND	ND	ND	0.5	ND	1.1	0.7	ND
Sacha Inchi (n=1)	4.1	0.9	1.1	1.1	1.6	ND	1.4	1.6	ND	1.1	ND	ND	2.8	3.4	ND
Wheat (n=2)	2.8	0.7	1.6	2.5	1.0	ND	ND	ND	0.5	0.6	ND	ND	0.5	ND	ND
Faba Bean (n=2)	3.2	1.6	0.9	ND	ND	ND	ND	2.0	1.6	2.6	ND	ND	ND	1.9	ND
Potato (n=3)	2.9	ND	1.8	1.5	0.8	ND	ND	ND	ND	2.5	0.8	ND	ND	ND	0.5
Hemp (n=3)	3.1	1.2	ND	2.2	ND	ND	2.2	ND	ND	2.1	ND	ND	ND	1.6	ND
Mung Bean (n=1)	3.0	ND	ND	ND	ND	ND	ND	ND	ND	2.8	ND	2.7	ND	2.5	2.3
Chia (n=1)	5.0	2.5	ND	ND	ND	ND	ND	ND	ND	4.7	ND	ND	ND	ND	ND
Pumpkin (n=2)	3.1	ND	ND	3.1	ND	1.3	ND	ND	ND	ND	ND	1.9	2.6	ND	ND
p-value	< 0.0001	0.027	0.132	0.002	0.0003	0.355	< 0.0001	0.0003	0.826	< 0.0001	0.881	< 0.0001	< 0.0001	< 0.0001	< 0.0001
MSE	0.4	0.9	1.4	0.8	0.4	0.6	0.7	0.4	0.9	0.2	0.6	0.05	0.5	0.5	0.03

Flavor intensities were scored using a 0 to 15-point universal Spectrum™ intensity scale where 15 = very high intensity of attribute and 0 = absence of attributes
ND= not detected
MSE=Mean square error:
Standard Error (Diff)=sqrt(MSE*(1/n1+1/n2)); MSD= minimum significant difference= t(0.025, degrees of freedom)* Standard Error(Diff)

Table 2.4 (continued)

	Painty	Cooked Cereal/ grain	Tortilla	potato	Earthy/ Soil	metallic	Fruity	Oxidized	Salty	bitter	Sour	Umami	Sandy	Astringent
Rice (n=9)	1.3	0.7	ND	ND	ND	ND	ND	1.0	ND	1.8	ND	ND	0.9	3.9
Pea (n=26)	ND	ND	ND	ND	ND	ND	ND	ND	1.3	1.7	ND	2.1	ND	3.4
Soy (n=7)	ND	ND	ND	ND	ND	ND	0.5	0.7	0.7	0.9	ND	1.1	ND	3.0
Sacha Inchi (n=1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.6	ND	1.5	1.1	3.3
Wheat (n=2)	0.8	3.2	ND	ND	ND	ND	ND	ND	ND	1.1	1.3	ND	ND	3.8
Faba Bean (n=2)	ND	ND	ND	ND	ND	2.4	1.3	ND	ND	3.4	ND	ND	ND	3.5
Potato (n=3)	ND	ND	ND	0.6	ND	ND	ND	ND	ND	1.4	1.1	ND	0.5	4.7
Hemp (n=3)	ND	ND	ND	ND	0.6	ND	ND	ND	ND	1.2	ND	0.6	1.0	3.2
Mung Bean (n=1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.3	ND	ND	ND	3.2
Chia (n=1)	1.2	ND	ND	ND	ND	ND	1.4	ND	ND	ND	ND	ND	1.0	1.2
Pumpkin (n=2)	ND	ND	1.4	ND	ND	ND	ND	ND	ND	ND	ND	3.3	1.0	3.0
p-value	0.009	<0.0001	<0.0001	0.019	0.019	<0.0001	0.874	0.474	<0.0001	0.054	0.001	<0.0001	0.022	0.011
MSE	0.5	0.1	0.1	0.04	0.04	0.01	0.1	0.1	0.5	1.1	0.4	0.7	0.5	0.5

ND= not detected
MSE=Mean square error

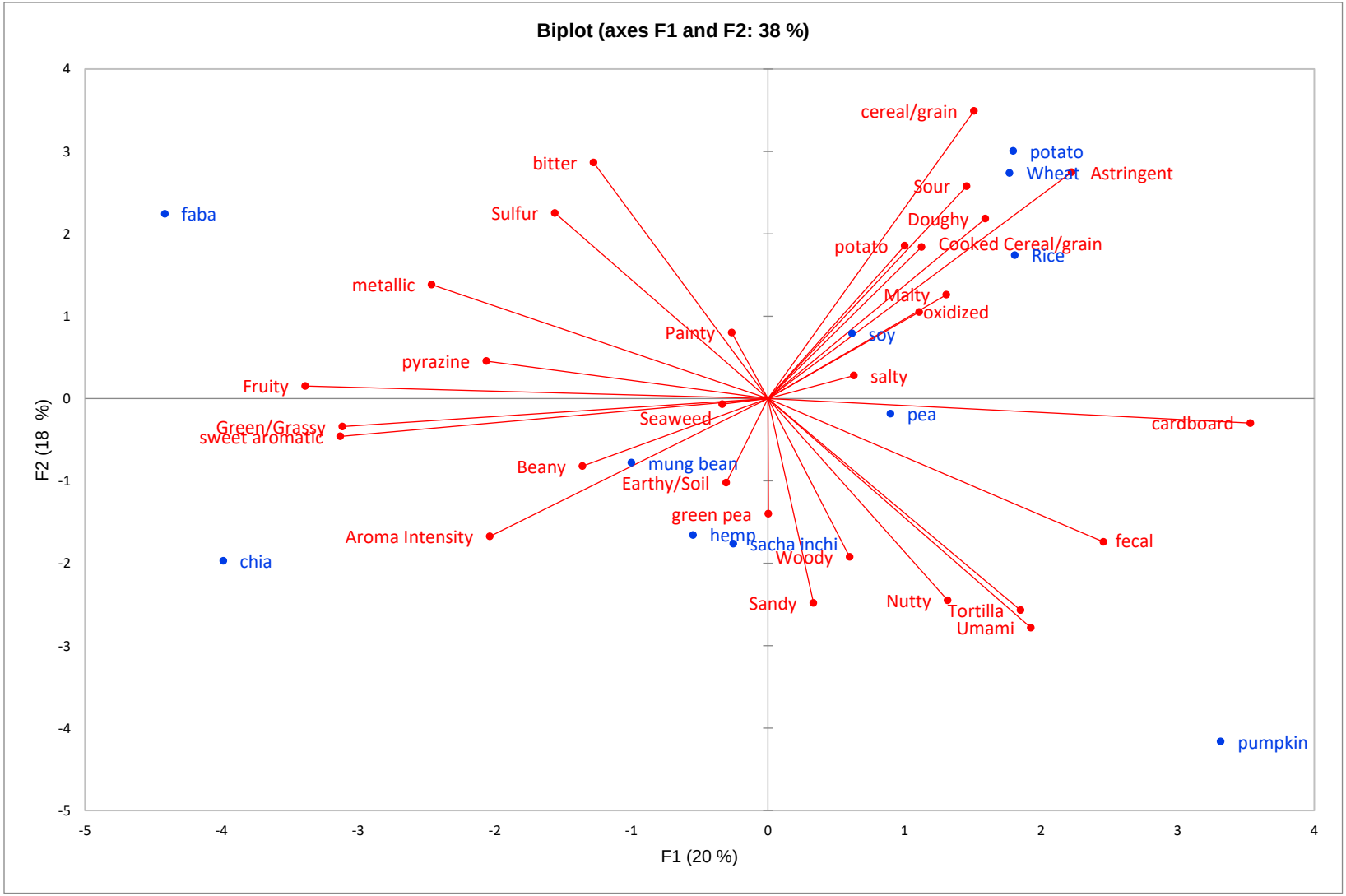

Figure 2.1: Principal component biplot of descriptive analysis for average sensory profiles for each rehydrated plant protein group (9 protein types, n=66 samples, Table 2.1) (PC1 and 2)

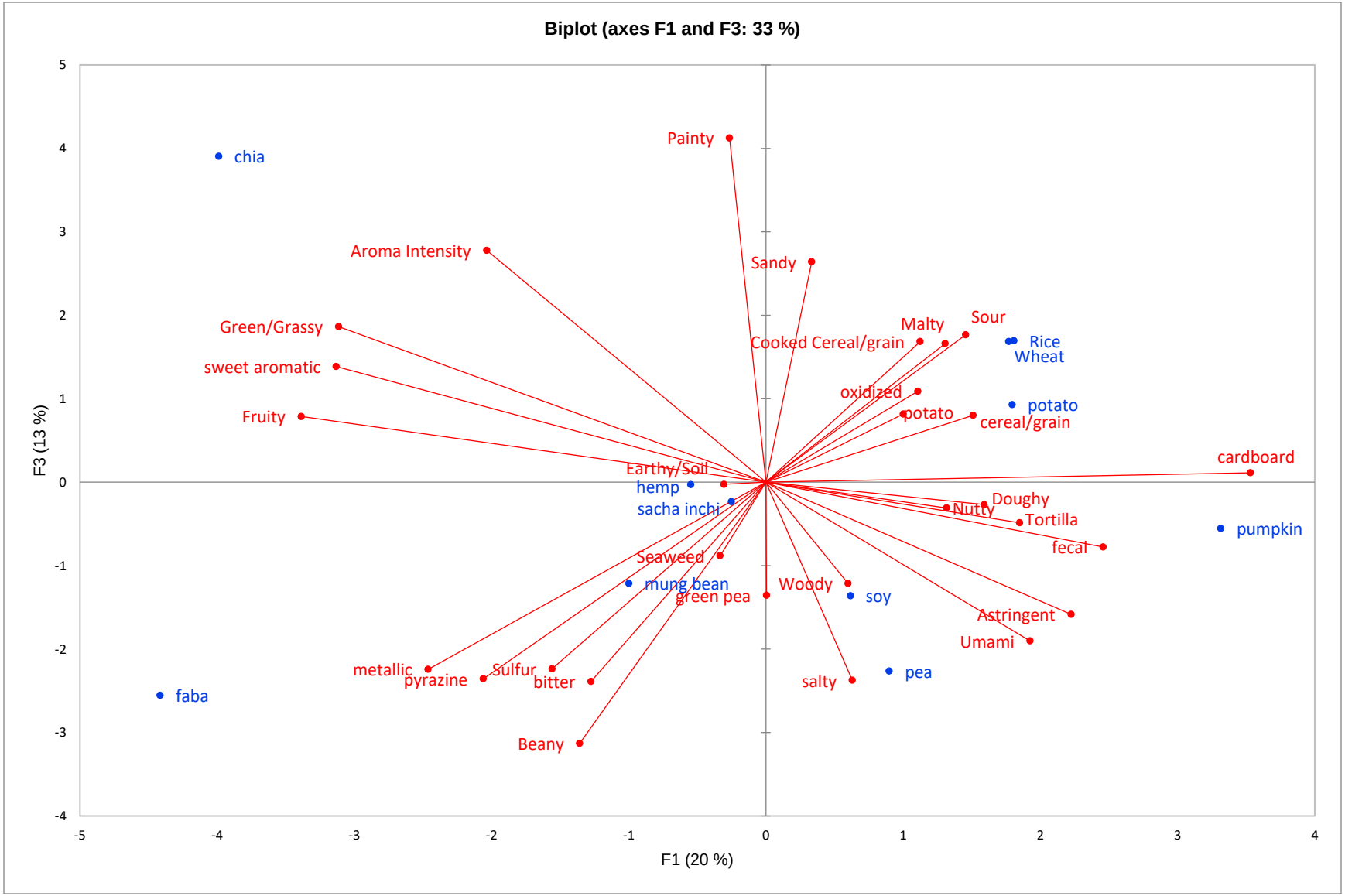

Figure 2.2: Principal component biplot of descriptive analysis for average sensory profiles for each rehydrated plant protein group (9 protein types, n=66 samples, Table 2.1) (PC1 and 3)

Table 2.5: Factor loadings for all rehydrated proteins.

Attributes	Factor 1	Factor 2	Factor 3	Factor 4
Aroma Intensity	**0.816**	0.199	0.105	-0.376
sweet aromatic	**0.519**	0.013	-0.287	-0.312
cereal/grain	-0.447	**-0.704**	0.086	0.192
cardboard	**-0.867**	0.174	-0.148	-0.184
fecal	-0.596	**0.640**	-0.224	0.038
green pea	0.111	0.249	**0.258**	0.025
Nutty	-0.225	0.537	**0.588**	-0.319
Beany	0.352	0.038	**0.856**	0.196
Painty	0.167	-0.217	-0.445	**-0.460**
pyrazine	0.203	-0.234	**0.718**	0.376
Malty	-0.025	-0.263	**0.721**	-0.597
Green/Grassy	**0.919**	-0.126	-0.169	0.035
Cooked Cereal/grain	-0.305	-0.415	-0.031	**-0.590**
Woody	0.028	**0.538**	0.071	0.173
Doughy	**-0.472**	-0.366	-0.347	0.191
Fruity	**0.677**	-0.205	-0.212	0.277
bitter	-0.148	**-0.599**	0.132	0.634
Salty	-0.290	-0.182	**-0.475**	0.164
Sour	-0.285	**-0.586**	-0.055	-0.548
Umami	-0.466	**0.754**	0.054	-0.002
Astringent	**-0.674**	-0.168	0.511	0.120
Sandy	-0.125	-0.336	**0.424**	-0.338

Numbers in bold are believed to be of primary importance

Table 2.6: Correlations between descriptive sensory attributes of all wheat proteins (n=10)

	Aroma Intensity	Swt Arm	Cereal / grain	Cdb	Malty	Sulfur	Green / Grassy	Nutty	Beany	Painty	Cooked Cereal /grain	Salty	Sour	bitter	Umami	Sandy	Ast
Aroma Intensity	1.00	0.08	-0.47	0.12	-0.01	0.37	0.10	-0.52	0.10	0.09	-0.02	0.10	-0.06	0.04	-0.03	0.10	0.43
Swt Arm		1.00	-0.47	-0.17	**0.93**	0.51	0.43	-0.50	0.43	**-0.70**	-0.24	-0.26	**0.67**	0.61	-0.39	0.45	0.28
cereal/ grain			1.00	0.38	-0.47	-0.28	0.13	**0.75**	0.12	0.46	0.60	-0.31	0.08	-0.29	0.61	0.13	-0.23
Cdb				1.00	-0.21	-0.40	-0.01	0.08	-0.01	**-0.67**	0.17	**-0.68**	0.05	-0.24	0.40	-0.01	0.32
Malty					1.00	0.42	0.43	-0.49	0.43	**-0.69**	-0.31	-0.25	0.61	0.59	-0.38	0.42	0.44
Sulfur						1.00	0.61	-0.40	0.61	-0.56	0.15	0.30	0.59	0.31	-0.31	0.61	0.45
Green/ Grassy							1.00	-0.21	1.00	-0.29	-0.01	-0.11	**0.84**	0.15	-0.16	1.00	0.46
Nutty								1.00	-0.21	0.24	0.30	-0.21	-0.24	-0.57	0.21	-0.21	-0.43
Beany									1.00	-0.29	-0.02	-0.11	0.84	0.14	-0.16	**0.99**	0.46
Painty										1.00	0.19	-0.29	-0.36	-0.45	0.55	-0.30	-0.15
Cooked Cereal/ grain											1.00	0.01	0.04	0.22	**0.74**	-0.01	0.03
Salty												1.00	-0.33	0.06	-0.16	-0.11	-0.45
Sour													1.00	0.30	-0.21	**0.85**	**0.67**
bitter														1.00	0.26	0.15	0.21
Umami															1.00	-0.17	-0.19
Sandy																1.00	0.46
Ast																	1.00

Numbers in bold represent significant correlations ($p<0.05$)
Swt Arm=sweet aromatic, Cdb=Cardboard, Ast=Astringent

Table 2.7: Correlations between descriptive sensory attributes of all rice proteins (n=9)

	Aroma Intensity	Swt Arm	Cereal / grain	Cdb	fecal	pyrazine	Sulfur	Doughy	Nutty	Beany	Painty	Cooked Cereal / grain	Onion	Oxd	bitter	Sandy	Ast
Aroma Intensity	1.00	0.35	0.41	0.44	-0.28	-0.30	-0.30	0.43	0.43	-0.30	**0.93**	0.65	-0.30	-0.36	0.41	-0.47	-0.45
Swt Arm		1.00	0.01	0.13	-0.34	-0.17	-0.17	**0.89**	-0.17	-0.17	0.41	-0.26	-0.17	-0.02	0.10	0.03	0.11
cereal/ grain			1.00	-0.25	0.24	0.37	0.18	0.27	0.28	-0.09	0.48	0.31	0.13	**-0.78**	**0.78**	**-0.80**	**-0.83**
Cdb				1.00	-0.41	-0.31	-0.22	0.02	0.24	**-0.68**	0.43	0.36	-0.23	0.33	-0.27	0.27	0.34
fecal					1.00	0.49	-0.24	-0.24	-0.24	0.36	-0.27	-0.37	-0.24	-0.34	0.29	-0.46	-0.24
pyrazine						1.00	-0.12	-0.12	-0.12	-0.12	-0.29	-0.18	-0.12	-0.17	0.43	-0.23	-0.31
Sulfur							1.00	-0.12	-0.12	-0.12	-0.30	-0.19	**0.98**	-0.17	-0.17	0.22	0.02
Doughy								1.00	-0.12	-0.12	0.58	-0.19	-0.12	-0.17	0.34	-0.24	-0.19
Nutty									1.00	-0.12	0.39	0.61	-0.12	-0.17	0.07	-0.23	-0.31
Beany										1.00	-0.29	-0.18	-0.12	-0.17	-0.04	-0.23	-0.13
Painty											1.00	0.60	-0.30	-0.41	0.53	-0.57	-0.52
Cooked Cereal/ grain												1.00	-0.19	-0.26	0.33	-0.36	-0.47
Onion													1.00	-0.17	-0.16	0.23	0.01
Oxd														1.00	**-0.72**	**0.84**	**0.83**
bitter															1.00	**-0.84**	**-0.86**
Sandy																1.00	0.61
Ast																	1.00

Numbers in bold represent significant correlations ($p<0.05$)
Swt Arm=sweet aromatic, Cdb=Cardboard, Oxd= Oxidized, Ast=Astringent

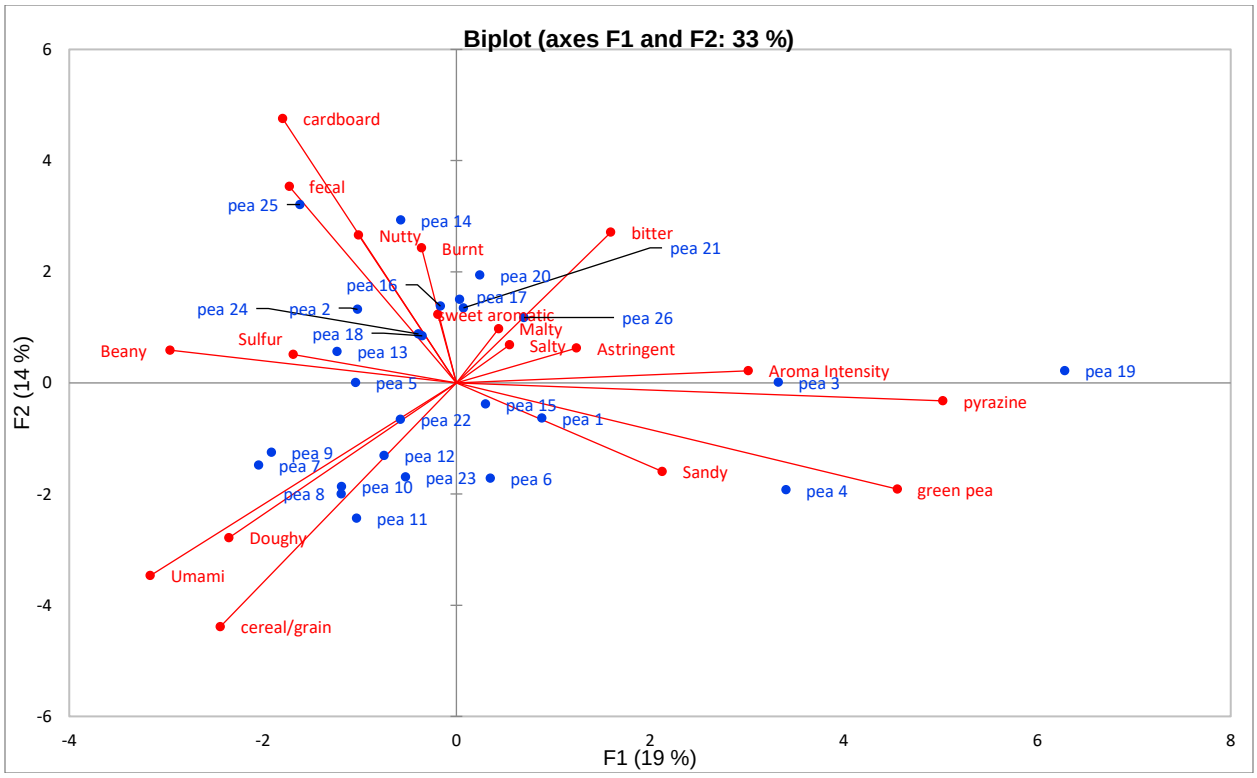

Figure 2.3: Principal component biplot of sensory profiles of rehydrated pea proteins (n=26) (PC1 and 2)

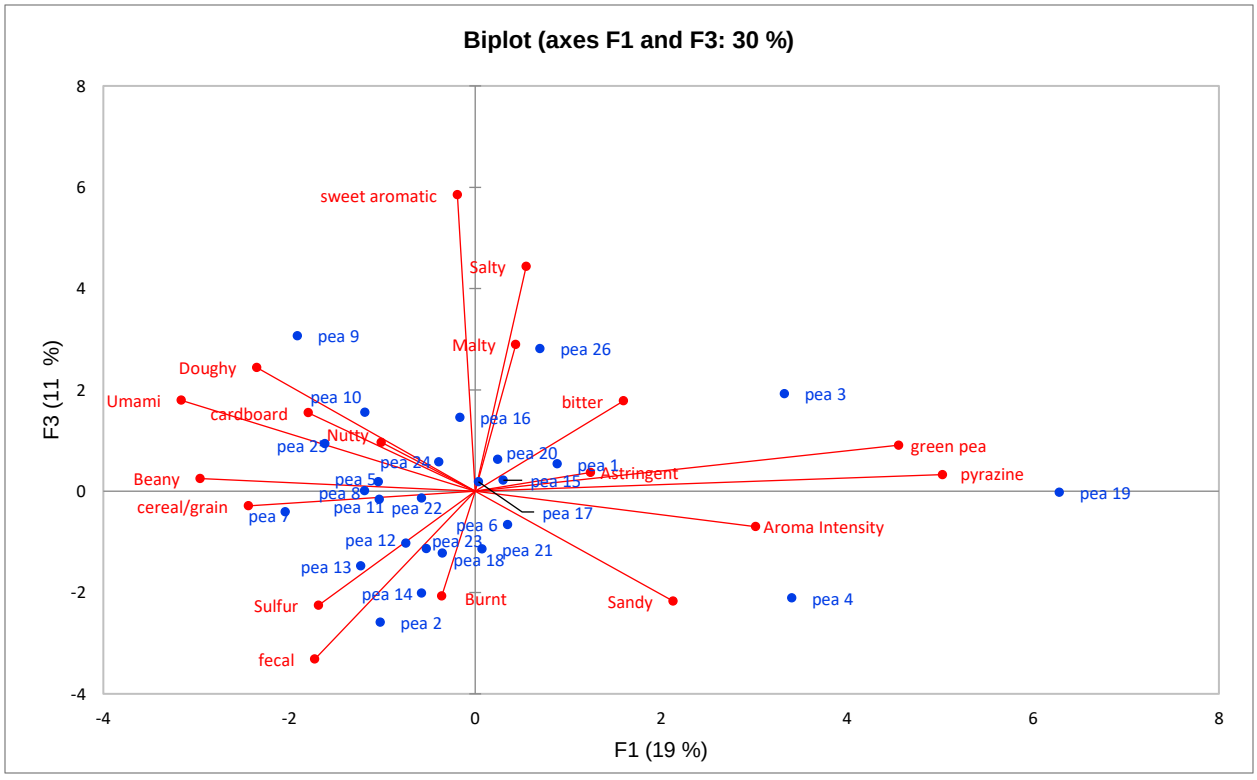

Figure 2.4: Principal component biplot of sensory profiles of rehydrated pea proteins (n=26) (PC1 and 3)

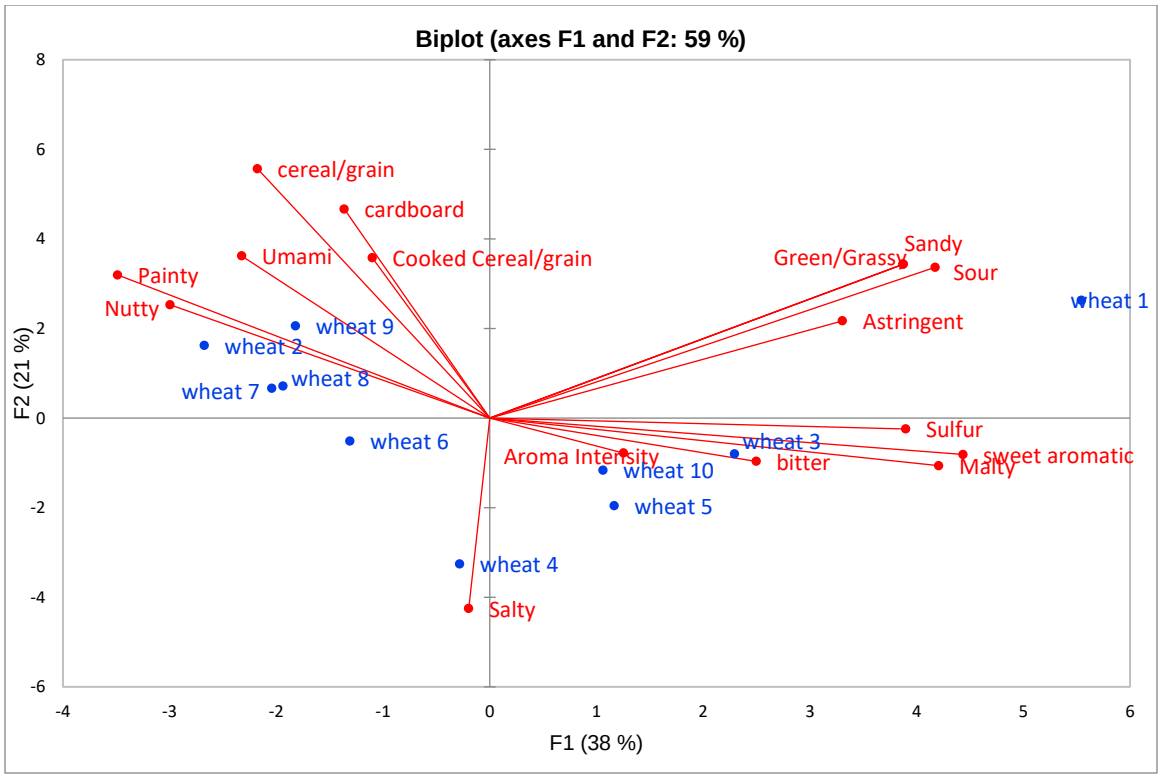

Figure 2.5: Principal component biplot of sensory profiles of rehydrated wheat proteins (n=11) (PC1 and 2)

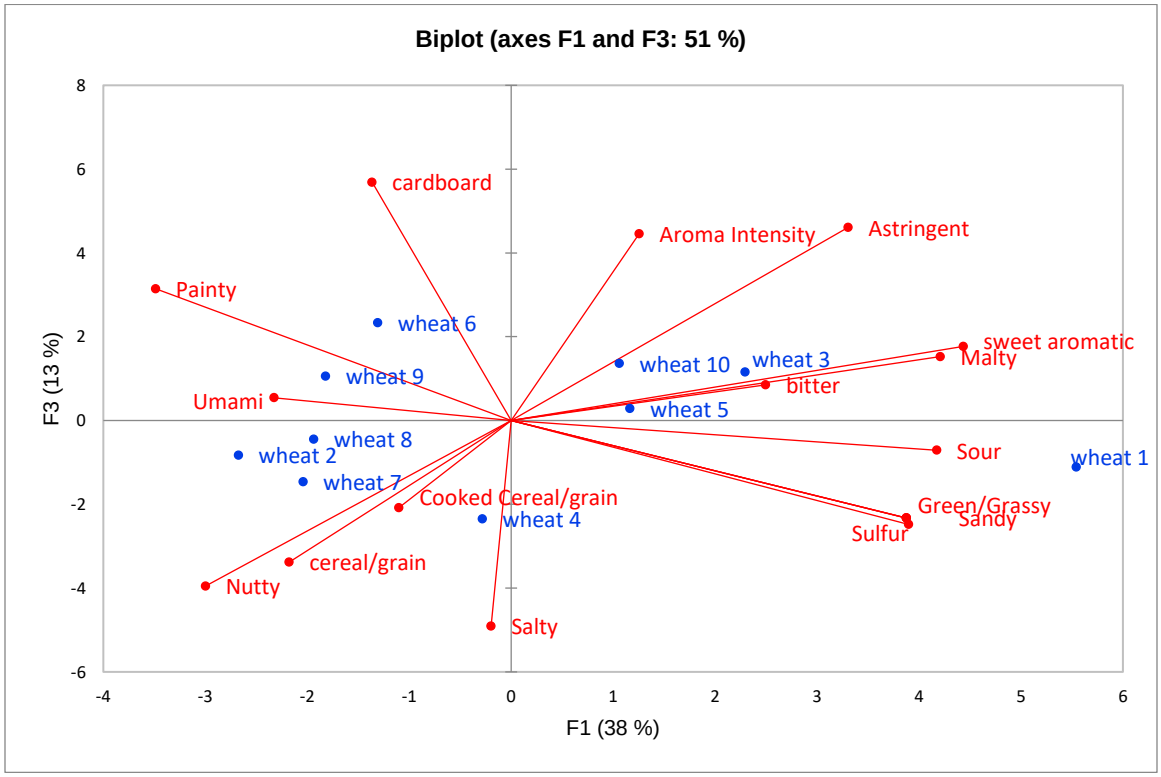

Figure 2.6: Principal component biplot of sensory profiles of rehydrated wheat proteins (n=11) (PC1 and 3)

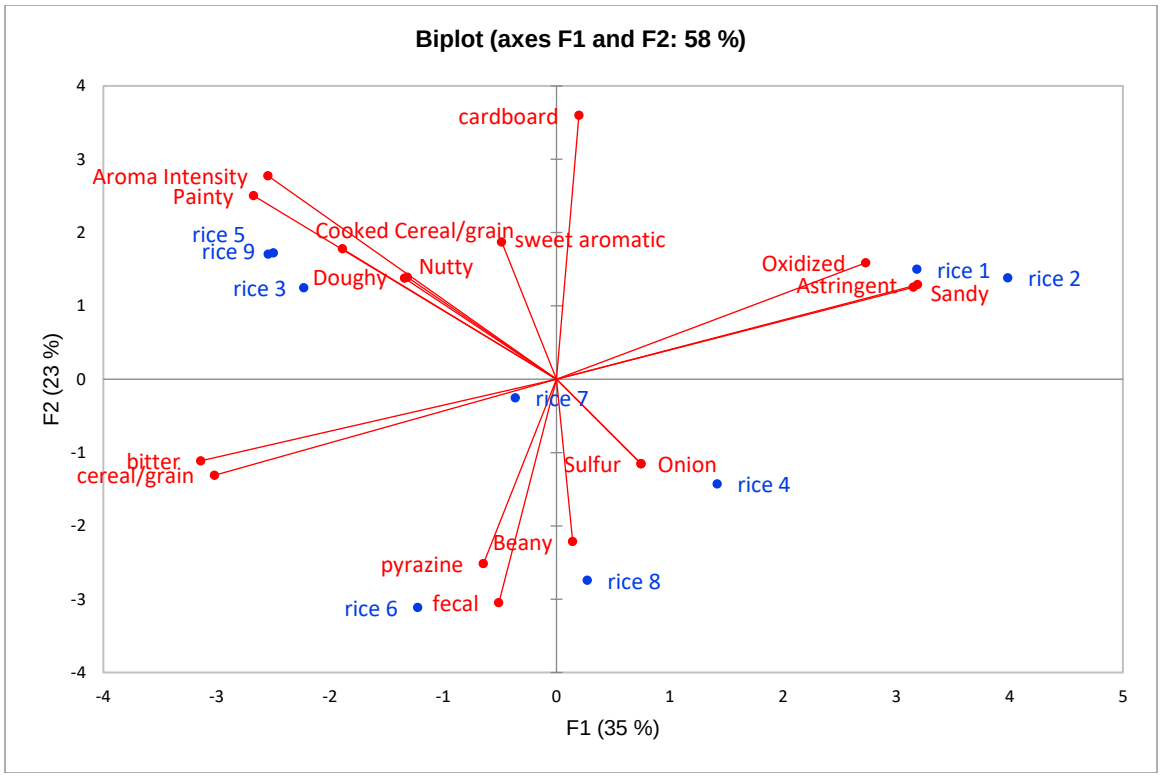

Figure 2.7: Principal component biplot of sensory profiles of rehydrated rice proteins (n=11) (PC1 and 2)

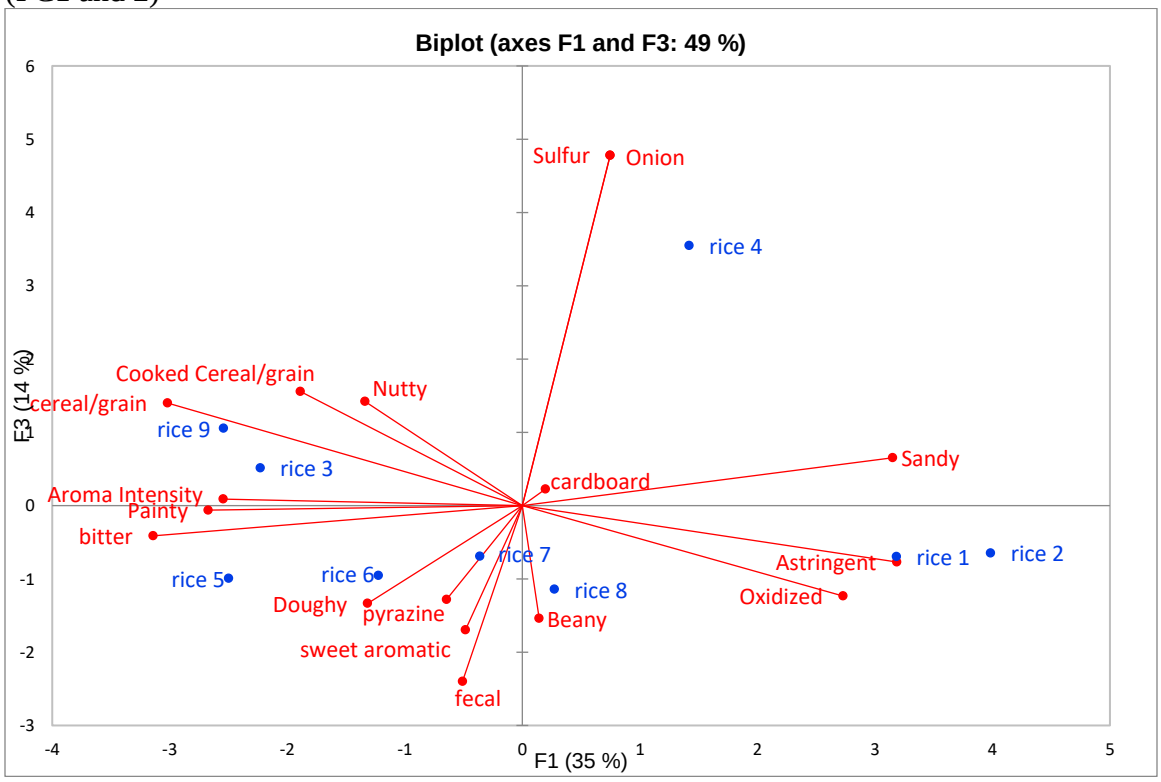

Figure 2.8: Principal component biplot of sensory profiles of rehydrated rice proteins (n=11) (PC1 and 3)

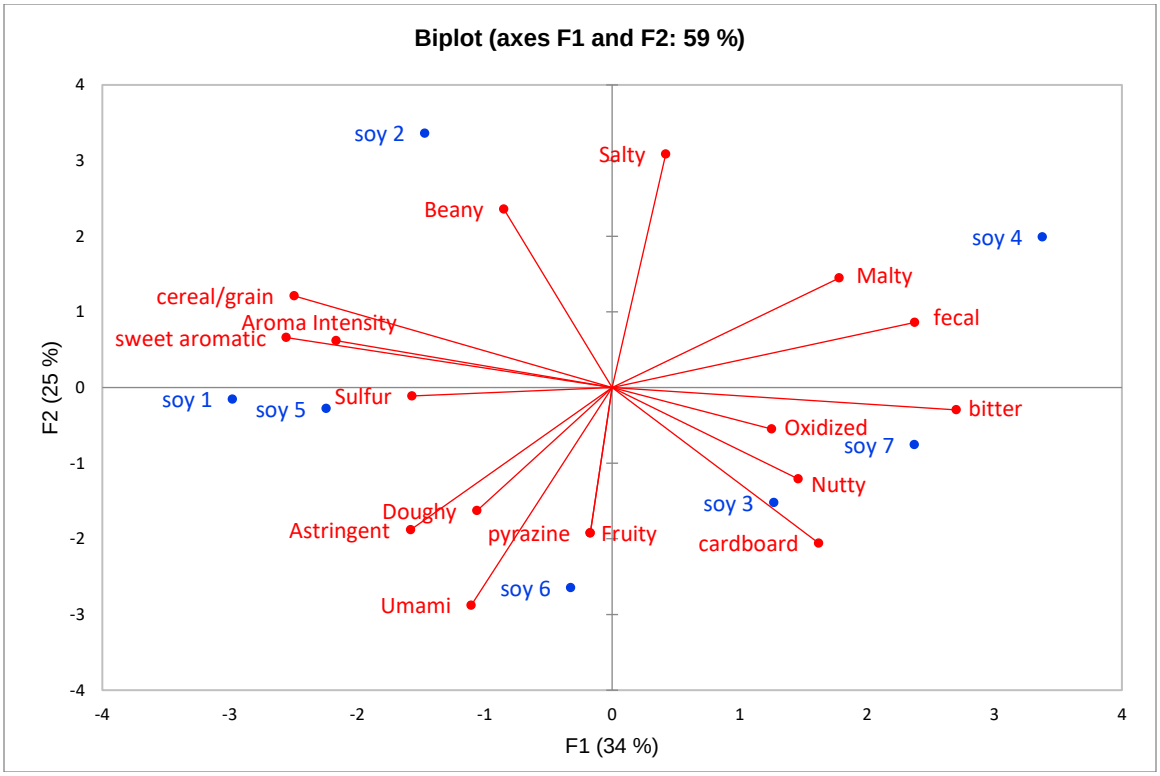

Figure 2.9: Principal component biplot of sensory profiles of rehydrated soy proteins (n=7) (PC1 and 2)

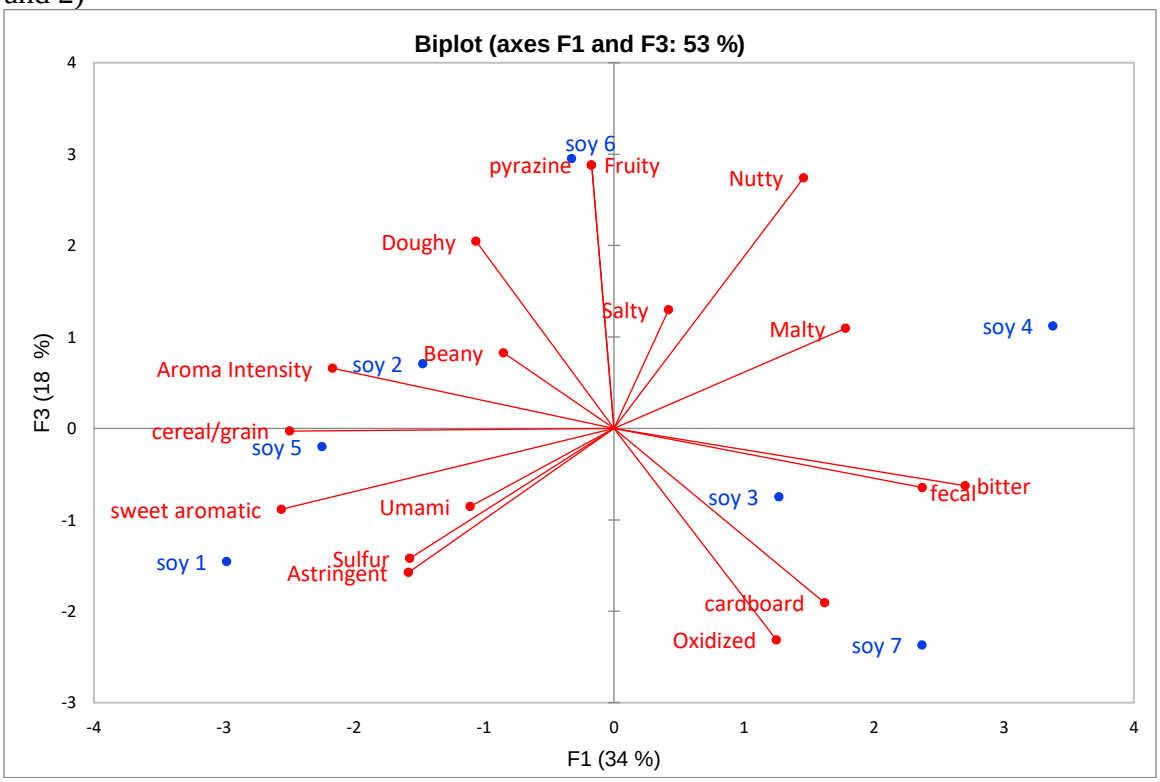

Figure 2.10: Principal component biplot of sensory profiles of rehydrated soy proteins (n=7) (PC1 and 3)

Table 2.8: Mean sensory attributes of selected rehydrated proteins and milk protein before and after heat treatment

Treatment Type	Protein Type	Aroma Intensity	Swt Arm	cereal / grain	Cbd	fecal	green pea	Sulfur	Nutty	Beany	Milky	Cooked Cereal /grain	Malty	Green/ Grassy	Woody
Rehydrated	Chia	3.0	2.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.6	ND
Heat Treated	Chia	5.0*	2.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.5*	ND
Rehydrated	Faba Bean	2.8	1.1	ND	1.0	ND	ND	ND	ND	1.1	ND	ND	ND	1.0	ND
Heat Treated	Faba Bean	3.0	ND*	ND	1.5	ND	ND	ND	ND	1.0	ND	ND	ND	3.0*	ND
Rehydrated	Hemp	3.4	2.0	ND	ND	ND	1.7	ND	ND	1.7	ND	ND	ND	2.9	ND
Heat Treated	Hemp	4.0	1.5	ND	ND	ND	3.5*	ND	ND	1.0	ND	ND	ND	2.5	ND
Rehydrated	Milk	2.0	1.0	ND	2.4	ND	ND	0.5	ND	ND	2.4	ND	ND	ND	ND
Heat Treated	Milk	2.0	0.5	ND	2.0	ND	ND	1.2*	ND	ND	2.5	ND	ND	ND	ND
Rehydrated	Mung Bean	4.3	ND	ND	2.8	ND	ND	ND	ND	2.2	ND	ND	ND	2.0	1.3
Heat Treated	Mung Bean	3.0	ND	ND	2.5	ND	ND	ND	ND	2.0	ND	ND	ND	2.5	2.3
Rehydrated	Pea	3.1	0.5	ND	ND	ND	1.9	2.5	ND	2.1	ND	ND	ND	ND	ND
Heat Treated	Pea	4.5*	ND	ND	ND	ND	4.2*	ND*	ND	1.1*	ND	ND	ND	ND	ND
Rehydrated	Potato	3.3	1.5	ND	1.3	ND	ND	ND	ND	ND	ND	ND	ND	1.5	ND
Heat Treated	Potato	2.8	ND*	ND	2.2*	ND	ND	ND	ND	ND	ND	ND	ND	3.8*	ND
Rehydrated	Pumpkin	2.0	1.3	ND	1.9	ND	ND	ND	1.1	ND	ND	ND	ND	ND	ND
Heat Treated	Pumpkin	3.0*	ND*	ND	3.0*	1.7*	ND	ND	2.2*	ND	ND	ND	ND	ND	1.0*
Rehydrated	Rice	2.8	1.4	1.6	2.4	ND	ND	1.6	ND	ND	ND	ND	1.6	ND	ND
Heat Treated	Rice	2.5	ND*	2.5*	2.3	1.6*	2.0*	ND*	ND	ND	ND	ND	ND*	ND	ND
Rehydrated	Sacha Inchi	4.0	1.3	2.0	0.5	ND	1.7	ND	1.3	3.7	ND	ND	ND	ND	ND
Heat Treated	Sacha Inchi	5.3*	ND*	1.3*	1.5*	ND	2.0	ND	3.3*	3.0	ND	ND	3.3*	ND	ND
Rehydrated	Soy	3.3	3.0	ND	1.2	1.5	ND	ND	ND	1.5	ND	0.9	ND	ND	ND
Heat Treated	Soy	2.0*	1.9*	3.0*	1.2	ND*	ND	ND	ND	1.5	ND	1.0	ND	ND	ND
Rehydrated	Wheat	2.9	1.0	1.5	2.1	ND	ND	ND	ND	ND	ND	3.0	ND	ND	ND
Heat Treated	Wheat	3.0	1.0	2.0	2.6	ND	ND	1.8*	ND	2.3*	ND	3.0	2.0*	2.5*	ND

Flavor intensities were scored using a 0 to 15-point universal Spectrum™ intensity scale where 15 = very high intensity of attribute and 0 = absence of attributes
ND= not detected
Swt Arm=sweet aromatic, Cbd=Cardboard
*Asterisks indicate significant difference ($p<0.05$) following heat treatment as determined by 2-sample t-test analysis.

Table 2.8 (continued)

Treatment Type	Protein Type	Tortilla	Seaweed	Earthy/Soil	Fruity	Salty	Sour	bitter	Umami	Astringent	Sandy
Rehydrated	Chia	ND	ND	ND	1.9	ND	ND	0.5	ND	3.0	ND
Heat treated	Chia	ND	ND	ND	1.0	ND	ND	ND	ND	3.5	ND
Rehydrated	Faba Bean	ND	ND	ND	1.0	ND	ND	1.4	ND	2.1	ND
Heat treated	Faba Bean	ND	ND	ND	2.1	ND	ND	4.0	ND	4.3	ND
Rehydrated	Hemp	ND	ND	ND	2.0	ND	ND	1.4	2.0	2.9	2.5
Heat treated	Hemp	ND	ND	ND	ND*	ND	ND	1.5	1.7	3.0	ND*
Rehydrated	Milk	2.1	ND	ND	ND	ND	ND	ND	ND	3.1	ND
Heat treated	Milk	2.0	ND	ND	ND	ND	ND	ND	ND	3.3	ND
Rehydrated	Mung Bean	ND	2.3	2.5	ND	ND	ND	1.6	1.8	3.9	ND
Heat treated	Mung Bean	ND	1.8	ND	ND	ND	ND	1.5	1.8	3.8	ND
Rehydrated	Pea	ND	ND	ND	ND	ND	ND	2.1	3.0	3.5	ND
Heat treated	Pea	ND	ND	ND	ND	ND	ND	4.3*	ND*	4.5*	ND
Rehydrated	Potato	ND	ND	1.6	ND	ND	ND	1.8	ND	3.1	2.8
Heat treated	Potato	ND	1.0*	1.1	ND	ND	ND	2.0	ND	4.0*	3.0
Rehydrated	Pumpkin	ND	ND	ND	ND	ND	ND	ND	ND	2.8	1.9
Heat treated	Pumpkin	ND	ND	ND	ND	ND	ND	ND	3.0*	3.0	ND*
Rehydrated	Rice	ND	ND	ND	ND	ND	1.1	1.0	ND	3.4	3.0
Heat treated	Rice	ND	ND	ND	ND	ND	1.0	1.3	ND	3.6	ND*
Rehydrated	Sacha Inchi	ND	ND	ND	ND	ND	ND	ND	ND	3.5	3.0
Heat treated	Sacha Inchi	ND	ND	ND	ND	ND	ND	1.0*	ND	3.8	ND*
Rehydrated	Soy	ND	ND	ND	1.0	2.7	ND	0.8	ND	2.0	ND
Heat treated	Soy	ND	ND	ND	ND*	3.1	ND	0.9	ND	2.3	ND
Rehydrated	Wheat	ND	ND	ND	ND	ND	2.3	1.6	1.9	4.7	1.4
Heat treated	Wheat	ND	ND	ND	ND	ND	4.5*	1.4	2.0	4.9	1.6

Flavor intensities were scored using a 0 to 15-point universal Spectrum™ intensity scale where 15 = very high intensity of attribute and 0 = absence of attributes
ND= not detected
Ast=Astringent
*Asterisk indicates significant difference ($p<0.05$) following heat treatment as determined by 2-sample t-test analysis.

Table 2.9: Significant changes (P<0.05) for rehydrated proteins following heat treatment

Protein	Attribute			
	Decreased	**Increased**	**Not detected following heat treatment**	**Detected following heat treatment**
Chia	ND	aroma intensity, green/grassy	ND	painty
Faba Bean	ND	green/grassy, bitter, astringent	Sweet aromatic	ND
Hemp	ND	green pea	fruity, sandy	ND
Milk	ND	Sulfur	ND	Sulfur
Mung Bean	ND	nothing	earthy/soil	ND
Pea	Beany	green pea, bitter, and astringent	sweet aromatic, umami, sulfur	ND
Potato	cardboard	green/grassy, astringent	sweet aromatic	seaweed
Pumpkin	ND	nutty, cardboard	sweet aromatic, sandy	fecal, woody, umami
Rice	ND	cereal/grain	sweet aromatic, sulfur, malty, and sandy	fecal, green pea
Sacha Inchi	cereal/grain	aroma intensity, cardboard, nutty	sweet aromatic, sandy	malty, bitter
Soy	aroma intensity, sweet aromatic	nothing	fecal, fruity	cereal/grain
Wheat	ND	sour	ND	sulfur, beany, malty, green/grassy

Changes in attributes represent significant differences (p<0.05) as determined by 2- sample t-test analysis
ND= no change

Table 2.10: Factor loadings for selected rehydrated proteins before heat treatment

Attributes	Factor 1	Factor 2	Factor 3	Factor 4
Aroma Intensity	**0.604**	-0.526	0.317	0.106
sweet aromatic	**-0.777**	-0.348	0.140	0.378
cereal/grain	0.116	**0.597**	0.585	0.093
cardboard	0.291	**0.598**	-0.589	0.190
fecal	-0.449	-0.338	-0.104	**0.537**
green pea	0.134	-0.261	**0.724**	-0.450
Sulfur	0.120	**0.306**	0.224	-0.202
Milky	-0.258	0.358	**-0.591**	-0.423
Nutty	-0.138	0.147	0.370	**-0.475**
Beany	**0.452**	-0.435	0.381	-0.307
Cooked Cereal/grain	0.075	0.400	0.140	**0.716**
Malty	-0.018	**0.487**	0.151	0.137
Green/Grassy	0.235	**-0.672**	-0.105	-0.008
Woody	**0.784**	-0.243	-0.416	0.016
Tortilla	-0.258	0.358	**-0.591**	-0.423
Potato	0.096	-0.087	**-0.123**	0.090
Seaweed	-0.416	-0.243	**0.784**	0.016
Earthy/Soil	**0.740**	-0.262	-0.434	0.066
Fruity	-0.389	**-0.670**	0.105	0.146
Sour	0.184	**0.695**	0.230	0.593
bitter	**0.582**	-0.208	0.126	0.423
Umami	**0.612**	-0.103	0.289	0.088
Salty	-0.449	-0.338	-0.104	**0.537**
Astringent	**0.687**	0.522	0.209	0.093
Sandy	0.014	0.294	**0.556**	-0.088

Numbers in bold are believed to be of primary importance

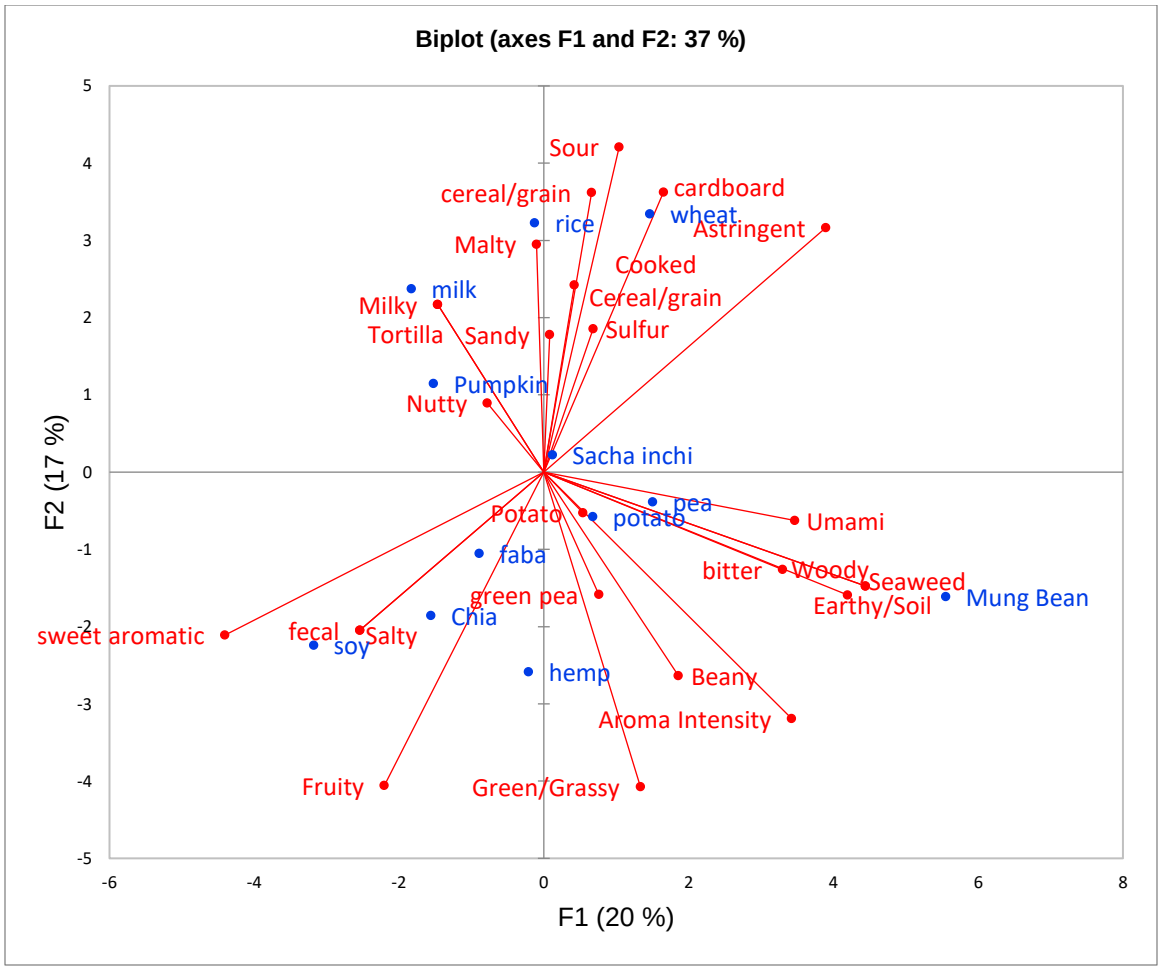

Figure 2.11: Principal component biplot of the selected rehydrated proteins before heat treatment (PC 1 and 2)

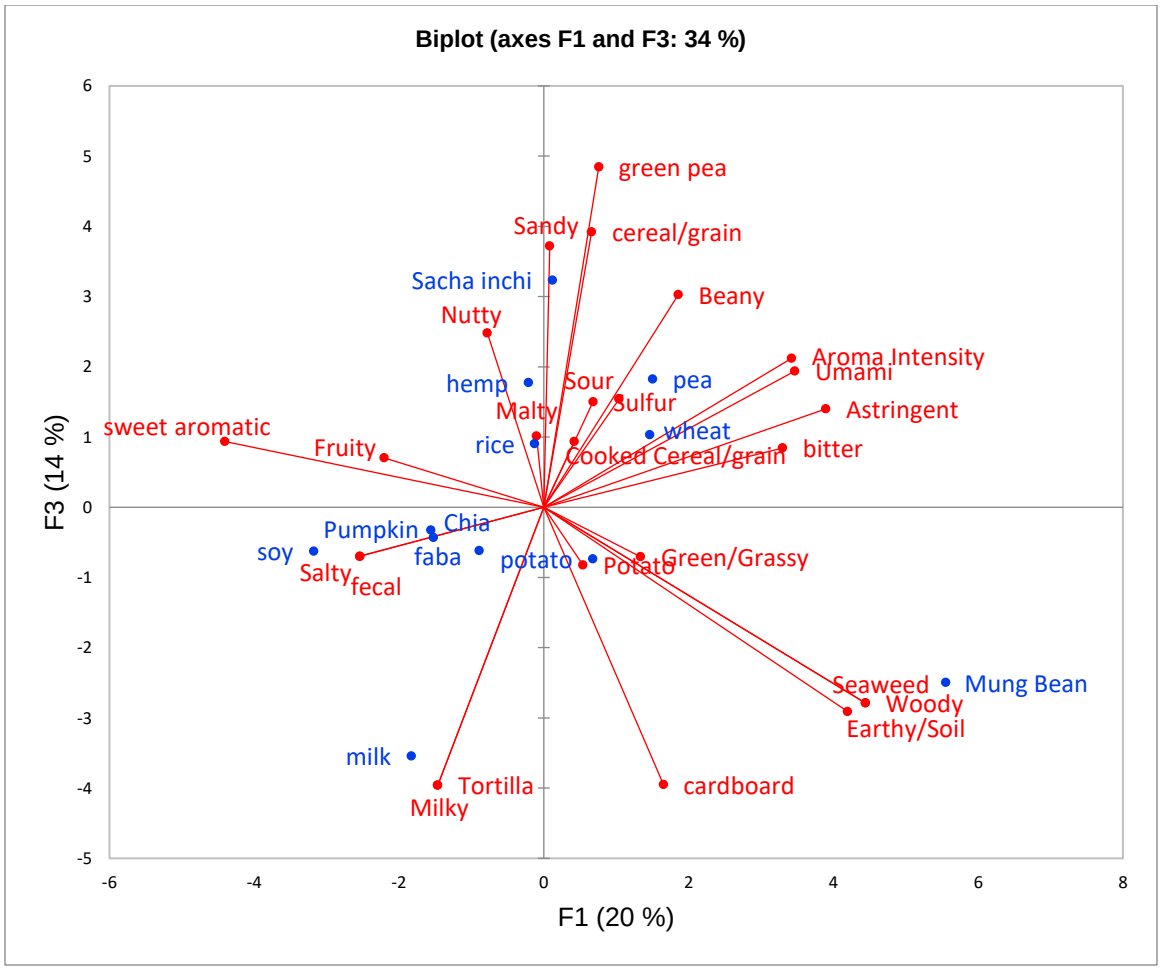

Figure 2.12: Principal component biplot of the selected rehydrated proteins before heat treatment (PC 1 and 3)

Table 2.11: Factor loadings for selected rehydrated proteins after heat treatment

Attributes	Factor 1	Factor 2	Factor 3	Factor 4
Aroma Intensity	**-0.699**	-0.231	0.275	-0.002
sweet aromatic	0.124	-0.238	-0.324	**0.773**
cereal/grain	0.410	**-0.546**	0.074	0.223
cardboard	**0.727**	0.116	0.163	-0.502
fecal	0.224	-0.112	-0.313	**-0.522**
green pea	**-0.569**	-0.340	0.089	-0.169
pyrazine	**-0.726**	-0.311	0.382	-0.196
Sulfur	**0.650**	-0.231	0.365	0.312
Doughy	0.143	**0.778**	0.241	-0.027
Painty	-0.262	0.130	-0.231	**0.517**
Nutty	-0.129	-0.426	0.088	**-0.571**
Beany	-0.055	-0.539	**0.582**	-0.093
Cooked Cereal/grain	**0.601**	-0.347	0.542	0.410
Malty	0.052	-0.547	**0.612**	-0.102
Green/Grassy	-0.106	**0.640**	0.290	0.452
Woody	0.189	0.182	-0.091	**-0.605**
Tortilla	0.300	0.006	**-0.417**	0.076
Seaweed	0.176	**0.598**	0.145	-0.377
Earthy/Soil	0.143	**0.778**	0.241	-0.027
metallic	**-0.396**	0.198	0.163	0.158
Fruity	**-0.488**	0.244	0.047	0.382
Milky	0.300	0.006	**-0.417**	0.076
Salty	0.208	-0.275	-0.336	**0.348**
Sour	0.567	-0.285	**0.631**	0.269
Umami	**0.413**	-0.110	0.088	-0.403
bitter	**-0.556**	0.180	0.497	0.002
Sandy	0.402	**0.581**	0.552	0.127
Astringent	-0.128	0.149	**0.849**	0.007

Numbers in bold are believed to be of primary importance

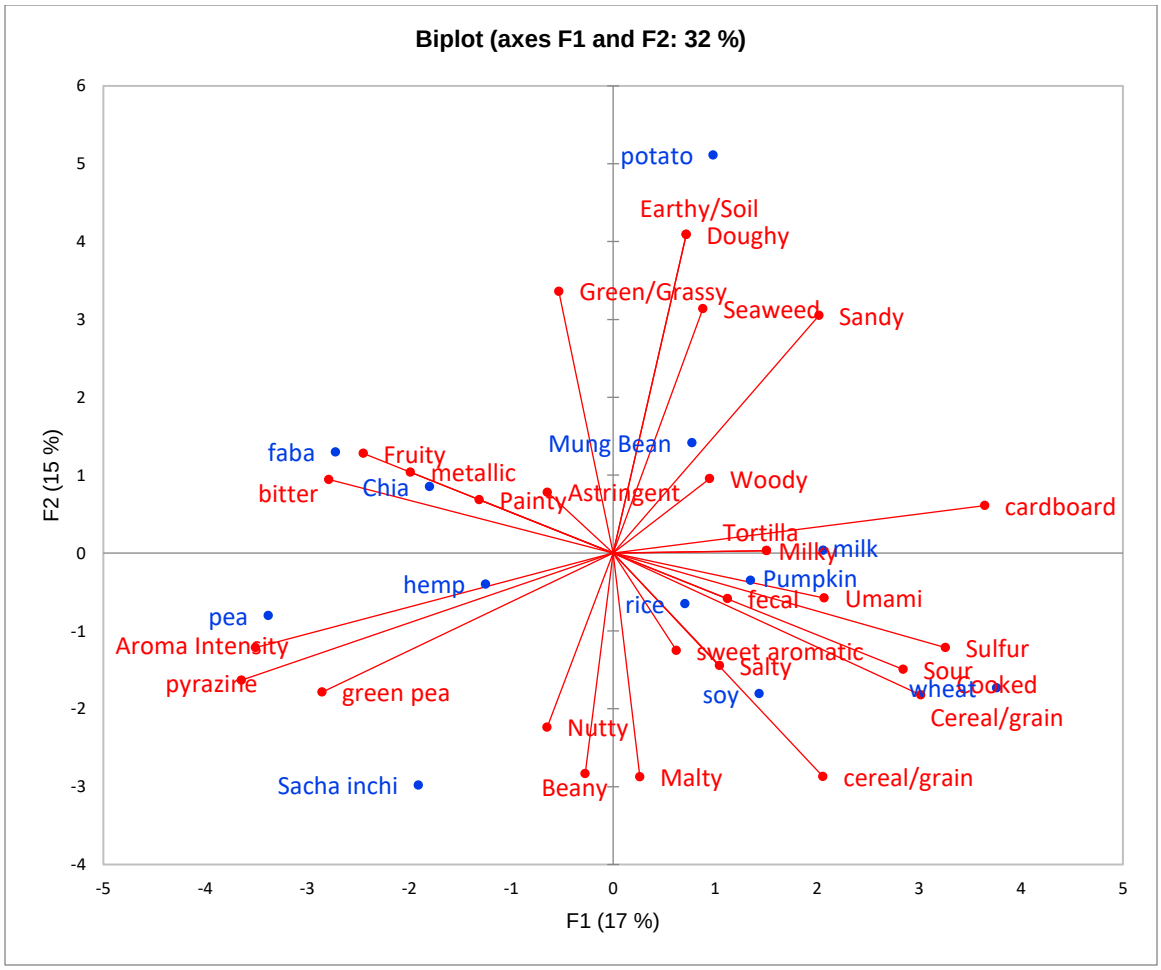

Figure 2.13: Principal component biplot of the selected rehydrated proteins after heat treatment (PC 1 and 2)

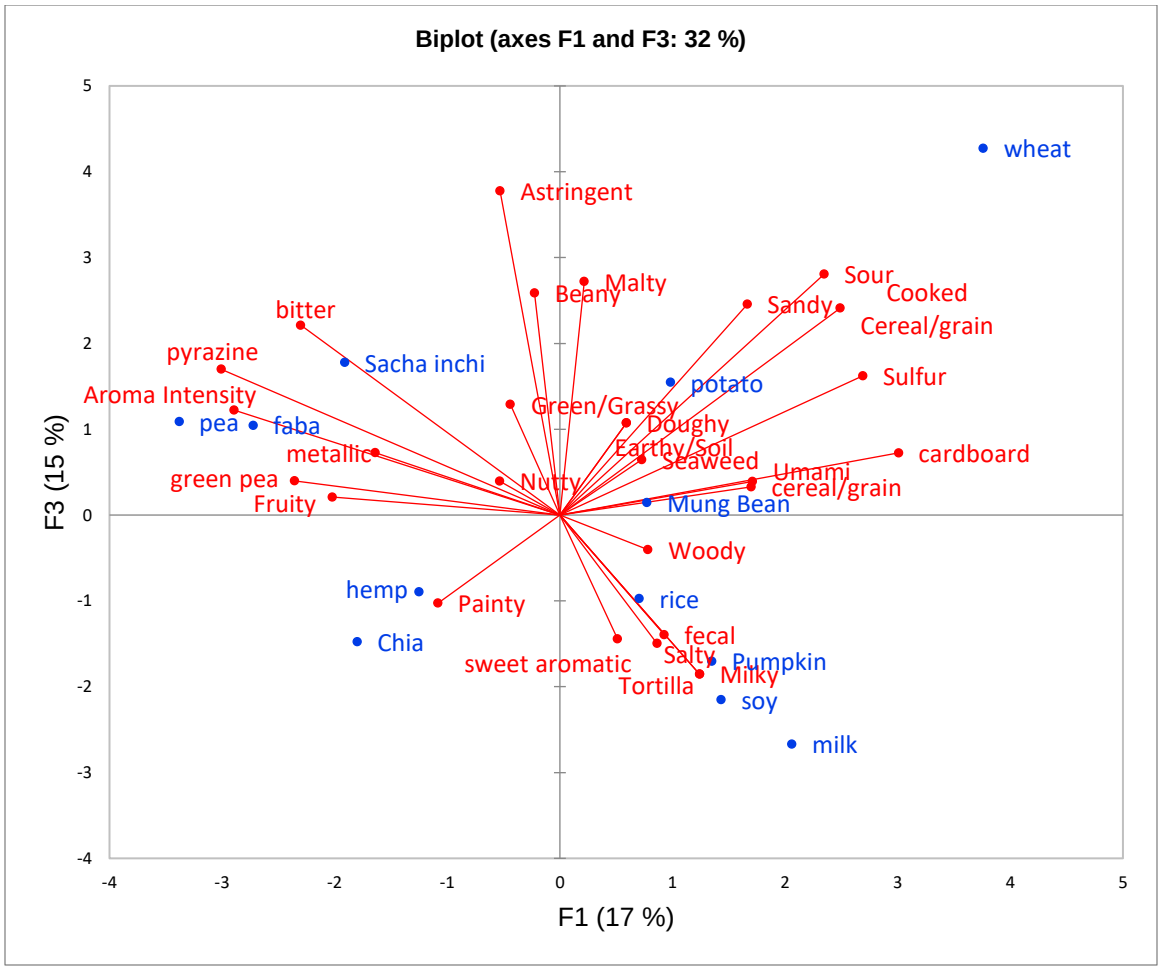

Figure 2.14: Principal component biplot of the selected rehydrated proteins after heat treatment (PC 1 and 3)

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APPENDIX

Appendix 1: Correlations between descriptive sensory attributes of all plant proteins (n=66)

	Aroma Intensity	Swt arm	cerea/ grain	Cdb	Malty	fecal	green pea	pyrazine	Sulfur	Green/ Grassy	Doughy	Woody	Nutty	Beany	Seaweed
Aroma Intensity	1.00	0.07	-0.30	-0.23	0.06	0.01	0.32	0.35	0.04	0.14	-0.06	-0.01	-0.11	0.17	-0.03
Swt Arm		1.00	-0.05	-0.07	0.12	**-0.56**	-0.06	-0.01	0.09	0.16	0.17	-0.15	-0.14	0.19	-0.12
cereal/ grain			1.00	-0.09	-0.14	-0.10	-0.27	-0.18	0.07	-0.15	0.16	-0.20	0.12	-0.08	-0.16
Cdb				1.00	-0.05	0.14	-0.08	-0.38	-0.08	-0.35	-0.01	-0.05	0.11	-0.21	-0.20
Malty					1.00	-0.14	-0.06	0.15	0.04	0.06	-0.14	-0.07	0.14	0.01	-0.06
fecal						1.00	-0.10	-0.11	0.04	-0.23	-0.05	0.12	0.00	0.10	-0.09
Green pea							1.00	0.38	-0.08	-0.14	-0.04	-0.15	-0.18	0.32	-0.12
pyrazine								1.00	-0.02	0.05	-0.05	-0.07	0.19	0.04	-0.06
Sulfur									1.00	-0.01	-0.10	-0.09	-0.14	0.13	-0.07
Green/ Grassy										1.00	0.01	0.14	-0.12	0.01	0.43
Doughy											1.00	-0.08	-0.06	0.04	0.18
Woody												1.00	0.24	-0.01	0.66
Nutty													1.00	-0.09	-0.08
Beany														1.00	0.05
Seaweed															1.00
Painty															
Cooked Cereal/grain															
Onion															
Tortilla															
Potato															
Earthy/ Soil															
metallic															
Fruity															
Oxd															
Burnt															
Salty															
Sour															
bitter															
Umami															
Ast															
Sandy															

Numbers in bold represent significant correlations ($p<0.05$)

Swt Arm=sweet aromatic, Cdb=Cardboard, Oxd= Oxidized, Ast=Astringent

Appendix 1 (continued)

	Painty	Cked crl	Onion	Tortilla	Potato	Earthy/ Soil	metallic	Fruity	Oxd	Burnt	Salty	Sour	bitter	Umami	Ast	Sandy
Aroma Intensity	0.23	-0.09	-0.01	-0.01	-0.01	-0.13	0.02	0.05	-0.19	0.04	0.005	-0.11	0.09	-0.01	0.05	-0.01
Swt arm	-0.05	-0.08	-0.09	-0.09	-0.09	0.12	0.07	0.04	-0.03	0.19	0.11	0.08	-0.03	0.04	-0.11	-0.02
cereal/ grain	0.21	0.22	0.03	-0.12	0.06	-0.12	-0.07	-0.09	-0.08	-0.02	-0.02	0.14	-0.23	0.10	-0.02	-0.13
cardboard	0.25	0.25	-0.06	0.15	0.05	0.01	-0.33	-0.31	0.23	-0.17	-0.06	0.16	0.02	0.14	0.18	0.11
Malty	-0.13	**0.66**	-0.04	-0.04	0.34	-0.04	-0.06	-0.07	-0.07	-0.06	-0.16	**0.66**	0.08	-0.23	0.31	-0.01
fecal	-0.10	-0.24	-0.07	0.02	-0.06	-0.06	-0.09	-0.11	-0.02	0.10	0.10	-0.17	0.11	0.08	-0.07	-0.17
Green pea	-0.27	-0.32	-0.09	-0.09	-0.09	-0.09	-0.12	-0.15	-0.14	-0.05	0.43	-0.22	0.17	0.35	-0.12	-0.16
pyrazine	-0.13	-0.15	-0.04	-0.04	-0.04	-0.04	0.41	**0.60**	-0.07	0.17	-0.01	-0.11	0.30	-0.13	0.05	-0.07
Sulfur	-0.16	0.04	0.19	-0.05	-0.05	-0.05	0.15	-0.09	-0.09	0.29	0.05	0.14	-0.04	0.10	0.03	-0.01
Green/ Grassy	-0.08	-0.10	-0.05	-0.05	0.17	0.16	**0.56**	**0.63**	-0.09	0.10	-0.32	0.20	0.00	-0.30	-0.08	0.25
Doughy	0.11	-0.18	-0.05	-0.05	-0.05	-0.05	-0.07	0.07	-0.08	-0.07	0.06	-0.13	0.10	0.33	-0.06	0.09
Woody	-0.08	-0.10	-0.03	0.37	-0.03	-0.03	-0.04	-0.04	-0.04	-0.04	-0.16	-0.07	-0.17	0.12	-0.11	-0.07
Nutty	0.10	0.10	-0.06	0.35	-0.05	-0.05	-0.08	0.11	-0.09	-0.08	-0.18	-0.05	-0.23	0.08	-0.21	-0.03
Beany	-0.36	-0.35	-0.12	-0.12	-0.12	0.06	0.03	-0.20	-0.20	0.29	0.30	-0.13	0.01	0.33	-0.14	-0.10
Seaweed	-0.06	-0.08	-0.02	-0.02	-0.02	-0.02	-0.03	-0.04	-0.04	-0.03	-0.12	-0.05	0.02	-0.15	-0.09	0.23
Painty	1.00	0.42	-0.05	-0.05	-0.05	-0.05	-0.07	-0.01	-0.08	-0.07	-0.28	0.03	0.09	-0.28	-0.08	-0.12
Cked crl		1.00	-0.06	-0.06	-0.06	-0.06	-0.08	-0.09	-0.09	-0.08	-0.21	**0.56**	-0.07	-0.31	0.12	-0.07
Onion			1.00	-0.02	-0.02	-0.02	-0.02	-0.03	-0.03	-0.02	-0.09	-0.04	-0.02	-0.11	0.06	0.25
Tortilla				1.00	-0.02	-0.02	-0.02	-0.03	-0.03	-0.02	-0.09	-0.04	-0.15	0.21	-0.06	-0.04
Potato					1.00	-0.02	-0.02	-0.03	-0.03	-0.02	-0.09	0.42	0.07	-0.11	0.41	-0.04
Earthy /Soil						1.00	-0.02	-0.03	-0.03	-0.02	-0.09	-0.04	-0.15	-0.11	0.08	-0.04
metallic							1.00	**0.63**	-0.04	0.39	-0.13	-0.05	0.32	-0.16	0.05	-0.06
Fruity								1.00	-0.04	-0.04	-0.15	-0.07	0.12	-0.12	-0.09	-0.07
Oxd									1.00	-0.04	-0.15	-0.07	-0.13	-0.13	0.28	0.50
Burnt										1.00	-0.03	-0.06	0.18	-0.07	-0.13	-0.06
Salty											1.00	-0.23	0.10	0.31	-0.24	-0.22
Sour												1.00	0.00	-0.26	**0.59**	0.08
bitter													1.00	-0.06	0.00	-0.20
Umami														1.00	-0.17	-0.19
Ast															1.00	0.28
Sandy																1.00

Numbers in bold represent significant correlations ($p<0.05$)

Swt Arm=sweet aromatic, Cdb=Cardboard, Oxd= Oxidized, Ast=Astringent, Cked crl = cooked cereal

Appendix 2: Correlations between descriptive sensory attributes of all pea proteins (n=26)

	Aroma Intensity	Swt Arm	cereal / grain	Cbd	Malty	fecal	green pea	pyrazine	Sulfur	Doughy	Nutty	Beany	Burnt	Salty	Umami	bitter	Ast
Aroma Intensity	1.00	0.14	-0.14	-0.23	-0.02	0.06	0.25	**0.54**	-0.05	-0.26	-0.03	0.10	-0.01	0.06	-0.23	-0.05	0.27
Swt Arm		1.00	-0.20	0.28	0.37	-0.23	-0.06	0.01	-0.19	0.13	0.17	0.21	-0.13	0.37	0.15	0.08	-0.04
cereal/ grain			1.00	-0.31	-0.17	-0.15	-0.21	-0.29	0.11	0.37	-0.17	-0.01	-0.18	0.05	0.49	-0.41	-0.09
cbd				1.00	0.04	0.32	-0.38	-0.28	0.10	-0.09	0.32	0.07	0.10	0.12	-0.12	0.25	0.04
Malty					1.00	-0.14	0.01	-0.06	-0.09	-0.10	-0.04	-0.02	-0.04	0.00	-0.07	0.08	0.08
fecal						1.00	-0.46	-0.23	0.18	0.00	0.14	0.15	0.36	-0.10	-0.24	-0.05	0.07
green pea							1.00	**0.71**	-0.27	-0.19	-0.20	-0.45	-0.12	0.22	-0.22	0.03	0.00
pyrazine								1.00	-0.16	-0.17	-0.06	-0.20	-0.07	0.10	-0.37	0.28	0.32
Sulfur									1.00	-0.11	0.34	-0.12	-0.10	-0.13	0.16	-0.20	-0.08
Doughy										1.00	-0.10	0.11	-0.10	0.09	**0.62**	-0.02	0.11
Nutty											1.00	0.04	-0.04	0.24	0.03	0.02	-0.04
Beany												1.00	0.11	-0.17	0.26	-0.28	0.06
Burnt													1.00	-0.05	-0.11	0.19	-0.17
Salty														1.00	-0.07	0.02	-0.13
Umami															1.00	-0.12	-0.08
bitter																1.00	0.16
Ast																	1.00

Numbers in bold represent significant correlations ($p<0.05$)
Swt Arm=sweet aromatic, Cdb=Cardboard, Ast=Astringent

Appendix 3: Correlations between descriptive sensory attributes of all soy proteins (n=7)

	Arm Int	Swt arm	cereal / grain	cbd	Malty	fecal	pyrazine	Sulfur	Doughy	Nutty	Beany	Fruity	Oxd	Salty	Umami	bitter	Ast
Arm Int	1.00	0.46	0.62	**-0.67**	-0.0004	-0.36	-0.0004	0.54	0.42	-0.30	0.42	-0.0004	-0.53	-0.0006	0.13	-0.60	0.52
Swt arm		1.00	**0.70**	-0.45	**0.71**	**-0.72**	-0.16	0.40	**0.71**	-0.60	0.33	-0.16	-0.16	0.03	0.16	-0.58	0.29
cereal/ grain			1.00	-0.62	-0.37	-0.44	-0.10	0.43	0.09	-0.52	0.38	-0.13	-0.19	0.16	-0.08	-0.63	0.21
cbd				1.00	-0.14	0.26	-0.11	-0.28	-0.10	0.17	-0.56	-0.11	0.56	-0.62	0.44	0.63	0.19
Malty					1.00	**0.75**	-0.16	-0.16	-0.25	0.41	0.13	-0.16	-0.16	0.47	-0.59	0.57	-0.40
fecal						1.00	-0.25	-0.25	-0.39	0.13	-0.11	-0.25	0.53	0.24	-0.53	0.48	-0.36
pyrazine							1.00	-0.16	0.64	0.61	-0.32	1.00	-0.16	-0.25	0.24	-0.13	-0.02
Sulfur								1.00	-0.26	-0.34	-0.32	-0.16	-0.16	-0.25	0.24	-0.46	0.60
Doughy									1.00	0.20	0.21	0.64	-0.25	-0.39	0.48	-0.25	0.36
Nutty										1.00	-0.35	0.60	-0.34	-0.03	-0.01	0.34	-0.31
Beany											1.00	-0.31	-0.32	0.60	-0.38	-0.28	-0.29
Fruity												1.00	-0.16	-0.25	0.23	-0.13	-0.02
Oxd													1.00	-0.25	-0.04	0.58	-0.02
Salty														1.00	-0.60	-0.04	-0.59
Umami															1.00	-0.25	0.47
Bitter																1.00	-0.29
Ast																	1.00

Numbers in bold represent significant correlations ($p<0.05$)

Arm Int- Aroma Intensity, Swt Arm=sweet aromatic, Cdb=Cardboard, Oxd=Oxidized, Ast=Astringent