ABSTRACT

BEST, ANITA RAE. Sources of Soapy Off-flavor in Clear Acidic Whey Protein Beverages (Under the direction of Dr. MaryAnne Drake).

Clear acidic protein beverages (CAPB) are made with whey protein isolate (WPI) due to its solubility at low pH (pH 3.3). These beverages can have a soapy off-flavor, yet soapy flavor is not detected in neutral pH WPI solutions. Our hypothesis was that medium chain fatty acids associated with the WPI are the source of this off flavor under acidic conditions. Fourteen commercial WPI powders were sourced from 4 different manufacturers in duplicate lots (10 neutral pH, 4 pre-acidified). WPIs were rehydrated to 10% solids (w/v); pH at 21°C was measured and the rehydrated WPI were evaluated by descriptive analysis (DA) with a trained panel. Subsequently, 6 WPIs (3 neutral, 3 pre-acidified) were selected for further analyses. Solvent assisted flavor evaporation (SAFE) with GCMS and gas chromatography olfactometry (GCO) were performed on selected WPI followed by quantitation of octanoic, decanoic and dodecanoic acids using stable isotope dilution assays (SIDA) with deuterated standards. Retronasal and orthonasal sensory thresholds of the three acids were determined in buffered solutions at pH 6.3 and pH 3.3. Model systems of WPI at pH 6.3 and pH 3.3 with the average concentrations of the three acids were generated to confirm soapy flavor. As expected, WPI at pH 6.3 exhibited no soapy flavor, whereas WPI at pH 3.3 had soapy flavor. The average concentrations of acids in selected WPI were: C8 = 14.39 ± 2.0 ppm; C10 = 27.78 ± 2.39 ppm; C12 = 24.71 ± 2.20 ppm. Sensory thresholds for all three acids at pH 3.3 were lower than at pH 6.3 ($p<0.05$). Trained panel profiling of model systems demonstrated that a combination of octanoic and decanoic acids at pH 3.3 caused a soapy flavor and this flavor was not detected in the same model beverage at pH 6.3. Identification of the sources of soapy off flavor in CAPB

provide the baseline to reduce or eliminate this off flavor. To eliminate or reduce soapy flavor in CAPB, residual free fatty acid content of WPI must be minimized.

Sources of Soapy Off-flavor in Clear Acidic Whey Protein Beverages

by
Anita Rae Best

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Food Science

Raleigh, North Carolina
2023

APPROVED BY:

_______________________________ _______________________________
Dr. MaryAnne Drake Dr. April Fogleman
Committee Chair

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Dr. Dana Hanson

DEDICATION

First and foremost, to my parents who have done nothing but support and love me through this journey. It may have taken longer than we all expected, but I still made it through. To my sister for giving me the wisdom and motivation to persevere as well as keeping me in line. To Will, for always supporting and believing in me. To Dr. Drake for pushing me to do my best and to believe in myself. I would not have made it this far without her guidance. Lastly, to the MAD lab, for we all know the obstacles that we must overcome to get to this point.

BIOGRAPHY

Anita Best was born on July 2, 1997 in Berea, KY. While her family moved around a bit in her early childhood, Anita spent most of her youth in Cookeville, TN. Her interest in agriculture was sparked at a young age, as her father and Opa spent each summer growing heirloom beans and tomatoes. Anita graduated high school in 2015 and headed off to the University of Tennessee, Knoxville to pursue a degree in Food Science. While Anita knew that she wanted to expand her education beyond a Bachelor's Degree, it was not until her senior year that she got to work in a food chemistry and sensory lab that sparked her interest in pursuing a graduate degree in Food Science. Upon graduating in May 2019, she moved to Raleigh, NC where she pursued a Master's degree in Food Science under the direction of Dr. MaryAnne Drake. She managed to convince her whole family to move there as well, with her father getting a job at NCSU and her sister attending nursing school at UNC - Chapel Hill. Her mother and childhood pets came along for the journey as well. Once completing her Master's she will continue to pursue her PhD at North Carolina State University. While not at work, Anita enjoys cooking, gardening, and spending time with her family and pets.

ACKNOWLEDGMENTS

First, I would like to thank my committee members, Dr. Drake, Dr. Hanson and Dr. Fogleman for their guidance and help in obtaining this degree. I would not have survived this program if it were not for Dr. Drake's continued support and encouragement. There were a few times where I was not sure if I would be able to finish this project, but I am thankful that I had Dr. Drake to always be there to push me along in the right direction. I would also like to thank every lab member who has been around during my time in the Drake lab. Thank you to Dylan and Tess and everyone else in analytical for helping my overcome every obstacle that stood in my way while working towards this degree. This lab has allowed me to learn so much more beyond the scope of my project and I am very fortunate that I have had this opportunity to learn and grow.

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INTRODUCTION

The global protein market was valued at $38.5 billion in 2020 and is expected to grow by 10.5% by 2028 (Grand View Research, 2021). Part of the drive behind the increase in demand is in part due to an increase in health-conscious consumers that want products that aid in functions like satiety, muscle repair, and energy balance (Grand View Research, 2021). In the past, whey protein was seen as a waste product in the industry (Pesta et al., 2007); however, as of 2016, the United States is now the world's largest exporter of whey products, producing 212 thousand metric tons of whey protein concentrate and 50 thousand metric tons of whey protein isolate (US Dairy Council Exports, 2018). Whey protein is now used as an ingredient across the food industry. Its uses range from an ingredient in dairy foods, processed meats, seasonings, infant formula, protein beverages and protein bars (Anandharamakrishnan et al., 2007; US Dairy Council Exports, 2018). Whey proteins are also used in the pharmaceutical industry as an encapsulating agent (Anandharamakrishnan et al., 2007). Whey protein that is not used for human consumption can be used as animal feed or spread as a fertilizer. More specifically, it has been used as supplemental feeding for pigs in order to wean piglets and has been proven to increase performance of pigs when used as 10-30% of their starter diet (Meat and Livestock Commission, 2003; Swine, 2019)

Whey proteins are versatile products that not only have numerous functional qualities that aid in the production of products, but also have a wide range of nutritional properties (Morr et al., 1993). Whey proteins, like all animal proteins, contain all the essential amino acids, which makes it a complete protein, and compared to other protein sources have a higher concentration of branched chain amino acids (leucine, isoleucine and valine) (Anand et al., 2013; Hoffman and

Falvo, 2005). The human body is incapable of synthesizing the essential amino acids, which is why it is essential to eat foods that contain them (Hoffman and Flavo, 2005).

There is more than one way to measure the quality of the protein in a food. Not all proteins have the same quality or function in the human diet. The Biological Value (BV) measures protein quality through the calculation of nitrogen utilized to form tissue divided by the nitrogen that is absorbed from the food (Hoffman and Falvo, 2004). According to the USDA, whey protein has a biological value (BV) that exceeds that of egg protein by 15 percent and other high protein foods, like meat, soy and casein (USDA, 2015). An egg has a BV of 0.9-1.00 (USDA, 2015). Animal protein sources normally have a higher BV than plant sources. One of the faults of BV is that it does not take into account the interaction of the protein with protein and other foods during digestion and how that effects absorption (Hoffman and Falvo, 2004). An additional way to measure protein quality is through the Protein Efficiency Ratio (PER). PER determines how effective a protein source is by measuring the growth of an animal (Hoffman and Falvo, 2004). To use this method, rats are fed the test protein source and are measured in grams as to how much weight they gain. The weight gain is compared to the standard value of 2.7, the value of casein protein. If the value exceeds 2.7 the protein source is considered an excellent protein source. The biggest drawback of PER is that it does not necessarily correlate to human growth needs (Hoffman and Falvo, 2004). Additionally, Protein Digestibility Corrected Amino Acid Score (PDCAAS) can be used (Hoffman and Falvo, 2004). PDCAAS compares the concentration of the first limiting essential amino acid to a test protein to the concentration of amino acids in a reference (Schaafsma, 2000). However, PDCAAS, like the other methods, also has its limitations, which includes not being a good estimate for the elderly as well as antinutritional factors (Hoffman and Falvo, 2004). A newer way to measure the quality of protein sources is through the Digestible Indispensable Amino Acid Score (DIAAS) (Craddock et al., 2021). This method was introduced by the Food Agriculture Organization (FAO) of the United Nations to replace PDCAAS (Craddock et al., 2021). The DIAAS method takes the ratio of digestible amino acid content (mg/g protein) in food and compares it to a reference pattern of the same amino acid looking specifically at the age-specific requirements for amino acid consumption (Agropur, 2022). This value is then converted to a percent. A score of less than 75% means that there is no protein claim. A score of 75-99% means that it has good protein quality, and a score of 100% or more means it is high quality protein (Agropur, 2022). Whey protein concentrate has a DIAAS value of 1.10 and whey protein isolate has a value of around 1.40, which is higher than soy or pea protein (Agropur, 2022). In addition to being a good protein source, whey protein offers additional health benefits that can help with the following health goals: maintaining a healthy weight, reducing appetite, aiding in muscle repair, and building muscles (US Dairy Exports, 2018).

Due to the many benefits that come from consumption of whey protein, it is used in a variety of high protein products. According to the Code of Federal Regulations, a product is "high" in protein if it contains at least 20% or more of the recommended dietary intake (RDI) (FDA, 2022a). The current RDA (Recommended Dietary Allowance) for protein is 0.8 g protein per kg of body weight (Wu, 2016). Not only does the addition of whey protein as an ingredient provide health benefits to consumers, but it also has many functional characteristics that assist in creating a product that is liked by consumers. Protein beverages are a growing market with the US functional beverage market, projected to have a compound annual growth rate of 6.6% between 2020 and 2025 (Glanbia Nutritionals, 2022). Sports drinks, including ready- to-drink (RTD) or meal replacements, make up a combined $16.2 billion of the $48.4 billion functional beverage market (Glanbia Nutritionals, 2022). While there are a variety of protein types that can be used for neutral pH protein beverages, which have a pH range of 6.8-7.0, acidic protein beverages, which have a pH range of less than 3.5, need an acid stable protein (Carter et al., 2020; Mulvihill and Ennis, 2003). Whey proteins are acid stable, which makes them unique for clear, acidic protein beverages (Carter et al., 2020).

However, with new product development there are always obstacles to overcome. It is important to make a product that meets consumer needs so that it will do well on the market. Whey protein is an ideal ingredient to be added to a product for its functional properties, like increased protein (Carter and Drake, 2018). However, certain processes that whey protein undergoes can amplify or even create off flavors and these off flavors are present in the end product application, creating an undesirable product for consumers.

WHEY PROTEIN AND ITS MANUFACTURE

Milk contains casein and whey, which are the two primary proteins in dairy products (Walzem et al., 2010). According to the Code of Federal Regulations, whey "is the liquid substances obtained by separating the coagulum from milk, cream, or skim milk in cheesemaking" (FDA, 2022b). Whey contains around 25% of the protein found in milk (Suarez et al., 1992; Zadow, 2003). Whey can either be obtained during cheesemaking, once the whey has been drained of the curd, or by acid or rennet precipitation of casein (Suarez et al., 1992; Zadow, 2003) or by microfiltration of fluid skim milk to separate casein and serum (whey) protein (Carter and Drake, 2018). Fluid whey is approximately 6% solids, most of which is lactose and minerals. The protein content of fluid whey is less than 1% so manufacture processes to concentrate protein from fluid whey are critical.

Whey Classifications

Fluid whey can be classified into three main categories: sweet, medium acid, and acid whey. Sweet whey typically has a pH between 5.8-6.6 (Zadow, 2003). It is obtained from Cheddar and mozzarella cheese manufacture (Nelson and Barbano, 2005). Sweet whey can be obtained from the manufacture of harder cheeses, like mozzarella and Cheddar cheese due to the higher pH needed for enzymatic clotting (rennet is used to coagulate milk in these cheeses) to occur (USDA, 2015). Medium acid whey typically has a pH of 5.0-5.8, and can be made from fresh acid cheese, like cottage cheese and ricotta (Zadow et al., 2003). Acid whey is created from fermentation or by direct acid addition during the manufacture of cottage cheese (USDA, 2015) and has a much lower pH of 4.5-4.8 (USDA, 2015; Zadow, 2003). The composition of sweet and acid whey can be found in Table 1.

Whey Composition

Whey is around 6.5% total solids with 75% of that being lactose (Zadow, 2003). Both sweet and acid whey contain significant, yet variable concentrations of the following minerals: Ca+2, Mg+2, Citrate (Cit-3) and phosphate (PO4,-3) (Morr and Ha, 1993). However, acid whey contains higher concentrations of these minerals than sweet whey (Morr and Ha, 1993). Whey protein is comprised of the following: B-lactoglobulin, a-lactalbumin, bovine serum albumin, immunoglobulin, lactoferrin, lactoperoxidase and glycomacropeptide (GMP) (Jeewanthi et al., 2015; Patel, 2015). beta-Lactoglobulin (beta-Lg) and alpha-lactoglobulin make up 80% of the weight of whey protein (Swaisgood, 2017). The proteins found in fluid whey are the most valuable components, which is why most of the processing operations done to fluid whey are focused on concentrating the protein stream (Zadow, 2003). beta-Lg is globular and makes up more than half of the weight of whey protein and 12% of the total protein. beta-Lg is the primary whey protein in buffalo, sheep and goat milk in addition to cow's milk (O'Mahony and Fox, 2013). Beta-Lg protein is the cause of allergic reactions, like milk intolerance (Singh et al., 2022). beta-Lg is not present in human milk (Swaisgood, 2017). The structure of beta-lactoglobulin can be affected based on the pH of the system that it is in; at a pH of 3 and greater, it exists as a monomer (Dziuba et al., 2021). At a pH of 5.2-3.5 it exists as an octamer (Dziuba et al., 2021).

alpha-lactalbumin (alpha LA), which is found in both human and bovine milk, is a source of essential and branched chain amino acids (Sharma, 2019). alpha-LA has three genetic variants: A, B and C (Juarez and Ramos, 2003). Its native form is stabilized via 4 disulfide bonds at a pH between 5.4 and 9.0 (Permyakov and Berliner, 2000). As stated previously, whey protein is a good source for essential amino acids, branched-chain amino acids and peptides (Jeewanthi et al., 2015). Leucine, which is a branched-chain amino acid, is plentiful in whey protein and aids in regulating the synthesis of skeletal muscle proteins (Jeewanthi et al., 2015). Bovine serum albumin has a large molecular weight and is a source of essential amino acids (Sharma, 2019). Its structure allows it to bind free fatty acids along with other lipids (Sharma, 2019). Immunoglobulins are antibodies; there are five different classes of them, which are IgA, IgD, IgE, IgG and IgM (Sharma et al., 2019). 10-15 percent of the total whey protein is composed of immunoglobulins (Gupta et al., 2016). Lactoferrin, which is an iron-binding glycoprotein, is made up of 689 amino acid residues and functions as a non-enzymatic antioxidant in whey protein (Gupta et al., 2016). It is present in high concentrations in breast milk and colostrum (Superti, 2020). Lactoperoxidase can be found in the milk of most animals as well as body fluids like tears and saliva (IDF, 2013). The lactoperoxidase system is a chemical reaction that extends the shelf life of milk by inactivating the metabolic enzymes of bacteria (IDF, 2013) and has also been harnessed to bleach Cheddar whey during whey protein processing.

The lactoperoxidase system is useful for raw milk that cannot be chilled, which can be the case for smaller dairy farms that are located in more rural areas, as it inhibits the growth of bacteria (IDF, 2013; Gupta et al., 2016). Lactoperoxidase is reasonably heat stable and remains active in pasteurized fluid whey. Previous research has also demonstrated that the lactoperoxidase system can be utilized to enzymatically bleach colored (Cheddar) whey during whey protein processing (Carter and Drake, 2018; Campbell and Drake, 2014; Campbell et al., 2012). Campbell et al. (2012) demonstrated that enzymatic bleaching occurs in a very short amount of time (<15 min), with lactoperoxidase activity declining sharply after 30 minutes of bleaching fluid whey. Lactoperoxidase can be an effective bleaching enzyme (Campbell et al., 2012). For optimal results, lactoperoxidase in the presence of 20 mg of H_2O_2/kg added to fluid whey gave the best results when compared to the traditional chemical bleaching method of using only 250 mg of H_2O_2/kg. Campbell et al. (2014) investigated bleaching methods further and determined that exogenous peroxidase can be added as a bleaching aid when lactoperoxidase is not completely active. Campbell et al. (2014) found that exogenous peroxidase is not affected by storage temperature (4 to 60°C), and as the pH decreased and temperature increased of fluid whey, the rate of reaction of exogenous peroxidase increased. For whey protein retentate, a higher pH (6.5 instead of 5.5) was needed for bleaching with the lactoperoxidase system (Campbell et al., 2014). Bleaching with the lactoperoxidase system did not decrease the off flavors in whey protein. Campbell et al. (2012) found that lactoperoxidase bleached WPC80 had increased lipid oxidation compounds compared to unbleached WPC80.

Whey Processing

The typical processing steps of whey are clarification, separation, pasteurization, concentration of solids, fractionation, and spray drying (O'Mahony and Fox, 2013). Figure 1 shows the processes needed to fraction whey into whey-based ingredients. Once whey has been fat separated and pasteurized, protein and other components can be separated via membrane separation, which include nanofiltration, microfiltration, and ultrafiltration (O'Mahony and Fox, 2013).

Membrane filtration, which arrived in the 1970s, allowed whey proteins to be separated and fractionated while still having the ability to be soluble (Onwulata et al., 2008). In order to concentrate whey protein, ultrafiltration (UF) is the most common membrane step used (Suarez et al. 1992). UF is the most common process in the dairy industry; it is used to concentrate large particles and macromolecules (Swaisgood, 2017). UF is designed to retain particles between 1-50 nm in size (Cheryan, 2000). Through the use of UF, the end product results in a product that is higher in protein than the original and lower in lactose. The UF pore size is specific to molecular weight, such that salts (minerals) and lactose pass through the membrane (permeate), while the protein stays due to its higher molecular weight (retentate) (Zadow, 2003). While UF is not the only filtration method used by the dairy industry, it is the most common process for manufacture of whey protein concentrates (WPC) from sweet whey (Nelson and Barbano, 2005).

Another common filtration process used in the dairy industry is microfiltration (MF). MF is used to remove particles such as bacteria, yeast cells and fat globules (Bylund, 2003; France et al., 2021). MF pore size ranges from 0.1 to 10 uM; smaller molecules such as water, minerals, lactose and whey protein are able to permeate through the membrane while fat and casein are retained (Huisman, 2000; France et al., 2021). MF is used in the dairy industry in order to reduce the bacteria in skim milk, whey and brine (Bylund, 2003), and is more commonly used for defatting whey that is to be used for whey protein isolate (WPI) (Bylund, 2003).

Whey can be used in a liquid form or further processed into a dried powder form. Spray drying has been used for over 170 years but didn't really take off until the early 1900s and is still the most frequently used method today; drum drying is also an available option (Henning et al., 2006). During the spray drying process, air is filtered to remove unwanted particles and heated to the desired temperature (Henning et al., 2006). This is followed by the liquid concentrate being sprayed out as small droplets by a pressure nozzle or wheel in the drying chamber (Henning et al., 2006). This process results in the evaporation of liquid and leaves only dried particles that are separated via an airstream (Anandharamakrishnan et al., 2007). Whey powder can be manufactured into many different forms from dried whey to WPC to WPI, which all have a wide range of protein concentrations (Lucey, 2009).

Liquid whey is only 0.6% (w/v) protein. When liquid whey is processed by filtration, the protein content can be increased to create WPC, which is 25-80% protein, or whey protein isolate (WPI), which is more than 90% protein (Foegeding et al., 2002). WPC and WPI are typically made from sweet whey rather than acid whey (Jelen, 2010). Sweet whey comes from the manufacture of cheese and casein; acid whey comes from destabilizing milk casein by direct addition of acid to a pH less than 5.0 (Jelen, 2010). Acid whey is not ideal for WPC and WPI production due to its low pH, high lactic acid concentration, and high calcium phosphate content, along with the fact that the high amount of lactic acid makes it more difficult to dry acid whey into a powder (Dec and Chojnowski, 2006). Whey protein powders with a lower protein level can be used in animal agriculture as feed (Pineda-Quiroga et al., 2017). Pineda-Quiroga and others (2017) found that feeding broilers WPC80 improved the broiler growth and feed efficiency as well as their digestibility of calcium and phosphate. WPC and WPI also have various uses in food and healthcare industries (Khaire and Gogate, 2019). While WPC and WPI started out as a supplement for body builders, they are now utilized in everyday protein supplements like beverages and bars (Khaire and Gogate, 2019). Whey proteins provide immunity support, boost metabolism and increase muscle mass (Khaire and Gogate, 2019). Whey proteins are also added to infant formula in order to improve gastrointestinal immunity. The presence of lactoferrin in whey protein increased the levels of *Bifidobacteria* in bottle-fed babies (Khaire and Gogate, 2019; Solak and Akin, 2012).

WHEY PROTEIN FUNCTIONALITY

The functional properties of proteins are classified based on the physicochemical properties that relate and contribute to desired characteristics of food products, which include the appearance, texture, viscosity, mouthfeel, or flavor retention (Morr and Ha, 1993). A protein's functional properties are dependent on protein primary, secondary, and tertiary structure (Swaisgood, 2017). The key functional properties can be classified into two groups: hydration and surface related properties (Morr and Ha, 1993). Whey protein functional properties can largely be influenced by external factors, like the concentration of protein, pH, ionic environment, state of whey protein, heat treatments and presence of lipids (Walzem et al., 2002). The ability of proteins to aid in these functions is called protein functionality (Foegeding et al., 2002). The ability for a protein to be able to act in ways that aid the product largely depends on how the whey protein is processed as well as what other ingredients it is combined with (Foegeding et al., 2002).

Solubility

WPI and WPC are soluble across a pH wide range, thus making them ideal to be used in sports drinks as well as liquid meal replacements (Walzem et al., 2002). Making powders that can easily dissolve in cold water can be done through instantization (Kelly et al., 2003). Instantization is achieved through agglomeration which is the process of making larger clumps of powders with greater density (Kelly et al., 2003). The process of agglomeration can either be done via a fluid bed or on a drying chamber with or without the addition of an instantizing agent (Bylund, 2003). The use of the instantizing agent increases wettability by decreasing surface tension. Agglomeration with steam involves air drying the particles and then humidifying them. This makes the surface of the particles sticky, which causes them to agglomerate into larger particles. Using a fluid bed for this process makes it so that the powder is pushed along from the spray tower to the humidifier. Air flow along with vibrations push the powder along the bed to different drying sections of the conveyor. Agglomeration takes place at the first step of drying and then is exposed to different temperatures along the bed that dries the powder back out to the ideal residual moisture level. Agglomeration via a drying chamber is a much simpler method as the instantization occurs immediately after the drying chamber (Bylund, 2003). Instantized products are agglomerated with lecithin; soy or sunflower lecithin are the most common lecithin sources used in whey proteins (Wright et al., 2009). Leicithins aid in the dispersibility and solubility of whey protein by decreasing surface tension and increasing wettability (Wright et al., 2009). However, the use of instantizers can negatively impact shelf life of the whey protein powders. Wright et al. (2009) found that instantized and agglomerated products had higher lipid oxidation related flavors and volatile compounds compared to their non-agglomerated counterparts, showing that they have a shorter shelf life.

Gelation

The structures formed in gelation can aid in the appearance, texture, water-holding and flavor of a gel (Foegeding et al., 1998). Gels are intermediate structures between solids and liquids that are formed by the cross-linking of protein strands (Foegeding et al., 1998). There are two main ways that gels can be created in whey proteins, and they are through heating and enzymatic treatments (Spotti et al., 2017). Age thickening can be a problem with unstable proteins (Deeth and Hartanto, 2009). The ability for whey protein gelation to occur largely depends on the characteristics of gel solution, like the pH, ionic strength, and protein concentration (Rodrigues et al., 2020). Due to the isoelectric point of casein being approximately 4.6, unheated milk can start to gel at a pH of 4.9 (Anema, 2009). The isoelectric point of whey proteins is at around pH 5.3, so gelation can be observed at different pHs due to the proteins present in the milk (Anema, 2009).

The interaction between protein molecules during the process of gelation are important in determining the properties of the end product (Rodrigues et al., 2020). Pre-heating milk before concentration can denature the whey proteins and minimize age thickening and protein instability (Deeth and Hartanto, 2009). Additionally, stabilizers or buffering salts can be added to the protein, like calcium chloride, before the product is sterilized to decrease thickening and gelation (Deeth and Hartanto, 2009). Proteolysis of K-casein can cause gelation in dairy products (Nieuwenhuijse and van Boekel, 2003). Studies have shown that this is happens when there is a high count of psychotropic bacteria present in the milk product before the milk is sterilized. K-casein breaks down into para-K-casein and the gel that forms from this scenario is similar to rennet curd (Nieuwenhuijse and van Boekel, 2003). Being aware of what factors affect gelling of proteins is important in product development because depending on pH, different proteins may be involved or need to be used.

Foaming

In order for proteins to act as foaming agents they need to be absorbed at an air/water interface and reduce the surface tension by reorganizing (Arntefield and Maskus, 2011). They also must have both hydrophobic and hydrophilic areas on the protein surface (Arntefield and Maskus, 2011). Whey protein and caseins have a high surface activity (Dickinson, 2003). Foaming properties of a protein can be improved by protein unfolding (Firebaugh and Daubert, 2005; Kinsella and Whitehead, 1989). A food product that contains a protein can form a foam by the dispersion of air through the solution. Dairy proteins with higher fat contents have a much lower ability to form a foam (de Witt, 1998). Carter et al. (2018) evaluated the difference of functional properties between spray drying and liquid whey proteins, milk proteins and micellar casein concentrates. They did not find any differences in foaming ability between liquid versus spray dried proteins. The foaming properties of dairy proteins are important because they can be used as functional ingredients in foods, such as baked goods and other confectionary products (de Wit, 1998). Whey proteins can be a substitute for eggs due to their foaming properties.; however, modifications may need to be made in order for whey proteins to perform to the same level of egg proteins (Foegeding et al., 2006). Foegeding et al. (2006) illustrated that in order for whey protein to serve as a substitute for egg protein in angel cake, the whey protein solution must be heated prior to baking or xanthan gum must be added. Defatted WPC60 can produce a meringue of a similar structure to that of one made out of eggs (de Wit, 1998). Foaming properties of dairy proteins can be harnessed to act as ingredient substitutes.

Emulsifications

An emulsion is defined as "colloidal dispersions of two immiscible liquids"; however, food emulsions are much more complex in nature (Morr and Ha, 1993). According to Masson and Jost (1986), most food emulsions are created through a combination of proteins, which are water soluble macromolecules, and oils. Whey proteins can stabilize oil-in-water emulsions, but many factors play into emulsion stability, including type of homogenizer, degree of homogenization, concentration of the protein, oil volume fraction, pH and ionic strength (Masson and Jost, 1986). The ability of whey protein to act as an emulsifier makes it an ideal ingredient to be used in protein drinks, salad dressings, and artificial coffee creamers (Walzmen et al., 2002). Diffusion, molecular unfolding, denaturation, and adsorption and interfacial activity are the chemical and physicochemical properties that give whey proteins the ability to act as emulsifiers (Morr and Ha, 1993).

WHEY PROTEIN FLAVOR

Spray dried whey protein has the potential for numerous off flavors based on a number of factors including milk source, starter cultures, cheese type, light exposure, storage, heat treatment, bleaching, acidification and spray drying (Carter and Drake, 2018; Carter et al., 2018; Park et al., 2014; Jervis et al., 2012; Whitson et al., 2010; Croissant et al., 2009; Carunchia Whetstine et al., 2005). Off flavors present in whey protein can negatively affect consumer liking of the products that whey protein is used in. In order to understand the off flavors present in whey protein, it is first essential to know the typical flavor profile of whey protein. Ideally, whey protein should have no flavor contributions. However, whey proteins have both dairy and non-dairy flavors (Carunchia Whetstine et al., 2005; Drake, 2007). Russell et al. (2006) developed a lexicon for whey and soy proteins; they identified 24 descriptors that assessed the appearance, flavor, and texture/mouthfeel of these proteins. Carunchia Whetstine et al. (2005) performed descriptive analysis, solvent-assisted-flavor-evaporation (SAFE) and aroma active dilution analysis (AEDA) on 7 commercial WPC80 and 8 commercial WPI to characterize sensory and aroma active volatile compounds in whey proteins. WPI and WPC80 had similar flavor profiles, but WPC80 more delicate dairy flavors such as sweet aromatic and cooked, which were not documented in WPI (Carunchia Whetstine et al., 2005). Forty one aroma active compounds were documented in WPC80 and 28 aroma active compounds in WPI (Carunchia Whetstine et al., 2005).

Smith et al. (2016) investigated the flavor profile of WPI from various whey sources. WPI were manufactured from Cheddar cheese, cottage cheese and mozzarella whey and rennet casein. All fluid wheys were concentrated to a total solids of 18% and protein content of 90% (wt/wt) before spray drying (Smith et al., 2016). Both Cheddar cheese and cottage cheese WPI had a higher aldehydes concentration than mozzarella and rennet casein WPI (Smith et al., 2016). These differences between Cheddar and Mozzarella WPI were consistent with previous studies on WPC and liquid whey (Liaw et al., 2010; 2011; Campbell et al., 2011a, 2011b). The rennet WPI had the lowest volatile load among all WPI in the study; rennet whey does not include the addition of a starter culture, which plays a role in whey protein flavor (Campbell et al., 2011a, 2011b). Cottage cheese WPI also had potato brothy and sour notes in it that were not detected in other WPI; Cheddar, mozzarella and rennet WPI all had sweet aromatic flavor, which were absent in cottage cheese WPI. This study shows that whey source can largely impact the flavor profile of WPI due to the different starter cultures used as well as other processing conditions that may affect the flavor profile.

Milk Sources

The fluid milk that the whey protein is ultimately sourced from is a contributor to whey protein flavor. Croissant et al. (2007) documented differences in milk flavors based on whether the cows were fed a conventional total mixed ration (TMR) diet or if they were fed on a pasture-based (PB) system. Milk collected from both farms was processed and standardized to 1.5% fat (Croissant et al., 2007). Sensory analysis of the milks between the two feeding options showed significant differences between feed types, but not between breed (Croissant et al., 2007). Conventional (TMR) milk was described as having a malty flavor, higher sweet aromatic, and sweet taste; it did not have grassy or mothball flavors, which were associated with PB milk (Croissant et al., 2007). Consumer testing revealed that consumers were not able to detect difference between the two milks (Croissant et al., 2007). However, there have been other studies that show that milk sources can impact consumer acceptance. Krause et al. (2007) showed that butter with a grassy flavor, caused by the grass and feeds that the cows consume, had variable liking among different groups of consumers. Additionally, Faulkner et al. (2018), O'Callaghan et al. (2018), Garvey et al. (2020), and Cheng et al. (2020) investigated how different cattle feeding systems affected the chemical composition and consumer acceptance of products made from cow's milk such as butter, skim milk powder and pasteurized milk. Collectively, these studies showed that feed type had a direct impact on the flavor profile and chemical composition of dairy products. Feed type can change the nutritional value, viscosity, aroma profile, and chemical composition of milk. These changes can affect consumer acceptance of the end products.

Harwood and Drake (2020) investigated how consumers perceive milk that was produced using conventional, organic, pasture-raised or locally farmed methods to determine if consumer beliefs of these different methods align with their preferences. Prior to consumer testing, it was determined that consumers have negative beliefs about conventional milk and that there was a positive bias for organic, local and pasture-raised milks (Harwood and Drake, 2020). The preference testing that Hardwood and Drake (2020) performed showed that both groups of consumers (blinded vs. primed) had sensory preferences regarding fluid milk. In blinded evaluations, organic milk was preferred over conventional milk and locally farmed was preferred over pasture-raised (Harwood and Drake, 2020). Within the primed group of consumers there were not any significant differences among any milk pairings, which shows that priming of the samples caused consumers to make independent assumptions about the milks unrelated to their flavor (Harwood and Drake, 2020). These two sets of consumers illustrate that flavor plays a large role in consumer preference and that conventional milk is still preferred over other types of milk (Harwood and Drake, 2020). Additionally, descriptive analysis of the milks showed that there were meaningful sensory differences among the four different milk sources (Harwood and Drake, 2020). These differences could be related to the way that the cows were fed within each of the four categories; however, the milks were processed and packaged differently as well, which could also lead to sensory differences (Harwood and Drake, 2020).

Another variable to consider when looking at the role of milk sources and how they can affect the product is the time of milking and level of milk fat. Bergamaschi and Bittante (2018) investigated the effects of using 4 different types of milk in cheesemaking that were collected from a herd of 148 cows across 4 different breads (Brown Swiss, Holstein Friesian, Simmental, and Rendena). The four milks collected were whole evening, skim evening, whole morning and mixed in the vat; these different types of milks were analyzed based on their levels of volatile organic compounds to determine their differences. Bergamaschi and Bittante (2018) found that the skimming process, followed by the time of day that the milking occurred had the greatest impact on the level of volatile organic compounds as well as the presence of different compounds. Most of the volatile organic compounds decreased in concentration after skimming (Bergamaschi and Bittante, 2018). More than half of the volatile organic compounds identified were affected by the time of day that milking occurred; however, even though differences were present most were small (Bergamaschi and Bittante, 2018).

Starter Culture

The type or lack of starter culture can influence the flavor profile of whey proteins. This effect has been demonstrated in a number of studies (Tomaino et al., 2004; Liaw et al., 2011; Campbell et al., 2011a, 2011b; Smith et al., 2016). Whey proteins made from rennet casein whey (no starter culture) had lower volatiles compared to whey protein from mozzarella, cottage, or Cheddar whey. Mesophilic (Cheddar) and thermophilic (mozzarella) starter cultures also result in whey with distinct sensory profiles and distinct volatile compounds which contribute to whey protein with distinct flavor. Within different strains of mesophilic starter cultures, distinct flavors and oxidative volatile compound profiles were documented. These differences in fluid whey flavor can all impact flavor of spray dried whey protein.

Bleaching

In order for whey protein to be used as a functional ingredient, it not only needs to have a bland flavor, but also should not impart color. A large volume of whey proteins are produced from annatto-colored Cheddar cheese whey. The whey from colored cheese retains residual annatto colorant. This is undesirable when used as a functional ingredient, so the liquid whey is typically bleached (Kang et al., 2010). The process of bleaching the residual annatto colorant (a carotenoid) is an oxidative process and as such, bleaching can also have negative effects on the flavor profile of the whey and subsequent whey protein. Currently, bleaching is accomplished by chemical or enzymatic means. There are two approved bleaching chemicals in the United States: benzoyl peroxide (BP) and hydrogen peroxide (HP) (Kang et al., 2010). Croissant et al. (2009) and Jervis et al. (2012) experimented with different bleaching agents (hydrogen peroxide and benzoyl peroxide) on whey products. Enzymatic bleaching is done through lactoperoxidase and exogenous hydrogen peroxide (Carter and Drake, 2018). The lactoperoxidase system is made up of 3 components, which are lactoperoxidase, thiocyanate and hydrogen peroxide (Campbell et al., 2012; Seifu et al., 2005). When the system is activated, which can be done by adding a small amount of hydrogen peroxide, hypothiocyanite is created, which is an oxidizing agent (Carter and Drake, 2018; Kang et al., 2010). Campbell et al. (2012) found that bleaching whey proteins increased lipid oxidation and specific sensory flavors including cardboard, fatty and cabbage flavors.

Acidification

While whey protein has good solubility over a wide range of pH, the use of acidified whey protein is growing due to its clarity and heat stability (Lee et al., 2008; Miller et al., 2007). Whey protein is acid stable, which make it unique for clear acidic protein beverages (pH <3.5) (Carter et al., 2020). Acidified protein beverages also have a more appealing protein system due to shelf stability through mild heat treatment (Carter et al., 2020; Ahmadi et al., 2018; Childs et al., 2007). However, astringency and soapiness are off-flavors that are present in acidified whey protein beverages, which negatively effects consumer liking (Childs et al., 2007). Whey protein powders can be acidified when rehydrated or the whey protein can be acidified prior to spray drying.

The acidification of food products can affect their flavor profile. Lee et al. (2008) determined that the astringency of whey protein beverages increased when they were acidified (Lee et al., 2008). Acids are known to have astringent characteristics (Thomas and Lawless, 1995). Thomas and Lawless (1995) evaluated the astringency and sourness of the following acids: hydrochloric, lactic, citric, acetic, fumaric, and malic acids. Hydrochloric acid was rated highest for roughing and drying, while the organic acids all scored similarly (Thomas and Lawless, 1995). Compared to the inorganic acids, the organic acids were found to be more sour than astringent (Thomas and Lawless, 1995). As the concentration of the acids increased, astringency, puckering, roughing, drying and sourness also increase across all acids (Thomas and Lawless, 1995).

White et al. (2013) determined what off flavors were caused by heat and acidification treatments of WPI. For this experiment, commercial, non-instantized WPI were rehydrated at 10% (wt/vol) and underwent three treatments: control, acidified, heated, and acidified and heated. The acidified treatment was acidified to a pH of 3.2 with a 2M 1:1 blend of phosphoric and citric acid to replicate industry practices (White et el., 2013). Of the three treatments (acid, heat, and a combination of acid and heat), the combined acid and heat treatment had the highest levels of the following attributes: overall aroma, cardboard, potato/brothy, soapy and astringent (White et al., 2013). Soapy aftertaste was only found in the combined heat and acid treatment (White et al., 2013). The soapy aftertaste that was detected was described as medium chain free fatty acid-like in follow up sensory panel sessions. The acidified treatment had increased sour and astringency, as expected (White et al., 2013). Evans et al. (2010) evaluated whey protein and serum protein concentrates at 80% protein. Volatile compound analysis was conducted on the pilot plant manufactured WPC80 and SPC80. Medium chain free fatty acids were aroma active in SAFE extracts from both protein concentrates. When these proteins were incorporated into acidic beverages (6% protein, a very low protein load for beverages), soapy flavors were documented in the acidic beverages. In contrast, soapy flavor was not documented in these proteins when they were rehydrated in water at neutral pH.

Park et al. (2014) also evaluated how acidification affected off-flavors of liquid and spray dried WPC80 at three different pHs. Spray drying liquid whey at acidic pH affected the flavor of the dried powder. Samples spray dried at pH 6.5 and 5.5 had increased overall aroma and cardboard flavors, and at pH of 6.5, had an increased sweet aromatic flavor compared to pH 3.5 (Park et al., 2014). WPC acidified to a pH of 3.5 prior to spray drying had increased soapy flavor compared to those spray dried at pH 5.5 or 6.5 (Park et al., 2014). WPC spray dried at pH 6.5 and 5.5 had increased concentrations of hexanal, pentanal, decanal, nonanal as well as DMDS, (Park et al., 2014). WPC that had been acidified to a pH of 3.5 prior to spray drying had lower concentrations of hexanal, heptanal, nonanal and dimethyl trisulfide (DMTS) compared to pH 5.5 and 6.5 (Park et al., 2014).

Drying

The purpose of drying is to take away any liquid in a product, dried milk and whey protein powders typically have a water content of between 1.5-5% (Bylund, 2003). A lower water content means that microorganisms are less likely to be able to grow in the product, which extends the shelf life (Bylund, 2003). Whey protein powder is also easier to transport than liquids (Carter et al., 2018). There are two main methods to drying, which are spray drying and roller drying (Bylund, 2003). Spray drying is the most common procedure today in the United States.

Multiple studies have shown that spray drying impacts flavors in whey proteins (Carter et al., 2018; Whitson et al., 2010; Park et al., 2014, Kuhn et al., 2008). Carter et al. (2018) investigated flavor differences between WPC80, WPI, MPC85, and MCC liquid with their spray dried counterparts. Spray dried powders had higher aromatics than their liquid counterparts (Carter et al., 2018). The spray dried powders had increased cardboard flavor, likely caused by the application of heat via spray drying (Carter et al., 2018). The spray dried powders also had higher concentrations of aldehydes, like hexanal, octanal and pentanal than their liquid counterparts, which correlates with the increased cardboard flavor found (Carter et al., 2018).

Storage of dried protein powder

The reported shelf life of WPI and WPC80 varies by supplier but is between 12 to 36 months (Wright et al., 2009). Wright et al. (2009) evaluated sensory and volatile compound of WPI and WPC80 over 18 months at 21℃ and 50% relative humidity (Wright et al. 2009). Storage time affected non agglomerated and agglomerated powders in a similar manner, as storage time increased, cardboard flavor intensity increased, while sweet aromatic flavor decreased (Wright et al., 2009). Flavors associated with Maillard reactions, like cabbage/brothy flavor, were detected in WPI samples as storage time increased (Wright et al., 2009). Hexanal and trans-2-hexenal were the most abundant compounds found in fresh WPC80 (Wright et al., 2009). As storage time increased so did the amount of lipid oxidation flavors and volatiles; off flavors from agglomerated whey proteins were detected by trained panelists in as early as 4 to 6 months, and nonagglomerated whey proteins had off flavors that were detectable as early as 6 to 10 months (Wright et al., 2009) Changes in quality of whey protein powders happened more quickly in agglomerated and instantized powders than in non agglomerated powders (Wright et al., 2009). This study illustrated that non agglomerated WPC80 and WPI have an optimum shelf life of 12 to 15 months when stored at 21°C, and steam-agglomerated and lecithin-agglomerated WPC80 and WPI have a shelf life of 8 to 12 months when stored at 21°C (Wright et al., 2009).

SENSORY AND VOLATILE FLAVOR ANALYSIS

To fully understand and analyze the flavor profile of whey protein, one needs to be able to understand how to use sensory tools and instrumental methods as they pertain to flavor chemistry. True flavor chemistry makes the connection between the volatile compounds found from performing gas chromatography or other analytical volatile compound analysis and descriptive sensory analysis. It is important to note that the concentration of volatile compounds doesn't correlate with how strong a flavor or aroma is in the product; some volatiles are in concentrations as small as parts per billion (ppb) or even parts per trillion (ppt), but that doesn't mean that they don't play a vital role in the flavor profile of a product.

Descriptive Analysis

Two main types of descriptive analysis (DA) were initially developed: Quantitative Descriptive Analysis (QDA) and the Spectrum method (Drake and Civille, 2003). QDA was first developed in the mid-1970s in order to quantify sensory descriptions through the use of a continuous line-scale; results from the line scale can be presented as a spider plot (Gacula, 1997). The Spectrum Method was developed at around the same time and uses an anchored line scale; Spectrum uses a universal scale where intensities of attributes are scored the same across the all products (Gacula, 1997; Drake and Civille, 2003). The QDA scale is product specific, which means the scale will vary based on the product being analyzed, while Spectrum is universal and can be more easily replicated. Today, there are hybrid techniques that use pieces from both approaches. Each type of DA needs a similar panel size, ranging from 8 to 12 panelists (Drake and Civille, 2003) and a sufficient amount of training to adequately document differences among products. The use of a published lexicon provides a clear roadmap for a product category. Otherwise, a lexicon will have to be developed by the panel. Approaches for lexicon development have been reviewed (Drake and Civille, 2003; Drake, 2007).

Drake et al. (2007) studied how different lexicons impacted trained panel profiles of the same cheeses from different countries (USA, New Zealand and Ireland). Each country had its own lexicon developed so the goal was to see if the lexicons across different countries created different flavor profiles of the identical cheeses selected and sent to each country (Drake et al., 2005). This study showed that although each sensory panel had distinct terms for Cheddar cheese attributes, the panels came to a similar conclusion, which is in part due to the amount of training. It also shows that different trained panels can work together to compare work as long as there is clear communication on the panel differences in documenting the product characteristics (Drake et al., 2005). When used correctly, DA can be used as a bridge to connect what is found through volatile compound analysis and either off-flavors detected in quality assurance, or consumer liking data.

FFA Flavors/ Ways to Quantify FFAs

Free fatty acids can play a role in the flavor profile of dairy products. They are created through the hydrolysis of triglycerides (Singh et al., 2003). Free fatty acids C4-C12 have specific aromas and the intensity of these off flavors is not solely dependent on the concentration of that particular FFA, but also the pH, presence of cations, protein degradation products, and distribution of aqueous and fat phases (Singh et al., 2003; Adda et al., 1982). pH is a major factor when it comes to FFA flavor, as with a higher (more neutral) pH, FFAs are less aroma active since they are ionized and polar (Singh et al., 2003). FFAs that contain less than 14 carbons have a larger impact on the flavor profile of a product than longer chain FFAs (Baldwin et al., 1973;

Bills et al., 1969). FFA analysis can be used to determine quality of dairy products, as milk that contains elevated levels of FFAs can have a rancid off flavor due to the short chain free fatty acids present (Mannion et al., 2015). Elevated FFA levels are indicative of mishandling of the raw milk.

Solid-Phase Microextraction (SPME) with gas chromatography (GC) is one method used to quantify FFA in dairy products (Gonzalez-Cordova et al., 2001). However, accurately quantifying the amount of FFAs via SPME can be difficult due to the volatility of these acids. While salt can be used with SPME in order to help drive out the volatiles into the headspace, as seen in Gonzalez et al. (2001), it is still not an effective method to quantify the amount of FFA in products. Previous papers have been able to identify free fatty acids in whey products through SPME. Tomaino et al. (2001) was able to analyze FFA concentration in liquid whey using SPME GCMS. Tomanino et al. (2001) built a standard curve to quantify the concentration of FFA in liquid whey; the ranges of FFA in the liquid whey were as low as 0.23 ug/g liquid whey to 15.23 ug/ g liquid whey (Tomaino et al., 2001). Tomaino et al. (2001) did not state the limit of quantitation (LOQ) for this experiment.

Methylation is another route that can be used to quantify FFA. This method has been proven useful in both cheese and milk. Melilli et al. (2004) was able to measure the amount of FFA in Ragusano cheese using methylation. However, methylation has only been used in dairy products that have a low protein content, like milk (8 g per cup) and cheese (7 g per slice) (USDA, 2018). There are currently no published studies that use methylation to quantify the amount of the FFA in high protein dairy products, like WPI. The range of FFA content found in cheese ranged from 0.67 mg per 100 g of cheese to 119.68 g per 100 g of cheese (Melilli et al., 2004).

The copper soap method is another way to determine free fatty acids in dairy products. However, this method, like methylation, is typically used with dairy products that have a lower protein content compared to WPI, which has a protein content of >90%. This method involves sodium diethyl dithiocarbamate solution as a color reagent in order to be read on a spectrophotometer at a wavelength of 440 nm (Shipe et al., 1980). This method determines the total amount of FFA/g of product and is unable to determine the amount of individual FFA in a product (Shipe et al., 1980). Newer methods can give more distinct results as it pertains to individual FFA. Melilli et al. (2004) also performed the copper soap method on the same cheese samples and the reported range of total FFAs for this portion of their experiment ranged from approximately 30 mg per 100 g of cheese to 200 mg per 100 g of cheese.

Another method utilized to measure the amount of volatile acids in dairy products in Solvent Assisted Flavor Evaporation (SAFE). SAFE has been used to measure volatile compounds in a broad range of dairy products, including WPI and WPC (Carunchia Whetstine et al., 2005). Before performing a SAFE, a Direct Solvent Extraction (DSE) needs to be performed. DSE is most useful in foods that have a low fat content, like WPI. This is because during the extraction process, lipids are co-extracted with the volatile compounds and then need to be separated further in order to do volatile analysis (Reineccius, 2006). A DSE is one of the most basic types of solvent extractions; solvents can be collected in a few steps by adding the solvent to the food sample, shaking and then taking off the desired solvent layer (Reineccius, 2006). One of the main disadvantages of evaporative techniques is that the loss of different components and classes will not be uniform (Reineccius, 2006). This means that even when using multiple internal standards and multiple replicates of samples, results are still likely to vary among replicates (Reineccius, 2006). However, if standard curves are used in order to measure each individual compound of interest, a more accurate quantification can be obtained. SAFE is then applied to separate the fat from the extracted volatiles.

There have been numerous studies that have utilized SAFE for compound identification in dairy products (Wherry et al., 2019; Sonmezdag, 2018; Smith et al., 2016; Drake et al., 2010; Wright et al., 2006). One of the main reasons that SAFE is commonly used for volatile compound identification and quantification in dairy products is because of the ability to deal with fat in SAFE. Engel et al. (1999) found that SAFE was a more effective extraction method than those previously used, such as high vacuum transfer (HVT), for fatty matrices (greater than 50%).

Karagul-Yuceer et al. (2001) identified the presence of FFA in nonfat dry milk through SAFE. Acetic, pentanoic, hexanoic, octanoic, and decanoic acids were detected through aroma extract dilution analysis (AEDA) (Karagul-Yuceer et al., 2001). These acids were also quantified using a standard curve and had concentrations ranging from 18-28mg/100g of acetic acid, 1150 to 1857mg/100g butanoic acid, 2.6-4.3mg/100g pentanoic acid, 501-677mg/100g hexanoic acid, and 201-314mg/100g decanoic acid (Karagul-Yuceer et al., 2001). Karagul-Yuceer et al. (2002) again identified the presence of FFA in stored nonfat dry milks (NFDM). The following FFA were identified on solvent extracts collected from SAFE: acetic, propionic, isobutyric, butanoic, 2-/3-methylbutyric acid, pentanoic, hexanoic, octanoic, nonanoic, decanoic, dodecanoic and phenylacetic acid (Karagul-Yuceer et al., 2002). Butanoic acid had the highest concentration ranging between 905 to 2380mg/100g; and dodecanoic had some of the lowest concentrations of 0.2 to 0.7mg/100g (Karagul-Yuceer et al., 2002). Karagul-Yuceer et al. (2003) identified and quantified butanoic and hexanoic acids in liquid wheys. Butanoic acid ranged from 96,279 to 181,169 ug/L and hexanoic acid ranged from 32,286 to 43,166 ug/L of liquid whey. Butanoic and hexanoic acids were quantified using a 5 point standard curve that utilized aliquots of stock solutions of each acid (Karagul-Yuceer et al., 2003). Solvent samples obtained from SAFE were also subjected to gas chromatography-olfactometry (GCO) and acetic, butanoic, pentanoic, and hexanoic were all detected through solvent sniffing as well (Karagul-Yuceer et al., 2003).

Carunchia Whetstine et al. (2005) performed SAFE on WPI and WPC. They found acetic, butanoic, pentanoic, hexanoic, heptanoic, octanoic, 4-methyl octanoic, nonanoic, and decanoic acid in both WPI and WPC (Carunchia Whetstine et al., 2005). The concentrations of these acids ranged from 20 part per billion (ppb) to 108,000 ppb; however, compounds were analyzed as relative abundance, which uses the area ratio of the internal standard versus the area of the compound multiplied by the concentration of the internal standard to determine the relative concentration of the compound versus using a standard curve. Carunchia Whetstine et al. (2005) was able to positively identify acetic, butanoic and hexanoic acid through AEDA. These studies all show that FFA can be positively identified and quantified through SAFE on a variety of dairy products including whey proteins. Using a standard curve along with SAFE is an acceptable measure to determine FFA concentration in whey proteins.

APPLICATIONS IN WHEY PROTEIN ISOLATE

Whey protein has many uses within the food industry. Different types of whey proteins have different types of application based on the amount of protein they contain, as that affects their chemical properties. Today, whey protein can be found in sports drinks, protein bars, infant formula, yogurt and others. Whey protein isolate is commonly used in protein beverages and has been designed to be appealing to a wide range of markets due to the high quality proteins they contain (Minj and Anand, 2020).

Health Benefits

The protein components of whey protein have many functional properties that help in the food industry, there has also been a lot of research on how whey protein can aid in numerous medical issues. Lactoferrin is found in both breast milk and bovine milk; its key function in milk is immune defense (Superti, 2020). Some of its most common functions include being antianemic, anti-inflammatory, antimicrobial, antioxidant, and having anticancer activities (Superti, 2020).

A study was performed on mice to determine if WPC and lactoferrin given orally on a daily occurrence would decrease diethylnitrosamine (DEN) effects, a natural carcinogen (Ramadan et al., 2020). High doses (60 mg) of lactoferrin and WPC helped to modulate body changes, such as body and liver weights, in the mice that were dosed with DEN (Ramadan et al., 2020). Another portion of Ramadan et al (2020) study aimed to determine if WPC and lactoferrin alleviated anemia in mice. This portion of the study also showed that consuming WPC and lactoferrin aided in controlling anemia (Ramadan et al., 2020).

Whey protein, specifically WPI due to its high protein concentration, can also be consumed pre or post workout in order to aid in muscle mass and strength (Chalé et al., 2012). Macnaughton et al. (2016) studied the myofibrillar protein synthesis (MPS) of participants who consumed 40 g and 20 g of whey protein. The study found that participants who consumed 40 g of whey protein followed by whole-body resistance exercise had more stimulated MPS than those who only consumed 20 g (Macnaughton et al., 2016).

Whey protein has also been shown to help prevent cancer as well as benefit those who have cancer (Patel, 2015; Marshall, 2004). Attaallah (2012) studied how the use of untreated whey protein versus whey protein hydrolysate affected the growth of tumors in rats with induced colon cancer (Attaallah et al., 2012; Patel, 2015). They found that rats fed with whey protein hydrolysate developed fewer tumors than those fed with untreated whey protein. Dillon et al. (2012) conducted a study on a 48 year old Caucasian female who had recurrent cervical cancer; she received 10 g of whey protein three times a day as well as receiving an injection of testosterone enanthate before undergoing chemotherapy. They found that her overall quality of life, lean muscle mass and physical activity was improved as a result of the treatment. While there is promise for whey protein to help aid in the prevention and recovery of cancer, more clinical studies need to be done in the future. Studies have been done on individual whey proteins as well.

Ready-To-Drink Protein Beverages

Whey proteins are typically used as a protein source in protein beverages due to their nutritional qualities, bland flavor, digestibility, and distinctive functionality (U.S. Dairy Exports Council, 2018). In 2020 the ready-to-drink protein beverage market reached $1.34 billion; it is expected to grow to $2.26 billion by 2027 (MarketWatch, 2022). Protein beverages can be made at a neutral pH (6.8-7.0), in which the end product is opaque and typically comes in flavors such as chocolate, vanilla, coffee, or caramel (Carter et al., 2020). Neutral protein beverages are a much larger beverage category than acidified protein beverages. This is due to the larger numbers of proteins that can be used in these beverages, including plant proteins. In regards to dairy proteins used for neutral protein beverages, the heat stability of casein allows for the use of both MPC and MPI to be used for this beverage category; whey proteins are not typically used due to their lack of heat stability (Carter et al., 2020). In contrast, whey proteins are soluble and provide clarity under acidic conditions, making them ideal for acidified protein beverages.

Acidified protein beverages (pH <3.5) are clear and typically have fruity flavors, such as fruit punch and grape that align more with the low pH (Carter et al., 2020; Beecher et al., 2008). The acid stability of whey protein makes them an ideal protein source for this type of protein beverage. Oltman et al. (2015) identified the key attributes in protein beverages that consumers liked. Protein beverage consumers were most concerned with what type of sweetener was used in the product as well as the amount of protein, satiety and flavor. Oltman et al. (2015) reported that consumers had a higher liking for acidified protein beverages with a lower amount of protein in them. The higher liking was likely partially due to the decrease in protein related off flavors and viscosity (Oltman et al., 2015).

Protein Bars

Protein bars or meal replacement bars are an expanding market in the US and across the world with a value of 4.66 billion USD in 2019 and is expected to almost double by 2027 (Fortune Business Insights, 2022). Protein bars can utilize a variety of protein sources, whether that be different types of dairy proteins or plant proteins. Harwood and Drake (2019) determined that consumers preferred a protein bar that was made up of whey protein, chocolate in flavor, contained 20-29 g of protein, was all-natural and sweetened with stevia. Childs et al. (2007) performed a taste test that compared prototype protein bars and found that consumers had a higher acceptance for protein bars made with whey protein or a mixture of whey and soy protein over products made with only soy protein. Childs et al. (2007) evaluated consumer perception of protein beverages versus bars and found that both those who are exercisers as well as non-exercisers preferred protein bars over beverages. These results were consistent with those of Harwood and Drake (2019).

Infant Formula

One of the main uses for whey protein is to be used in infant formula as it is a source of essential amino acids (Fenelon et al., 2019). While breast milk is still the most ideal, when it is not available, infant formula with whey protein is one of the best substitutes (Fenelon et al., 2019). Infant formula may use a higher density protein, like whey protein, in order to compensate for it still not being as beneficial as the protein that comes from consuming breast milk (Jost et al., 1999). However, not all dairy proteins are created equal when it comes to formulating infant formula. Alexander and Cabana (2010) found that infant formula with partially hydrolyzed 100% whey protein reduced the risk of atopic dermatitis in infants compared to infants who were fed an intact protein cow's milk formula. Early on in the development of bovine based infant formula, the ratio of caseins and whey protein was lowered from 80:20 to 40:60 in infant formula with the supplementation of desalted whey or WPC so that the protein and mineral content of the infant formula more closely matched the composition of human milk (de Wit, 1998).

CONCLUSIONS AND OBJECTIVE

Whey protein has a wide range of functional and nutritional properties that make it a desirable ingredient in food products; however, off-flavors are present that make products less desirable to consumers. Clear acidified beverages have a soapy flavor. This flavor is documented in both pre-acidified WPI and WPI that is acidified post-spray drying to a pH <3.5, so it can be concluded that WPI is the source of the flavor. The objective of this study is to determine if medium-chain free fatty acids are the cause of soapy off-flavors in clear acidic protein beverages. This study will help aid in the future development and improvement of clear acidic protein beverages.

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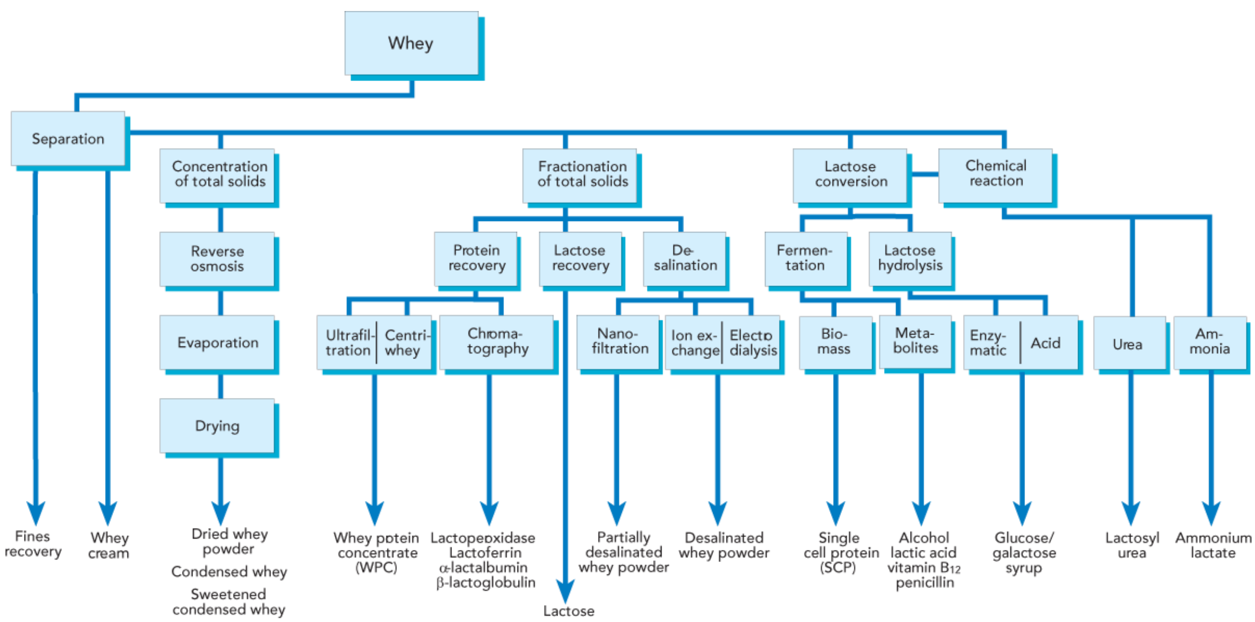
Table 1.1 Fluid Whey Composition

Component	Sweet Whey	Acid Whey
Total solids	63.0-70.0%	63.0-70.0%
Lactose	46.0-52.0%	44.0-46.0%
Protein	6.0-10.0%	6.0-8.0%
Calcium	0.4-0.6%	1.2-1.6%
Phosphate	1.0-3.0%	2.0-4.5%
Lactate	2.0%	6.4%
Chloride	1.1%	1.1%

(Jelen, 2010).

Figure 1.1 Processing Steps Taken to Turn Whey Into Whey Products



(Tetra Pak, 2022).

CHAPTER 2:

SOURCES OF SOAPY OFF-FLAVOR IN CLEAR ACIDIC WHEY PROTEIN BEVERAGES

AUTHORS: A.R. Best[1], Y. Liu[1], M.A. Drake[1]

[1]Department of Food, Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, Box 7624, North Carolina State University, Raleigh, NC, 27695-7624

The contents of this chapter will be submitted to:
Journal of Food Science

ABSTRACT

Clear acidic protein beverages are made with whey protein isolate (WPI) due to its solubility at low pH (pH 3.3). These beverages can have a soapy off-flavor, yet soapy flavor is not detected in neutral pH WPI solutions. Our hypothesis was that medium chain fatty acids associated with the WPI are the source of this off flavor under acidic conditions. Fourteen commercial WPI powders were sourced from 4 different manufacturers in duplicate lots (10 neutral pH, 4 pre-acidified). WPIs were rehydrated to 10% solids (w/v); pH at 21°C was measured and the rehydrated WPI were evaluated by descriptive analysis (DA) with a trained panel. Subsequently, 6 WPIs (3 neutral, 3 pre-acidified) were selected for further analyses. Solvent assisted flavor evaporation (SAFE) with GCMS and gas chromatography olfactometry (GCO) were performed on selected WPI followed by quantitation of octanoic, decanoic and dodecanoic acids using stable isotope dilution assays (SIDA) with deuterated standards. Retronasal and orthonasal sensory thresholds of the three acids were determined in buffered solutions at pH 6.3 and pH 3.3. Model systems of WPI at pH 6.3 and pH 3.3 with the average concentrations of the three acids were generated to confirm soapy flavor. As expected, WPI at pH 6.3 exhibited no soapy flavor, whereas WPI at pH 3.3 had soapy flavor. The average concentrations of acids in selected WPI were: C8 = 14.39 ± 2.0 ppm; C10 = 27.78 ± 2.39 ppm; C12 = 24.71 ± 2.20 ppm. Sensory thresholds for all three acids at pH 3.3 were lower than at pH 6.3 ($p<0.05$). Trained panel profiling of model systems demonstrated that a combination of octanoic and decanoic acids at pH 3.3 caused a soapy flavor and this flavor was not detected in the same model beverage at pH 6.3. Identification of the sources of soapy off flavor in clear acidic protein beverages provides the baseline to reduce or eliminate this off flavor. To eliminate or reduce soapy flavor in these beverages, residual free fatty acid content of WPI must be minimized.

INTRODUCTION

The global sports foods and drinks market was valued at $79.8 billion in 2020 and is expected to grow to $115.2 billion by the year 2026 (Businesswire, 2022). Protein beverages, a portion of this growing market, comprised $1.34 billion in 2020 and are expected to reach $2.26 billion by 2027 (Marketwatch, 2022). Protein beverages consist of both ready-to-drink (RTD) and ready-to-mix (RTM) products and as well as neutral pH and acidified beverages within the RTD category (Singh et al., 2022; Carter et al., 2020). Neutral protein beverages fall between a pH of 6.8-7.0 while acidified protein beverages are lowered to a pH of less than 4.6 (Carter et al., 2020; Singh et al., 2022). Neutral pH protein beverages encompass a wide array of dairy and plant protein sources. In contrast, acidified RTD beverages are limited to whey protein due to its unique solubility and clarity under acidic conditions. These clear acidic protein RTDs are typically flavored with fruity flavors congruent with a low pH; whey proteins are acid stable, and due to their pH, can be shelf stable with a mild hot fill heat treatment (Singh et al., 2022). Whey protein isolate (WPI) is the whey protein ingredient used in these beverages.

As with other consumer products, flavor is a paramount attribute in RTD beverages and protein-related flavors are a challenge (Oltman et al., 2015; Harwood and Drake, 2019). Both plant and dairy proteins, including whey protein, can contribute undesirable flavors to ingredient applications (Carter et al., 2020; Liu et al., 2023; Russell et al., 2006). Identifying sources of off flavors in RTD beverages or their protein sources can be useful to identify methods to reduce off flavors. For example, cardboard flavor is a ubiquitous flavor in dairy proteins – and proteins in general – and has been sourced to lipid oxidation products (Whitson et al., 2010; Carter and Drake, 2018; Liu et al., 2023). Minimizing process steps or storage to reduce oxidation can reduce the intensity of this off flavor in dried protein ingredients (Carter and Drake, 2018).

Sources of cabbage and sulfur/eggy flavor have been similarly identified (Wright et al., 2006; Whitt et al., 2022). Soapiness is an off-flavor in whey protein that has been attributed to solutions of whey protein, but more prevalently in clear acidic protein beverages made with WPI (Oltman et al., 2015; Park et al, 2014; White et al., 2013; Evans et al., 2010).

Carunchia Whetstine et al. (2005) was one of the first studies to document the sensory profiles and flavor chemistry of WPI and 80% whey protein concentrates (WPC80). Soapy flavor was only documented in 2 of the WPI evaluated. These WPI had a higher pH than other WPI and a distinct mineral content. Carunchia Whetstine et al. (2005) identified octanoic and decanoic acids and suggested that they might contribute to soapy/waxy flavor. White et al. (2013) demonstrated that acidified of WPI (pH 3.2) increased in soapy flavor intensity and prevalence, and Park et al. (2014) demonstrated that WPC80 that was pre-acidified to a pH of 3.5 had a soapy flavor that was not evident at neutral pH. There have been several studies that have noted how different variables (eg. milk type, starter culture, addition of enzymes) can affect the prevalence of FFAs in WPI or liquid whey (Campbell et al., 2011a; Liaw et al., 2011; Tomaino et al., 2001). Short chain FFAs are naturally occurring in milk at low levels (Cadwallader et al., 2007), and they can increase through the addition of lipase or selection of starter culture during cheese manufacture (Campbell et al., 2011a; Drake et al., 2009). As such, FFAs are present in fluid whey and whey protein.

The source of soapy off-flavor present in acidified whey protein has not been clearly identified. Although previous studies have documented soapy flavor occasionally in whey protein, soapy flavor is prevalent in acidified whey protein solutions and acidic whey protein beverages. There appears to be a relationship between pH and the presence of a soapy off-flavor (Park et al., 2014; White et al., 2013). We hypothesize that soapy flavor is caused by medium chain free fatty acids associated with WPI that have low flavor activity under neutral pH but are more flavor active under acidic conditions. The objective of this study was to determine if free fatty acids, specifically octanoic, decanoic, and/or dodecanoic acids, which are known to have soapy/waxy flavors and aromas, are the cause of soapy off flavor in acidified WPI.

MATERIALS AND METHODS

Samples and Experimental Overview

Fourteen commercial WPI powders (n=4 pre-acidified, n=5 neutral pH, n=5 neutral pH instantized) were sourced from 4 different manufacturers in duplicate lots. WPI were stored at 21°C in a dark environment prior to analysis (less than 60 d). All sensory testing was done in accordance with North Carolina State University Institutional Review Board regulations (NCSU IRB). Each WPI was rehydrated at 10% solids (w/v) with deionized water to confirm the pH at 21°C. Rehydrated WPI were subjected to descriptive sensory analysis and were evaluated at their original pH, as well as pH adjusted to 6.3 (pre-acidified) or to 3.3 (neutral) at 21°C. Neutral (pH 6.3) WPI solutions were acidified to pH 3.3 (21°C) with 0.5 M phosphoric and 0.5 M citric acids. Acidic (pH 3.3) WPI solutions were neutralized to pH 6.3 (21°C) with 2.5 M NaOH. Subsequently, 6 WPI (n=3 neutral pH and n=3 pre-acidified) were selected for further analyses. Selected WPI were subjected to solvent assisted flavor evaporation (SAFE) with gas chromatography mass spectrometry (GCMS) and gas chromatography olfactometry (GCO) to characterize aroma-active compounds. Quantitation of selected compounds was determined using stable isotope dilution assays (SIDA) with deuterated standards. Retronasal and orthonasal thresholds of the selected compounds of interest were determined in both neutral (pH 6.3) and acidic (pH 3.3) conditions. Lastly, model WPI beverages at neutral (pH 6.3) and acidic (pH 3.3) conditions were created and spiked with the average concentrations of selected compounds quantified from SAFE. These model beverages were evaluated by the trained sensory panel (Figure 1).

Chemicals

Diethyl ether (anhydrous, 99.7%), methanol, hydrochloric acid (HCl, 36.5%), sodium hydroxide (NaOH, 98.7%), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), sodium sulfate (granular, anhydrous 99%), and ethanol were purchased from Fisher Scientific (Hampton, NH). Deuterated reference standard (octanoic d-15 acid) and other chemicals, citric acid, phosphoric acid, dibasic sodium phosphate, octanoic acid, decanoic acid, and dodecanoic acid were obtained from Sigma-Aldrich (St. Louis, MO). Other deuterated reference standards (decanoic-d19 acid and dodecanoic d-23 acid) were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). An n-Alkane series (C7–C26) was purchased from Sigma-Aldrich.

pH

The pH of rehydrated WPI were determined at 21°C. WPI were rehydrated at 10% solids (wt/wt) in triplicate using room temperature (21℃) deionized (DI) water and held overnight at 4°C to allow for complete rehydration. Fifty mL of rehydrated WPI was tempered to 21°C in a water bath. Subsequently, the tempered solution was placed in a 100 mL glass beaker with a stir bar on a stir plate (VMS C-4, VWR) and stirred at a constant rate while pH was taken at 21℃ using a SympHony B10P pH meter (VWR) and sympHony pH probe 89231-596 (VWR). The pH meter was calibrated at 21℃ prior to sample measurement.

In order to evaluate the role of pH on flavor of WPI, neutral pH WPI were also acidified to pH 3.3 (the average pH of commercial clear acidic whey protein beverages) and pre-acidified WPI were neutralized to pH 6.3 for sensory evaluation. Neutral pH WPI were acidified to a pH of 3.3 using 0.5 M phosphoric acid or 0.5 M citric acid at 21°C. Both of these acids were used as acidulants with the WPI followed by sensory evaluation since both are used in commercial clear acidic protein beverages. Similarly, pre-acidified (pH 3.3) WPI were neutralized to pH of 6.3 using 2.5 M NaOH at 21°C. Prior to acidification and neutralization, preliminary experiments to determine the volumes of acid or base required to achieve the target pH for each WPI were conducted as described by White et al. (2013). The volume of 0.5 M citric acid or 0.5 M phosphoric acid to achieve a pH of 3.3 was determined and that volume was subtracted from the total liquid needed to retain a 10% solids solution with each WPI (White et al., 2013). The same procedure was done for 2.5 M NaOH with each WPI to reach a pH of 6.3. The pH of the WPI were evaluated and confirmed using the previously described methods.

Descriptive Analysis

WPI were rehydrated to 10% solids (w/v) in deionized (DI) water. The rehydrated WPI were evaluated by a highly experienced sensory panel (n=7, 4 females, 3 males, ages 23-53 y), each with a minimum of 60 h previous experience in the descriptive analysis of dried dairy ingredients. An established sensory language (White et al., 2013; Wright et al., 2009) was used in conjunction with an intensity scale consistent with the Spectrum descriptive analysis method (Lawless and Heymann, 2010). The WPI solutions were dispensed (30 mL) into 64 mL lidded souffle cups with 3-digit codes and evaluated at 21°C. WPI solutions were evaluated in duplicate by each panelist using an online ballot within the NCSU secure server network. Subsequently, 6 representative WPI lots were selected for instrumental volatile analysis, 3 pre-acidified WPI and 3 neutral pH WPI. These WPI were selected based on their sensory profiles; the selected neutral WPI had no or minimal soapy flavor and the pre-acidified WPI displayed distinct soapiness.

Direct Solvent Extraction with Solvent Assisted Flavor Evaporation (DSE-SAFE) of 6 Selected WPI

Volatile components of acidified rehydrated WPI were extracted by direct solvent extraction with solvent assisted flavor evaporation (DSE-SAFE) using a modified method from Carunchia Whetstine et al. (2005). Duplicate extractions were performed on each of the selected WPI. Acidified WPI solutions were analyzed since our focus was soapy off flavor in clear acidic beverages and because previous research has demonstrated that flavor active fatty acids are best extracted from matrices under acidic conditions (Drake et al., 2010; Carunchia Whetstine et al., 2005). Twenty (20g) grams of WPI was rehydrated with DI water in a 10% (w/w) solution and lowered to a pH of 1.5 using 18% HCl (Carunchia Whetstine et al., 2005). The acidified WPI solution was evenly distributed between 6 (250 mL) glass bottles (VWR). Sixty (60g) grams of NaCl (Sigma Aldrich) and 200 mL of diethyl ether (Sigma Aldrich) was also added and distributed among the 6 bottles. Five mL of internal standards (2-methyl pentanoic acid at 10,000 ppm and heptanoic acid at 10,000 ppm in methanol) (Sigma) were equally distributed among the centrifuge bottles. The mixtures were shaken for 30 min at 200 rpm using an OrbiShaker (Benchmark Scientific, Sayreville, NJ) to separate the nonpolar solvent phase from the mixture. The bottles were then centrifuged at 1300 x g for 15 min (Thermo Lynx 6000, Thermo, Rochester, NY) to separate the ether layer. The ether layer was collected. The extraction was repeated two more times. All three extracts were combined and filtered through sodium sulfate (Sigma Aldrich) to remove remaining water left in the ether layer. Each solvent extract was then concentrated to 200 mL using a gentle stream of nitrogen and subjected to solvent-assisted flavor evaporation (SAFE). The SAFE apparatus (Ace Glassware, Vineland, NJ) was assembled and performed as described by Engel et al. (1999) and Cadwallader et al. (2022). A 1L round flask was placed in a circulating water bath (Ace Glassware) that was set to 40°C to maintain a constant temperature. The SAFE head apparatus had 2 headspace traps, both of which were submerged in Nalgene buckets filled with liquid nitrogen and refilled every 20 min to maintain temperature. The entire SAFE apparatus was then brought to a vacuum of 10^{-5} torr. Once the system equilibrated, the sample was introduced dropwise through the stopcock of the apparatus head across a 30 min span. When the sample volume had been fully introduced into the system, the system was kept at a constant vacuum and temperature for 2 h. After 2 h, the system was shut down and the sample that was collected in the primary trap was thawed at room temperature and collected in a 250 mL amber glass jar. Phase separation procedures were followed as described by Carunchia Whetstine et al., (2005). After SAFE, the distillate was concentrated under a stream of nitrogen to 20 mL. The concentrated distillate was washed with sodium bicarbonate (0.5 M) three times and shaken vigorously for 5 min using a Multi Reax vibrating test tube shaker (Heidolph Instruments, Schwabach, Germany). The water phase (bottom) was removed and collected in a separate test tube. The distillate was then washed with 2 mL saturated sodium chloride solution and shaken vigorously for 5 minutes, 3 times. The water phase (bottom layer) was removed and collected in the same tube. The upper layer remaining was the neutral/basic fraction. This fraction was discarded since our focus was flavor active fatty acids. For the acidic fraction, the water layer was acidified to a pH of 2.0-2.5 using 18% hydrochloric acid. Five (5mL) mL of ether was added 3 times to the water phase and shaken vigorously for 10 minutes using the Multi Reax vibrating test tube shaker (Heidolph Instruments, Schwabach, Germany). The ether layer containing the extracted acidic volatiles layer was collected and was dried over anhydrous sodium sulfate. The extracted acidic volatiles were then concentrated under a stream of nitrogen to 0.25 mL.

Gas Chromatography-Mass Spectrometry (GCMS) of Solvent Extracts

Gas chromatography-mass spectrometry analysis of the solvent extracts was performed using a 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA). Separations were performed using a ZB-Waxplus (30 m length×0.25 mm i.d. × 0.25 μm film) (Phenomenex, Torrance, CA). The method for analysis was based on Carunchia Whetstine et al. (2005) with minor modifications. Helium gas was used as a carrier at a constant flow of 1 mL/min. Oven temperature was programmed from 40 to 200°C at a rate of 5°C/min with initial and final hold times of 5 and 45 min, respectively. Mass selective detector conditions were as follows: capillary direct interface temperature, 250°C; ionization energy, 70 eV; mass range, 33 to 330 amu; scan rate, 5scans/s. Two microliters of each acidic fraction were injected in the splitless mode with a 3 min solvent delay. Triplicate injections were conducted on each extract.

Gas Chromatography-Olfactometry (GCO)

The aroma-active components of the solvent fractions were determined by gas chromatography olfactometry. Each fraction was injected on two GCO systems (6850 GC, Agilent Technologies Inc, Santa Clara, CA), each with a with a flame ionization detector (FID) with a sniffer port but different columns, ZB-WAX plus and a ZB-5ms (30 m length × 0.25 mm i.d. × 0.25 μm) (Phenomenex). Five microliters of each sample was injected onto each column. The GCO method followed Whetstine et al. (2005) with minor modifications. Column effluent was split 1:1 between the FID and sniffing port. The oven was programmed to 40 to 200°C with a rate of increase of 10°C/min with a start hold time of 3 min and a final holding time of 20 min. The FID was maintained at a temperature of 250°C. Each acidic fraction was evaluated in duplicate on both columns by two trained sniffers, each with more than 75 hours of experience.

Sniffers described the odor and rated the odor intensity on a 5-point numerical intensity scale (Carunchia Whetstine et al., 2005).

Compound Identification and Quantitation

Compound identification was determined based on mass spectra, aroma character, retention indices on both polar and non-polar columns, and the use of authentic standards. Stable isotope dilution assays (SIDA) with deuterated standards were used to determine concentration of selected compounds. The response factors of the selected compounds were quantified through a 5-point internal standard curve created using deuterated compounds (Sigma and CDN Isotopes). A stock solution of each of the deuterated standards was made in methanol (Fisher Scientific). The deuterated standard stock solutions were added to rehydrated WPI that was acidified to pH 1.5 as described previously in duplicate. Each standard curve was generated by separate extractions and were injected on the same GC-MS using the same method described previously. The selected compounds were quantified by utilizing the response factor with the area ratio of the compound to internal standards. The standard curve for octanoic acid had an R^2 0.9925 and decanoic acid had an R^2 of 0.9929. Due to the low solubility of dodecanoic acid, the standard curve created for decanoic acid was used to quantify dodecanoic acid.

Threshold Testing

Orthonasal Threshold Testing

The orthonasal Best Estimate Thresholds (BET) of octanoic, decanoic and dodecanoic acids (Sigma Aldrich) were determined orthonasally in acidic (pH of 3.3) and neutral (pH 6.3) conditions. Octanoic and decanoic acids were dissolved in water-based buffer solutions. Due to low solubility in water (less than 1 ppm) (American Chemical Society, 2022) dodecanoic acid was dissolved in a 40% ethanol (Fischer Scientific) buffer solution. To maintain a constant pH for threshold determinations, a citric acid-dibasic sodium phosphate buffer was made (Baldwin et al., 1973). The buffer was made using 0.1 M citric acid (Sigma Aldrich) and 0.2 M dibasic sodium phosphate (Sigma Aldrich) solutions in DI water to generate acidic (pH 3.3) and neutral (pH 6.3) matrices. For a 3,000 mL solution of neutral water (pH 6.3), 118.2 mL of 0.1 M citric acid and 181.8 mL of 0.2 M dibasic sodium phosphate was added to 2,700 mL of DI water. For a 3,000 mL solution of neutral water (pH 3.3), 110 mL of 0.1 M citric acid and 23 mL of 0.2 M dibasic sodium phosphate was added to 2,850 mL of DI water.

All compounds were dissolved in the appropriate matrix on the day of the test and stock solutions were serially diluted by a step factor of 2. Fifteen (15 mL) milliliters of each signal was poured into clean, labeled 56-mL plastic souffle cups and lidded (Solo cup, Highland Park, IL). All cups were labeled with randomized 3-digit codes. Blank samples of the corresponding matrix were added in the same amount to souffle cups and lidded. Samples were presented to consumers at 21 °C. Panelists who participated in the threshold testing were instructed prior to tests (Leksrisompong et al., 2010) on consistent orthonasal and retronasal procedures.

Sample presentation and calculations were based on the American Society for Testing and Materials (ASTM) procedure E679–9 (ASTM, 2004). Samples were presented to panelists in a series of three with 2 blanks and one signal cup. Seven ascending series were tested each time. Each series was presented in a randomized order. Panelists were instructed to open the souffle cups within each series, sniff them in the corresponding presentation order and then circle which sample they thought was different from the other two. After picking which coded sample they thought was different, panelists were then asked to indicate whether or not they were sure that the sample selected was the signal cup. The individual BETs were taken as the geometric mean of the last concentration with an incorrect response along with the first concentration that had a correct response. If the panelist indicated "not sure" on the correct choice, then a factor of 1.41 was used as an adjustment from the panelist choosing correctly by chance (Lawless et al., 2000). Group thresholds were taken as the geometric mean of the calculated individual BETs.

Retronasal Thresholds

Retronasal thresholds were also determined for octanoic and decanoic acids. Dodecanoic acid retronasal thresholds were not performed due to the lack of solubility of dodecanoic acid and potential interference and fatigue (compound dissolved in a 40% ethanol solution). Retronasal thresholds were prepared the same way that orthonasals were prepared. Panelists were provided with a nose clip (Drake, 2007). Panelists were instructed to put on the nose clip prior to sampling of the blank and signal solutions. Panelists were instructed to place the sample in their mouth and manipulate the sample for 2-3 sec. Panelists then took off the nose clip before expectorating. Calculations for retronasal BET values were calculated in the same manner as orthonasal BET values.

Model System Confirmation

To confirm the contributions of octanoic and/or decanoic acids to soapy flavor in acidic whey protein beverages, sensory evaluation of model systems of rehydrated WPI (10% w/v) were performed at neutral (pH 6.3) and acidic (pH 3.3) conditions. The trained sensory panelists described previously evaluated the model systems. WPI utilized for model systems were selected from previous trained panel profiling to have low or no soapy flavor. Neutral models were determined using a neutral pH WPI (pH 6.3) and by neutralizing a pre-acidified WPI to pH 6.3 with 2.5 M NaOH. Acidic models were performed using pre-acidified WPI (pH 3.3) and by acidifying a neutral pH WPI to an acidic pH with 0.5 M citric or 0.5 M phosphoric acids. Model systems were not performed on dodecanoic acid due to its low solubility in water.

The average concentration of octanoic and decanoic acids was used to spike acidified WPI (pH 3.3) and neutral WPI (pH 6.3) solutions. Stock solutions of each acid were made and added to rehydrated WPI. Octanoic and decanoic acids were evaluated separately and in combination. The following references were provided: a rehydrated pre-acidified WPI that was previously determined by the trained panel to have a soapy aroma and flavor (soapy reference) and a rehydrated neutral WPI powder that had no soapy characteristics and blank (unspiked) solutions of the acidic and neutral WPI that were selected for model system matrices. The baseline sensory profile of the acidified and neutral WPI were confirmed by the trained panel prior to the addition of the compounds. Each compound or compound combination in each WPI model along with a coded unspiked solution (blind control) was dispensed into lidded soufflé cups with three-digit codes and was then evaluated for soapy flavor intensity using the 0 to 15 point intensity scale described previously. Each coded sample was also scored on a similarity scale of 0-10 with 0 being not similar at all and 10 being highly similar compared to the pre-acidified soapy WPI (soapy reference). Each WPI model was evaluated in duplicate by each panelist at room temperature conditions (21°C).

Statistical Analysis

Analysis of variance (ANOVA) was performed on trained panel and compound quantification data. Statistical analyses were performed with XLSTAT (2022, Addinsoft Inc., New York, NY) and carried out using a 5% significance level.

RESULTS AND DISCUSSION

pH Readings of Commercial WPI

The average pH of the 4 pre-acidified WPI at 21°C was 3.3±0.1, and the average pH of the 10 neutral (or regular) WPI at 21°C was 6.3 ±0.3. These reported values and the pH range are similar to other studies that examined the pH of commercial WPI under acidic and neutral conditions (Park et al., 2014; White et al., 2013).The pH of the selected WPI for further analysis are shown in Table 1. Neutral ion-exchange WPI had a significantly higher ($p<0.05$) pH than the other neutral WPI (7.1 ±0.1 vs 6.2±0.4).

Sensory Profiles of Rehydrated WPI

Sensory profiles of the 10 neutral rehydrated WPI were generally similar with variable intensities of cardboard and sweet aromatic flavors and astringency, typical of WPI (Appendix 1) (Wright et al., 2009). Pre-acidified WPI were characterized by cardboard, sour aromatic, and soapy flavors, sour taste, and astringency (Appendix 2), also consistent with previous work (Park et al., 2014). Six WPI (3 neutral and 3 pre-acidified) were selected for further analyses. Pre-acidified WPI were selected based on soapy flavor intensity. Neutral pH counterparts from the same manufacturer were also selected. The pre-acidified WPI had low but distinct soapy flavor (mean 1.2-1.6 on 0 to 15 point intensity scale) when rehydrated (pH 3.3). However, when these WPI were neutralized to pH 6.3, soapy flavor was not detected (Table 2, Figure 2). The neutral WPI (pH 6.3) had no soapiness except for WPI 6 (Table 3, Figure 3). This WPI had a pH on rehydration of 7.1 ±0.1, higher ($p<0.05$) than the other WPI (6.2±0.4) which may explain the perceived soapy flavor. This WPI was also an anion exchange and the soapy flavor (and higher pH) may also be due to the distinct manufacture process which involves a salt or mild pH adjustment rather than traditional microfiltration-produced WPI (Carter and Drake, 2018). When the neutral pH WPI were acidified with phosphoric or citric acid, a soapy flavor was evident, and the soapy flavor increased in WPI 6 (Table 3, Figure 3).

Previously, Park et al. (2014) reported that when liquid WPC80 retentate was acidified to pH 3.5 with a blend of phosphoric and citric acids prior to spray drying, soapy flavor was evident in the reconstituted pH 3.5 powder and was not detected in the same liquid WPC80 retentate acidified to pH 5.5 or 6.5 prior to spray drying. Concurrently, the WPC80 powders that were spray dried at pH 5.5 or 6.5 had soapy flavor when the reconstituted powder was acidified to pH 3.5 prior to sensory evaluation. Park et al. (2014) noted that the process of pre acidification reduced neutral volatile aldehydes and cardboard flavor in the spray dried (pH 3.5) powder. They hypothesized that the process of pH reduction increased partitioning of volatile neutral aldehydes into the headspace and allowed more loss of these compounds during spray drying. Park et al. (2014) also noted that the source of soapy flavor was unknown. Soapy flavor has been previously documented in WPC80, WPI and serum protein concentrates that were not under acidic conditions (Drake et al., 2009; Evans et al., 2010), as also documented in WPI 6 in the current study. Acidic conditions (namely the acidulants) are not the source of the soapy flavor. The acidulants in the current study were evaluated at pH 3.3 prior to use with WPI, and neither were determined to have a soapy flavor (results not shown). Soapy flavor was documented in pre acidified WPI, but also when neutral pH WPI were acidified with different acidulants (phosphoric and citric acid) to pH 3.3. For the single neutral pH WPI that had a low intensity of soapy flavor at a neutral pH, acidification increased soapy flavor intensity (Tables 2, 3 and Figures 1, 2). Decreased pH increased the prevalence and intensity of soapy flavor in the current study and in other studies (Park et al., White et al., 2013).

Identification and Quantitation of Octanoic, Decanoic and Dodecanoic Acids

Medium chain FFAs have been documented previously in whey and serum proteins (White et al., 2013; Evans et al., 2010; Carunchia Whetstine et al., 2005; Mortenson et al., 2008). These acids have a distinct soapy aroma and flavor (Carunchia Whetstine et al., 2005; McDaniel et al., 1969) and have been used as trained panel references for soapy flavor (Cadwallader et al., 2007; Whetstine et al., 2005). These three acids were the focus of the current study. Concentrations of octanoic, decanoic and dodecanoic acids were different among WPI but were within a similar range in both pre acidified and neutral WPI (Table 4). All three acids were also aroma-active in the 6 selected WPI (Table 5) and each acid was described as soapy in aroma at the sniffer port.

Carunchia Whetstine et al. (2005) reported that WPI that had a higher relative abundance of octanoic and decanoic acids had a higher intensity of soapy flavor. Their results suggested that these two acids contributed to the soapy flavor of WPI (Carunchia Whetstine et al., 2005). However, Carunchia Whetstine et al. (2005) did not evaluate pre-acidified WPI nor did they quantify these medium chain fatty acids. They also did not report acids higher than C6 as aroma active. Carunchia Whetstine et al. (2005) reported the relative abundance of octanoic and decanoic acids in WPI from .089 to 39 ppm and 9.5 to 157 ppm, respectively. These previous relative abundance results are within the range of the quantitation results in the current study (Table 4).

Short-chain FFAs are present naturally in milk fat at very low concentrations (Cadwallader et al., 2007) and are also present in fluid whey (Karagül-Yüceer et al., 2006; Tomaino et al., 2001). Campbell et al. (2011b) reported that skim milk and wheys from skim milk had higher concentrations of FFA than whole milk and wheys from whole milk; it has also been reported that skim milk has a higher amount of phospholipids than whole milk (Campbell et al., 2011b; Morr and Ha 1993). Carunchia Whetstine et al. (2003) confirmed that FFA concentration can be affected by a number of factors including milk source (percent fat, cow breed, feed type), and starter culture (mesophilic vs thermophilic) along with processing and handling (Campbell et al., 2011a; Croissant et al., 2007; Carunchia Whetstine et al., 2003). Drake et al. (2009) confirmed that the starter cultures used for cheesemaking (thermophilic vs mesophilic) will change the sensory perception and volatile compound make-up of the liquid whey. Tomaino et al. (2001) showed distinct differences in liquid whey depending on what starter cultures were used; liquid whey made with *Lactococcus lactic* subsp. *Lactis* had the highest concentration of FFAs of the starter cultures evaluated in their study (Tomaino et al., 2001). Liaw et al. (2011) studied flavor and flavor chemistry differences of wheys from Cheddar and Mozzarella production and found that Cheddar whey had the highest levels of lipid oxidation. While levels of lipid oxidation do not directly relate to FFA concentration, it further shows how starter culture and cheese type can affect the compositional make up of liquid whey (Liaw et al., 2011). FFA content in dairy products and fluid whey can also increase with the addition of lipases, which may be used in Italian and Feta cheese manufacture (Drake et al., 2009). Lipases can increase the concentration of FFAs present in the liquid whey along with imparting undesirable flavors (Drake et al., 2009).

Threshold Determination of Octanoic, Decanoic and Dodecanoic Acids

Orthonasal detection best estimate thresholds (BET) for octanoic and decanoic acids in water-based buffer solutions were lower than previously reported at similar pH ranges (Baldwin et al., 1973, Table 6). Baldwin et al. (1973) did not lid their samples after pouring, nor did they use an established threshold procedure such as the American Society for Testing and Materials

(ASTM) procedure E679–9 (ASTM, 2004) utilized in the current study. Orthonasal thresholds for dodecanoic acid were higher than thresholds for decanoic or octanoic acids, as expected (Table 6). Dodecanoic acid has a higher molecular weight than octanoic and decanoic acids, making it less volatile than shorter chained fatty acids. Dodecanoic acid also has lower solubility in water and was dissolved in a 40% ethanol for orthonasal threshold testing. Retronasal thresholds for dodecanoic acid were not determined due to the interference of alcohol burn that would result from evaluating a sample with such a high alcohol content. Thresholds for octanoic and decanoic acids were higher for retronasal conditions than for orthonasal conditions (Tables 6 and 7). Other studies with thresholds have also reported some compounds having a higher retronasal threshold than orthonasal BET values (Piornos et al., 2019). These align with the theory of the duality of the olfactory sense, which essentially states that the route of detection changes the perception of the odor (Rozin, 1982). As expected, the BET values of the three acids were lower under acidic conditions than at neutral pH (Tables 6 and 7). FFAs are more easily detectable at a lower pH because the anions are protonated in an acidified pH, which increases volatility of FFAs by decreasing polarity (Bills et al., 1969). The concentration of these acids in WPI (Table 4) fall within a similar range regardless of initial WPI pH. Taking into account sensory thresholds, these acids could be flavor active at low pH and not detected at a neutral pH.

Model System Confirmation

Whey protein model systems containing added concentrations of the targeted FFA (octanoic and decanoic) were prepared to further confirm specific contributions to soapy flavor in clear acidic whey protein beverages. The model systems were evaluated by a trained sensory panel at neutral (pH 6.3) and acidic (pH 3.3) conditions with and without the addition of each fatty acid alone and in combination. The fatty acids were added at the average concentration found in WPI. Commercial pre-acidified WPI was used as a control and point of reference for the trained panel. Addition of C8 or C10 to pre-acidified WPI increased soapy flavor intensity and neutralization of these same solutions to pH 6.3 resulted in no detectable soapy flavor (Table 8). Neutral pH WPI acidified with either citric or phosphoric acid had a low intensity of soapy flavor, presumably due to native fatty acids present in the WPI. When C8 or C10 were added to neutral pH WPI and acidified, soapy flavor intensity increased compared to WPI that was acidified only (Table 8). The panel described octanoic acid as having an earthy/soapy flavor, whereas decanoic acid had an ivory soap bar flavor and aroma. Dodecanoic acid was not tested in model systems due to its low solubility. Trained panelists noted that decanoic acid alone was the closest sensory match in soapy character to the pre-acidified reference WPI ($p<0.05$). Addition of these acids alone or in combination to neutral pH WPI elicited no detectable soapy flavor (Table 8). These WPI model systems confirm the role of octanoic and decanoic acids as contributors to soapy flavor in acidified whey protein beverages.

Soapy flavor was evident in acidified WPI, regardless of the acidulant used (Table 8). WPI acidified with citric acid to pH 3.3 that contained C8+C10 matched closer to the pre-acidified soapy WPI reference than when the acids were added individually to WPI acidified with citric acid ($p<0.05$). In contrast, all combinations of acids added to WPI acidified with phosphoric acid were similar to the reference pre-acidified WPI ($p>0.05$). Soapy intensity was the highest for the WPI that was acidified (pH 3.3) with phosphoric acid and spiked with C8+C10 ($p<0.05$), pre-acidified (pH 3.3) WPI spiked with C8+C10 was second highest ($p<0.05$), and WPI acidified with citric acid (pH 3.3) spiked with C8+C10 was the third most intense soapy flavor ($p<0.05$).

Previous studies have shown that pH plays a large role in the flavor profile of dairy foods. Woo et al. (1984) analyzed the FFA concentration of various cheeses. Limburger cheese was found to have around the same amount of FFA as other cheeses evaluated; however, the flavor of the Limburger cheese did not indicate a presence of FFA (eg rancid flavor); this was likely due to the pH of this cheese being much higher than the others at 6.97 (Woo et al., 1984). The higher pH results in a greater amount of the ionized form of the fatty acids which makes them less flavor active (Woo et al., 1984). Under acidic conditions (pH 1-1.5), FFA aromas were much more abundant, presumably due to the acids being primarily in protonated form (Woo et al., 1984). White et al. (2013) reported that WPIs that were heated and acidified (pH 3.2) had higher soapy flavor than a neutral pH control. Evans et al. (2010) manufactured acidic protein beverages using WPC80 and reported low intensities of soapy flavor; however when the same WPC80 was rehydrated and adjusted to a neutral pH, there was no soapy flavor documented (Evans et al., 2010). These studies show a direct relationship between soapy flavor and pH in whey protein that was evident with FFAs and pH in cheeses (Woo et al., 1984). The current study demonstrates a similar relationship between medium chain FFAs, namely octanoic and decanoic acids, in WPI and pH. These acids are present at variable concentrations in spray dried whey proteins. At their concentrations under neutral pH, they are below sensory detection. However, under acidic conditions, concentrations may be sufficient to be at flavor active concentrations (eg concentrations > sensory BET values). More in depth studies relating to modifications of whey protein processing could be done to identify possible processing changes to minimize soapy off flavor in pre-acidified WPI. One key component of whey processing that can be evaluated is the starter cultures used for cheesemaking and/or addition of lipases. When choosing the type of whey to use to produce pre-acidified WPI, manufacturers should choose whey that is known to have a lower fatty acid profile. These results can specifically aid in the production of pre-acidified WPI for appealing clear acidic protein beverages.

Conclusion

Soapy flavor was present in WPI under acidified conditions (pH 3.3) with and without addition of octanoic and decanoic acids and regardless of acidulant used. Results from this study suggest that the soapy flavor in acidified whey protein beverages is caused by a mixture of low concentrations of octanoic and decanoic acids associated with the (spray dried) whey protein. While dodecanoic acid has been found to have a soapy aroma and flavor, it is likely not the cause of this off flavor due to its low solubility in water. Orthonasal and retronasal threshold results along with sensory analysis of model systems show that these acids are not flavor active at neutral pH, but when the pH of a whey protein solution is decreased to pH 3.3, a soapy flavor is evident.

Acknowledgements

Funding provided in part by Dairy West (Meridian, ID) and Dairy Management, Inc. (Rosemont, IL).

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Table 2.1. pH of selected rehydrated WPI (10% w/v) at 21°C

Condition	Sample	pH
Pre-acidified	1	3.3c
	2	3.4c
	3*	3.3c
Neutral	4	6.2b
	5	6.3b
	6*	7.1a

pH readings of each rehydrated sample were taken in triplicate from duplicate lots
Different letters in columns following means signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$).
*Indicates ion exchange WPI

Table 2.2. Trained panel soapy flavor intensity of selected rehydrated (10% w/v) pre-acidified (pH 3.3) WPI at acidic and neutral pH at 21°C

Sample	at pH 3.3	at pH 6.3
1	1.6a	ND
2	1.2c	ND
3	1.5b	ND

Samples were evaluated by a trained panel (n=7) in duplicate using a 0-15 point intensity scale
Different letters in columns following means signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$). ND – not detected

Table 2.3. Trained panel soapy flavor intensity of selected rehydrated (10%w/v) neutral WPI at acidic and neutral pH evaluated at 21°C

Sample	at pH 6.3	at pH 3.3 (Phosphoric Acid)	at pH 3.3 (Citric Acid)
4	ND	1.5b	1.3b
5	ND	0.8c	1.3b
6	1.0	2.6a	1.6a

Samples were evaluated by a trained panel (n=7) in duplicate using a 0-15 point intensity scale at 21°C
Different letters in columns following means signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$). ND – not detected

Table 2.4. Mean concentrations of selected volatile free fatty acids in WPI

	WPI	Concentration of acid (ppm) mg/kg		
		Octanoic	Decanoic	Dodecanoic
Pre-Acidified WPI	Sample 1	16.06a ± 0.30	28.84ab ±1.55	25.92a ± 0.84
	Sample 2	13.66bc ± 1.21	27.35bc ± 1.58	26.52a ± 2.58
	Sample 3	13.02c ± 0.55	26.44c ± 2.11	23.66b ± 0.69
Neutral WPI	Sample 4	16.49a ± 2.10	30.21a ± 0.93	22.71b ± 0.72
	Sample 5	13.57bc ± 2.55	26.7bc ± 3.47	25.83a ± 3.39
	Sample 6	14.85ab ± 1.67	28.41abc ± 1.21	23.76b ± 0.83

Samples were evaluated by triplicate extractions and injections

Different letters in columns following means signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$).

Table 2.5. Mean aroma intensity of selected volatile free fatty acids identified in WPI by SAFE-GCO

Compound	Odor[1]	RI		Pre-Acidified (pH 3.3) Powders Signal Intensity						Neutral (Ph 6.3) Powders Signal Intensity					
				1		2		3		4		5		6	
		DB-5	Wax	DB 5	Wax	DB5	Wax	DB5	Wax	DB5	Wax	DB5	Wax	DB5	Wax
Octanoic Acid	FFA, acidic, green, soapy	1191	2074	2.5	1.8	2.4	2.0	3.0	2.4	2.0	2.4	2.8	1.7	4.0	2.0
Decanoic Acid	sweaty, acidic, soapy, FFA, waxy	1391	2294	1.6	1.8	1.8	1.9	2.8	2.0	2.6	2.0	2.5	1.8	2.5	1.8
Dodecanoic Acid	FFA, green, sour, soapy	1562	2513	1.5	2.4	1.7	1.5	1.8	1.8	1.8	1.8	2.2	1.4	2.5	1.5

[1]Odor description at the gas chromatograph (GC) sniffing port.
Each sample was evaluated in duplicate on each column by 2 trained sniffers, each with >50 hours of training
Sniffing intensities were scaled using a 1-7 intensity scale where 1=very weak and 7=very strong.
RI- Retention index

Table 2.6. Orthonasal best estimate thresholds (BET) (mg/kg) results at 21°C

Compound	Orthonasal BET Acidic (pH 3.3) (mg/kg)	Orthonasal BET Neutral (pH 6.3) (mg/kg)
Octanoic Acid	0.42b ± 0.32	2.34a ± 0.42
Decanoic Acid	0.12b ± 0.38	2.82a ± 0.41
Dodecanoic Acid	28.98b ± 0.41	141.35a ± 0.36

Different letters in rows following means signify significant differences ($p < 0.05$)
Octanoic acid (n=45); decanoic acid (n=60); dodecanoic acid (n=40)

Table 2.7. Retronasal best estimate thresholds (BET) (mg/kg) results at 21°C

Compound	Retronasal BET Acidic (pH 3.3) (mg/kg)	Retronasal BET Neutral (pH 6.3) (mg/kg)
Octanoic Acid	0.90b ± 0.41	3.91a ± 0.12
Decanoic Acid	0.45b ± 0.55	3.05a ± 0.23

Dodecanoic acid retronasal thresholds were not performed due to low solubility of C12 in water.
Different letters in rows following means signify significant differences ($p < 0.05$)
Octanoic acid (n=45); decanoic acid (n=35)

Table 2.8. Mean descriptive sensory profiles of rehydrated WPI (10% w/v) model systems at 21°C

Sample	Soapy Intensity	Similarity to Reference Pre-Acidified (pH 3.3) WPI (0-10)
Pre-Acidified WPI (pH 3.3)	1.1i	-
Neutral WPI (pH 6.3)	ND	0.0f
WPI acidified with citric acid (pH 3.3)	1.1i	8.5e
WPI acidified with phosphoric acid (pH 3.3)	1.7g	9.0cd
Neutral WPI (pH 6.3) with C8	ND	0.0f
Neutral WPI (pH 6.3) with C10	ND	0.0f
Neutral WPI (pH 6.3) with C8+10	ND	0.0f
Neutralized Pre-Acidified WPI (pH 6.3)	ND	0.0f
Neutralized Pre-Acidified WPI (pH 6.3) with C8	ND	0.0f
Neutralized Pre-Acidified WPI (pH 6.3) with C10	ND	0.0f
Neutralized Pre-Acidified WPI (pH 6.3) with C8+10	ND	0.0f
Pre-Acidified WPI (pH 3.3) with C8	2.0f	9.9a
Pre-Acidified WPI (pH 3.3) with C10	2.9d	10.0a
Pre-Acidified WPI (pH 3.3) with C8+10	3.4b	10.0a
WPI acidified with citric acid (pH 3.3) with C8	1.4h	8.9d
WPI acidified with citric acid (pH 3.3) with C10	2.5e	9.2c
WPI acidified with citric acid (pH 3.3) with C8+10	3.1c	9.6b
WPI acidified with phosphoric acid (pH 3.3) with C8	1.4h	9.5b
WPI acidified with phosphoric acid (pH 3.3) with C10	3.5b	9.6b
WPI acidified with phosphoric acid (pH 3.3) with C8+10	3.8a	9.6b

Samples were evaluated by a trained panel (n=7) in duplicate
Means in the same column followed by a different letter differ ($P<0.05$)
Amounts of each acid added to WPI solution for each combination: C8= 14.39 mg/kg; C10=27.78 mg/kg
ND = not detected

Figure 2.1. Experimental Overview

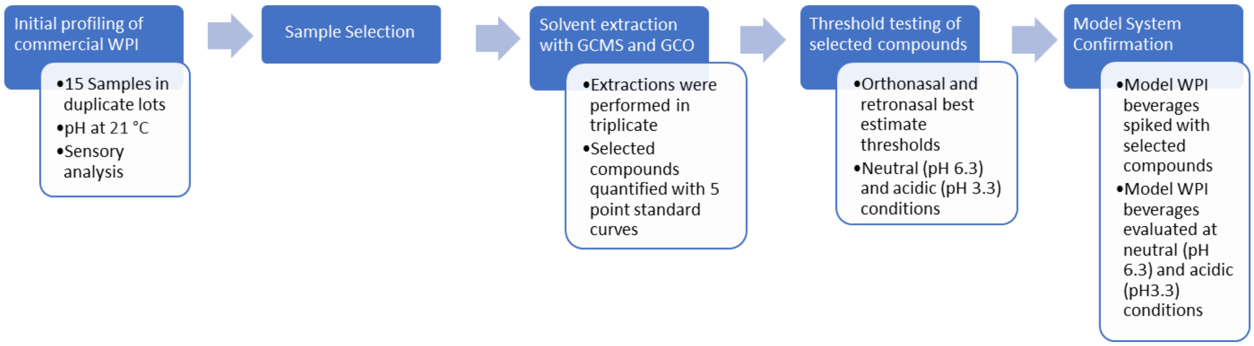
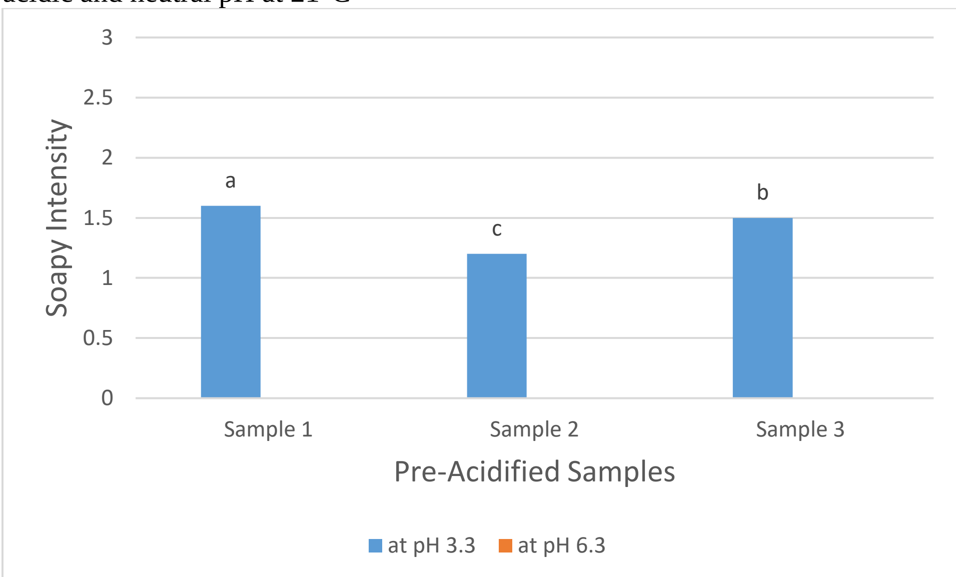
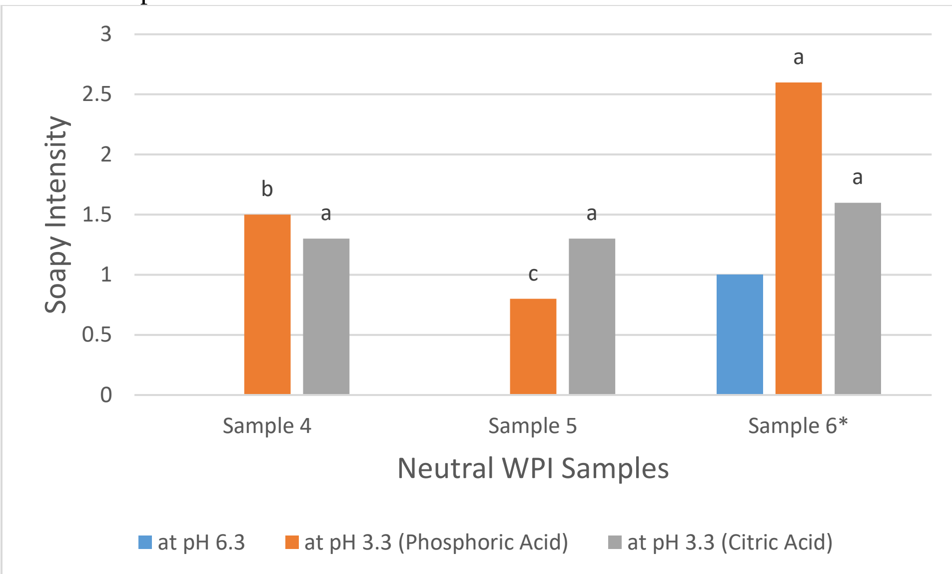


Figure 2.2. Trained panel soapy flavor intensity of selected rehydrated (10%w/v) pre-acidified WPI at acidic and neutral pH at 21°C



Samples were evaluated by a trained panel (n=7) in duplicate using a 0-15 point intensity scale at 21°C
Different letters within each set of data signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$).
*Indicates ion exchange WPI

Figure 2.3. Trained panel soapy flavor intensity of selected rehydrated (10%w/v) neutral WPI at acidic and neutral pH evaluated at 21°C



Samples were evaluated by a trained panel (n=7) in duplicate using a 0-15 point intensity scale at 21°C
Different letters in columns following means signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$).
*Indicates ion exchange WPI

APPENDICES

Appendix 1. Trained panel sensory attribute means of rehydrated (regular) commercial WPI

Sample	Aromatics/Flavor						Basic Tastes			
	Aroma Intensity	Sweet Aromatic	Sour Aromatic	Card board	Potato Brothy	Cabbage Brothy	Soapy	Sour	Bitter	Astringency
1*	2.4a	ND	ND	2.6a	1.0a	0.6a	ND	ND	ND	1.9bcd
2*	2.0c	0.8a	ND	2.1c	ND	ND	ND	ND	ND	1.7e
3*	1.0f	ND	ND	1.1h	ND	ND	1.0a	ND	1.0a	2.0bc
4	1.8d	0.5b	ND	1.6fg	ND	ND	0.5b	ND	ND	1.9cd
5	2.4a	ND	ND	2.4b	ND	ND	ND	ND	ND	2.5a
6	1.5e	ND	ND	1.7ef	ND	ND	0.9a	ND	0.7b	2.0b
7	1.8d	0.5b	ND	1.7de	ND	ND	ND	ND	0.6c	1.9cd
8	1.9c	0.9a	ND	1.8d	ND	ND	ND	ND	ND	1.9d
9	2.1b	ND	ND	2.3b	ND	ND	ND	ND	ND	2.5a
10	1.5e	ND	ND	1.5g	ND	ND	ND	ND	ND	1.7e

*indicates sample that was chosen for further analysis. Samples 3 and 6 are ion exchange WPI.
Samples were evaluated by a trained panel (n=7) in duplicate using a 0-15 point intensity scale
Different letters in columns following means signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$). ND – not detected

Appendix 2. Trained panel sensory attribute means of rehydrated pre-acidified commercial WPI

Sample	Aromatics/Flavor						Basic Tastes			
	Aroma Intensity	Sweet Aromatic	Sour Aromatic	Card board	Potato Brothy	Cabbage Brothy	Soapy	Sour	Bitter	Astringency
1*	2.5a	ND	ND	2.0b	ND	ND	1.6a	3.3b	ND	3.7b
2*	2.0c	ND	1.1a	1.6c	ND	ND	1.2c	3.7a	0.9a	3.5c
3*	1.8d	ND	1.0a	1.0d	ND	ND	1.5b	3.8a	ND	4.0a
4	2.1b	ND	ND	2.2a	ND	ND	1.4b	3.2b	ND	4.0a

*indicates samples that were chosen for further analysis. Sample 3 is ion exchange WPI.
Samples were evaluated by a trained panel (n=7) in duplicate using a 0-15 point intensity scale
Different letters in columns following means signify significant differences ($p < 0.05$). If same letters occur, then there is no significant difference between those samples ($P > 0.05$). ND – not detected

Appendix 3. Standard Curves
Standard Curve for Quantitation of Octanoic acid

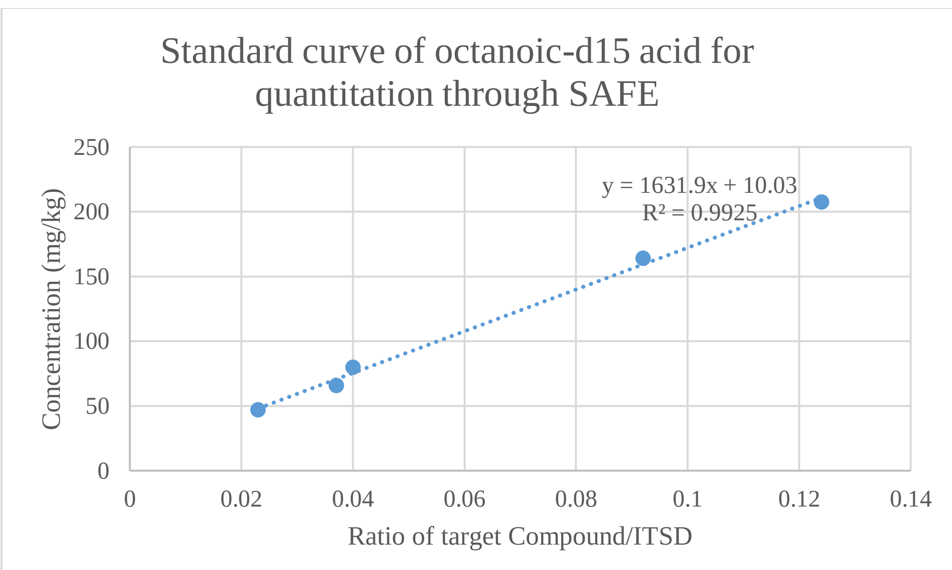
Standard: Octanoic acid-d15

CAS: 69974-55-6

Mfg/Reference: Sigma-Aldrich (St. Louis, MO)

Concentration of Octanoic-d15 acid (mg/kg)	Avg Ratio of Analyte/ ITSD
47	0.023
65.8	0.037
79.9	0.04
164	0.092
207.5	0.124

Extractions for each concentration was performed in duplicate
Triplicate injections of each replicate were performed



Extractions for each concentration was performed in duplicate
Triplicate injections of each replicate were performed

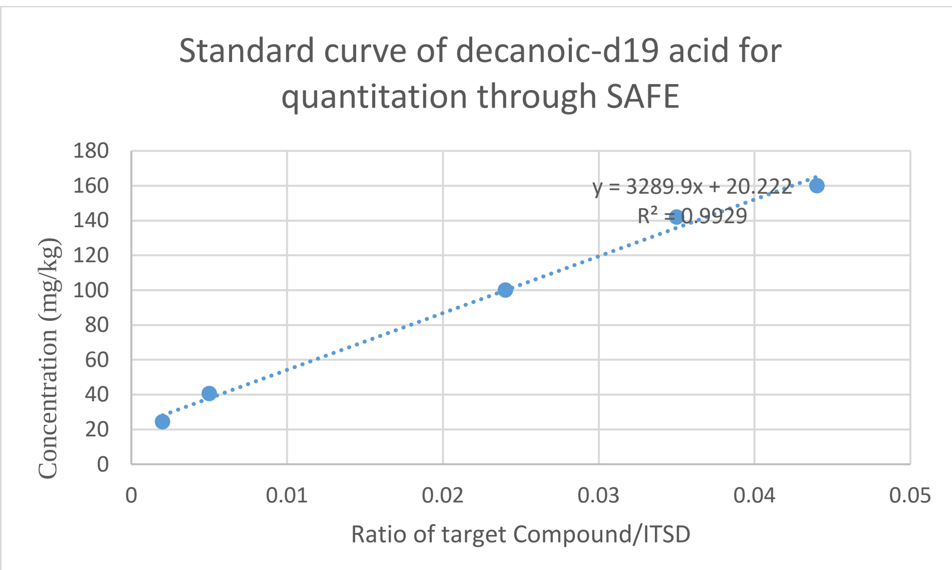
Standard Curve for Quantitation of Decanoic and Dodecanoic Acids

Standard: Decanoic-d19 acid
CAS: 88170-22-3

Mfg/Reference: CDN Isotopes (Pointe-Claire, Quebec, Canada)

Concentration of Decanoic-d19 acid (mg/kg)	Avg Ratio of Analyte/ ITSD
24.3	0.002
40.5	0.005
100	0.024
142	0.035
160	0.044

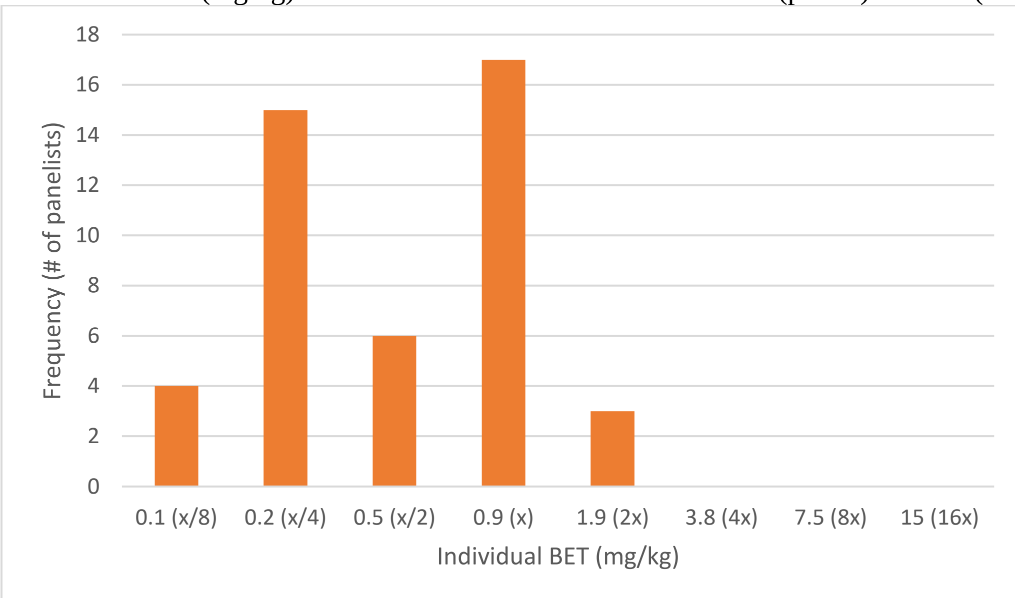
Extractions for each concentration was performed in duplicate
Triplicate injections of each replicate were performed



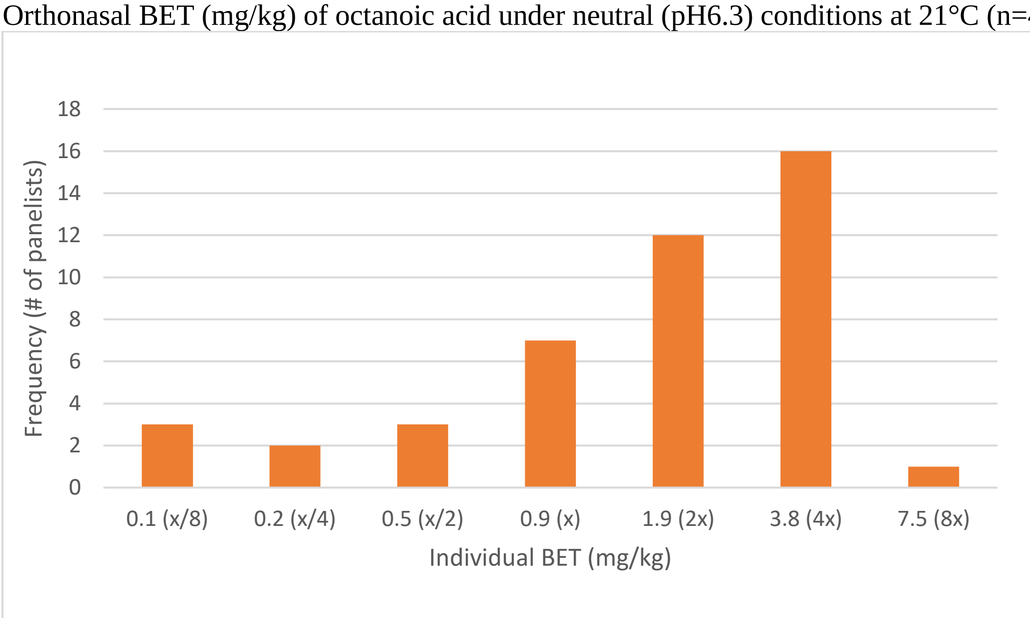
Extractions for each concentration was performed in duplicate
Triplicate injections of each replicate were performed

Appendix 4. Figures of Orthonasal Threshold Results for Target Acids

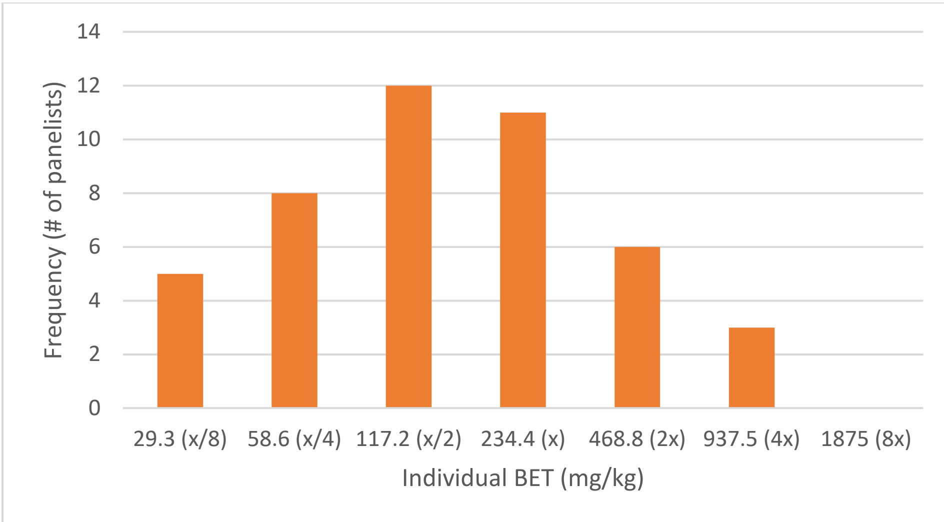
Orthonasal BET (mg/kg) of octanoic acid under acidic conditions (pH3.3) at 21°C (n=45)



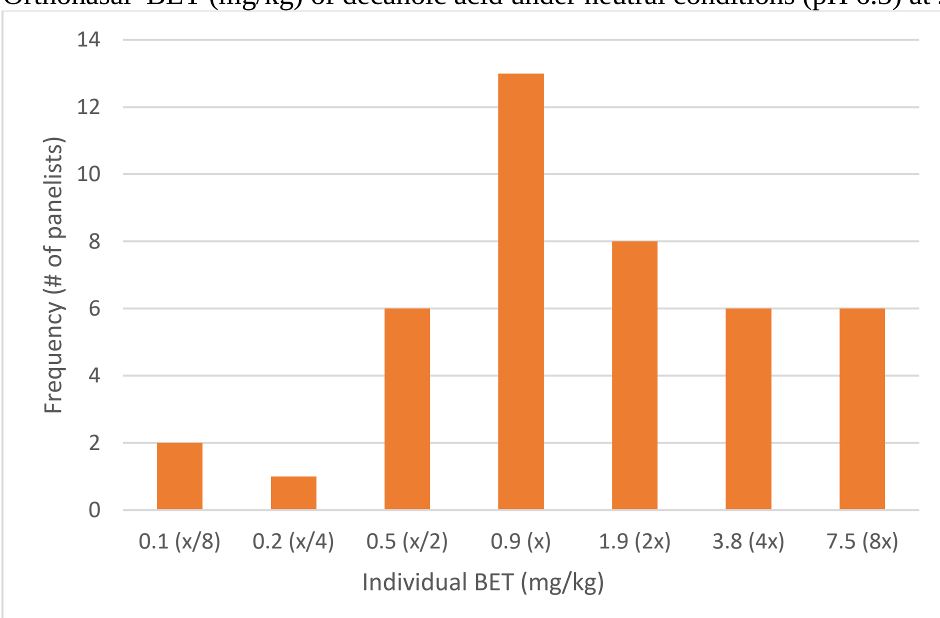
Orthonasal BET (mg/kg) of octanoic acid under neutral (pH6.3) conditions at 21°C (n=45)



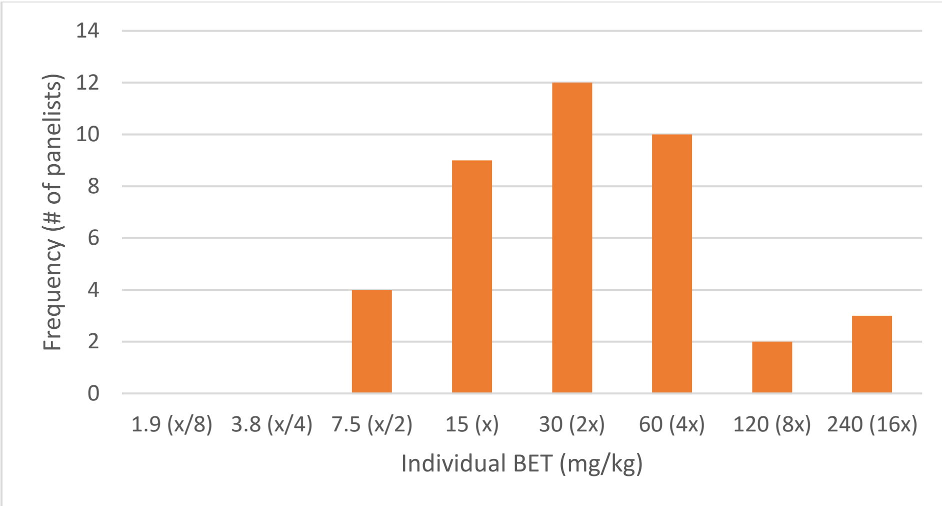
Orthonasal BET (mg/kg) of decanoic acid under acidic conditions (pH 3.3) at 21°C (n=60)



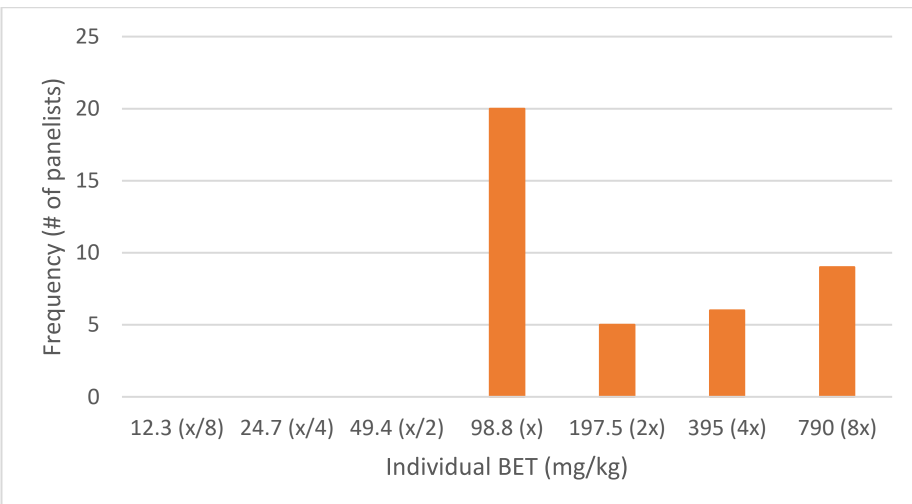
Orthonasal BET (mg/kg) of decanoic acid under neutral conditions (pH 6.3) at 21°C (n=60)



Orthonasal BET (mg/kg) of dodecanoic acid under acidic conditions (pH 3.3) at 21°C (n=40)

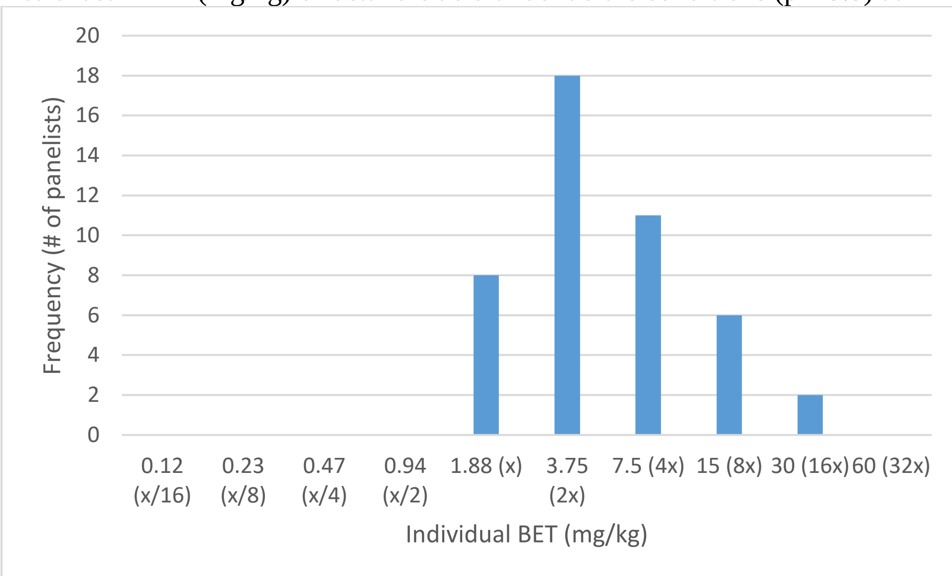


Orthonasal BET (mg/kg) of dodecanoic acid under neutral conditions (pH 6.3) at 21°C (n=40)

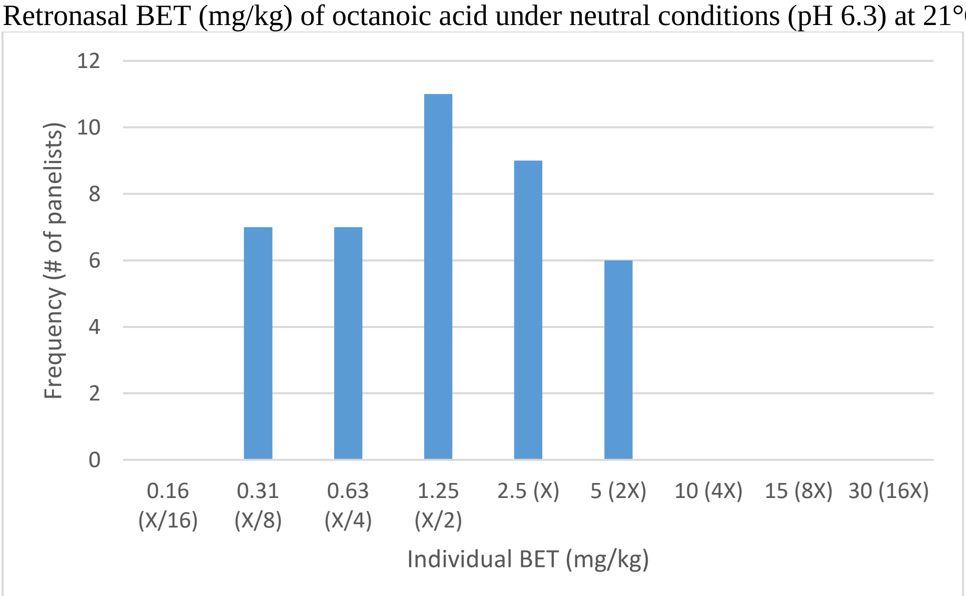


Appendix 5. Figures of Retronasal Threshold Results for Target Acids

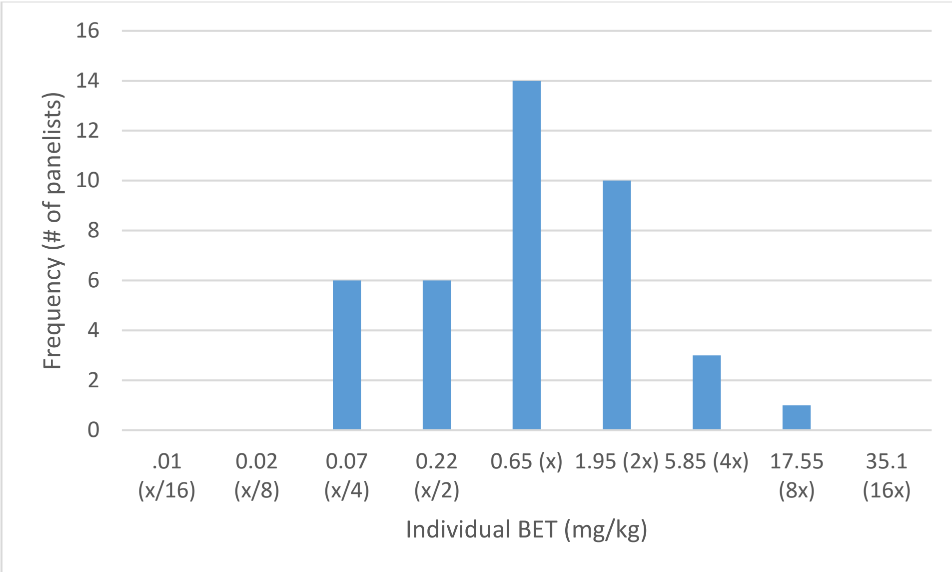
Retronasal BET (mg/kg) of octanoic acid under acidic conditions (pH 3.3) at 21°C (n=45)



Retronasal BET (mg/kg) of octanoic acid under neutral conditions (pH 6.3) at 21°C (n=40)



Retronasal BET (mg/kg) of decanoic acid under acidic conditions (pH 3.3) at 21°C (n=40)



Retronasal BET (mg/kg) of decanoic acid under neutral conditions (pH 6.3) at 21°C (n=35)