Comparing Texture and Mouthfeel Characteristics of Plant and Animal-Based Beverages, Relating Them Back to Oral Tactile Sensitivity

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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2024

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Abstract

In response to high consumer demand, the food industry is increasingly focused on developing plant-based beverages (PBMA) that mimic the desirable sensory properties of animal-based beverages. While extensive research has characterized the differences in appearance, aroma, taste, and flavor between PBMAs and cow's milk, limited research exists that comprehensively characterizes the textural and mouthfeel differences between these two types of beverages. Moreover, the complexity of texture and mouthfeel perception poses a challenge in formulating these beverages. Formulation changes that have minimal impact on analytical measurements can still result in significant perceptual differences in texture and mouthfeel sensations. Finally, there has been little research exploring the mechanical underpinnings of textural and mouthfeel perception of food within oral cavity. To address these gaps, this dissertation aimed to comprehensively characterize textural and mouthfeel differences between PBMAs and cow's milk and relate these sensations back to oral tactile sensitivity.

Utilizing a "top-down" approach, trained panelists were used to develop a comprehensive texture and mouthfeel lexicon to characterize sensory differences between animal and plant-based beverages. Sixteen unique texture and mouthfeel attributes were identified and used by trained panelists to evaluate 14 different liquid beverages, categorized by protein content: low protein (LP; 8g of protein/8fl. oz) and high protein (HP; 13g of protein/8fl. oz). Each beverage group included two types of animal-based beverages (commercial skim milk [CSM] and milk protein isolate [MPI]) and five types of plant-based beverages (pea protein isolate [PPI], soy protein concentrate [SPC] and three types of soy protein isolates [SPI 1-3]). Textural and mouthfeel similarities were

evident between LP animal-based beverages, while only nuanced differences were observed within the LP-SPIs. In contrast, LP-SPC was significantly different in 8 out of the 16 attributes compared to other LP beverages. Similarly, HP animal-based beverages exhibited comparable textural and mouthfeel characteristics, with small differences observed within the HP-SPIs. HP-SPC significantly differed in 9 out of the 16 attributes compared to other HP beverages. Overall, the trends observed among the different protein in LP beverages were reflected in HP beverages. These findings emphasize that textural and mouthfeel differences between plant and animal-based beverages are mainly driven by the type of protein used, rather than by protein concentration.

A "bottom-up" approach was then used to explore the relationship between oral tactile sensitivity and texture perception (Chapter 4). Thirty-four panelists assessed the astringency, mouth coating, and smoothness of LP-SPC and LP-CSM, along with suprathreshold oral tactile sensitivity to lingual/rugal roughness, lingual punctate pressure, thickness, and grittiness. Significantly correlations were observed between rugal roughness sensitivity and perceived astringency ($r=0.45$, $p=0.001$), tongue roughness sensitivity and perceived mouth coating ($r=0.38$, $p=0.02$), stimulus thickness sensitivity and perceived mouth coating ($r=0.44$, $p=0.01$), and stimuli grittiness sensitivity and perceived smoothness ($r=-0.38$, $p=0.03$). These findings suggest that suprathreshold mechanosensitivity of oral tissues contributes to the perception of astringency, mouth coating, and smoothness in beverages, emphasizing the importance of considering multiple oral surfaces and tactile stimuli in texture and mouthfeel research.

Lastly, Chapter 5 built upon the findings in Chapter 4 by delving deeper into the mechanisms underpinning astringency perception in the human oral cavity. Chapter 5

investigated the role of transient receptor potential (TRP) channels in astringency perception within the oral cavity. Thirty-seven panelists underwent unilateral lingual desensitisation of TRPA1 and TRPV1 channels using mustard oil and capsaicin, respectively. Subsequently, panelists evaluated four astringent stimuli: epicatechin (EC), epigallocatechin gallate (EGCG), potassium alum (Alum), and tannic acid (TA) using 2-AFC and intensity ratings. When TRPA1 receptors were desensitized via mustard oil, no significant differences were observed between the treated and untreated sides for both 2-AFC and intensity ratings. Similarly, when TRPV1 receptors were desensitized via capsaicin, no significant differences were observed between the treated and untreated sides for both 2-AFC (except TA) and intensity ratings. These findings challenge previous suggestions that TRP channels playing a pivotal role in astringency perception in the human oral cavity.

In summary, this dissertation addresses the critical gap in understanding the textural and mouthfeel differences between plant and animal-based beverages. By utilizing both “top-down” and “bottom-up” approaches, we provide a comprehensive understanding behind sensory differences between these beverages. Overall, these findings provide valuable insight to guide the development of the next generation of PBMAs as suitable alternatives to cow’s milk.

Dedication

To my loving parents who has never given up on me once, despite all my struggles and failures, you always believed in me. The journey of your immigrant life will be forever remembered and will live on through my life.

To all the people, young and old, who just need a second chance in life, that chance will come. When it does, grab onto it for dear life and run with it. Don't let anyone tell you that you can't do something, because with passion, perseverance, and a little bit of luck, anything is possible. Never give up… Hwaiting!!!

Acknowledgements

Reflecting on my journey, its astonishing to see how far I've come. From nearly drooping out as an undergrad and graduating with a 1.7. GPA, the turnaround from such a low point to reaching the highest level of academic education is nothing short of miraculous. None of this would have been achievable without the support of my community who have helped get past through the hard times and celebrated with me in the good times. With that said, I want to thank everyone who has made this incredible journey possible.

First and foremost, I'd first like to extend my deepest gratitude to my advisor Dr. Christopher T. Simons, for his unwavering and endless support. You are someone that I respect, admire, and who inspires me to be the best researcher I can be. I cannot thank you enough for the amazing opportunity you have given me, and I will never forget your guidance, encouragement, and camaraderie throughout the last three years. I would also like to thank my committee members for Dr. Devin G. Peterson, Dr. Osvaldo Campanella, and Dr. Rafael Jimenez-Flores, for their guidance and time. I would also like to thank my dearest friends back home in Canada (Sean, Nicole, and Doren) and to the new friends I've made here in Ohio (Ellia, Josh, Alice, Ben, and John). I never thought I would say this, but thanks to the memories created here, I will always cherish Ohio as my second home. I would also like to thank my lab members, past and present, for the memories made at conferences and always sharing and listening to my passion for sensory science. I would also like to thank all the FST staff members, particularly Kelly Elisar and Peggy Grgich, without whom I would have never been able to run any of my studies.

I would also like to thank my family. My brother Kenny, although we've had our differences, you have always been there to support me in any way that you can. To my mom and dad, thank you for immigrating to Canada and leaving everything behind in Korea to give your children a better life. Without your sacrifices, I would have never learned the skills to think critically, be independent, and most importantly, have the courage to pursue this academic journey. Your unconditional love and dedication to support me, making sure I always have delicious food on the table, have helped me become the person I am today.

Lastly, I would like to thank my partner, Kym. You push me to grow every single day. Without you challenging and pushing me towards excellence, I would never have the physical, emotional, and intellectual growth I have developed over these past few years. Without your trust and support in by ability to succeed, I would never have been able to complete this dissertation. You make me want to become a better person. Thank you, and I love you.

Vita

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2. **Kim, M.S.,** Duizer, L.M., Grygorczyk, A. (2022). Application of a Texture Analyzer friction rig to evaluate complex texture attributes in apples. *Postharvest Biology and Technology,* 186. 111820. https://doi.org/10/1016/j.postharvbio.2021.111820

3. **Kim, M. S.,** Walters, N., Martini, A., Joyner, H. S., Duizer, L., & Grygorczyk, A. (2020). Adapting tribology for use in sensory studies on hard food: the case of texture perception in apples. *Food Quality and Preference, 86*. 103990. https://doi.org/10/1016/j.foodqual.2020.103990

In Preparation

1. Kim, M.S., Nattress, L., & Simons, C.T. Utilization of a texture and mouthfeel lexicon to differentiate plant and animal-based beverages. *Manuscript in preparation*
2. Kim, M.S., Nattress, L., & Simons, C.T. Relating texture perception to suprathreshold oral tactile sensitivity in plant and animal-based beverages. *Manuscript in preparation*
3. Kim, M.S., & Simons, C.T. The role of TRPA1 and TRPV1 in the perception of astringency. *Manuscript in preparation*
4. Kim, M.S., Bao, R., Peterson, J., Tello, E., Peterson D.G., & Simons, C.T. Sensory properties and consumer acceptance of pea protein beverages. *Manuscript in preparation*

Fields of Study

Major: Food Science and Technology

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List of Abbreviations

AFC – Alternate Forced Choice

Alum – Aluminum Potassium Sulfate

ANOVA – Analysis of Variance

AUC – Area Under the Curve

CMC – Carboxymethyl Cellulose

CSM – Commercial Skim Milk

DA – Descriptive Analysis

EC – Epicatechin

EGCG – Epigallocatechin Gallate

FPD – Fungiform Papillae Density

gLMS – Generalized Labelled Magnitude Scale

HP – High Protein

HSD – Honestly Significant Difference

HTST – High Temperature Short Time

JND – Just Noticeable Difference

LTLT – Low Temperature Low Time

MF - Microfiltration

MPI – Milk Protein Isolate

NF - Nanofiltration

PBMA – Plant Based Milk Alternative

PCA – Principal Component Analysis

PPI – Pea Protein Isolate

PRPs – Proline Rich Proteins

QDA – Quantitative Descriptive Analysis

r – Pearson's Correlation Coefficient

RAI – Rapidly Adapting Type I

RAII – Rapidly Adapting Type II

RO – Reverse Osmosis

SAI – Slowly Adapting Type I

SAII - Slowly Adapting Type II

SDA – Spectrum Descriptive Analysis

SPC – Soy Protein Concentrate

SPI – Soy Protein Isolate

TA – Tannic Acid

TRPA1 – Transient Receptor Potential Ankyrin 1

TRPV1 – Transient Receptor Potential Vanilloid 1

UF – Ultrafiltration

UHT – Ultra High Temperature

Chapter 1. Introduction

The food industry has recently shifted to developing a more plant-based food system due to high consumer demand. The interest is driven by factors such as environmental impact, sustainability, dietary restrictions, allergies, and ethical concerns (Haas et al., 2019; McCarthy et al., 2017; Schiano et al., 2020; Sethi et al., 2016). As a result, the past decade has seen significant growth in the diversity of plant-based food and beverage products, with plant-based milk alternatives (PBMA) holding the largest market share, generating $2.9B in sales in the United States in 2023 (The Good Food Institute, 2024). Despite the increased consumption of PBMAs, consumers remain hesitant to fully adopt these beverages over cow's milk due to the undesirable sensory properties (McClements et al., 2019). Consequently, mimicking the desirable sensory characteristics of animal-based proteins remains a challenge as plant-based proteins are comprised of different ingredients and processing methods compared to cow's milk.

The inherent composition of cow's milk (e.g., protein, fats, and sugars) significantly impact its sensory properties. Cow's milk consists of approximately 87% water, 4-5% lactose, 3-4% fat, 3% protein, 0.8% minerals, and 0.1% vitamins (Chalupa-Krebzdak et al., 2018). Different processing methods (e.g., homogenization, pasteurization, and fractionation/separation) are applied depending on the application to change the functionality of cow's milk. These processes not only alter the milk's physicochemical properties, but also significantly affects its sensory characteristics. Despite these factors, cow's milk is typically described as a low viscous fluid, with an opaque creamy white appearance, and a bland flavor profile (Schiano et al., 2017).

PBMAs can be made from various plant-based sources, including, almonds, coconuts, flaxseed, oats, rice, soy, and peas (Sethi et al., 2016). Generally, PBMAs are designed to have similar characteristics to cow's milk, in terms of their appearance, aroma, taste, flavor, texture, and mouthfeel, and are formulated using four primary ingredients: the raw plant source, water, emulsifiers, and additives (e.g., oils, stabilizers, sweeteners, and thickeners) (McClements, 2020; Reyes-Jurado et al., 2021). The sensory characteristics of PBMAs vary significantly based on the plant-based source used and the processing methods used to enhance the functionality of the beverage. Extensive research has characterized the range of appearance, aroma, taste, and flavor differences in PBMAs (Abou-Dobara et al., 2016; Day N'Kouka et al., 2004; Jeske et al., 2019; Liu et al., 2021; Moss et al., 2022; Pointke et al., 2022; Pramudya et al., 2019; Torres-Penaranda & Reitmeier, 2001). However, less emphasis has been placed on identifying the textural and mouthfeel characteristics. This is important as replacing animal-based proteins with plant-based proteins in food and beverages can lead to changes in textural and mouthfeel (Martins & Pliner, 2005; Sha & Xiong, 2020). Therefore, to develop the next generation of PBMAs as suitable alternatives to cow's milk, it is essential to gain a better understanding of the differences in sensory properties between these two types of beverages.

Texture and mouthfeel are complex concepts encompassing various multi-dimensional complex attributes. These attributes play a crucial role in driving consumer acceptance and liking, as well as influencing food aversion (Pellegrino & Luckett, 2020; Spence et al., 2013). Texture is typically described as "the sensory and functional manifestation of the structural, mechanical, and surface properties of food detected

through the senses of vision, hearing, touch, and kinesthetics" (Szczesniak, 1963, 2002), whereas mouthfeel, includes additional sensations that are perceived in food/beverages by somatosensory receptors within the oral cavity (Guinard & Mazzucchelli, 1996).

Previous literature on food texture and mouthfeel research has focused on two strategies. The first is a "top-down" approach, where food product evaluations are made using trained panelists to identify specific sensory characteristics of interest (Linne & Simons, 2017). This strategy combines and averages panelists' data to provide a summary of differences between products for attributes of interest (Piggot et al., 1998). However, this approach offers little information about individual variability or the underlying physiological and psychological mechanisms underpinning these sensations. Alternatively, a "bottom-up" approach focuses on the physiological and/or psychological mechanism that are responsible for eliciting these sensations (Kravchuk et al., 2012; Linne & Simons, 2017). As texture and mouthfeel perception are complex sensations influenced by both the structural breakdown of the food and the processing of these sensations in the human oral cavity, combining the "top-down" and a "bottom-up" approaches will provide a more comprehensive understanding of these sensations.

Chapter 3 will begin by utilizing the "top-down" approach to help identify and characterize the subtle differences unique to plant and animal-based beverages. Specifically, we investigate how the type and concentration of protein affect the texture and mouthfeel sensations of these beverages. A trained descriptive panel will develop a sensory lexicon solely focusing on texture and mouthfeel attributes. Emphasis will be placed on creating unique and distinct descriptions and references to avoid confusing different attributes (Lawless & Heymann, 2010; Lawless & Civille, 2013). Using these

descriptors, the panel then characterized the differences between 14 different liquid beverages grouped by protein content: low protein (8g of protein/8fl. oz) and high protein (13g of protein/8fl. oz). Each beverage group included two types of animal-based beverages (commercial skim milk and milk protein isolate) and five types of plant-based beverages (pea protein isolate, soy protein concentrate, and three types of soy protein isolates). Understanding the nuanced differences in texture and mouthfeel driven by protein concentration and type will provide valuable guidance to product developers. This knowledge will help them focus on specific attributes that differentiate these products, enabling the development of plant-based beverages that more closely resemble their animal-based counterparts. However, this approach only provides insight on the differences in textural and mouthfeel characteristics between plant and animal-based beverages without exploring the underlying mechanisms. For example, while the compounds eliciting astringency are well known, the mechanism of action is highly debated and uncertain. To mitigate astringency in plant-based beverages, a better understanding of the mechanisms that elicit this sensation in critical. Therefore, Chapters 4 and 5 will utilize the "bottom-up" approach to investigate the potential mechanisms subserving these complex sensations.

Oral tactile sensitivity underpins texture and mouthfeel perception, but limited research has investigated oral mechanoreception in relation to these complex sensations. Due to the multi-dimensional nature of these attributes, it is unlikely that a single oral mechanoreceptor codes for a single sensation; rather, a combination of receptors is likely to underpin these complex sensations (Foegeding et al., 2015; Linne & Simons, 2017). The majority of our understanding of oral mechanoreception stems from studies in

glabrous (non-hairy) skin (Foegeding et al., 2015), where mechanosensory neurons process mechanical stimuli (Zimmerman et al., 2014). However, recent studies have quantified oral tactile acuity to various tactile stimuli including punctate pressure, roughness, two-point discrimination, and edge, point, and shape stimuli (Aktar et al., 2015a, 2015b; Bangcuyo & Simons, 2017; Breen et al., 2019; Cattaneo et al., 2020; Linne & Simons, 2017; Miles et al., 2018, 2020; Miles, Berkowitz, et al., 2022; Miles, Wu, et al., 2022; Nishimura et al., 2021). While these studies have characterized sensitivity of oral tissues to tactile stimuli, limited evidence exists linking oral tactile sensitivity to food texture perception. Most studies fail to associate significant relationships between oral mechanosensitivity and food texture and mouthfeel perception (Aktar et al., 2015b, 2015a; Lv et al., 2020). Several limitations may contribute to the lack of significant correlations. First, the majority of these studies measure threshold rather than suprathreshold measurements, whereas consumers typically experience food at suprathreshold levels (Liu et al., 2022). Second, these studies typically use food-like matrices, rather than real food, which may not accurately represent actual food textures. Therefore, Chapter 4 will address these limitations by assessing suprathreshold sensitivities to various oral tactile stimuli and evaluating texture and mouthfeel perception of animal and plant-based beverages. The selection of attributes is based on results from Chapter 3, focusing on attributes with the most variance among the different beverages (i.e., astringency, mouth coating, and smoothness). This chapter will also correlate the perceived intensity of these attributes with panelist suprathreshold sensitivity to lingual/rugal roughness, lingual punctate pressure, thickness, and grittiness. The findings from this chapter will help provide additional fundamental understanding of

the interplay between oral tactile sensitivity and texture and mouthfeel perception in beverages. This will offer insights into the potential mechanisms of oral mechanosensation in these sensations.

Transient receptor potential (TRP) channels can be activated by various temperatures and chemical stimuli. Among these receptors, TRPA1 and TRPV1 are known to be activated by stimuli such as mustard oil (Merrill et al., 2024) and capsaicin (Caterina et al., 1997), respectively. Interestingly, previous research has shown that *in vitro*, astringent compounds (e.g., epigallocatechin gallate [EGCG] and its auto-oxidation products) are potent activators of TRPA1 and TRPV1 (Kurogi et al., 2012, 2015; Takahashi et al., 2021). However, no research to date has investigated the role of TRPA1 and TRPV1 in astringency perception in the human oral cavity. Hence, Chapter 5 will focus on exploring the role of TRPA1 and TRPV1 in astringency perception in the oral cavity. By desensitizing either TRPA1 or TRPV1 channels and comparing the astringency perception to a control non-desensitized side, we can determine if, and how, astringency perception is affected when these TRP channels are desensitized. If TRP channels do play a significant role in astringency perception, desensitization of these receptors should decrease the intensity of astringency in comparison to the control side.

Chapter 2. Literature Review

2.1. The rise of popularity of plant-based milk alternatives

Over the past decade, there has been an increase in popularity of plant-based foods. In the United States (US), the sales of plant-based food products have grown from a $4.9B in 2018 to $8.1B in 2023 and is continuing to grow (The Good Food Institute, 2021, 2024). The interest in plant-based foods by consumers is due to several factors including these products being a healthier option, more unique, or in response to dietary restrictions (e.g., lactose intolerance, cow's milk allergy, hypercholesterolemia), and/or ethical concerns (animal abuse/slaughter) (Haas et al., 2019; McCarthy, Parker, et al., 2017; Schiano et al., 2020; Sethi et al., 2016). One other major reason may be due to environmental issues and wanting to find a more sustainable source of food. This is important because the global human population has been predicted to reach 10 billion by the year 2050 (McClements, 2019). With these ever-increasing numbers, there will be a struggle to meet the food demand of the growing population with the current food supply. In order to meet this demand, food production must increase, and this could have detrimental effects on the environment. This is especially the case when it comes to animal products (primarily meat, fish, egg, milk, and their derivatives). Increasing the production of such food products would require raising more livestock, which would lead to more pollution, greater greenhouse gas emissions, water use, and land use (Willett et al., 2019), equating to a more harmful effect on the environment. To address this issue, there has been increasing interest in developing more sustainable foods/sources that would have a less detrimental environmental impact but still meet the food demands of the growing population. These include a variety of products that are analogues (e.g., plant

based, lab-grown meat, etc.) of their animal-based food product alternatives that include milk, meat, fish, eggs, and other products used as ingredients in food. In the environmental sector, dairy products (e.g. milk) are known for being a large contributor of green-house gases, and the ecological and water footprint of milk production is higher than that of fruits and vegetables (Reyes-Jurado et al., 2021). For example, the carbon footprint of cow's milk on average is 1.39 CO_2 eq/kg, whereas other plant-based products such as soybean (0.88 CO_2 eq/kg) and almond (0.42 CO_2 eq/kg) "milk" have a much lower carbon footprint (Clune et al., 2017).

With the increased awareness and interest in these plant-based products, there has been an increase in product diversity to meet consumer demand (see Table 2.1.). In particular, plant-based milk alternatives (PBMA) are of interest due to their large market share of the plant-based food industry, having $2.9B (~36% of the market) in sales in 2023 in the United States (The Good Food Institute, 2024). However, one of the biggest challenges in replacing animal-based protein with plant-based protein has been mimicking their desirable sensory characteristics (McClements et al., 2019). In particular, appearance, taste, aroma, flavor, and texture of cow's milk depend on its macronutrient composition (McCarthy, Lopetcharat, et al., 2017). Mimicking the sensory attributes of cow's milk is a challenge as each plant-based ingredient has its own innate sensory attributes that are distinct and often different from those observed in cow's milk (Jeske et al., 2018). As a result, consumers still prefer the animal version due to the superior sensory profile, including its taste, flavor, aroma, and texture (Hoek et al., 2011; McClements et al., 2019). Beyond the sensory characteristics, nutritional profiling of cow's milk and plant-based alternatives differ in terms of sugar, fat, protein, and amino

acid compositions (Gorissen et al., 2018; Thorning et al., 2016; Vanga & Raghavan, 2018). In addition, different types of processing methods can also have a significant impact on the sensory properties of these beverages (Do et al., 2018; Schiano et al., 2017; Sethi et al., 2016), and also influencing its functional application as an ingredient (McClements et al., 2019).

Table 2.1 Category breakdown and value of plant-based foods sold in the US (2023). (The Good Food Institute, 2024)

Category	Sales
Plant-based milk	$2.9B
Plant-based meat and seafood	$1.2B
Plant-based creamer	$701M
Plant-based meal	$498M
Plant-based protein liquids and powders	$392M
Plant-based yogurt	$384M
Plant-based ice cream and frozen novelty	$351M
Plant-based butter	$292M
Plant-based bars	$253M
Plant-based ready-to-drink beverages	$247M
Plant-based cheese	$219M
Tofu, tempeh, and seitan	$201M
Plant-based ream cheese, sour cream, and dips	$129M
Plant-based baked foods and other desserts	$105M
Plant-based condiments and dressings	$90M
Plant-based eggs	$43M

2.1.1. Cow's milk processing

Mammalian milk (e.g., cow's milk) is a vital source of nutrition for infants, containing a blend of proteins, carbohydrates, fats, vitamins, and minerals essential for growth and development (Chalupa-Krebzdak et al., 2018; McClements et al., 2019; Pereira, 2014). The nutritional composition of cow's milk can vary due to factors such as the cow's age, breed, genetics, diet, and the season (National Research Council, 1988; Nickerson, 1995). Generally, cow's milk is composed of approximately 87% water, 4-5% lactose, 3-4% fat, 3% protein, 0.8% minerals, and 0.1% vitamins (Chalupa-Krebzdak et al., 2018). Cow's milk is rarely consumed in its raw form and undergoes several processing methods for commercial use, including homogenization, pasteurization, and fractionation/separation (Datta & Tomasula, 2015; McClements et al., 2019). These processes not only enhance the milk's functionality, but also significantly affects its sensory characteristics.

Raw milk contains large fat globules (4 – 5 μm) that tend to rise up to the surface during storage, as it is less dense than water, a process commonly known as creaming (Lopez et al., 2015; Tobin et al., 2015). Homogenization can break these large globules into much smaller droplets (<500nm) using mechanical stress. This process allows milk proteins, such as casein and whey, to stabilize the milk emulsion by covering the surface of the smaller fat droplets (Cano-Ruize & Richter, 1997). Different homogenization pressures can significantly impact the milk's physical properties. For example, previous research has shown that milk homogenized at 13.8 MPa had a higher viscosity compared to milk processed at 20.7 and 27.6 MPa (Li et al., 2018).

Bacteria and enzymes present in nutrient-rich raw milk can pose health risks if consumed and can cause milk spoilage. Thus, heat treatment (i.e., pasteurization) is utilized to minimize potential health hazards from pathogenic microorganisms and extend shelf life (Ryser, 2011). Common pasteurization techniques include low temperature long time (LTLT), which uses a minimum temperature of 62.8°C and a minimum time of 30 mins; high temperature short time (HTST), which uses a minimum temperature of 71.1°C and minimum time of 15s; and ultra-high temperature (UHT), which uses a minimum temperature of 135°C and a minimum time of 1 second (Ryser, 2011). These methods can impact the physicochemical properties of milk through processes such as whey protein denaturation, protein-protein interaction, lactose-protein interaction, isomerization of lactose, Maillard browning, sulfhydryl compound formation, formation of range of carbonyl and other flavorsome compounds, and formation of insoluble flavors (Cheng et al., 2019). These changes can have significant impact on the sensory properties of milk. For example, UHT milk has been shown to have higher viscosity in comparison to HTST milk (Li et al., 2018). Additionally, UHT milk often exhibits “off” notes such as “cooked” or “heated” flavors, and a chalky, astringent texture (Datta et al., 2002).

Fractionation of milk components can be executed through various processes such as filtration or centrifugation. Filtration processes include reverse osmosis (RO), nano filtration (NF), ultra filtration (UF), and microfiltration (MF). RO concentrates milk by removing water, whereas NF additionally removes monovalent salts and acids. UF results in a retentate of fat and protein, with the permeate containing minerals, non-protein nitrogen, and lactose, and MF further separates protein from fat (Cheryn, 1998; France et al., 2021). Centrifugation can also be used to separate milk into a fat-rich cream layer and

a fat-free serum layer. These two fractions can then be recombined to various ratios to result in skim, 1%, 2%, whole milk and creams. Previous research has shown that fat content significantly impacts sensory characteristics such as opacity, thickness, mouth coating, viscosity, milk fat flavor, creaminess, and yellow color which typically increase with fat content (McCarthy, Lopetcharat, et al., 2017; Phillips et al., 1995).

Despite various factors affecting the sensory properties of cow's milk, it is typically described as a low viscous fluid, with an opaque creamy white appearance, and a bland flavor profile (Schiano et al., 2017).

2.1.2. PBMA processing

PBMAs are broadly designed to have similar characteristics to cow's milk in terms of appearance, aroma, flavor, taste, and texture, enabling their use in similar applications (McClements, 2020). The sensory properties and functionality of PBMAs, like those of cow's milk, depend significantly on the ingredients and processing methods (McClements et al., 2019). Although PBMAs contain natural oil bodies with compositions and structures somewhat similar to those in cow's milk, and yet, they cannot fully replicate its desirable physicochemical and sensory properties. Variability in appearance, aroma, taste, flavor, and texture within PBMAs can be attributed to different molecules and structures in the ingredients.

PBMAs can be made from various plant-based sources, including, almonds, coconuts, flaxseeds, oats, rice, soy and peas (Sethi et al., 2016). Typically, PBMAs are formulated using four primary ingredients, the raw plant source, water, emulsifiers, and additives (e.g., oils, stabilizers, sweeteners, and thickeners) (Reyes-Jurado et al., 2021).

Although there are slight variations in processing methods, the general approach is similar which includes first disrupting plant tissues to isolate fat, protein, and sugars, and then homogenizing the mixture to create artificial fat globules from plant-based materials (Do et al., 2018; Mäkinen et al., 2016; McHugh, 2018; Sethi et al., 2016). This process begins with soaking the plant material to soften it, removing enzyme inhibitors, and improving nutrient digestibility and bioavailability, followed by grinding to extract the oil bodies, or through dry milling and extraction of the flour (Iwanaga et al., 2007; McHugh, 2018) (Figure 2.1.). Additionally, other steps in the processes may include centrifugation to remove unwanted plant debris, blanching to deactivate endogenous enzymes, pasteurization to eliminate spoilage and pathogenic bacteria, homogenization to reduce particle size and enhance beverage stability, and product formulation with the addition of sugars, flavors, stabilizers and thickeners (Iwanaga et al., 2007; Mäkinen et al., 2016; McClements et al., 2019; McHugh, 2018; Sethi et al., 2016).

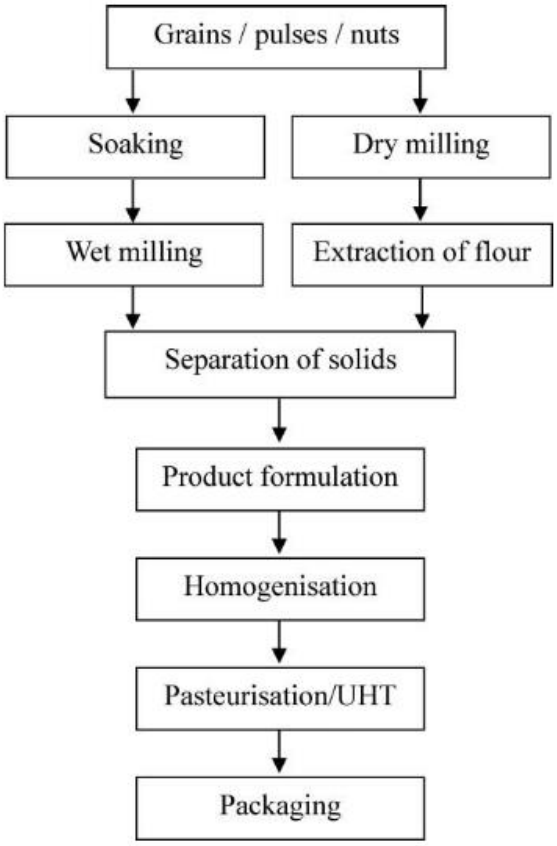

Figure 2.1 From Mäkinen et al., (2016). An example of a general manufacturing process of PBMAs

Although various processes are utilized to make plant-based beverages more palatable, it is challenging to replicate cow's milk due to the unique nutritional, sensory, and physicochemical properties of each plant-based source. Additionally, as cow's milk has a relatively bland flavor and light color, "off notes" and "off colors" exhibited in PBMA are easily detected by consumers. This presents a significant challenge for PBMAs that have distinctive flavors, textures, or pigments (Jeske et al., 2018).

PBMAs are often regarded as a healthier choice due to their nutritional profiles and the health benefits linked to certain plant-based ingredients (see Sethi et al., (2016) for a complete review). However, their nutritional content varies significantly compared to cow's milk. For example, soy and pea-based PBMA are two of the few plant sources with protein content comparable to that of cow's milk (Mäkinen et al., 2016; Schuster et al., 2018; Vanga & Raghavan, 2018). On the other hand, almond, coconut, oat and rice PBMAs have higher sugar and fibre content than cow's milk (Mäkinen et al., 2016; Vanga & Raghavan, 2018). Additionally, plant-based protein isolates have generally been shown to have lower levels of essential amino acids, particularly methionine and lysine, compared to animal based proteins (Gorissen et al., 2018). PBMAs also lack key micronutrients such as vitamin A, vitamin B12, vitamin D, vitamin E, and calcium. (McClements, 2020). Previous research has also shown that, overall, PBMAs contain less saturated fats and more mono and unsaturated fats (excluding coconut milk) in comparison to 2% cow's milk (Chalupa-Krebzdak et al., 2018). Therefore, there has been emphasis on enhancing and balancing PBMAs' nutritional profile to better mimic those of cow's milk (McClements, 2020).

From a function and application perspective, the structural differences in the protein from plant-based sources are different from those of cow's milk which impact its use. For example, casein are flexible chains and can form a homogenous network of interconnected strands when formed into gels in yogurt (Dalgleish & Corredig, 2012). On the contrary, proteins from plant-based sources (i.e., soy or pea) are tight knit compact structures that hinder the solubility and can impact the formation of a well structured network when forming gel (Queirós et al., 2017). It is speculated that this may be why

yogurt made from soy, almond, or coconut yogurts do not have the same creaminess that cow's milk yogurts have (McClements et al., 2019). Other limitations include PBMA curdling when added to coffee, potentially due to the different isoelectric points of the proteins (Brown et al., 2019) or the production of less stable foams (Zakidou et al., 2022).

The sensory characteristics of PBMA vary significantly depending on the plant-based source used. For example, Jeske et al., (2017) showed differences in the whiteness index when comparing cow's milk and various PBMAs, indicating that the PBMAs appear less white than cow's milk. Furthermore, extensive research has characterized the of aroma, taste, and flavor differences in PBMA (Abou-Dobara et al., 2016; Day N'Kouka et al., 2004; Jeske et al., 2019; Liu et al., 2021; Moss et al., 2022; Pointke et al., 2022; Pramudya et al., 2019; Torres-Penaranda & Reitmeier, 2001). For instance, pea and soy proteins are known to have "beany" and "painty" off flavors, often attributed to lipoxygenase activity during storage or manufacturing processes (Kwok & Niranjan, 1995; Liu et al., 2021; Torres-Penaranda & Reitmeier, 2001). Other common flavor attributes in PBMAs include cardboard, cereal, cheesy, grassy, and green pea, which can negatively impact consumer liking (Liu et al., 2021; Pointke et al., 2022; Pramudya et al., 2019; Torres-Penaranda & Reitmeier, 2001). In addition, bitterness is a prevalent taste attribute in PBMAs that can be a deterrent in consumer acceptance (Ongkowijoyo et al., 2023; Pointke et al., 2022; Pramudya et al., 2019).

While extensive research has focused on the aroma, taste, and flavor profiles of PBMAs, less emphasis has been placed on identifying their textural and mouthfeel characteristics. The aforementioned studies have typically examined fewer than five textural and mouthfeel attributes, compared to dozens of aroma and flavor attributes. This

is noteworthy because replacing animal-based proteins with plant-based alternatives in food and beverages can lead to changes in texture and mouthfeel, which are critical for consumer acceptance and preference (Sha & Xiong, 2020). Therefore, a more comprehensive investigation into the textural and mouthfeel properties of PBMAs is necessary.

2.2 Texture and Mouthfeel Perception

Texture and mouthfeel encompass a broad range of complex, multi-modal, and multi-dimensional sensory attributes (Szczesniak, 2002; van der Stelt et al., 2020). These attributes are crucial, not only driving consumer acceptance and liking, but also influencing food aversion (Pellegrino & Luckett, 2020; Spence et al., 2013). Research indicates that consumers have specific textural expectation of food and beverages, and when these expectations are not met, they often lead to rejection (Scott & Downey, 2007). Given that new product failure rates range from 50-85% (Costa & Jongen, 2010; Dijksterhuis, 2016), understanding consumer preferences in textural and mouthfeel characteristics is vital for developing successful plant-based products.

Texture is typically described as "the sensory and functional manifestation of the structural, mechanical, and surface properties of food detected through the senses of vision, hearing, touch, and kinesthetics" (Szczesniak, 1963, 2002), whereas mouthfeel, includes additional sensations that are also perceived in food/beverages by somatosensory receptors within the oral cavity (Guinard & Mazzucchelli, 1996). The complexity of these sensations can vary; for example, hardness/firmness/softness are related to the resistance of the food to the applied compressive forces (Szczesniak, 2002). Other textures such as creaminess, are comprised of multiple subqualities – related to thickness (dependent on

the physical viscosity) and smoothness (dependent on physical frictional forces) (Guinard & Mazzucchelli, 1996). Additionally, some textures like crispness are multidimension sensations, influenced by both the force and sound produced during the breakdown of a product (Costa et al., 2011; Edmister & Vickers, 1985).

Texture and mouthfeel perception is highly product dependent. For instance, both apples and potato chips can product a crisp sensation, yet the underlying structures differ (Edmister & Vickers, 1985). Potato chips exhibit a “dry crisp” with air-filed cells, whereas apples exhibit a “wet crisp” due to liquid filled cells (Duizer, 2001). Therefore, understanding the physical structures of a product is essential for predicting and characterizing its textural and mouthfeel properties (Foegeding et al., 2015). This can be done either through instrumental measurements or sensory evaluation.

2.2.1. Instrumental Measurements for Texture and Mouthfeel Perception

Despite the complex, multi-modal nature of texture and mouthfeel perception, food scientists have sought to instrumentally measure these sensations (Stokes et al., 2013). Historically, instruments like texture analyzers, rheometers, and viscosimeters have been used to measure these properties. These instruments typically assess the rheological properties of the food, focusing on its physical properties in relation to deformation. A common technique known as the “Texture Profile Analysis” has shown correlations between instrumental measurements and texture attributes like hardness, cohesiveness, springiness, adhesiveness, fracturability, etc., (see Pons & Fiszman for a complete review (1996)). This technique involves measuring the mechanical responses of a product during a double compression, mimicking the first and second bites of food (Stokes et al., 2013). However, texture and mouthfeel involves more than just rheology ;

it also includes tactile mechanosensation from the interaction between food, food residue, and oral surfaces (Stokes et al., 2013). While there is generally good correspondence between some instrumental measurements and textural and mouthfeel perceptions, these correlations are limited to certain attributes that are less complex (e.g., hardness or firmness). The dynamic and complex nature of food requires that testing parameters be optimized for each product to accurately reflect the human experience of food manipulation and perception in the mouth (Szczesniak & Hall, 1975).

Another instrumental measurement that can better mimic tactile some aspect of mechanosensation is tribology, which considers the forces associated with interacting surfaces in relative motion (Kim et al., 2020; Stokes et al., 2013). The majority of tribological research related to food texture and mouthfeel has focused on liquids and semi-solids. For example, studies have shown that correlations between friction measurements and creaminess perception in milk (Chojnicka-Paszun et al., 2012) and between the friction coefficient and attributes such as smoothness, lumpiness, and graininess in yogurt (Laiho et al., 2017). More recently, research has also demonstrated correlations between tribological measurements and the texture of solid foods. For instance, correlations were found between friction measurements and crispiness, juiciness, and mealiness in apples (Kim et al., 2020).

Despite the existing correlations between various instrumental measurements and texture and mouthfeel perception, a deeper understanding of the psychophysical mechanisms underpinning these sensory percepts will better equip future research to predict these sensations accurately.

2.2.2. Importance of Sensory Lexicon Development

Different methodologies are utilized to assess the sensory attributes of food. In the early stages, the Flavor Profile Method (Caul, 1957) was developed as a means to evaluate flavor, aroma, and aftertaste intensities utilizing a 7-point scale. Subsequently, the Texture Profile Method (Brandt et al., 1963) was developed to characterize mechanical, geometric, fat, and moisture properties of food. A more recent addition is the Spectrum Descriptive Analysis (SDA) method (Meilgaard et al., 2006) designed to provide greater discrimination (15-point scale) and establish absolute intensity references. This method requires extensive panelist training and validating, which can be quite costly. Conversely, the Tragon Quantitative Descriptive Analysis (QDA) method (Stone et al., 1974) is designed for panelists to provide evaluations based on relative judgements rather than absolute intensities. One advantage of QDA compared to SDA is that it requires much less panelist training. However, it should be noted that for QDA, intensity ratings can differ among panelists, and distinctions among products are measured relatively, often overlooking the significance of absolute scale values. Other sensory evaluation methodologies have adaptations stemming from QDA and SDA methodologies to meet the demands of specific study objectives. A common feature of all these methodologies is the use of a sensory lexicon.

Sensory lexicons are lists of standardized vocabularies developed to objectively characterize sensory properties across diverse audiences (Lawless & Civille, 2013; Suwonsichon, 2019). Numerous lexicons have been developed to describe and characterize various sensory characteristics of food products. The majority of these lexicons are fairly product dependent, encompassing various sensory modalities including

appearance, aroma, taste, flavor, texture, and mouthfeel. In developing such lexicons, it is important to have clear and distinct sensory attributes that are not confusable with one another (Lawless & Heymann, 2010; Lawless & Civille, 2013). This is important as different groups (i.e., consumers, product developers, and scientists) use their own terminology to describe sensory properties (van der Stelt et al., 2020). This is especially important for complex attributes, such as texture and mouthfeel. As such, references for descriptive analysis techniques are important and enable the accurate and repeatable evaluation by ensuring consistency within the panel (Suwonsichon, 2019). However, a common occurrence in literature is using the attribute name within its own definition. For example, chalky is described as "a chalky, cloying powdery sensation in the mouth" (Ciron et al., 2011), and softness is described as the "degree of softness as opposed to hardness" (Patterson et al., 2021). Having these ambiguous descriptions does not help clarify the complex sensation of the product being evaluated. Therefore, future research on developing lexicons should emphasize creating unique and distinct definitions that do not incorporate the attribute name within its own definition. For more detailed information on the development of sensory lexicons, see Lawless & Heymann (2010) and Lawless & Civille (2013).

Research on food texture and mouthfeel can be explored through two primary approaches. The first being a "top-down" approach, where food product evaluations are conducted using panelists to identify and measure specific sensory characteristics of interest (Linne & Simons, 2017). Alternatively, a "bottom-up" approach focuses on the physiologic and/or psychological mechanisms that are responsible for eliciting these sensations (Kravchuk et al., 2012; Linne & Simons, 2017). For a thorough understanding

of food texture and mouthfeel, it is essential to employ a combination of both these approaches.

2.3. Somatosensation

Somatosensation encompasses the perception of tactile, thermal, nociceptive, and proprioceptive sensations. In the oral cavity, these sensations are processed by trigeminal and chorda tympani afferents (Dunn et al., 2015; Klein, 2019; Mistretta & Bradley, 2021). Mechanoreceptors relay information in response to various mechanical stimuli including, touch, pressure, vibration and proprioception (Foegeding et al., 2015; Johansson et al., 1988; Mats Trulsson & Johansson, 2002; Zimmerman et al., 2014). Thermoreceptors relay information regarding the change in temperature, and lastly, nociception involves the detection of noxious mechanical, thermal, or chemical stimuli via nociceptors (Haggard & de Boer, 2014). Additionally, chemesthesis, which pertains to the sensory perception of chemical irritants, is mediated by the activation of the trigeminal nerve, contributing to the overall sensory experience in the oral cavity.

2.3.1. Mechanosensation

Despite the critical role of mechanosensation in food texture and mouthfeel perception, research on oral mechanoreception is lacking. The groundwork of our understanding of human oral mechanoreceptors stems from studies in glabrous (non-hairy) skin (Foegeding et al., 2015), where mechanosensory neurons process mechanical stimuli (Zimmerman et al., 2014). These mechanoreceptors are classified based on their adaptation rate, fiber myelination, and receptive field size (Bukowska et al., 2010; Jacobs et al., 2002; Roudaut et al., 2012; Trulsson & Essick, 1997). There are four specialized mechanoreceptive nerve fibers (Figure 2.2): slowly adapting (SA) receptors, including

SAI (associated with Merkel disk cells and have small receptive fields) and SAII (associated with Ruffini endings have large receptive fields), and rapidly adapting (RA) receptors, including RAI (associated with Meissner corpuscles and have small receptive fields) and RAII (associated with Pacinian corpuscles and have large perceptive fields) (Abraira & Ginty, 2013; Chambers et al., 1972; Johnson & Hsiao, 1992). Merkel disks respond to light pressure and can detect corners, edges, and curves, whereas Ruffini endings are involved in skin stretch and proprioception (Abraira & Ginty, 2013; Foegeding et al., 2015; Roudaut et al., 2012). Meissner corpuscles are associated with rapid skin movement or "flutter", whereas Pacinian corpuscles are associated with vibration and roughness (Abraira & Ginty, 2013).

Previous research has shown that the oral cavity's surfaces are innervated by the same nerve fibers as the glabrous skin (Moayedi et al., 2021; Trulsson & Essick, 2010; Trulsson & Johansson, 2002). Research has identified the presence of SAI, SAII, and RAI in response to mechanical stimuli in the human oral cavity (Trulsson & Essick, 2010; Trulsson & Johansson, 2002), however, some of the specialized mechanoreceptive nerve endings differ. For instance, recently, Moayedi et al., (2021) showed that in the tongue, filiform papillae are innervated by end bulbs of Krause, subepithelial neuronal densities, and NFH+ free neurons. On the other hand, fungiform papillae house Meissner corpuscles and NFH+ free neurons. In contrast, the hard-palate more so resembles the glabrous skin, showing innervation by Meissner corpuscles, Merkel cell-neurite complexes, and glomerular endings. Although studies have identified SAI, SAII, and RAI fibers in the oral cavity, no research has yet detected RAII mechanoreceptors in oral surfaces (Moayedi et al., 2021; Trulsson & Essick, 2010; Trulsson & Johansson, 2002).

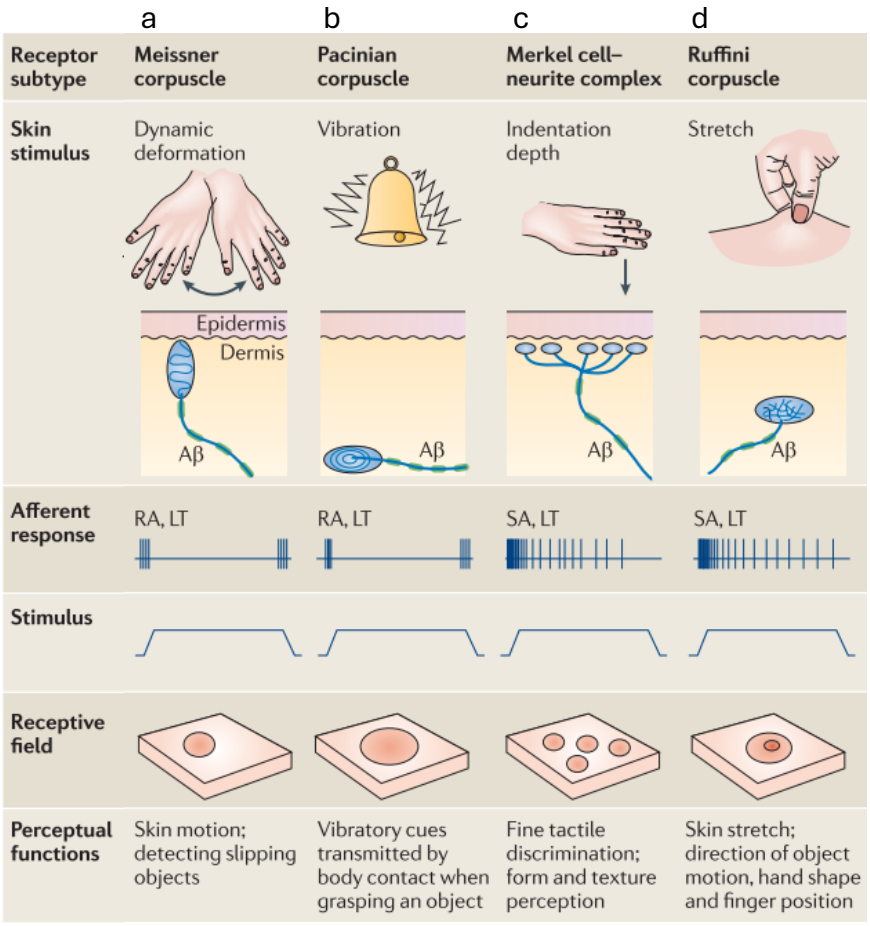

Figure 2.2 Adjusted from (Delmas et al., 2011) Cutaneous somatosensory mechanoreceptors in mammals. A) Meissner corpuscles are rapidly adapting (RA), low threshold (LT) mechanoreceptors that are associated with rapid skin motion and "flutter" movements. B) Pacinian corpuscles are RA, LT mechanoreceptors that are associated with vibration. C) Merkel cell-neurite complexes (disks) are slowly adapting (SA), LT mechanoreceptors associated with pressure and fine tactile discrimination such as corners, edges, and curves. D) Ruffini corpuscle/endings are SA, LT mechanoreceptors associated with skin stretch and proprioception.

2.3.2. Nociception and Thermoreception

Thermoreceptors translate information about temperature changes to the body. Transient receptor potential (TRP) channels, which can be activated by both temperature and chemical stimuli, play a key role in this process. Different TRP channels are activated by specific temperature ranges. TRPM8, V3, and V4 generally respond to non-noxious temperatures (though some overlap occurs: see Figure 2.3.), whereas TRPA1, V1, and V2 respond to noxious temperatures (~below 15°C or above ~45°C) (Story, 2006; Vriens et al., 2014). For example, TRPV1 is activated by noxious heat (≥42°C) (Caterina et al., 1997), whereas TRPA1 is activated by noxious cold temperatures ≤17°C (Story et al., 2003).

The types of chemical stimuli that can activate these channels also vary. Capsaicin is a well-known agonist of TRPV1 (Caterina et al., 1997), whereas menthol (Peier et al., 2002) or mustard oil (Merrill et al., 2024) have shown to be agonists of TRPA1. Certain TRP channels (e.g., TRPV1 and A1) can also undergo sensitization and desensitization, which affect their responsiveness. Sensitization occurs when repeated, short interval application of a stimulus leads to an increase in the perceived intensity of the irritant sensation (Caterina et al., 1997; Green, 1989). In contrast, desensitization occurs when a delay in stimulation following a high concentration of the stimulus results in a reduced intensity of the sensation upon subsequent application (Green, 1989). These phenomena have been demonstrated in TRPV1 and TRPA1 channels using capsaicin and mustard oil, respectively.

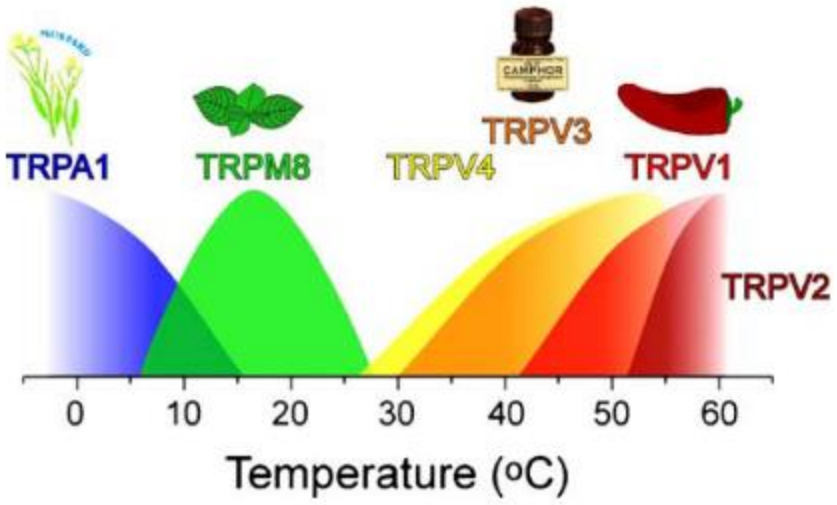

Figure 2.3 From Mckemy (2007). Mammalian TRP ion channels respond to a broad range of temperatures and chemical stimuli. TRPM8, V3, and V4 respond to non-noxious temperatures, whereas TRPA1, V1, and V2 respond to noxious temperatures (~below 15°C or above ~45°C). TRPA1 can be activated by compounds such as mustard oil, cinnamaldehyde, and menthol. TRPM8 can be activated by compounds like menthol, eucalyptol, and ilicin. TRPV3 can be activated by camphor. TRPV1 can be activated by capsaicin.

Nociception is the physiological process by which intense thermal, mechanical, or chemical stimuli are detected by nociceptors (Basbaum et al., 2009). It is important to note that nociception is not pain, but rather pain is a sensory experience associated with nociception (Julius & Basbaum, 2001). In the face, nociceptors are located in the trigeminal ganglion and comprise of various subtypes, including those that feature TRP channels like TRPV1, A1, and M8 (Basbaum et al., 2009). This can blur the line between

thermoreception and nociception, as these receptors respond to both thermal and noxious stimuli. Indeed, cross-modulation between thermoreception and nociception exists. For example, extreme temperatures can lead to thermoreception and nociception through the activation of TRP channels (e.g., TRP channels) (Basbaum et al., 2009; Julius & Basbaum, 2001; Vriens et al., 2014). Consequently, activation of these receptors can result in hyperalgesia (increased sensitivity to pain) (Caterina et al., 1997; Koltzenburg et al., 1992) and allodynia (pain in response to normally non-painful stimuli) (Story, 2006).

As the process of consuming food is a dynamic, tissues in the oral cavity are exposed to a wide range of sensory stimuli, including the mechanical breakdown of food, changes in temperature, and chemesthesis. Consequently, multiple somatosensory pathways can be activated simultaneously, contributing to the perception of texture and mouthfeel. Understanding how these various mechanisms interact and influence each other is critical for comprehending the overall sensory experience of food.

2.4. Tactile sensitivity

Texture and mouthfeel play an important role in consumer acceptance and food intake, with individual differences influencing affecting textural and mouthfeel preferences (Jeltema et al., 2015; Pellegrino & Luckett, 2020; Spence et al., 2013). However, research on the underlying factors driving these individual differences is still limited. Investigating these differences is challenging because texture and mouthfeel perceptions are multifaceted. No single oral mechanoreceptor likely codes for a single sensation, rather, a combination of these various mechanoreceptors underpin these complex sensations (Foegeding et al., 2015; Linne & Simons, 2017). Consequently, a single method to measure texture and mouthfeel sensitivity is inadequate (Liu et al., 2022). Thus, various

instruments and techniques have been developed to evaluate responses to physical stimuli, which may be linked to texture and mouthfeel sensitivity (Galler & Varela, 2023; Liu et al., 2022).

2.4.1. Psychophysics of Threshold and Suprathreshold Evaluation

To better understand the mechanical and biological underpinnings of human sensory perception, various psychophysical methods can be used to quantify such sensations in response to different stimuli. Consequently, there are numerous methods to measure threshold and suprathreshold differences in individuals, which can influence individual texture and mouthfeel perception.

The concept of threshold is a statistical measure indicating the concentration needed to be detected or recognized 50% of the time (Bartoshuk, 1978). Several types of thresholds exist, including detecting threshold, recognition threshold, and difference threshold. The detection threshold is the lowest intensity at which a sensation can be detected. The recognition threshold is the lowest intensity at which a sensation can identified. The difference threshold, also known as the just noticeable difference (JND), refers to the minimum intensity difference required to distinguish between two stimuli. In contrast, suprathreshold refers to stimulus intensities that exceed the threshold required to elicit a perceivable sensation. Moreover, as threshold and suprathreshold measurements do not necessarily correlate (Bartoshuk, 1978; Lawless & Heymann, 2010), it is recommended to evaluate both to obtain a complete characterization of a percept. The threshold and suprathreshold measurements of individuals can be assessed using various physical stimuli, some of which are discussed below.

2.4.2. Oral Punctate Pressure Sensitivity

The assessment of oral punctate pressure can be assessed using various tactile stimuli such as Von-Frey hairs, Semmes-Weinstein monofilaments, or, more recently, Cochet-Bonnet aesthesiometers (Galler & Varela, 2023; Liu et al., 2022). Von-Frey hairs and Semmes-Weinstein monofilaments consists of various filaments, each with a constant length but varying in the thickness (Liu et al., 2022). Monofilaments are applied perpendicularly to a surface until the filament bends, exerting the pressure. Use of these devices enables the delivery of a consistent, repeatable pressure stimulus. Notably, in both instruments the lowest allocated force is 0.0008g of force (0.08mN).

Previous studies have utilized monofilament stimuli to determine punctate pressure sensitivities in the oral cavity (Aktar et al., 2015b, 2015a; Appiani et al., 2020; Breen et al., 2019; Lv et al., 2020; Nishimura et al., 2021; Santagiuliana et al., 2019). For example, Aktar et al., (2015a) established punctate pressure detection thresholds for both the tongue (0.021g) and fingertip (0.023g), but found no significant differences. However, a subsequent study by Aktar et al., (2015b) revealed much lower punctate pressure detection threshold for the tongue (0.013g) compared to the fingertips (0.028), indicating greater sensitivity of the tongue. Breen et al., (2019) demonstrated variability in sensitivity among individuals in discriminating punctate pressure from monofilaments, and showed associations between particle size discrimination in chocolate and punctate pressure sensitivity in the tongue. Nonetheless, most studies linking punctate pressure sensitivity to texture and mouthfeel sensitivity in food models (e.g., viscosity, hardness, particle texture, and elasticity) have been unsuccessful (Aktar et al., 2015b, 2015a; Lv et

al., 2020; Santagiuliana et al., 2019), possibly due to the limitations of monofilaments (see below) for threshold detection in the oral cavity.

Alternatively, the Cochet-Bonnet aesthesiometer, a device used to measure corneal sensitivity (Chao et al., 2015; Grobnicu et al., 2018), can also assess oral punctate pressure. These monofilaments have a fixed diameter and a continuous slider to adjust the length, with longer lengths resulting in a decreased applied force. Cochet-Bonnet aesthesiometers offer several advantages over Von-Frey/Semmes-Weinstein monofilaments. First, monofilaments may not be sufficiently sensitive for comprehensively evaluating oral tactile sensitivity. The lowest available monofilament is 0.08mN, which exceed the tongue's reported sensitivity threshold of 0.03mN (Trulsson & Essick, 1997). In contrast, Cochet-Bonnet aesthesiometers can measure forces as low as 0.04mN, providing a closer match to the tongue's sensitivity. Secondly, there is inter-device variability with monofilaments (Bell-Krotoski et al., 1995), while Cochet-Bonnet aesthesiometers offer adjustable punctate pressure forces within a single device, reducing variability. Lastly, diameter variability among the monofilaments can affect the number of neuronal receptive fields activated, with larger filaments stimulating more receptors (Miles et al., 2018). Cochet-Bonnet aesthesiometers vary force based on the filament length while maintaining activation of the same receptive field, allowing for a more accurate measurement of punctate pressure (force per unit area) (Miles et al., 2018). However, a limitation with the Cochet-Bonnet aesthesiometer is that the thin nylon fiber and tip are easily damaged, which can compromise the accuracy of sensitivity of the resulting measurements (Murphy et al., 1996).

To date, only two studies have utilized Cochet-Bonnet aesthesiometers to assess oral punctate pressure sensitivity. Miles et al., (2018) compared the punctate pressure sensitivity of the tongue compared to the fingertips by measuring JND thresholds. The findings indicated significantly lower JNDs with the tongue than with the fingertips, suggesting that the tongue is more sensitive to punctate pressure sensations than the fingertips. In a follow-up study, Miles et al., (2022) expanded the investigation to compare punctate pressure sensitives of various locations within the oral cavity. Results from this study showed lower JND thresholds for the tongue compared to the gums and palate, while JNDs between the gums and palate did not significantly differ.

Despite extensive research into threshold detection of punctate pressure sensitivity in the oral cavity, there remains a lack of understanding regarding the mechanical underpinnings of this sensation. Previous research has associated Merkel cells with pressure sensitivity in the hand (Delmas et al., 2011; Roudaut et al., 2012), however, evidence of Merkel cells within the tongue is lacking (Moayedi et al., 2021). Instead, evidence was found of Meissner-like corpuscles and myelinated afferents surrounding the fungiform papillae in the tongue, suggesting that the latter structure could generate SAI responses. The same study also discovered that Merkel cell-neurite complexes innervate the hard palate, suggesting a potential significant role in texture and mouthfeel perception involving punctate pressure sensitivity. Therefore, further research is needed to better understand the specific mechanisms of punctate pressure in the oral cavity.

2.4.3. Oral Stereognosis Sensitivity

Stereognosis involves assessing and recognition of shapes and forms. In its most basic form, stereognosis involves the assessment of edges and points. As such, when oral tactile sensitivity using this approach, the majority of tests focus on edge, shape, and letter and shape stimuli (Bangcuyo & Simons, 2017; Essick et al., 1999; Haggard & de Boer, 2014; Kremer et al., 2005; Miles et al., 2018, 2020).

The majority of these studies have shown that interindividual variability of oral stereognosis exists with letter recognition (Bangcuyo & Simons, 2017; Essick et al., 1999; Kremer et al., 2005; Olarte Mantilla et al., 2022). Notably, Miles et al., (2018) demonstrated that differences in stereognosis letter recognition sensitivity exists between the tongue and fingertip, with fingertips displaying heightened sensitivity indicated by lower recognition thresholds. However, the assessment of oral stereognosis evaluation involves both tactile and cognitive components (shape or letter identification) (Miles et al., 2018, 2020), introducing a confounding variable when gauging tactile sensitivity.

As discussed in Miles et al., (2018), fMRI data (Fujii et al., 2011) demonstrates that stereognosis tasks involving fingertips activate the visual association cortex, which is associated with mental imagery formation during processing (Zeki, 1993). This suggests that tactile tasks with the finger may involve visualization, unlike oral tasks. Consequently, the cognitive aspect may augment the stereognosis sensitivity, potentially skewing assessments of "tactile" stereognosis sensitivity. To address this issue, Miles et al., (2020) developed "pure-tactile" stimuli to assess edge sharpness using both the fingertip and tongue, eliminating the cognitive component present in previous stereognosis methods. Results from this study utilizing the new "pure tactile" stimuli

indicated that the tongue exhibited greater sensitivity, as evidenced by lower JND thresholds for edge sharpness compared to the finger. By focusing on a more primitive characteristic like edge sharpness rather than complex stimuli, such as shape and edge orientation, this approach minimizes the cognitive component in stereognosis evaluation.

2.4.4. Oral Roughness Sensitivity

Unlike oral stereognosis, and punctate pressure discrimination, surface roughness sensitivity in the oral cavity is vastly understudied. Consequently, there is no standardized stimulus set for evaluating this sensation. To date, only four studies have investigated roughness sensitivity in the oral cavity, using stainless steel bars roughened with sandpaper of various grits, with stimuli roughness values (Ra) ranging from 0.177 – 0.465μm (Linne & Simons, 2017; Miles et al., 2018; Miles, Berkowitz, et al., 2022; Ricci et al., 2024).

The studies conducted by Miles et al., (2018; 2022) provide fascinating insights into how the sensitivity to roughness perception can vary depending on location. In the 2018 study, Miles et al., (2018) investigated the roughness sensitivity of the tongue compared to the fingertips by measuring JND thresholds. The findings revealed that subjects had significantly lower JNDs with their tongue compared to their fingertips, indicating that the tongue is more sensitive to roughness sensations than the fingertips. Building on these findings, Miles, Berkowitz et al., (2022) expanded the investigation to compare the roughness sensitivities of the tongue with those of the gums and palate. Results from this study demonstrated that the tongue had significantly lower JND thresholds for the tongue compared to the gums and palate, in addition to the JNDs obtained from the palate being significantly lower than the gums.

In a complementary study, Linne & Simons (2017) explored whether sensitivity to lingual tactile roughness would be an indicator of astringency sensitivity. Subjects were subdivided into groups based on their roughness sensitivity detection thresholds. The high sensitivity group showed significantly greater sensitivity to the astringency evoked by epigallocatechin gallate (EGCG) but not to tannic acid (TA), compared to the low sensitivity group. Additionally, individual suprathreshold assessment of surface roughness perception was strongly associated with suprathreshold sensitivity to EGCG but not TA astringency. Recognizing that mechanosensitivity may be influenced by temperature, a recent study by Ricci et al., (2024) assessed the influence of thermal sensations on lingual roughness sensitivity. Using the same roughened stainless-steel bars, they found that a cold temperature (8°C) significantly reduced tongue sensitivity to surface roughness (higher JND) compared to ambient stimulus temperature (21°C). However, at warm (35°) and hot (45°C) temperatures, there were no significant differences compared to thresholds obtained at ambient temperature. To further investigate whether this decrease in roughness sensitivity at lower temperatures was mediated by TRPM8 channels, they compared roughness thresholds obtained after lingual application of Evercool 190 (a TRPM8 agonist (Furrer et al., 2008)) to those obtained following a control application of water. Interestingly, there was no significant difference between the two conditions, suggesting that the decrease in lingual roughness sensitivity at cold temperatures is not TRPM8 dependent.

Results from these studies underscore the complexity of lingual roughness perception. However, the specific mechanisms of roughness perception in the oral cavity remain unclear. While previous research in glabrous skin has identified Pacinian

corpuscles (RAII) as the underlying mechanisms for detecting surface roughness (Bensmaïa & Hollins, 2005; Brisben et al., 1999), Pacinian corpuscles have not been identified in any human oral tissues to date (Bukowska et al., 2010; Moayedi et al., 2021; Trulsson & Essick, 1997). Although it is speculated that end bulbs of Krause my serve this function in the tongue, further research is needed to elucidate the specific mechanisms of roughness perception in the oral cavity.

2.4.5. Biological Parameters Affecting Human Oral Tactile Mechanosensitivity

Biological differences between individuals makes each human unique. Consequently, variability in biological factors (e.g., age, sex, filiform and fungiform papillae density [FPD], and salivary flow) have been systemically explored in relation to oral tactile sensitivity.

Previous research has investigated the impact of sex differences on lingual mechanosensitivity. Michon et al., (2009) observed that females have greater sensitivity to lingual stereognosis, though their evaluation methods and scoring methods were unconventional and somewhat controversial. More recently, Appiani et al., (2020) found significant sex differences only in lingual grating orientation sensitivity, but found no significant sex differences in punctate pressure sensitivity. Similarly, other studies have reported no significant sex differences in threshold sensitivity and suprathreshold sensitivity of lingual stereognosis (Bangcuyo & Simons, 2017; Essick et al., 1999).

As aging is a biological process of humans, the decline in orosensory functions has been linked to decreased neural pathway efficiency (Kremer et al., 2005; Liu et al., 2022). Various studies have shown differences in oral mechanosensitivity between age groups.

For instance, Kremer et al., (2005) reported a decline in oral stereognosis and size discrimination in elderly (60-85 years) compared to young adults (18-35 years). Similarly, Bangcuyo & Simons (2017) found that panelists aged 40 years or older had significantly higher stereognostic threshold averages than younger panelists aged 18-29 years. However, they also noted that suprathreshold sensitivity for oral stereognosis did not differ significantly between age groups. Likewise, Appiani et al., (2020) observed no-age related differences in lingual tactile sensitivity (grating and punctate pressure) between adults (19-33 years), children (6-13 years), and their parents (32-58). Furthermore, Linne & Simons (2017) did not find significant age group differences for threshold and suprathreshold sensitivity in lingual roughness sensitivity. Overall, these findings suggest that oral mechanosensitivity in relation to age is highly dependent on the specific age groups and oral tactile stimuli studied.

Mechanical stimuli in the oral cavity activate mechanoreceptors associated with fungiform and filiform papillae, leading to the speculation that papillae density may influence oral mechanosensitivity. As such, numerous studies have explored the relationship between lingual papillae anatomical characteristics and oral tactile sensitivity. Research by Zhou et al., (2021) found a positive correlation with fungiform papillae density and tactile sensitivity to punctate pressure. Similarly, Bangcuyo & Simons (2017) observed that oral stereognosis threshold sensitivity is correlated with FPD, with higher densities resulting in increased tactile sensitivity. However, they noted that FPD was not significantly correlated with suprathreshold oral stereognosis sensitivity, similar to findings related to age. Likewise, Linne & Simons (2017) did not find significant associations between FPD and either threshold or suprathreshold lingual

roughness sensitivity. More recently, Miles et al., (2022) discovered that sensitivity to high-viscosity solutions is significantly correlated with both the length and density of filiform papillae, but not with diameter. These findings suggest that both fungiform and filiform papillae density can influence certain aspects of oral tactile sensitivity, however, its impact may vary depending on the specific type of mechanosensitivity being measured.

Chapter 3. Utilization of a texture and mouthfeel lexicon to differentiate plant and animal-based beverages

Modified from: Min Sung Kim, Laura Nattress, and Christopher T. Simons, Manuscript in Preparation

3.1 Abstract

Recent high consumer demand has driven the food industry towards developing the next generation of plant-based beverages. One major challenge for this initiative has been mimicking the desirable textural and mouthfeel properties of their animal-based counterparts. Despite playing a key role in consumer acceptance, there is limited research investigating textural and mouthfeel differences between plant and animal-based beverages. This study developed a comprehensive sensory lexicon solely focusing on texture and mouthfeel attributes to characterize the differences between animal and plant-based milk beverages. A total of 16 different texture and mouthfeel attributes were generated with unique descriptors and references. Sixteen assessors evaluated 14 different liquid beverages that were grouped by protein content: low protein (LP; 8g of protein/8fl. oz) and high protein (HP; 13g of protein/8fl. oz). Each beverage group included two types of animal-based beverages (commercial skim milk [CSM] and milk protein isolate [MPI]) and five types of plant-based beverages (pea protein isolate [PPI], soy protein concentrate [SPC] and three types of soy protein isolates [SPI 1-3]). Similarities in textural properties were observed between LP animal-based beverages, while small differences were observed within LP-SPIs. LP-SPC was significantly different for 8 out of the 16 attributes compared to all other LP-beverages. As with the LP-beverages, similarities in textural properties were observed between HP animal-based beverages, while small differences were observed within HP-SPIs. HP-SPC was significantly

different for 9 out of the 16 attributes compared to all other HP-beverages. Overall, trends observed amongst the various protein sources within the LP-beverages were consistently mirrored in the HP-beverages. These findings underscore that textural and mouthfeel differences between plant and animal-based beverages are predominantly influenced by the type of protein used rather than protein concentrations within the range of 8-13 g/8 fl. oz.

3.2 Introduction

Over the past decade, there has been an increase in popularity of plant-based foods. In the United States (US), the sales of plant-based food products have grown from $4.9B in 2018 to $8.1B in 2023, and are continuing to rise (The Good Food Institute, 2024). The rapidly growing interest in plant-based foods can be attributed to a number of factors including environmental impact and sustainability, dietary restrictions, allergies, and ethical concerns (Sethi et al., 2016). To meet consumer demand, a variety of plant-based foods have been developed, ranging from dairy to meat alternatives. Of those, plant-based milk alternatives (PBMA) are of interest as they contribute the highest proportion of plant-based food sales in the US (The Good Food Institute, 2024). Although consumption of PBMAs has increased, there is hesitation from consumers to fully adopt these products over their animal-based counterparts due to undesirable sensory attributes (McClements et al., 2019). Indeed, sensory properties are one of the most important drivers of food choice in plant-based food products (Martins & Pliner, 2005). Therefore, to develop the next generation of PBMAs as suitable alternatives to cow's milk, it is essential to gain a better understanding of the differences in sensory properties between these two types of beverages.

In general, PBMAs are produced by the breakdown of plant material extracted in water to isolate oil bodies and other colloidal matter and homogenized with other fluids, resulting in a product that can be comparable to cow's milk in taste and appearance (Bocker & Silva, 2022; McClements & Grossmann, 2021; Sethi et al., 2016). Nevertheless, clear differences are still apparent as changes in the physical and chemical properties of PBMAs can lead to differences in the functional, structural, and sensory properties compared to their animal-based counterparts (Sha & Xiong, 2020). A few functional limitations of PBMAs compared to their animal-based counterparts are curdling when added to coffee (Brown et al., 2019) and producing less stable foams (Zakidou et al., 2022). To potentially overcome these functional limitations, addition of emulsifiers (e.g., alginates, gelatin, vegetable gums) (Sethi et al., 2016) or pH buffering agents (e.g., sodium citrate or sodium phosphate) (Whitaker, 1931) can be utilized. Although these additions may improve PBMAs from a functional perspective, these additions can have significant implications on the sensory properties of these beverages, which may negatively impact consumer liking/acceptance.

As the replacement of animal-protein with plant-protein in foods and beverages can lead to changes in texture and mouthfeel properties, it can have a significant impact on food acceptance and liking (Sha & Xiong, 2020). Texture generally relates to the physical properties associated with the breakdown of food/beverages (Szczesniak, 2002), whereas mouthfeel includes additional sensations that are also perceived in food/beverages by somatosensory receptors within the oral cavity (Guinard & Mazzucchelli, 1996). Despite texture and mouthfeel being key drivers in food acceptance, these sensations are poorly understood and understudied relative to taste and smell (Jeltema et al., 2015). This is

further exemplified in PBMA, as the majority of research has focused on the taste, aroma, and flavor differences between plant and animal-based beverages, with little emphasis on identifying textural and mouthfeel differences (Day N'Kouka, et al., 2004; Jeske et al., 2019; Liu et al., 2021; Pointke et al., 2022; Pramudya et al., 2019). Within the studies that have assessed texture and mouthfeel, none have examined more than 5 different texture and mouthfeel attributes. Rather, most studies only evaluated one or two terms, with many additionally assessing over a dozen aroma and flavor attributes. As texture and mouthfeel are complex sensations that are comprised of multiple underlying dimensions and attributes (van der Stelt et al., 2020), a single or a few attributes describing the "texture" or "mouthfeel" of a product is inadequate to fully characterize the nuanced differences that may be impacted by the different types of protein used. In addition, the majority of these studies did not control for the amount of protein in each beverage as the protein content of commercialized PBMAs are inherently different than cow's milk (Mäkinen et al., 2016; Vanga & Raghavan, 2018). As such, some of the differences exhibited between these beverages may be attributed to the amount of protein rather than the source of protein itself.

Therefore, the objectives of this present study were to: 1) develop a lexicon that thoroughly captured the various complex texture and mouthfeel attributes that can be perceived when consuming either an animal or plant-based beverage, and 2) investigate the effect of protein type and amount on the textural and mouthfeel properties of plant and animal-based milk.

3.3 Methods

3.3.1 Products

A total of 14 different liquid beverages were selected to be evaluated by the panel. Liquid beverages were grouped into low protein (LP; 8g of protein/8fl. oz), and high protein (HP;13g of protein/8fl. oz) to mimic the protein content of commercially available cow's milk. Each beverage group consisted of two animal-based options: commercial skim milk (CSM) and milk protein isolate (MPI), as well as five plant-based options: pea protein isolate (PPI), soy protein concentrate (SPC), and three varieties of soy protein isolates (SPI 1-3). Commercial skim milk was used as a comparison instead of other commercial milks to maintain consistency in fat content given that none of the other beverages contain fat. The LP-CSM used was Kroger fat free skim milk (The Kroger Company, USA) whereas the HP-CSM used was Fairlife fat free ultra filtered lactose free milk (The Coca-Cola Company, USA). Commercial milks were purchased within 7 days of evaluation. The other beverages were manufactured by the Archer Daniels Midland Company in Kentucky, USA. Beverages were produced (except CSM) from protein powders with varying levels of protein concentrations: SPC 70.3%, PPI – 82.9%, SPI 1 – 92.3%, SPI 2 – 92.9%, and SPI 3 – 94.9% (Figure 3.1). In addition, SPI 1 and 2 were enzyme modified, with SPI 1 having a higher degree of enzyme modification (i.e., hydrolysis). Additives for the soy and pea protein beverages included tricalcium phosphate, guar gum, gellan gum and maltodextrin. Similarly, additives to the MPI also included tricalcium phosphate, guar gum, gellan gum, maltodextrin, and silicon dioxide. Visual differences between the low and high protein variants of the beverages were not apparent. All products were stored at refrigeration temperature (4°C) prior to evaluation.

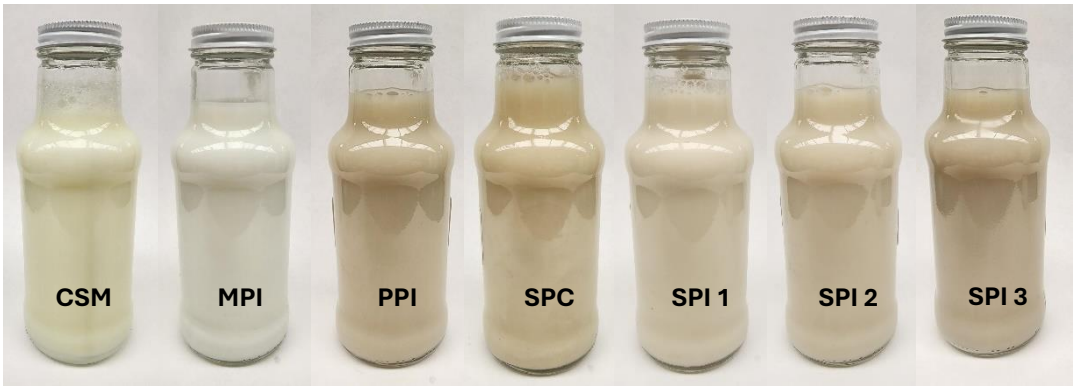

Figure 3.1 Depiction of the various LP beverages used. From left to right: commercial skim milk (CSM), milk protein isolate (MPI), pea protein isolate (PPI), soy protein concentrate (SPC), and soy protein isolates (SPI) 1-3.

3.3.2 Trained sensory panel evaluation

A trained panel consisting of 16 assessors (5 males, 11 females) was recruited from members of The Ohio State University Food Science and Technology department via email. Exclusion criteria included persons with oral tactile deficits (e.g., denture implants, trigeminal neuropathies, dysphagia, etc.), tongue piercings, or oral lesions. Participating subjects were asked to refrain from consuming food and smoking for at least 1 hour prior to the start of each session. All panelists were also consumers of animal and/or plant-based milk. Each panelist gave their written consent to participate in the project that was approved by The Ohio State University Institutional Review Board

(2021E1103). Panel sessions took place twice per week in 1-hour sessions. The goal of the panel was to develop an initial sensory lexicon related to the texture and mouthfeel sensations observed in liquid products and then use these descriptors to differentiate between the various plant- and animal-based products. Over 20 sessions, panelists were trained to identify and discriminate between textural and mouthfeel properties of the animal and plant-based beverages. All training sessions were conducted in a classroom setting, where panelists could freely communicate with each other.

To solely focus on the texture and mouthfeel properties of the products and references, nose-clips (AM-Systems, Sequim, WA, USA) were provided and used for each evaluation. A preliminary texture and mouthfeel lexicon of 63 attributes were compiled based on a review of 47 studies from the literature (data not shown), covering evaluations of various types of foods (i.e., liquids, solids, and semi-solids). Panelists used the check-all-that-apply and rate-all-that-apply techniques to identify the attributes most relevant for the liquid beverages used in this study, reducing the list to 16 terms. Over 14 subsequent sessions, definition refinement and reference development were completed. Emphasis was placed to avoid including the attribute's name within its own definition. In addition, no reference sample overlapped between attributes. The final lexicon including definitions and references is included in Table 3.1.

Following training, descriptive analysis (DA) for all 14 beverages was conducted over 3 sessions, where the HP and LP variations of the products were evaluated in the same session. These sessions were conducted in semi-isolated booths in the Sensory Evaluation Center at The Ohio State University. Panelist data were collected using Compusense Cloud (Guelph, Ontario, Canada). All samples were kept in a refrigerator at

4°C and taken out 1 hr prior to evaluation to warm up to room temperature. Products were served in 2 oz. clear plastic cups labelled with a unique three-digit code. Samples were serial monadically presented under red light in a random, balanced design and assessed in replicate. Similar to the development of the sensory lexicon, panelists were provided nose-clips to use when evaluating the products and the references. Panelists rated the intensity of each attribute on an individual 10-point continuous line scale where "0" was no intensity, "1" was "weak" and "9" was "strong", with the exception of melting ("slow" [1] to "fast" [9]), and viscosity (anchored from "thin" [1] to "thick" [9]). Prior to evaluation, panelists were asked to swirl each sample a few times to ensure it was fully mixed. Although panelists had ad libitum access to filtered water and unsalted crackers, they were also specifically instructed to rinse their palates during a 30s break between sample evaluation to minimize sensory fatigue.

Table 3.1 Liquid based beverage lexicon used by trained panelists and their definitions, anchors, and references. 10-point continuous line scales were anchored from "weak" (anchored at "1") to "strong" (anchored at "9"). Number in parentheses in the reference column indicates the anchor intensity for the reference. All references were served in 2 oz. clear plastic cups.

Attribute	Definition	Reference
Adhesiveness	Degree to which the sample stick/adheres to any mouth surface (including teeth) during mastication	Jif creamy peanut butter (6.5)
Astringency	Sensation of roughness and/or drying/puckering in oral surfaces	0.285g/500mL aluminum sulfate (3)

Cohesiveness	Degree to which the product holds together in a mass	Kroger marshmallow crème (6.5)
Dissolving	Rate/degree that the sample breaks down and disappears	Kroger powdered sugar (7)
Drying	Saliva absorbing, free from moisture in the mouth	50g PB Fit peanut butter powder in 300mL of RO water (3)
Fatty/Oily	The oleaginous sensation perceived on the oral surface	Bertoli Extra Virgin Olive Oil Rich Taste (10)
Foamy	Perception of bubbles that disintegrate easily	2 Large egg whites with 0.85g of cream of tartar and whisk for 150s (4)
Melting	Rate of thinning of the product in the mouth (anchors: slow "1" to fast "9")	Kroger honey (6.5)
Mouth Coating	Cloying sensation of the product coating the oral surface	Simple Truth Organic Grade A maple syrup (5.5)
Powdery	Degree of fine particles perceived on the oral surface	30g Baney butter almond butter powder in 300mL of RO water (5)
Puckering	Reflex action of the oral surface being brought together and released in order to lubricate the oral surface	0.1%w/vol tartaric acid in RO water (2.5)
Residual Coating	Residuals left on the oral surface after swallowing the product	200g of melted semi sweet chocolate chips Kroger mixed with 40mL of Mazola corn oil (5.5)
Sliminess	Sticky and slippery product	3g carboxymethyl cellulose in 500mL H20 (7)
Slipperiness	Moves easily around the mouth and degree to which the sample slides across the tongue/palate	Kroger brand canned peaches syrup (6.5)
Smooth	Contains no particulate matter, even consistency	Non-fat vanilla Yoplait yogurt (7)
Viscosity	Resistance to flow of the product in the mouth. How it moves around the oral cavity (anchors: thin "1" to thick "9")	0.1%w/vol guar gum in RO water (2) 1% w/vol guar gum in RO water (8)

3.3.3 Statistical analysis

Data for the LP and HP beverages were initially analyzed separately to investigate the differences among the samples within each group. A three-way mixed ANOVA (panelist, product, replicate) was conducted to analyze the descriptive data. Data from both beverage groups were then combined to further analyze the effect of protein concentration on textural and mouthfeel properties using a four-way ANOVA (panelist,

product, replicate, protein concentration). Tukey's Honestly Significant Difference (HSD) ($\alpha = 0.05$) was applied to all data sets for post-hoc analysis using SPSS version 27 (IBM, Armonk, NY). Principal component analysis (PCA) was conducted using R Studio 2022.12.0 (R Core Team Vienna, Austria). The data used to create the PCA were the average attribute evaluations for each product obtained from the 16 panelists. For the purpose of this study, panel averages were used to interpret the data (Lawless & Heymann, 2010; Næs et al., 2021).

3.4. Results

3.4.1. Comparison of texture and mouthfeel attribute intensities of low-protein beverages

For the 7 LP beverages, ANOVA results indicated a significant product effect (p's<0.05) for all texture and mouthfeel attributes except dissolving ($p = 0.15$; see Table 3.2). Out of the 16 attributes, only 3 had mean scores that ranged greater than 2 points including powdery (LP-CSM:0.9±0.1 – LP-SPC: 3.9±0.4), smooth (LP-SPC: 3.5±0.3 – LP-CSM: 6.8±0.3), and viscosity (LP-SPI 3: 1.4±0.1 – LP-SPC: 3.7±0.3).

Table 3.2 F and p-values for the sensory attributes of the different beverages. LP and HP beverage F- and p-values were initially analyzed separately, and then combined for between protein-level analyses.

Attributes	LP beverages F- and p-values	HP beverages F- and p-values	LP and HP beverages F- and p-values
Adhesiveness	13.51 (<0.001)	32.56 (<0.001)	41.50 (<0.001)
Astringent	4.94 (<0.001)	4.85 (<0.001)	7.85 (<0.001)

Cohesiveness	8.93 (<0.001)	23.89 (<0.001)	37.40 (<0.001)
Dissolving	1.62 (0.15)	4.01 (<0.001)	0.22 (0.64)
Drying	29.88 (<0.001)	25.41 (<0.001)	9.21 (0.003)
Fatty/Oily	17.55 (<0.001)	8.36 (<0.001)	1.17 (0.28)
Foamy	4.62 (<0.001)	4.46 (<0.001)	0.75 (0.39)
Melting	4.81 (<0.001)	2.78 (0.02)	4.54 (0.04)
Mouth coating	17.35 (<0.001)	32.19 (<0.001)	15.52 (<0.001)
Powdery	71.52 (<0.001)	41.19 (<0.001)	12.75 (<0.001)
Puckering	5.44 (<0.001)	4.66 (<0.001)	3.94 (0.05)
Residual coating	19.76 (<0.001)	38.32 (<0.001)	18.19 (<0.001)
Sliminess	12.05 (<0.001)	21.07 (<0.001)	32.92 (<0.001)
Slipperiness	3.68 (<0.003)	9.04 (<0.001)	3.93 (0.05)
Smooth	30.75 (<0.001)	39.89 (<0.001)	10.23 (0.02)
Viscosity	42.61 (<0.001)	115.84 (<0.001)	44.63 (<0.001)

Tukey's HSD analyses indicated that none of the 16 attributes significantly differed between the two LP animal-based beverages (Table 3.3).

Significant differences were evident among some of the attributes for the LP-SPIs. Mouthcoating (LP-SPI 1: 2.8±0.3, LP-SPI 2: 2.2±0.2) and smooth (LP-SPI 1: 4.4±0.3, LP-SPI 2: 5.6±0.3) were found to be significantly different between LP-SPI 1 and SPI 2. LP-SPI 1 was also shown to be more powdery (LP-SPI 1: 2.3±0.3, LP-SPI 2: 1.5±0.2, LP-SPI 3: 1.6±0.1) and have higher residual coating (LP-SPI 1: 2.8±0.2, LP-SPI 2: 2.2±0.2, LP-SPI 3: 2.2±0.2) than LP-SPI 2 and 3. LP-SPI 1 (2.0±0.2) was significantly more viscous than LP-SPI 3 (1.4±0.1). For all other attributes, there were no significant differences among the 3 LP-SPI beverages.

Tukey's HSD analyses for LP-PPI showed that it was the most fatty/oily (2.1±0.2) among all LP beverages. It was also shown to be most similar to LP-MPI, where only fatty/oily was significantly different between the two beverages (LP-MPI: 1.6±0.2, LP-

PPI: 2.1±0.2). In comparison to the LP-CSM, LP-PPI was significantly different (p's<0.05) for 7 attributes including adhesive, astringent, dry, melting, powdery, puckering, and smooth (Table 3.3).

The LP-SPC beverage was the most different when compared to all other LP beverages where it differed significantly (p's <0.05) for 8 out of the 16 attributes including cohesiveness, drying, mouth coating, powdery, residual coating, sliminess, smooth, and viscosity (Table 3.3). It also had the highest intensity in 9 of the 16 attributes (adhesiveness, cohesiveness, drying, foamy, mouth coating, powdery, residual coating, sliminess, and viscosity), while being the least slippery, smooth, and the lowest in melting. In contrast, LP-CSM had the highest intensity in melting, slipperiness, smooth, with the lowest in adhesiveness, astringency, cohesiveness, drying, powdery, puckering, and sliminess, showing opposite trends to that of LP-SPC.

The LP-SPC beverage was the most different when compared to all other LP beverages where it differed significantly (p's <0.05) for 8 out of the 16 attributes including cohesiveness, drying, mouth coating, powdery, residual coating, sliminess, smooth, and viscosity (Table 3.3). It also had the highest intensity in 9 of the 16 attributes (adhesiveness, cohesiveness, drying, foamy, mouth coating, powdery, residual coating, sliminess, and viscosity), while being the least slippery, smooth, and the lowest in melting. In contrast, LP-CSM had the highest intensity in melting, slipperiness, smooth, with the lowest in adhesiveness, astringency, cohesiveness, drying, powdery, puckering, and sliminess, showing opposite trends to that of LP-SPC.

When the descriptive data of the LP-beverages were subjected to a PCA, additional insights were observed. LP principal component (PC) 1 explained 60.2% of the

total variance (Figure 3.2), where five of the 16 attributes had correlations ±0.3: cohesiveness (0.30), powdery (0.31), sliminess (0.31), slippery (-0.31), and viscosity (0.31). LP PC2 explained 18.7% of the total variation, where six of the 16 attributes astringency (-0.42), dissolving (0.39), foamy (0.43), melting (0.30), mouth coating (0.34), and puckering (-0.39). This PCA distinctly illustrates that LP-SPC substantially differs from other LP-beverages, as it is characterized by attributes such as drying, mouth coating, powdery, residual coating, sliminess, and viscosity. LP-CSM also differentiates itself from other LP-beverages, characterized by attributes such as dissolving, foamy, melting, slipperiness, and smooth. LP-MPI and LP-SPI 2 are closely grouped, characterized by astringency and puckering in the positive direction of dimension 1, and slipperiness and smoothness in the negative direction of dimension 2. Interestingly, although LP-SPI 3 shares an identical loading on dimension 1 with LP-SPI 2, they are differentiated on dimension 2 with LP-SPI2 being characterized as more astringent and puckering. Similarly, LP-PPI and LP-SPI 1 exhibit identical loading on dimension 1 but stand in stark contrast on dimension 2, with LP-PPI loading negatively, characterized by astringency and puckering, and LP-SPI 1 loading positively on dimension 2, characterized by attributes such as dissolving, foamy, melting, and mouth coating.

Table 3.3 Perceived texture and mouthfeel intensity ratings (mean ±SE) of low protein (8g protein/8fl.oz) beverages rated on a 10-point continuous line scale. Different letters in a column indicate significant differences ($p<0.05$). Continuous line scales were anchored from "weak" (anchored at "1") to "strong" (anchored at "9").

Products	Adhesiveness	Astringency	Cohesiveness	Dissolving	Drying	Fatty/Oily	Foamy	Melting
LP CSM	1.1a±0.1	1.0a±0.1	1.3a±0.1	3.2a±0.4	1.0a±0.1	1.9cd±0.2	1.1abc±0.1	4.3c±0.4
LP MPI	1.5ab±0.2	1.4ab±0.2	1.4a±0.2	2.9a±0.4	1.4ab±0.2	1.6bc±0.2	0.9a±0.1	3.8abc±0.4
LP PPI	1.8bc±0.2	1.6b±0.2	1.5a±0.2	3.0a±0.4	1.4b±0.1	2.1d±0.2	1.0ab±0.1	3.6ab±0.4
LP SPC	2.2c±0.2	1.5ab±0.2	2.2b±0.2	3.0a±0.3	2.6d±0.3	2.0cd±0.3	1.3c±0.1	3.4a±0.3
LP SPI 1	1.3a±0.1	1.6b±0.2	1.4a±0.1	3.3a±0.4	2.0c±0.2	1.2a±0.1	1.2bc±0.1	4.1bc±0.4
LP SPI 2	1.2a±0.1	1.9b±0.2	1.5a±0.2	3.2a±0.4	1.7bc±0.2	1.5ab±0.1	1.0ab±0.1	3.9abc±0.4
LP SPI 3	1.4ab±0.1	1.5ab±0.1	1.5a±0.1	3.6a±0.2	1.6bc±0.1	1.2a±0.1	1.0ab±0.1	3.7abc±0.2

Products	Mouth Coating	Powdery	Puckering	Residual Coating	Sliminess	Slipperiness	Smooth	Viscosity
LP CSM	2.8b±0.2	0.9a±0.1	0.8a±0.1	2.2a±0.2	1.7a±0.2	4.2b±0.4	6.8e±0.3	1.6ab±0.1
LP MPI	2.4ab±0.2	1.3ab±0.2	1.1ab±0.1	2.1a±0.2	1.8a±0.2	3.9b±0.3	6.0de±0.4	1.7ab±0.1
LP PPI	2.7ab±0.2	1.5b±0.2	1.3b±0.1	2.3ab±0.2	2.2a±0.2	3.9b±0.2	5.5cd±0.2	2.0b±0.1
LP SPC	3.8c±0.3	3.9d±0.4	1.2b±0.2	3.4c±0.2	3.1b±0.3	3.1a±0.3	3.5a±0.3	3.7c±0.3
LP SPI 1	2.8b±0.3	2.3c±0.3	1.2b±0.1	2.8b±0.2	1.8a±0.2	3.7ab±0.3	4.4b±0.3	2.0b±0.2
LP SPI 2	2.2a±0.2	1.5b±0.2	1.4b±0.1	2.2a±0.2	1.9a±0.2	4.2b±0.4	5.6cd±0.3	1.7ab±0.1
LP SPI 3	2.5ab±0.2	1.5b±0.1	1.2b±0.1	2.2a±0.2	2.1a±0.2	4.0b±0.4	5.0bc±0.3	1.4a±0.1

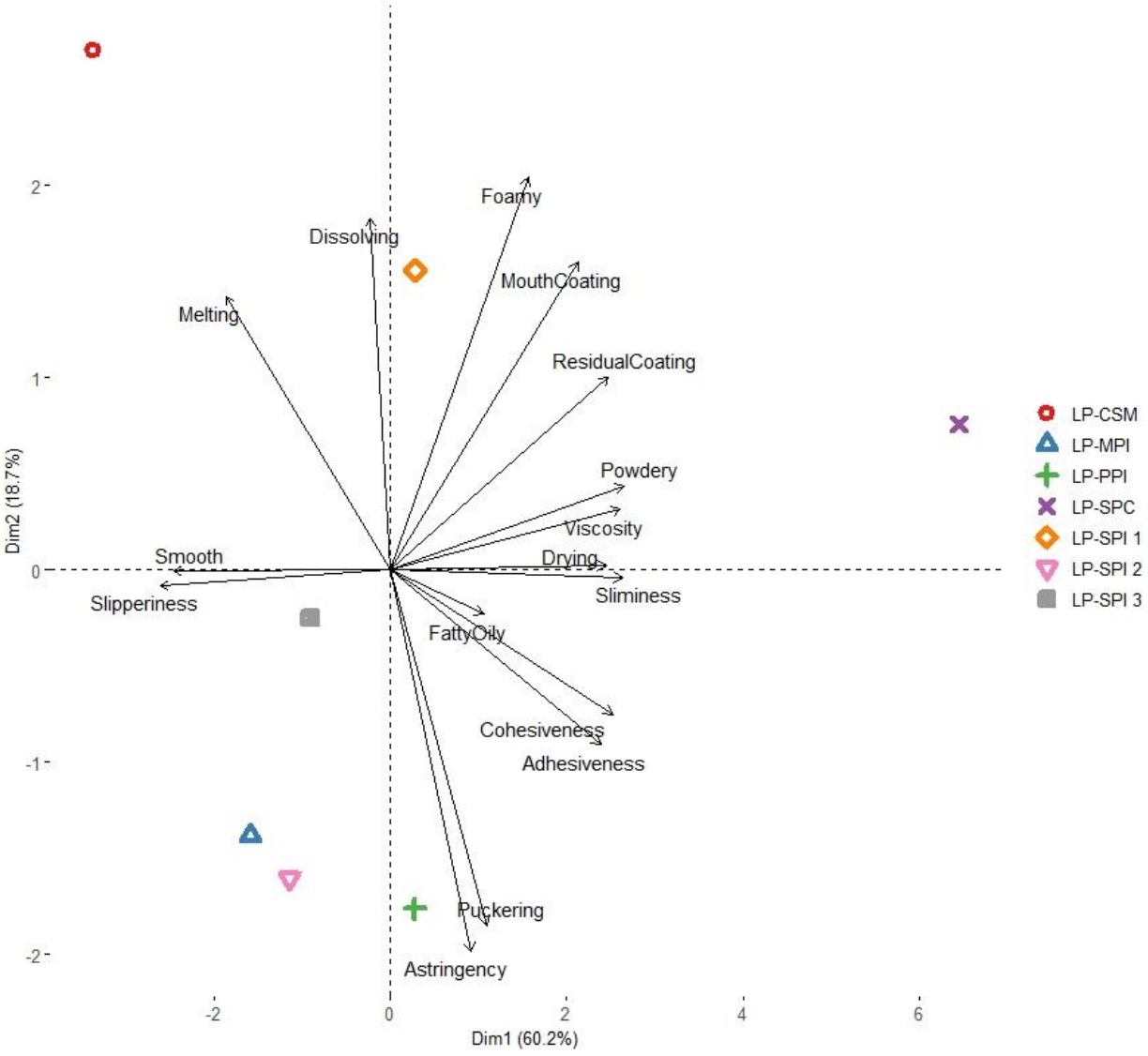

Figure 3.2 Biplot of the Principal Component Analysis of the attribute intensity data obtained from the trained panel for the seven LP beverages The figure depicts Factors 1 (60.2%) and 2 (18.7%) of the PCA which explain 78.9% of the total variation. Colored symbols indicate the LP beverages whereas attribute loadings are depicted by vectors.

3.4.2. Comparison of texture and mouthfeel attributes intensities of high protein beverages

ANOVA results indicated significant differences (p's<0.05) for all 16 textural and mouthfeel attributes across the HP beverages. Of the 16 attributes, 5 had ranges greater than 2 points including mouthcoating (HP-SPI 2/3: 2.5±0.2 – HP-SPC: 4.8±0.3), powdery (HP-CSM: 1.1±0.1 – HP-SPC: 4.4±0.4), residual coating (HP-MPI: 2.0±0.3 – HP-SPC: 4.2±0.3), smooth (HP-SPC: 3.2±0.2 – HP-CSM: 6.2±0.2), and viscosity (HP-CSM: 1.6±0.1 – HP-SPC: 5.1±0.3).

Tukey's HSD analysis indicated the HP animal-based beverages significantly differentiated (p's<0.05) from one another for dissolving (HP-CSM: 3.7±0.2, HP-MPI: 2.6±0.2) and fatty/oily (HP-CSM: 1.8±0.1, HP-MPI: 1.4±0.1). In comparison to HP-CSM, HP-PPI was significantly different for 7 attributes including astringency, drying, puckering, sliminess, slipperiness, smooth and viscosity (p's<0.05) (Table 3.4.).

Significant differences were evident among some of the attributes between the HP-SPIs. Tukey's HSD analysis indicated that HP-SPI 1 (2.2±0.2) was significantly drier than HP-SPI 3 (1.8±0.1), had more residual coating (HP-SPI 1: 2.8±0.2, HP-SPI 2: 2.2±0.2), and was less slippery (HP-SPI 1: 3.4±0.4, HP-SPI 2: 4.2±0.4) and smooth (HP-SPI 1: 4.3±0.2, HP-SPI 2: 5.2±0.4) than HP-SPI 2. HP-SPI 1 was also significantly more powdery (HP-SPI 1: 2.6±0.3, HP-SPI 2: 1.6±0.2, HP-SPI 3: 1.6±0.2) than HP-SPI 2 and 3. Fatty/oily was shown to be significantly different between HP-SPI 2 (1.7±0.2) and HP-SPI 3 (1.3±0.1). For all other attributes, there were no significant differences among the three HP-SPI beverages.

When compared to all other HP beverages, the HP-SPC beverage was significantly different for 9 out of 16 attributes including adhesiveness, cohesiveness, drying, mouth coating, powdery, residual coating, sliminess, smooth, and viscosity. It also had the highest intensity in 9 of the 16 attributes (adhesiveness, cohesiveness, drying, foamy, mouth coating, powdery, residual coating, sliminess, and viscosity), while being the least slippery, smooth, and lowest in melting and dissolving. In contrast, HP-CSM had the highest intensity in dissolving, smooth, and slippery, with the lowest in astringency, drying, powdery, puckering, sliminess, and viscosity, showing opposite trends to that of HP-SPC.

When the descriptive data of the HP-beverages were subjected to a PCA, additional insights were observed. HP PC 1 explained 66.6% of the total variance (Figure 3.3), with viscosity having a correlation of 0.3 with this factor. HP PC 2 explains 13.2% of the total variance, with astringency and puckering having correlations with factor 2 of (-0.59) and (-0.64), respectively. This PCA, similar to the LP PCA, distinctly illustrates that HP-SPC drastically differs from the other HP-beverages, as it is characterized by attributes such as adhesiveness, cohesiveness, foamy, mouth coating, powdery, residual coating, sliminess, and viscosity. HP-CSM also differentiates itself from the other beverages being characterized by slipperiness and smooth. HP-MPI and HP-SPI 3 are closely grouped and also characterized by slipperiness and smooth, but to a lesser degree than HP-CSM (higher loading on the negative direction of dimension 1 and on the positive direction on dimension 2). Similar to what was observed in the LP PCA, HP-SPI 2 and 3 have similar loadings on dimension 1 but differ on dimension 2, being driven by

astringency and puckering. Lastly, HP-PPI and HP-SPI 1 exhibit comparable loadings on dimension 1 and 2, also characterized by astringency and puckering.

Table 3.4 Perceived texture and mouthfeel intensity ratings (mean±SE) of high protein (13g protein/8fl. oz) beverages rated on a 10-point continuous line scale. Different letters in a column indicated significant differences ($p<0.05$). Continuous line scales were anchored from "weak" (anchored at "1") to "strong" (anchored at "9").

Products	Adhesiveness	Astringency	Cohesiveness	Dissolving	Drying	Fatty/Oily	Foamy	Melting
HP CSM	1.6ab±0.1	1.1a±0.1	1.6ab±0.1	3.7b±0.2	1.2a±0.1	1.8c±0.1	1.1a±0.1	3.9ab±0.4
HP MPI	1.5a±0.1	1.5ab±0.2	1.6ab±0.2	2.6a±0.3	1.4ab±0.1	1.4ab±0.1	1.0a±0.1	3.8ab±0.2
HP PPI	2.0b±0.1	2.0b±0.2	1.9b±0.2	3.3ab±0.2	1.7b±0.2	2.0c±0.2	1.1a±0.1	3.4ab±0.4
HP SPC	3.3c±0.3	1.7b±0.2	2.9c±0.3	2.6a±0.3	2.8d±0.3	1.8c±0.3	1.4b±0.2	3.2a±0.2
HP SPI 1	1.4a±0.1	1.8b±0.2	1.7ab±0.2	3.3ab±0.4	2.2c±0.2	1.4ab±0.2	1.2ab±0.1	3.8ab±0.4
HP SPI 2	1.5ab±0.1	2.0b±0.2	1.3a±0.1	3.3ab±0.4	1.8bc±0.1	1.7bc±0.2	1.0a±0.1	3.9b±0.4
HP SPI 3	1.7ab±0.1	1.5ab±0.2	1.6ab±0.1	3.5b±0.2	1.8b±0.1	1.3a±0.1	1.0a±0.1	3.4ab±0.2

Products	Mouth Coating	Powdery	Puckering	Residual Coating	Sliminess	Slipperiness	Smooth	Viscosity
HP CSM	2.8a±0.2	1.1a±0.1	1.0a±0.1	2.4abc±0.2	2.1a±0.2	4.3c±0.3	6.2d±0.2	1.6a±0.1
HP MPI	2.6a±0.2	1.5a±0.2	1.2a±0.1	2.0a±0.2	2.3a±0.3	3.6bc±0.4	5.9d±0.3	1.9abc±0.2
HP PPI	2.9a±0.2	1.5a±0.1	1.6b±0.2	2.6bc±0.2	3.2b±0.3	3.4ab±0.2	4.9bc±0.2	2.3c±0.1
HP SPC	4.8b±0.3	4.4c±0.4	1.2a±0.1	4.2d±0.3	3.9c±0.4	2.7a±0.3	3.2a±0.2	5.1d±0.3
HP SPI 1	3.1a±0.2	2.6b±0.3	1.4ab±0.2	2.8c±0.2	2.0a±0.2	3.4ab±0.4	4.3b±0.2	2.1bc±0.2
HP SPI 2	2.5a±0.2	1.6a±0.2	1.3ab±0.1	2.2ab±0.2	2.2a±0.2	4.2c±0.4	5.2c±0.4	1.8abc±0.1
HP SPI 3	2.5a±0.2	1.6a±0.2	1.2a±0.1	2.6bc±0.2	2.5a±0.3	3.9bc±0.3	4.8bc±0.2	1.8ab±0.1

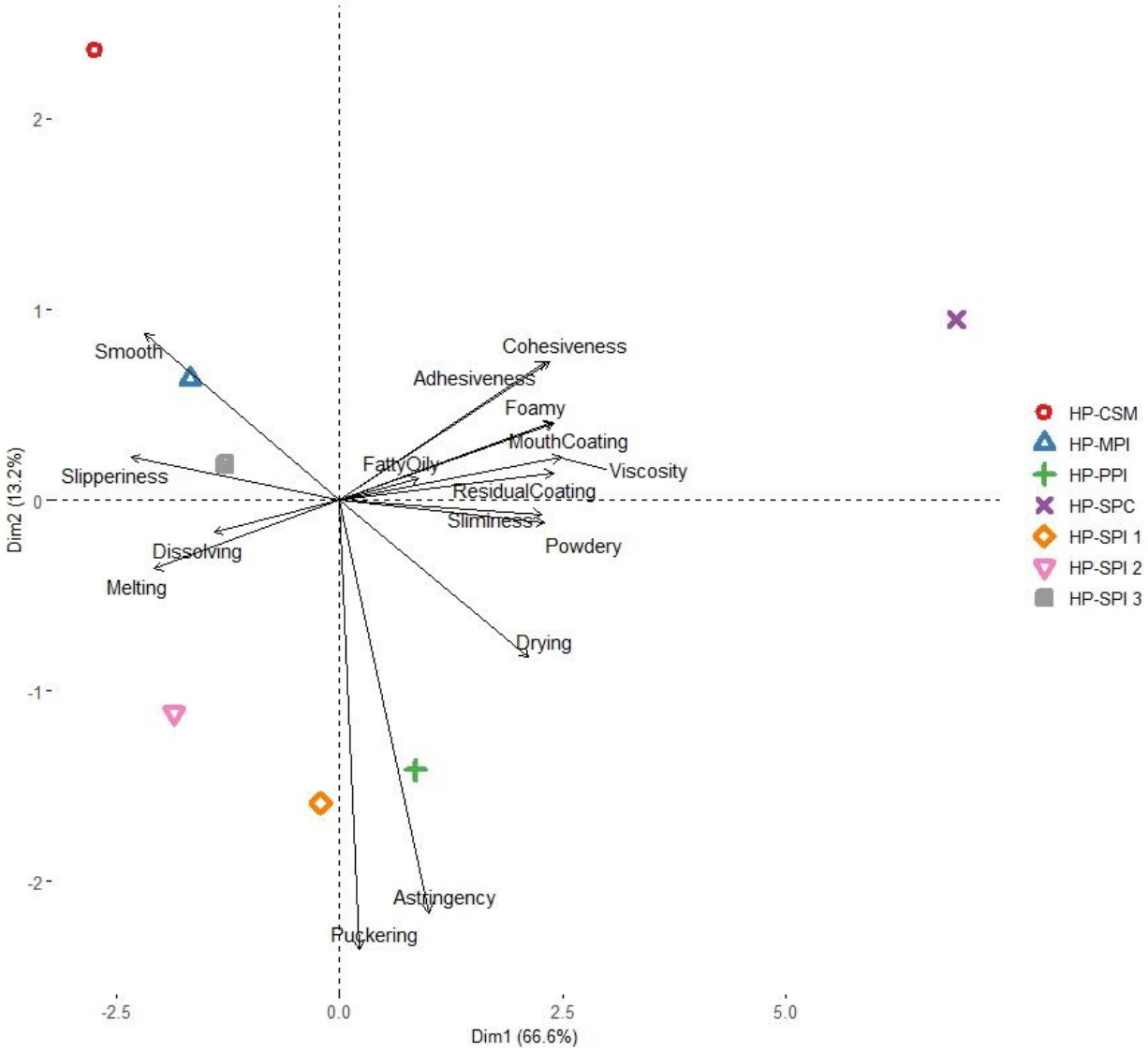

Figure 3.3 Biplot of the Principal Component Analysis of the attribute intensity data obtained from the trained panel for the seven HP beverages. The figure depicts Factors 1 (66.6%) and 2 (13.2%) of the PCA which explains 79.8% of the total variation. Colored symbols indicate the LP beverages whereas attribute loadings are depicted by vectors.

3.4.3. LP vs HP beverages

When attribute intensities were averaged across all LP or all HP products, ANOVA results showed a significant protein concentration effect (p's≤0.05) for all texture and mouthfeel attributes except for dissolving (p=0.64), fatty/oily (p=0.23), and foamy (p=0.39) (Table 3.2). For the other 13 attributes, overall trends showed that increasing protein concentrations decreased the intensity of melting, slippery, and smooth. Conversely, the intensities of the other attributed tended to increase with a higher protein concentration Additionally, the ANOVA results indicated four of the 16 attributes (adhesiveness, cohesiveness, mouth coating, and viscosity) had a significant product*protein concentration effect (Table 3.5.), indicating the effect of the protein concentration was not consistent across all beverages for these descriptors.

Table 3.5 F and p-values for the sensory attributes comparing the effect of protein level on the products

Attributes	Product*Protein Level
Adhesiveness	5.16 (<0.001)
Astringent	0.57 (0.75)
Cohesiveness	4.04 (<0.001)
Dissolving	1.26(0.28)
Drying	0.22 (0.97)
Fatty/Oily	1.55 (0.16)
Foamy	0.96 (0.46)
Melting	0.31 (0.93)
Mouth coating	2.41 (0.03)
Powdery	0.29 (0.94)

Puckering	1.20 (0.31)
Residual coating	2.08 (0.06)
Sliminess	1.65 (0.13)
Slipperiness	0.63 (0.70)
Smooth	0.68 (0.67)
Viscosity	8.31 (.001)

3.5. Discussion

Presently, we developed an extensive texture and mouthfeel lexicon to enable the comprehensive profiling of liquid beverage products. We then used this language to profile and compare the textural and mouthfeel sensations evoked by protein beverages made from animal and plant-based proteins. We found significant, but nuanced, texture and mouthfeel differences between animal and plant-based beverages that were generally consistent whether the products were developed with low or high protein concentrations.

3.5.1. LP beverages

The similarities in textural characteristics observed between the LP animal-based beverages were unsurprising as milk texture can be highly influenced by fat and protein content (Morison & Mackay, 2001; Phillips et al., 1995; Van Vliet & Walstra, 1979), which the two beverages were alike in. Indeed, neither product contained fat, and the LP-MPI beverage was specifically formulated to have the same protein level as seen in skim milk. Although LP-CSM and LP-MPI did not significantly differ for any of the 16 attributes, the PCA (Figure 3.2) indicates the LP-CSM is characteristically more dissolving, foamy, melting, more mouth coating (higher loading on dimension 1), smooth, and slippery (higher loading on dimension 2), while being less puckering and astringent (opposite direction of these attributes on dimension 2) than LP-MPI. These nuanced differences may be attributed to LP-MPI being created from a powder, with the

inclusion of various additives (e.g., tricalcium phosphate, guar gum, gellan gum, maltodextrin, and silicon dioxide) impacting texture, mouthfeel, homogeneity, and solubility of the beverage.

Despite the LP-SPIs having minor differences via Tukey's HSD analyses, the PCA shows that these products were differentiated on dimensions 1 and 2 (Figure 3.2). Most notably, LP-SPI 1 loads positively on dimension 1 and 2, whereas LP-SPIs 2 and 3 load negatively on both dimensions. One potential reason why LP-SPI 1 is different than LP-SPIs 2 and 3 may be attributed to it being highly hydrolyzed through enzyme treatment. Previous research has shown that whey protein undergoing a higher degree of denaturation via heating is also higher in mouthcoating, dry, and chalky attributes (Bull et al., 2017). Similar observations were shown for LP-SPI 1 in comparison to LP-SPI 2 and 3, as the PCA indicates that LP-SPI 1 was characteristically more dry, powdery (similar to chalky) (positive on dimension 1) and had more mouthcoating (positive on dimension 2). Indeed, similar to protein denaturing via heating, the enzymes used to hydrolyze and cleave the soy protein could affect the textural properties of the powder, leading to solutions that also exhibit reductions in smoothness and slipperiness (negative on dimension 2) with more residual coating (positive on dimension 2) compared to LP-SPI 2 and 3 (Figure 3.2). It should be noted that dimension 2 explains less variance than dimension 1, suggesting smaller differences between these two samples (when compared to the same degree of difference on dimension 1), as indicated by minimal differences in attributes between the two beverages via Tukey's HSD analysis. Nevertheless, differences observed in LP-SPI 2 may be attributed to how LP-SPI 2 was hydrolyzed, albeit to a lesser degree than LP-SPI 1, whereas LP-SPI 3 was not. LP-SPI 2 is more astringent and

puckering, which is highly driving differences in the negative direction of dimension 2, where LP-SPI 2 resides.

As depicted in the PCA (Figure 3.2), LP-PPI differentiated itself from the other LP beverages as the only product to load positively on dimension 1 and negatively on dimension 2, characterized by astringency and puckering (a sub-quality of astringency). This was not surprising as previous studies have shown that astringency is one of the distinctive characteristics of pea protein (Cosson et al., 2020; Lesme et al., 2024).

The unique sensory properties of LP-SPC cause it to have a substantial impact on dimension 1 of the PCA. LP-SPC was highest in adhesiveness, cohesiveness, drying, foamy, mouthcoating, powdery, residual coating, sliminess, and viscosity, which load high on PC 1. One reason contributing to the vastly different sensory profile of LP-SPC, compared to the other LP-beverages is the low protein content of the initial protein powder. While the other protein beverages (with the exception of LP-CSM) were ~83-95% protein in powder form, the LP-SPC was only 70% protein. Hence, to normalize the protein content (8g/8fl. oz) across all of the LP-beverages, LP-SPC powder was added at a much higher concentration. Increasing the solute concentration likely led to changes in textural properties that differentiated it from the other LP -beverages.

Similar to LP-SPC, LP-CSM was substantially different from all other beverages as shown in Figure 3.2. LP-CSM was the only LP beverage that was commercially made and not from a powder. However, LP-MPI still had similar textural characteristics to LP-CSM. Hence, creating a beverage from powder form may not be the only factor affecting texture and mouthfeel. In addition, the type of protein used to create a beverage

seemingly also has a substantial implication on the textural and mouthfeel properties of these products.

3.5.2. HP beverages

There were minor differences between the two HP animal-based beverages. It is noteworthy that HP-CSM and HP-MPI have comparable loadings on dimensions 1 and 2, similar to LP-CSM and LP-MPI (Figure 3.2). The similarities between the two HP animal-based beverages may be due to HP-CSM being processed through ultrafiltration, which increases the protein and mineral content (known to affect texture properties in milk) (Hadde et al., 2015) and eliminates sugars. However, HP-CSM loads more negatively on dimension 1 and more positively on dimension 2 suggesting HP-CSM to be characteristically more dissolving, melting, slippery, and smooth, albeit not statistically different than HP-MPI. These findings are similar to that found in the LP-variants. For instance, differences between the two animal-based beverages may be attributed to HP-MPI being created from a powder with the inclusion of additives (e.g., tricalcium phosphate, guar gum, gellan gum, maltodextrin, and silicon dioxide) that impact texture, homogeneity, and solubility of the beverage, leading to nuanced differences between the HP animal-based beverages.

Though small intensity differences were observed among the HP-SPI beverages (Table 3.4), the PCA shows that textural differences were apparent. HP-SPI 1 mainly differentiated from HP-SPI 2 on dimension 1, whereas it differentiated from HP-SPI 3 on dimensions 1 and 2. Some attributes that define HP dimension 1 are cohesiveness, mouth coating, powdery, residual coating, and viscosity, which load positively, whereas smooth, slippery, and dissolving load negatively. Similar to LP-SPI 1, HP-SPI 1 may be different

due to the intensive enzyme treatment that results in a high hydrolyzed product that is less smooth and slippery, while being more powdery, and having more mouth coating and residual coating compared to HP-SPI 2 and 3 (Figure 3.3). While the PCA might suggest noticeable differences between HP-SPI 2 and 3, particularly in PC 2 where they differ, its important to note that PC 2 only accounts for 13.2% of the variance. In contrast, PC 1 explains 66.6% of the variance, and HP-SPI 2 and 3 are closely aligned in this dimension. Furthermore, Tukey's HSD analysis showed that no significant differences were observed across most texture and mouthfeel attributes (except fatty/oily) between these two beverages. The similarity in textural characteristics is noteworthy as HP-SPI 2 underwent slight hydrolysis treatment whereas HP-SPI 3 did not. These differences and similarities observed amongst the HP-SPIs were also evident in the LP-SPIs, emphasizing the significant impact that the degree of hydrolyzation can have on textural properties of soy protein isolate-based beverages.

HP-PPI was significantly more astringent, fatty/oily, and puckering amongst the HP beverages. This is further illustrated in the PCA, where HP-PPI stands out by loading negatively on PC 2, which is particularly associated with astringency and puckering. This is similar to that of LP-PPI shown in Figure 3.2. However, in comparison to LP-PPI, these trends may have been further exaggerated in the HP-PPI variant with the increase in protein content, increasing the intensities of astringency and puckering that are commonly present in pea protein.

Unsurprisingly, HP-SPC's unique sensory properties causes it to have a substantial impact on dimension 1 of the HP-PCA. HP-SPC was highest in adhesiveness, cohesiveness, drying, foamy, grain, lumpy, mouthcoating, powdery, residual coating,

sliminess, and viscosity, which all load high on HP-PC 1. Similar to LP-SPC, due to the low protein content of the initial protein powder, HP-SPC powder was added at a much higher concentration compared to the other HP-beverages. Increasing the solute concentration likely led to the changes in textural properties that substantially differentiated it from the other HP-beverages.

3.5.3. Effect of protein concentration

Overall, results showed that there was a significant protein concentration effect for 13 of the 16 attributes. Among these, melting, slipperiness, and smooth attributes tended to decrease as protein concentrations increased. This was expected as previous research has shown that increasing casein concentration in milk protein beverages increased instrumental viscosity which can impact the perception of other attributes (Cheng et al., 2019). As these beverages become thicker due to the increase in protein concentration, they are less prone to thinning (reduced melting), more resistant to movement in the mouth (reduced slipperiness), and have an uneven consistency due to it being less homogenous (reduced smoothness). Conversely, with a higher protein concentration, attributes such as astringency, drying, mouth coating, and viscosity became more pronounced and detectable to panelists resulting in higher perceived intensities of these attributes. However, it should be noted that ANOVA results indicated only four of the 16 texture attributes had a significant product*protein level effect (Table 3.5.). This suggests that the effect of the protein level (LP vs HP) depended on the specific product. For example, a high concentration of protein impacted adhesiveness to a greater extent in the SPC (LP-SPC: 2.2±0.2, HP-SPC: 3.3±0.3) compared to other products such as SPI 3 (LP-SPI 3: 1.4±0.1, HP-SPI 3: 1.7±0.1). Despite this, trends

between the different types of proteins were consistent. For example, sliminess intensities among the SPIs increased between the low and high protein concentrations, but SPI 1 was the least slimy, whereas SPI 3 was the most slimy for both LP and HP SPIs. This suggests that distinct sensory characteristics linked to each protein-type persist consistently as concentrations increase. In other words, additional attributes do not emerge with higher protein concentrations. This finding carries implications for the formulation of functional beverages, as increasing protein content, within the ranges tested presently, to fortify beverages may not drastically change a product's texture and mouthfeel profile. However, it remains uncertain whether the subtle textural differences resulting from increasing the protein content are noticeable to consumers and whether it affects product acceptance. Additionally, a limitation of this study was its focus solely on texture and mouthfeel, overlooking other sensory modalities such as taste, aroma, and flavor. Therefore, further research should explore the effects of increasing protein concentration on these sensory aspects and consumer preference.

3.6. Conclusion

This study provided a comprehensive sensory profiling of the texture and mouthfeel properties of liquid beverages created from plant and animal-based protein sources. The developed lexicon consisted of 16 unique attributes, allowing for a detailed characterization of these beverages. This is particularly notable given that the majority of studies tend to focus on a limited number of attributes (Day N'Kouka et al., 2004; Liu et al., 2021; Pramudya et al., 2019). In contrast, this study demonstrated that texture is not confined to a handful of attributes, and the use of 16 different texture/mouthfeel attributes enables panelists to discern nuanced differences across various beverages.

Results from this study showed that textural properties of animal-based protein differed from plant-based protein beverages, with SPC exhibiting the most distinct sensory characteristics amongst all protein sources. Additionally, variations in the degree of hydrolysis via enzyme treatment were found to have a significant effect on the textural properties of SPI beverages. Importantly, the observed trends among the different protein sources in the LP beverages were consistently reflected in the HP beverages. This underscores that textural differences among these beverages are inherent to the type of protein used, rather than being dependent on protein concentration. These findings offer valuable insight for guiding product development strategies and contribute to a deeper understanding of the intricate relationship between protein characteristics and textural properties in liquid beverages.

Chapter 4. Relating Texture Perception to Suprathreshold Oral Tactile Sensitivity in Plant and Animal-Based Beverages

Modified from: Min Sung Kim, Laura Nattress, and Christopher T. Simons, Manuscript in Preparation

4.1. Abstract

Despite advancements in characterizing mechanosensitivity of oral tissues to tactile stimuli, limited research exists that links oral tactile sensitivity to food texture perception. The objective of the present study was to evaluate suprathreshold sensitivity to oral tactile stimuli and explore its relationship with texture sensitivities in beverages (soy protein concentrate [SPC] and commercial skim milk [CSM]). Thirty-four participants were recruited to assess the perceived intensity of astringency, mouth coating, and smoothness of SPC and CSM beverages. Oral tactile sensitivity of the participants was evaluated using roughness (rugae and tongue), punctate pressure (tongue), thickness (whole mouth), and grittiness (whole mouth) stimuli. Results indicated significant differences in texture perception between the two beverages, with SPC being more astringent and mouth coating, while CSM was perceived as smoother. Tactile sensitivity data were used to calculate area under the curve (AUC) as a measure of suprathreshold sensitivity. ANOVA results showed suprathreshold tactile sensitivity varied among panelists, showing significant correlations between rugal roughness sensitivity and perceived astringency ($r=0.45$, $p=0.001$), tongue roughness sensitivity and perceived mouth coating ($r=0.38$, $p=0.02$), stimulus thickness sensitivity and perceived mouth coating ($r=0.44$, $p=0.01$), and stimuli grittiness sensitivity and perceived smoothness ($r=-0.38$, $p=0.03$). These findings suggest that specific oral tactile sensitivities significantly

contribute to the perception of food texture, highlighting the importance of considering multiple oral surfaces and tactile stimuli in texture perception research.

4.2. Introduction

Texture perception plays a pivotal role in the consumer acceptance, intake, and preferences of food and beverages (Forde et al., 2013; Pellegrino & Luckett, 2020; Scott & Downey, 2007; Spence et al., 2013). Texture cannot be described by a single attribute, but encompasses a variety of multi-modal complex sensations (Szczesniak, 2002). As such, no single mechanoreceptor likely codes for a specific texture modality, but likely a combination of these signals produces these complex and nuanced sensations (Foegeding et al., 2015; Linne & Simons, 2017). Despite the derivation of these sensations being underpinned by oral tactile sensitivity, there is a lack of research investigating the mechanisms subserving texture perception.

There are numerous psychophysical approaches for examining the relationship between oral tactile sensitivity and texture perception, with the majority of research focusing on threshold and suprathreshold sensitivities. Threshold is a statistical measure that represents the point at which a difference between confusable stimuli intensities can be detected or recognized 50% of the time (Bartoshuk, 1978; Bi & Ennis, 1998). Threshold can be categorized into detection threshold, recognition threshold, and difference threshold. Detection thresholds indicates the lowest intensity at which a sensation can be perceived. Recognition thresholds indicates the lowest intensity at which a sensation can be identified. Finally, difference threshold, also known as just-noticeable-difference (JND), refers to the minimum intensity difference needed to distinguish

between two stimuli. On the contrary, suprathreshold refers to stimulus intensities that are above the threshold required to elicit a clearly perceptible sensation.

Previous studies have utilized these measurements to quantify oral tactile acuity to various tactile stimuli, including punctate pressure, roughness, two-point discrimination, and edge, point, and shape stimuli (Aktar et al., 2015a, 2015b; Bangcuyo & Simons, 2017; Breen et al., 2019; Cattaneo et al., 2020; Linne & Simons, 2017; Miles et al., 2018, 2020; Miles, Berkowitz, et al., 2022; Miles, Wu, et al., 2022; Nishimura et al., 2021). Furthermore, these studies have also shown that the tongue is more sensitive than the fingertip to tactile stimuli such as punctate, two-point discrimination, edge sharpness, and roughness (Aktar et al., 2015b; Miles et al., 2018, 2020). While these studies have characterized sensitivity of oral tissues to tactile stimuli, limited evidence exists linking oral tactile sensitivity to food texture perception.

Research by Linne and Simons (2017) found a strong relationship between both detection threshold and suprathreshold surface roughness sensitivity and the astringency elicited by epigallocatechin gallate (EGCG), but not by tannic acid (TA). Likewise, Breen et al., (2019) reported a significant relationship between punctate pressure discrimination thresholds and chocolate particle size perception. However, most studies investigating the relationship between oral tactile sensitivity to food texture perception have not reported significant correlations. For example, Aktar et al., (Aktar et al., 2015a) found no significant correlations between viscosity of syrups and lingual tactile sensitivity measured through detection thresholds for punctate pressure. In a follow-up study, they found no significant relationship between jelly firmness and elasticity and either two-point discrimination or punctate pressure detection thresholds (Aktar et al., 2015b).

Similarly, Lv et al., (2020) found no relationship between viscosity discrimination of maltodextrin samples and detection thresholds for punctate pressure or two-point discrimination.

Several limitations may account for these inconsistencies between texture perception and oral tactile sensitivity in these studies. First, many studies have focused on threshold rather than suprathreshold measurements. Consumers typically experience food at suprathreshold levels, making these measures more relevant (Liu et al., 2022). Second, using food-like matrices rather than real food products may not accurately represent actual food textures. These limitations are present in prior research; for instance, although Linne and Simons (2017) examined both threshold and suprathreshold measurements, they used a food-like matrix (EGCG and TA solutions in water) rather than real food. Conversely, Zhou et al., (2021) found a correlation between recognition threshold of punctate pressure (0.02g Von Frey Filaments) and biscuit hardness, but did not evaluate suprathreshold measures. Moreover, most studies have focused solely on lingual tactile sensitivity. Food texture perception results from the combination of the tactile inputs from various oral surfaces, not just the tongue (Engelen & Van Der Bilt, 2008; Miles, Berkowitz, et al., 2022). As such, the appropriate selection of tactile stimuli and assessment areas could yield better correlations. For example, Breen et al., (2019) observed a significant correlation between chocolate particle discrimination and punctate pressure discrimination on the central tongue but no on the lateral edges. Finally, the choice of stimuli to use in the assessment of oral tactile sensitivity is important, as different approaches will evaluate different surface fields and mechanoreceptors, which will influence the relevance to texture perception.

To address these prior limitations, the current study aims to evaluate perceived intensity of astringency, mouth coating, and smoothness in soy protein concentrate (SPC) and cow's skim milk (CSM), and correlate panelist sensitivity to specific suprathreshold measurements of oral tactile acuity including lingual/rugal roughness, lingual punctate pressure, thickness, and grittiness. We hypothesize that lingual tactile acuity to various stimuli will underpin sensitivity to specific textural attributes in SPC and CSM.

4.3. Methods

4.3.1. Participants

Thirty-four subjects (11 males, 23 females), all students from the Food Science and Technology department at The Ohio State University (Columbus, OH) were recruited for this study. Eligibility criteria required participants to consume plant-based and/or animal-based beverages and have no allergies to dairy or soy. Additionally, participants were screened out if they had any oral or sensorial deficiencies (e.g., denture implants, trigeminal neuropathies, dysphagia, etc.) that may impact their ability to evaluate texture and/or oral tactile cues. Participants were instructed to refrain from eating and smoking for 1 hour prior to each session. Each participant attended three 1-hour sessions and received $20 per session. The study was approved by the local Institutional Review Board (IRB2022B0179), and all data were collected under written informed consent from each participant.

4.3.2. Sensory evaluation of beverages

Training and sensory evaluation of the beverages was conducted at the Sensory Evaluation Center at The Ohio State University. Two beverages were evaluated: Kroger

(Cincinnati, OH) skim milk (CSM) and a soy protein concentrate (SPC) manufactured by the Archer-Daniels-Midland Company in Kentucky, USA. Both beverages contained 8g of protein per 8fl. oz and were fat-free.

In the first session, participants were provided training on using the generalized labeled magnitude scale (gLMS) (Bartoshuk et al., 2004) by rating three verbal scenarios: the brightness of a well-lit room, brightness of a dimly lit restaurant where the only light is from candles on the table, and the brightest light the panelist has ever seen. Additionally participants were introduced to definitions and references for three texture attributes that included astringency, mouth coating, and smoothness. Texture attribute definitions and references were previously developed in Chapter 3 (Table 4.1).

In the second session, participants evaluated the perceived intensity of astringency, mouth coating, and smoothness in the CSM and the SPC. Twenty mL of each sample was served in a 2 oz. clear plastic cup with a unique three-digit code. Samples were presented under red light in a randomized, balanced design, and evaluated monadically.

Table 4.1 List of the 3 texture attributes with definitions and references

Attributes	Definition	Reference
Astringency	Sensation of roughness and/or drying/puckering in oral surfaces	0.285g/500mL aluminum sulfate
Mouth Coating	Cloying sensation of the product coating the oral surface	Simple Truth Organic maple syrup
Smooth	Contains no particulate matter, even consistency	Non-fat vanilla Yoplait yogurt

4.3.3. Suprathreshold Evaluation of Tactile Stimuli

Oral tactile sensitivity of each subject was assessed using specific stimuli (see below) in the Psychophysical Lab at The Ohio State University. Participants were instructed to close their eyes during evaluation in order to avoid visual biases. The stimulus sets assessed perceived roughness on the palatine rugae (rugae), perceived roughness on the dorsal tongue surface, punctate pressure on the dorsal tongue surface, perceived thickness, and perceived grittiness within the whole mouth. For each set, stimuli were presented in a randomized, balanced design, and evaluated monadically. Participants were allowed to adjust ratings within a set but were not allowed to re-evaluate previous stimuli. All tactile stimuli intensities were screened to be at suprathreshold level and to be sufficiently different so as not to be confusable.

4.3.3.1. Roughness Stimuli

The set of roughness stimuli were created using epoxy (Adtech, USA) casts from a micro finish comparator (C-9 Cast Microfinish Comparator, GAR Elecrtoforming). The moulds were then attached to backside of a teaspoon, providing a slight curve in the stimuli to allow them to reach and rub against the palatine rugae (Figure 4.1). Roughness measurements (R_a) were taken with a TIME3221 Surface Roughness Tester (Beijing TIME Haofang Technology Co., Ltd, China), with R_a averages of 2.61, 5.68, 8.89, 22.72, and 26.42 μm. Instrumental measurements were collected over 10 replications.

Panelists were tasked with assessing stimulus roughness when stimuli were rubbed against the rugae and the tongue. With closed eyes, panelists received each stimulus and were directed to rub it back and forth 3-5 times across their palatine rugae. Following this, the stimuli was withdrawn, and panelists rated the intensity of roughness

using the gLMS. Once all given stimuli had been evaluated on the rugae, panelists proceeded to assess stimulus roughness using their tongue with a different presentation order of the roughness stimuli.

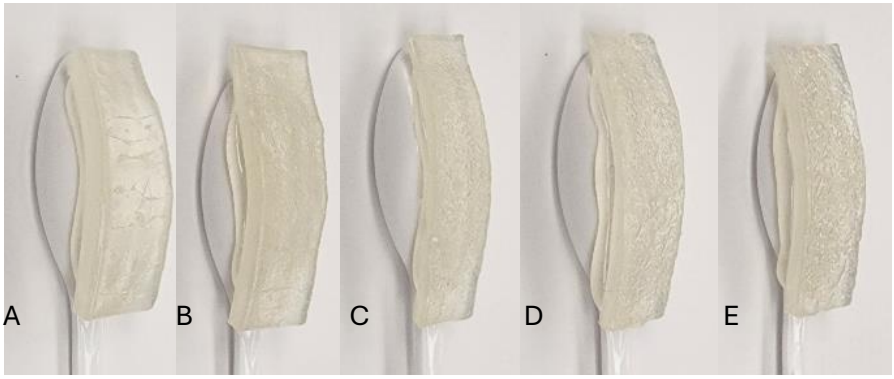

Figure 4.1 Roughness stimuli were created using epoxy casted from a micro finish comparator and attached to the backside of teaspoons. Roughness measurements of the stimuli (from left to right) were A) 2.61 µm, B) 5.68 µm, C) 8.89 µm, D) 22.72 µm, and E) 26.42 µm. Panelists were asked, to rub the stimuli back and forth 3-5 times against the rugae and tongue, evaluating the perceived roughness of the stimuli.

4.3.3.2. Punctate Pressure Stimuli

The set of punctate pressure stimuli consisted of five Semmes-Weinstein monofilaments (Fabrication Enterprises, USA) with force measurements of 0.00069, 0.0039, 0.0098, 0.020, and 0.039N (Figure 4.2). For evaluation, the administrator would press the monofilament perpendicular to the anterior dorsal surface of the tongue three

times, at the same location. Following this, panelists were asked to rate the punctate pressure intensity of each stimulus, individually, using the gLMS.

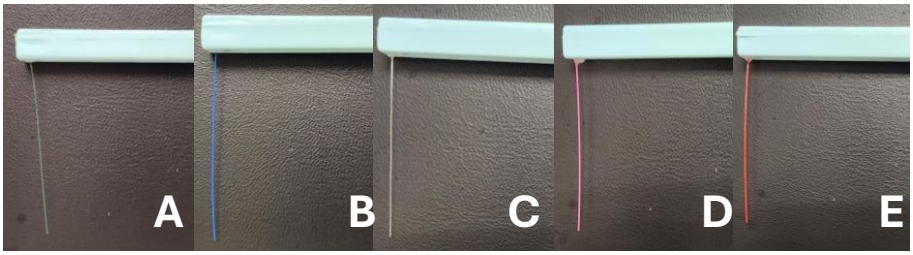

Figure 4.2 Punctate pressure stimuli consisted of five Semmes-Weinstein monofilaments with forces ranging (from left to right) A) 0.00069N, B) 0.0039N, C) 0.0098N, D) 0.020N, and E) 0.039N. The punctate pressure stimuli would be pressed onto the anterior dorsal surface of the tongue three times, and panelists were asked to evaluate the perceived punctate pressure of each stimulus.

4.3.3.3. Thickness Stimuli

The set of thickness stimuli was created using varying concentrations of carboxymethylcellulose (CMC) (Sigma-Aldrich, USA) from 0-2% w/v, with increments of 0.5%. Thickness measurements were measured using a viscometer with a RV06 attachment (DV2T Viscometer, AMETEK Brookfield, USA) in triplicate, with measurement averages being 20, 147, 1400, 5873, and 15053cP (Figure 4.3). Thickness

stimuli were filled into 10mL syringes (Chemglass Life Sciences, USA), and panelists were instructed to insert all 10mL of the solution into their mouth, and swirl it around for three seconds. After which, each panelist was asked to rate the stimulus thickness using the gLMS. Panelists were instructed to rinse with water between each thickness stimulus.

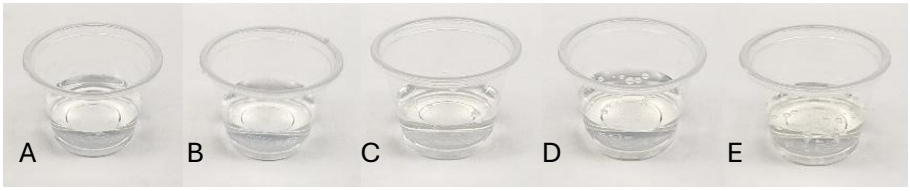

Figure 4.3 The set of thickness stimuli varied in concentrations of carboxymethyl cellulose solutions in water from 0-2% w/v, with increments of 0.5%. Thickness measurements for the stimuli are as follows (from left to right) A) 20cP, B) 147cP, C) 1400cP, D) 5873cP, and E) 15053cP. A 10mL solution for each stimulus would be placed in their mouth and panelists were asked to swirl it around for three seconds, evaluating the thickness perception of the solution.

4.3.3.4. Grittiness Stimuli

The set of grittiness stimuli consisted of aluminum oxide finishing media (Interactivia Incorporated, Canada) with varying diameters in sizes of 56, 89, 165, 254,

and 550 µm. Each sample (0.1g) was presented in 1 oz. plastic cups (Figure 4.4). Panelists were asked to place all 0.1g into their mouth, move it around their oral cavity for three seconds and evaluate the perceived grittiness sensation. Panelists were then asked to expectorate and rinse their mouths with either water and/or a 0.5% CMC solution between samples; some panelists felt using the 0.5% CMC solution aided in removing the residual particles remaining after expectoration. Once all of the previous grittiness sample was removed from the mouth, they would receive the next sample.

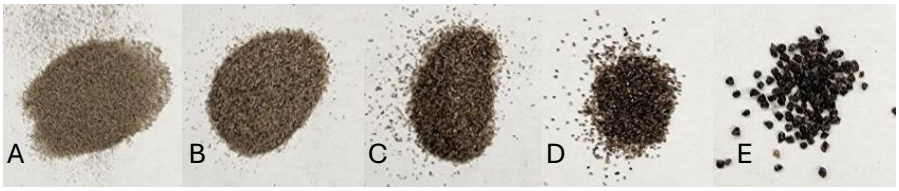

Figure 4.4 The grittiness stimuli set consisted of aluminum oxide finishing media that ranged in diameters (from left to right) A) 56µm, B) 89µm, C) 165µm, D) 254µm, and E) 550µm. Panelists were asked to place 0.1g of each stimulus into their mouth and move it around their oral cavity for three seconds and evaluate the perceived grittiness sensation of each sample.

4.3.4. Statistical Analysis

Variability in texture perception of the beverages were assessed by log-transforming gLMS values. The beverage with the higher perceived intensity range for

each texture attribute was selected to be correlated with suprathreshold tactile sensitivity (Figure 4.5). For astringency, the rating for SPC was used, whereas for mouth coating and smooth, the ratings obtained from CSM were used. To analyze the beverage texture data, a two-way (panelist, beverage main effects) analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) post-hoc analysis was conducted on each attribute using SPSS version 27 (IBM, Armonk, NY). For each suprathreshold stimulus set, perceived stimulus intensity was analyzed by log-transforming gLMS values and then subjected it to a two-way (panelist, stimulus level main effects) ANOVA with a Tukey's HSD post-hoc analysis. All data are presented as means ± SE.

Panelist intensity data for each stimulus was plotted against stimulus strength to generate individual psychophysical curves. Area under the curve (AUC) measurements were then calculated as a measure of suprathreshold sensitivity using the trapezoidal method in GraphPad Prism 5; Insight Partners, US. These values were then used to correlate to individual texture perception intensity for the beverage with the higher intensity range using Pearson's correlation coefficient (r).

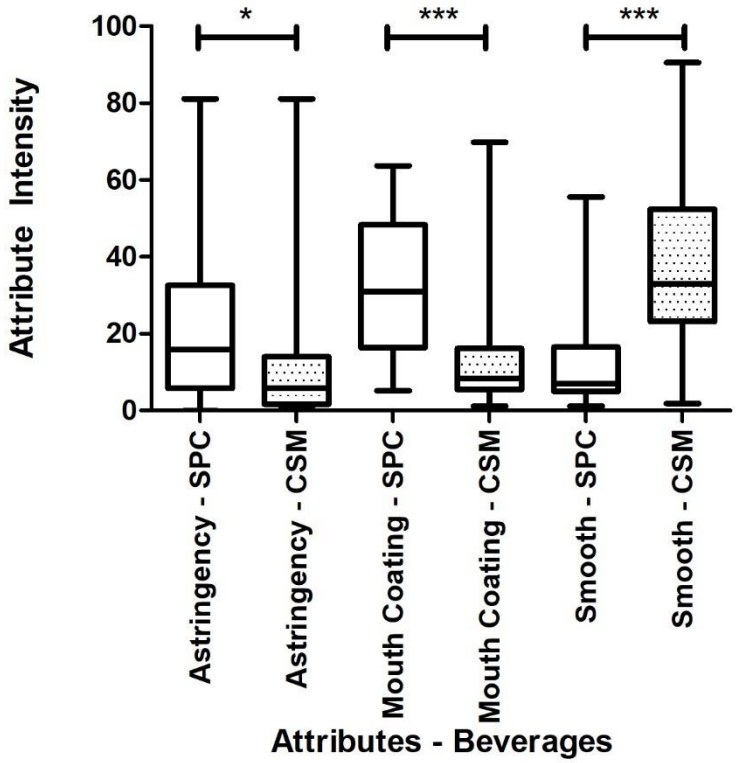

*Figure 4.5 Depiction of the boxplots for each texture attribute and beverages evaluated by the panelists. Intensity ratings were rated using the gLMS (0 – no sensation, 1.4 – barely detectable, 6 – weak, 17 – moderate, 34.7 – strong, 52.5 – very strong, 100 – strongest imaginable sensation of any kind). ANOVA results showed SPC and CSM differed significantly for all three texture attributes. SPC was significantly higher in astringency and mouth coating compared to CSM, whereas CSM had a higher intensity of smoothness compared to SPC. Significant differences of texture intensities between beverages were denoted as * significance at $p<0.05$, ** significance at $p<0.01$, and *** significance at $p<0.001$.*

4.4. Results

4.4.1. Sensory Evaluation of Texture Attributes of Beverages

Texture perception intensities were obtained from all 34 panelists for each beverage. ANOVA results indicated that SPC and CSM different significantly ($p's<0.05$) in the perceived intensity for all three texture attributes assessed (Table 4.2). SPC was significantly more astringent (SPC: 20.8±3.1, CSM: 11.7±2.7) and had higher mouth coating (SPC: 31.3±2.7, CSM:12.8±2.3) than CSM. The opposite was true for smoothness, where CSM (40.8±4.2) was significantly higher than SPC (13.5±2.3) (Figure 4.5).

Table 4.2 F (and P values) for panelist and product effect for texture evaluation of a soy protein concentrate beverage and cow's skim milk.

	Astringency	Mouth Coating	Smooth
Panelist	1.24 (0.27)	1.21 (0.29)	0.89 (0.63)
Product	5.54 (0.02)	29.8 (<0.001)	31.4 (<0.001)

4.4.2. Tactile stimuli evaluation

In general, for each stimulus set, ANOVA results indicated significant stimulus and panelist effects (Table 4.3). Within each set of stimuli, a significant stimulus effect

indicates that perceived stimulus intensity increased with the stimulus strength (Figure 4.6). As expected, the tongue was better at discriminating roughness compared to the rugae, as depicted by the larger intensity range within the stimulus set of roughness stimuli (Figure 4.6i). Overall, in comparison to the roughness and grittiness sets, stimuli within the punctate and thickness sets appeared to be better differentiated, as indicated by more Tukey's HSD letter allocations (Figure 4.6 ii. & iii). Moreover, a significant panelist effect indicates variability in panelist perceptions with some panelists perceiving a given stimulus to be stronger (or weaker) than other panelists (Table 4.3). The range in panelist's AUCs varied depending on the stimulus set: rugae roughness: 57.6 – 783.6, tongue roughness: 103.3 – 990.8, punctate pressure: 0.23 – 1.3, thickness: 236,744 – 1,334,000, and grittiness: 4,472 – 42,787.

Table 4.3 F (and P values) for the effect of stimulus strength and panelist for each tactile stimulus set

	Rugae Roughness	Tongue Roughness	Punctate Pressure	Thickness	Grittiness
Panelist	5.71(<0.001)	18.54(<0.001)	9.02(<0.001)	5.79(<0.001)	9.02(<0.001)
Stimuli	3.43(0.01)	66.42(<0.001)	196.88(<0.001)	68.74(<0.001)	47.3(<0.001)

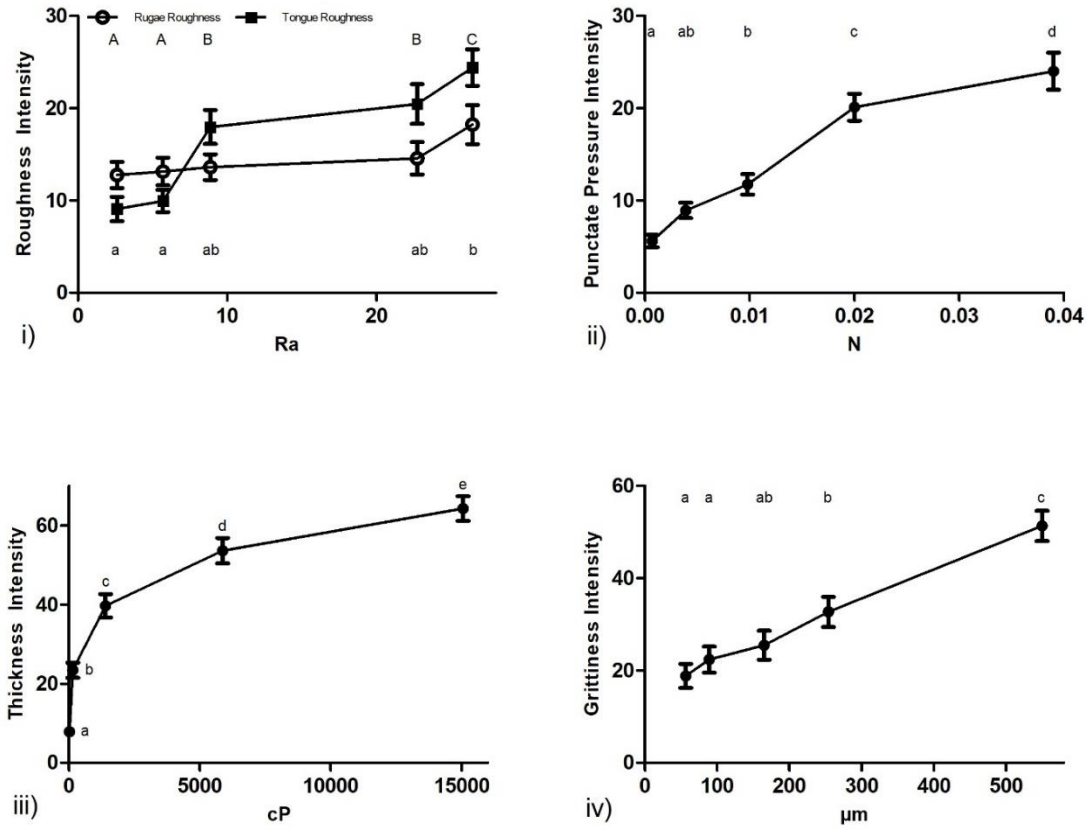

Figure 4.6 Average intensity ratings for each stimulus each set of tactile stimuli. Stimulus intensities were evaluated using gLMS (0 – no sensation, 1.4 – barely detectable, 6 – weak, 17 – moderate, 34.7 – strong, 52.5 – very strong, 100 – strongest imaginable sensation of any kind). Stimuli sets evaluated were i) roughness, ii) punctate pressure, iii) thickness, and iv) grittiness. Different letters of the same capitalization indicate significant differences via Tukey's HSD post-hoc analysis.

4.4.3. Correlations Between Suprathreshold Tactile Stimuli Evaluation and Texture Attribute Sensitivity

Correlations between oral tactile stimulus sensitivity and texture attributes of beverages are shown in Table 4.4. Significant associations were observed for rugae roughness sensitivity and SPC astringency (r=0.45, p=0.001) whereas the correlation for tongue roughness sensitivity and SPC astringency was marginally significant (r=0.32, p=0.06). Sensitivity to other tactile stimuli did not significantly correlate with perceived astringency of SPC. Mouth coating, as expected, was found to be significantly correlated with sensitivity to fluid thickness (r=0.44, p=0.01), as well as tongue roughness sensitivity (r=0.38, p=0.02). Sensitivity to other tactile stimuli did not significantly correlate with mouth coating of CSM. Finally, grittiness sensitivity was found to have a significant negative association with perceived smoothness of CSM (r=-0.38, p=0.03). A marginally significant negative correlation was also observed between lingual roughness sensitivity and smooth perception of CSM (r=-0.32, p=0.06). Other tactile stimuli did not significantly correlate with smooth perception of CSM.

Table.4.4 Correlation matrix between products with the largest attribute intensity range and tactile stimuli AUC, showing Pearson's correlation values and p-values in brackets.

Tactile Stimuli	Astringency SPC	Mouth coating CSM	Smooth CSM
Rugae Roughness	0.45* (0.001)	0.24 (0.17)	-0.05 (0.76)
Tongue Roughness	0.32 (0.06)	0.38* (0.02)	-0.32 (0.06)
Punctate Pressure	0.19 (0.28)	0.27 (0.12)	0.12 (0.49)
Thickness	0.09 (0.60)	0.44* (0.01)	-0.19 (0.28)
Grittiness	0.16 (0.37)	0.15 (0.39)	-0.38* (0.03)

*Denotes statistical significance at $p<0.05$

4.5. Discussion

The present study indicated that subjects varied in their sensitivity to oral tactile stimuli and that oral tactile sensitivity is associated with sensitivity to texture perception in beverages.

4.5.1. Roughness Sensitivity and Astringency

The significant correlations observed between oral roughness sensitivity and astringency perception were expected, given that the definition of astringency includes roughness as a subquality (Lee & Lawless, 1991). Interestingly, rugal sensitivity to roughness (as measured via AUC) showed a stronger and more significant correlation with perceived astringency than lingual roughness sensitivity (Table 4.4). In addition, at lower roughnesses, the rugae seemed to be more sensitive, reflecting higher intensities of perceived roughness (Figure 6. i). The stronger correlation of rugal sensitivity to roughness perception observed in this study may be attributed to the unique design of the roughness stimuli. Previous iterations of the roughness coupons were applied parallel to the surface (Linne & Simons, 2017; Miles, Berkowitz, et al., 2022), whereas the present stimuli are curved and can increase the area of contact, stimulating more mechanoreceptors. However, in general, the tongue was more sensitive to roughness stimuli than the rugae. This observation aligns with previous research that showed the anterior dorsal tongue is more sensitive than the rugae based on roughness JND

thresholds (Miles, Berkowitz, et al., 2022). The tongue's increased sensitivity to roughness perception may be attributable to its flexibility and absence of an epidermal/hard keratinized tissue layer, in contrast to the palatine rugae (Miles, Berkowitz, et al., 2022). These results underscore the significant role of the rugae in texture perception, as exhibited in previous research. In a recent study investigating lingual detection mechanisms for high-viscosity solutions, Miles, Berkowitz, et al., (2022) found that a palate blocked condition resulted in elevated JND compared to an unblocked condition. However, Engelen et al., (2002) found that the palate impeded the size estimation of large spheres (4-9mm) when compared to the evaluation using the tongue alone. Therefore, it is important to note that the role of the rugae in texture perception may be attribute dependent.

Previous research investigating lingual roughness sensitivity and perceived astringency focused solely on the tongue and found a significant correlation between epigallocatechin gallate (EGCG) astringency AUC and roughness AUC on the tongue (Linne & Simons, 2017). In addition, the present study suggests that rugal sensitivity to roughness may play a pivotal role in the mechanical underpinnings of astringency perception.

4.5.2. Correlation between mouthcoating and tactile sensitivity

The significant correlation between thickness sensitivity and mouth coating evoked by CSM was expected. Mouth coating is defined as the cloying sensation from the product coating oral surfaces. Therefore, a thicker product is likely to amplify this sensation by increasing the persistence of the fluid remaining on oral tissues. It should be noted that thickness is also a commonly used sensory attribute to describe beverage

viscosity (Liu et al., 2022). Previous research has explored the relationship between viscosity and mouth coating perception. For example, He et al., (2016) demonstrated a significant correlation between oral thickness perception and shear viscosity, as measured by a rheometer. Additionally, Wagoner et al., (2020) found that varying viscosity levels significantly impacted mouth coating in protein beverages, with higher viscosity levels being perceived as having increased mouth coating. While these studies provide evidence of the association between thickness/viscosity and mouth coating, they do not explain the variability in individual sensitivity. More recently, Miles, Berkotwitz, et al., (2022) found a relationship between the length and density of filiform papillae and sensitivity to high viscosity solutions. This suggests that individuals with longer and denser filiform papillae may be more sensitive to mouth coating. However, since the currently study did not measure individual filiform papillae features, further research is needed to confirm this association.

Interestingly, the current study showed a significant correlation between lingual sensitivity to surface roughness and perceived mouth coating from CSM, which is a novel finding as previous research has not explored this relationship. Although the mechanisms behind this correlation remain elusive, existing studies have firmly established a relationship between instrumental friction and perception of oral roughness (de Wijk & Prinz, 2005; de Wijk & Prinz, 2006; Pradal & Stokes, 2016). Furthermore, Carvalho-Da-Silva et al., (2013) observed a relationship between instrumental friction and mouth coating in milk chocolate, highlighting higher friction in chocolate samples with increased mouth coating.

These findings prompt speculation that increased friction or resistance of a solution, coupled with viscosity, could result in more solution adhering to oral surfaces, thus increasing mouth coating perception. Nonetheless, further research is needed to have a better understanding of the mechanisms underlying sensitivity of mouth coating perception.

4.5.3. Correlation between smoothness and mechanosensitivity

The observed significant negative association between grittiness sensitivity (measured via AUC) and smoothness perception of CSM was expected. Smoothness, by definition, infers to the absence of particulate matter, whereas increased perceived intensity of grittiness suggests more particulate content. Hence, heightened sensitivity to grittiness would enable greater detection of this sensation in products that would have decreased perception of smoothness. This correlation is further supported by prior research (Engelen et al., 2005), indicating the addition of small particles to custard reduced the smoothness perception of these products. Individuals more sensitive to grittiness may detect even minuscule particles (e.g., 2μm) in a relatively homogenous solution, particularly when rubbing oral surfaces together (Engelen et al., 2005). Furthermore, our results showed that tongue roughness sensitivity was marginally significant with individual smoothness perception of CSM. This implies that when oral surfaces rub against each other, especially with the tongue involved, the perception of smoothness may be influenced by roughness friction. This is consistent with previous research by Upadhyay & Chen (2019), which significantly correlated oral smoothness perception and friction force measured instrumentally using a tribometer mimicking tongue and palate surfaces.

4.6. Conclusion

This study aimed to address previous limitations by evaluating suprathreshold sensitivities of both texture perception in beverages and oral tactile sensitivity of various mechanical stimuli. The results indicated that variation in sensitivity to oral tactile stimuli was associated with the perception of some textures inherent to beverages. Associations were established between rugae roughness sensitivity and perceived astringency, lingual roughness sensitivity and perceived mouth coating, and grittiness sensitivity to perceived smoothness in beverages. These findings underscore the intricate interplay between oral tactile sensitivity and texture perception.

Future research should explore mechanisms underlying these associations, considering factors such as individual differences in anatomy (i.e., fungiform papillae density, and filiform papillae density and length) (Bangcuyo & Simons, 2017; Miles, Wu, et al., 2022) and biological features (i.e., salivary flow) (Linne & Simons, 2017) have been shown to have significant impact on oral tactile sensitivity. Furthermore, as threshold and suprathreshold measurements do not necessarily correlate (Bartoshuk, 1978; Lawless & Heymann, 2010), future studies can assess both measurements to get a complete characterization of a percept. By addressing these aspects, future studies can contribute to a deeper understanding of the role oral tactile sensitivity plays in texture perception.

Chapter 5. The role of TRPA1 and TRPV1 in the perception of astringency

Modified from: Min Sung Kim and Christopher T. Simons, Manuscript in Preparation

5.1 Abstract

Astringency, commonly described as a drying, roughening, and/or puckering sensation, associated with polyphenol-rich foods affects their palatability. While the compounds eliciting astringency are known, its mechanism of action is debated. This study investigated the role of transient receptor potential (TRP) channels A1 and V1 in astringency perception. If TRP A1 or V1 have a functional role in astringency perception, then desensitizing these receptors should decrease perceived astringency. Thirty-seven panelists underwent unilateral lingual desensitization of TRP A1 and V1 channels using mustard oil and capsaicin, respectively. Panelists then evaluated four astringent stimuli: epicatechin (EC), epigallocatechin gallate (EGCG), tannic acid (TA) and potassium alum (Alum), via 2-AFC and intensity ratings. When TRPA1 receptors were desensitized on one half of the tongue via mustard oil, no significant differences were observed between the treated and untreated sides for both 2-AFC and intensity ratings. Similarly, when TRPV1 receptors were desensitized on one half of the tongue via capsaicin, no significant differences were observed between the treated and untreated sides for both 2-AFC (except TA) and intensity ratings. These findings challenge the notion that TRP channels play a pivotal role in astringency perception.

5.2 Introduction

Astringency is commonly described as a drying, roughening, and/or puckering sensation in the mouth (Canon et al., 2021; Lee & Lawless, 1991). Astringency is frequently associated with foods high in polyphenols including teas, red wines, fruits, chocolates, and nuts (Bajec & Pickering, 2008; Lesschaeve & Noble, 2005), and can have adverse effects on the palatability of these products. While the compounds that elicit astringency are well known (Green, 1993; Thomas & Lawless, 1995), there is controversy regarding the specific mechanisms underpinning astringency perception. Historically, astringency was purported to be a taste sensation with some studies showing that astringent compounds can stimulate the *chorda tympani* taste nerve in animal models (Schiffman, Suggs, & Simons, 1992; Schiffman et al., 1992). However, subsequent research by Schöbel et al., (2014) indicated that lesion or lidocaine blockade of the *chorda tympani* resulted in no impairment of astringency perception in humans. Only simultaneous anesthetic block of the lingual and inferior alveolar nerves resulted in loss of astringency perception (Schöbel et al., 2014). These results suggest that astringency is a somatosensory sensation and, as such, most studies have investigated mechanisms involving physical and/or chemical activation of mechanoreceptors or chemoreceptors, respectively.

Currently, three potential mechanisms have been proposed to underpin astringency perception (for a recent review, see Wang et al., 2024). The first mechanism involves binding of astringent compounds to salivary proline-rich proteins (PRPs), which have a high affinity for polyphenols (Canon et al., 2015; Charlton et al., 2002; Jöbstl et al., 2004). Binding of polyphenols to the salivary PRPs causes the protein to coil, enabling

crosslinking of the PRP-polyphenols to create protein dimers that can aggregate to form large insoluble complexes that precipitate (Bajec & Pickering, 2008). The increase in precipitated PRP complexes may be perceived as discrete particles relating to roughness perception in the oral cavity (Rene A de Wijk & Prinz, 2006). Furthermore, the precipitation of these PRP-polyphenol complexes decreases salivary lubricity by rupturing the salivary film (Breslin et al., 1993), resulting in an increase of friction that can be detected by mechanoreceptors (Aken, 2010; Rinaldi et al., 2012).

The second proposed mechanism involves the mucosal pellicle, a viscoelastic gel that acts as a lubricant similar to the salivary film, preventing excessive abrasion between surfaces (Gibbins & Carpenter, 2013; Humphrey & Williamson, 2001). The mucosal pellicle is comprised of a layer of salivary proteins including MUC5B, MUC7, cystatins, and IgA (Gibbins et al., 2014) that directly bind to the oral epithelium and are stabilized by protein cross-linking (Bradway et al., 1992; Laguna & Sarkar, 2017). Of these salivary proteins, MUC5B is a major constituent that is able to form complexes with polyphenol compounds (Davies et al., 2014; Ployon et al., 2018). The aggregation of these complexes can disrupt the structure of the mucosal pellicle, causing a decrease of its lubrication properties (Davies et al., 2014). The decreased lubrication is thought to result in increased friction between oral surfaces (Ployon et al., 2018), which is detected by mechanoreceptors, leading to the perception of astringency.

The third and most recent proposed mechanism suggests that polyphenolic compounds evoke astringency by binding and activating the transient receptor potential (TRP) channels ankyrin 1 (A1) and vanilloid 1 (V1). Prior studies using human (Takahashi et al., 2021) and animal cell lines (Kurogi et al., 2012, 2015) indicate that

some astringent compounds (e.g., epigallocatechin gallate [EGCG] and its auto-oxidation products) are potent activators of TRPA1 and TRPV1. However, controversy exists around this theory as large astringent molecules would have very limited access to TRP channels, which are founds below the mucosal surface (Canon et al., 2018; Carpenter, 2013). Moreover, black tea polyphenols, which are known to be astringent, failed to activate TRP channels in immortalized human oral epithelial cells (Carpenter, 2013). Finally, no human sensory study has tested the hypothesis that TRP A1 or V1 have a functional role in astringency. Therefore, the purpose of this study was to investigate if TRP channels contribute to astringency perception in the human oral cavity.

TRP A1 and V1 are temperature-activated channels expressed by nociceptive cells that are commonly activated by mustard oil and capsaicin, respectively (Caterina et al., 1997; Jordt et al., 2004). One phenomenon associated with TRP A1 and V1 is desensitization. Desensitization results from prior exposure to mustard oil or capsaicin and manifests as a loss of sensitivity such that subsequent application of TRP A1 or V1 agonists evokes a substantially reduced sensory response (Green, 1989; Rozin et al., 1981; Simons et al., 2003). With that said, if astringents do bind to TRP A1 and/or V1, as suggested by the third theory of astringency perception, desensitizing these channels would result in a significant reduction in astringency perception. Therefore, the objective of this study is to desensitize both oral TRP A1 or V1 channels and compare perceived astringency to a control, non-desensitized state. We hypothesize that desensitization of both oral TRP A1 or V1 channels will not reduce astringency perception in comparison to a control, non-desensitized state.

5.3. Methods and Materials

5.3.1. Panelists

Thirty-seven panelists (10 male, 27 female) with ages ranging from 22-34 years old were recruited from the Food Science and Technology department at The Ohio State University (Columbus, Ohio). Panelists were non-smokers, had no history of chronic pain, no tongue, cheek, and/or lip piercings, and were free from any taste defects, or visible sores, wounds, wrinkles, scars, or surface deformations of the tongue or oral cavity. Panelists also reported with no sensitivities to capsaicin, mustard oil, or green tea (EGCG and epicatechin are commonly present in green tea). To avoid any pre-existing desensitization, participants were asked to refrain from consuming any spicy foods (e.g., chili peppers, wasabi, horseradish, etc.) 48 hrs prior to each session.

5.3.2. Training

The training protocol was adapted from research previously conducted by Linne and Simons (2017), where prior to evaluation of the astringent stimuli, panelists were taught the differences between astringency and bitterness to ensure they do not confuse the two sensations. Subjects were instructed that astringency may be perceived as any or all of the sub-qualities, including drying, roughening, or puckering. For all liquid samples, panelists were instructed to pour all 10 mL of the sample into their mouth, swish around their oral cavity for 3 s, expectorate into a spittoon, and then rub the anterior half of their tongue against their palatine rugae from front to back 3-5 times. The first part of training consisted of evaluating two samples that included 10mL of 1.20 mM aqueous K alum (Kroger, USA) and 10mL of 90 μM sucrose octaacetate (SOA) (Sigma-Aldrich, USA). Panelists were told that the first solution was astringent but not bitter, and the

second solution was bitter but not astringent. Once panelists were confident in their ability to differentiate the two sensations, they were given a pair of practice samples that included 10mL of 90 μM SOA (bitter only sample), and a second solution that was comprised of a 7:3 ratio of 1.2 mM potassium alum and 90 μM SOA (bitter and astringent). Panelists were told that both samples were bitter but only one was bitter and astringent and they were asked to select the astringent sample. If the panelists correctly selected the astringent sample and expressed confidence in their ability to distinguish them, the training was ended. If panelists were incorrect and/or were unsure, then they repeated the training until they correctly distinguished the samples and were confident in their ability to differentiate them.

5.3.3. Desensitization of TRP A1 and V1

In order to desensitize oral TRP A1 or V1 channels, mustard oil (0.5% in propylene glycol; Sigma-Aldrich, USA) or capsaicin (100 ppm 50% v/v ethanol; Enzo Lift Sciences, USA), respectively, was applied unilaterally to the anterior half of the dorsal lingual surface using a cotton-tipped applicator saturated in solution. This method delivers approximately 40 μL of solution to the lingual surface. The other half of the tongue received application of DI water as a control. DI was selected as the control as ethanol can activate TRPV1 (Trevisani et al., 2002), and propylene glycol is commonly known to have a faintly sweet taste and exothermic properties when mixed with saliva. After receiving the lingual treatment, panelists were initially asked to rate the intensity of "irritation" using a 10-point continuous line scale (anchored: 0 – none, 1 – weak, 9 – strong) on both sides of the tongues. They were then asked to sit quietly with their tongue resting on the floor of their mouth for a minimum of 10 mins or until the irritation had

subsided. At this time, desensitization was confirmed in each subject by re-applying a small amount (~10 µL from a newly saturated swab) of mustard oil (or capsaicin) to the previously treated side of the tongue and asking them to rate the intensity of "irritation". If the perceived irritation was lower than the initial rating, the area was deemed desensitized. If not, mustard oil (or capsaicin) was re-applied to the same previously treated side of the tongue and subjects would wait another 10-15 mins until desensitization was achieved.

The initial administration of either the mustard oil or capsaicin solution was counter-balanced among the panelists, and after a minimum washout period of 7 days, panelists would receive the other solution during their second session. In addition, the side of the tongue (left or right) receiving the mustard oil or capsaicin treatment was counterbalanced among the panelists, and the same side was applied for both sessions.

5.3.4. Astringent Stimuli Evaluation

Four astringent stimuli, diverse in chemical structure, were selected for evaluation. Two catechins were selected based on their difference in molecular size, including epicatechin (EC; 1mM) and epigallocatechin gallate (EGCG; 1.1 mM). Tannic acid (TA; 0.25mM) was selected as it is an astringent polyphenol but not a catechin. Lastly, potassium alum (Alum; 0.4 mM) was selected as it is an astringent salt (Linne & Simons, 2017). Compounds were dissolved in deionized water to the desired concentration. The concentration of each compound was selected based on preliminary testing to ensure solutions were of suprathreshold intensities and were approximately isointense amongst the four stimuli. For each solution, 10 mL were served in a 2 oz. clear plastic cup labeled with a unique three-digit code. Samples were presented monadically

in a random, balanced design. Panelists were given a 2-min time delay between samples and were instructed to rinse their mouth with filtered water between samples. All stimuli were prepared one week prior to evaluation and stored at 4°C. Prior to evaluation, samples were removed from the refrigerator and allowed to equilibrate to room temperature.

Once desensitization was confirmed for each panelist, they received a test sample to evaluate astringency. Similar to training, panelists placed all 10 mL of the solution in their mouth, swished it around for 3 s and then expectorated into a spittoon. Subjects were then instructed to rub both sides of the anterior portion of their tongue against their palatine rugae from front to back 3 – 5 times and assess the astringency intensity of the left and right side of their tongue. Panelists would then indicate which side was more astringent (2-AFC) followed by bilateral intensity ratings of perceived astringency using a 10-point continuous line scale (anchored: 0 – none, 1 – weak, 9 – strong).

5.3.5. Data Analysis

2-AFC data were analyzed using the binomial test (2-tail $\alpha = 0.05$) to determine if a significant majority of subjects chose the desensitized side (via capsaicin or mustard oil) to be less astringent compared to the control. In addition, a 2-tailed paired t-test ($\alpha = 0.05$) was used to compare if the side that was desensitized by either capsaicin or mustard oil had a significant effect on the corresponding astringency ratings on the tongue. Data are presented as mean ±SE.

5.4 Results

5.4.1. 2-AFC Astringency Ratings

Results from the 2-AFC task indicated that when mustard oil was administered to desensitize TRPA1 channels in the tongue, there was no significant effect on astringency perception for any of the astringent compounds. For each compound, there was a non-significant difference in the number of subjects selecting the desensitized over the non-desensitized side of the tongue as being less astringent (Figure 5.1 A): EC (18 of 37; p=1.00), EGCG (20 of 37; p=0.74), TA (18 of 37; p=1.00), and Alum (19 of 37, and p=1.00). Similarly, following capsaicin desensitization of TRPV1 channels, there was a non-significant difference in the number of subjects selecting the desensitized over the non-desensitized side of the tongue as being less astringent for all compounds except TA, where a significant majority of subjects selected the desensitized side of the tongue as being more astringent: EC (24 of 27; p = 0.10), EGCG (21 of 37; p=0.51), and Alum (24 of 37; p=0.10), and TA (26 of 37; p = 0.02).

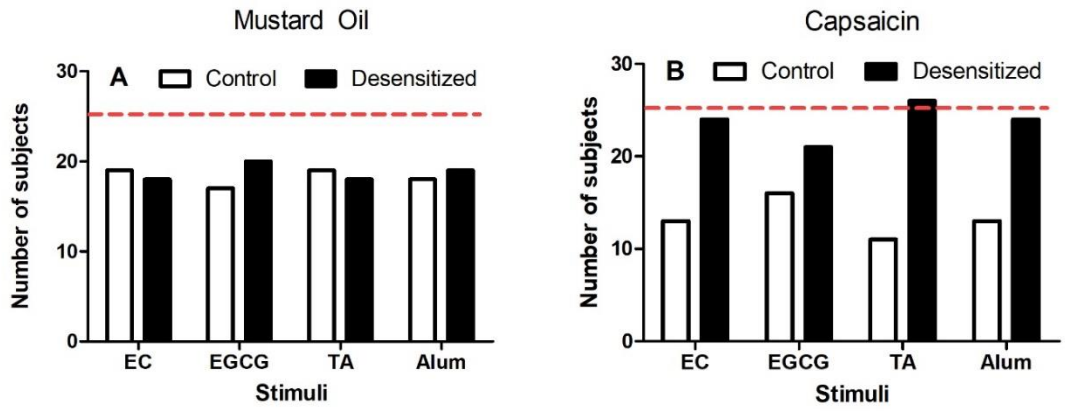

Figure 5.1 2-AFC results from the panelists' bilateral assessment of astringency perception following mustard oil (A) or capsaicin (B) desensitization. A minimum 25 out of 37 subjects (red dashed line in the figure) is needed for it to have a significant effect (a=0.05). White bars depict control, non-desensitized condition. Black bars depict treated, desensitized condition.

5.4.2. Astringency Intensity Ratings

Following mustard oil desensitization, the perceived astringency intensity ratings paralleled what was observed in the forced-choice task. Indeed, for each astringent solution evaluated, no significant difference was observed between the treated and control sides of the tongue (Figure 5.2 A): EC (2.56±0.34 vs. 2.79±0.34, respectively; p=0.67), EGCG (2.83±0.33 vs. 2.75±0.32, respectively; p=0.81), TA (2.96±0.37 vs. 2.67±0.38, respectively; p=0.31), and Alum (2.59±0.35 vs. 2.72±0.34, respectively;

p=0.29) (Figure 5.2). Similarly, following lingual capsaicin desensitization, the perceived astringency intensity of the treated and untreated sides of the tongue did not significantly differ (Figure 5.2 B): EC (2.60±0.36 vs. 2.38±0.28, respectively; p=0.82), EGCG (2.74±0.34 vs. 2.96±0.35, respectively; p=0.55), TA (2.74±0.34 vs. 2.64±0.42, respectively; p=0.71), and Alum (2.62±0.37 vs. 2.26±0.31, respectively; p=0.71) (Figure 5.2).

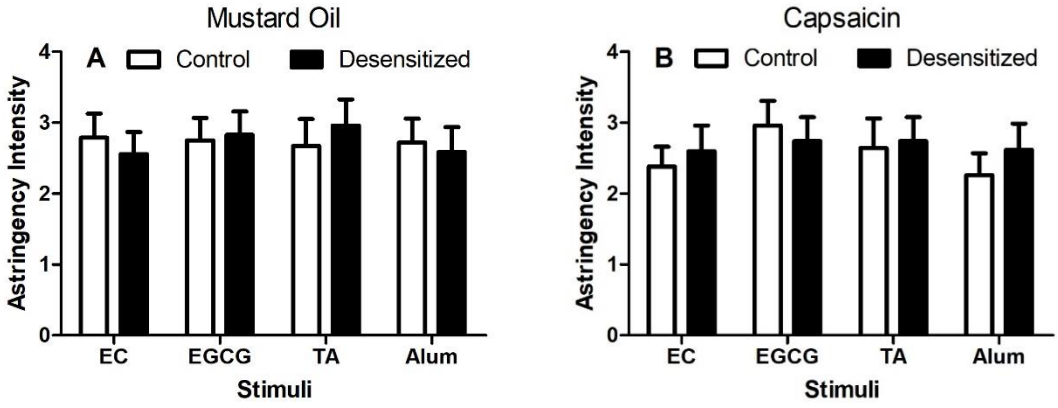

Figure 5.2 Bilateral mean astringency intensity rating ±SE (10-pt line scale anchored: 0 – none, 1 – weak, 9 – strong) following mustard oil (A) or capsaicin (B) desensitization. White bars depict control, non-desensitized condition. Black bars depict treated, desensitized condition.

5.5. Discussion

Whereas prior studies in cell-based assays (Kurogi et al., 2012, 2015; Takahashi et al., 2021) indicate that some astringent compounds may activate TRP A1 and V1 receptors, it does not appear that prior desensitization of these channels impacts the perception of astringency. The present results, therefore, suggest that TRP A1 and V1 do not have a functional role in the perception of oral astringency.

5.5.1 Role of TRPA1 in Astringency Perception

When mustard oil was applied topically to the tongue surface to desensitize TRP1 channels, no significant differences in perceived astringency were noted between the treated and control sides of the tongue for all four astringent stimuli. Similarly, astringency intensity ratings were not different between the treated and control sides of the tongue for all four astringent stimuli. If TRPA1 channels did play a significant role in astringency perception as suggested by previous studies (Kurogi et al., 2012, 2015; Takahashi et al., 2021), then desensitizing them to further activation should have resulted in a decrease in perceived astringency intensity. Although previous studies have indicated that some polyphenols activate TRPA1, experimental constraints limit their ability to suggest that TRPA1 contributes to the perception of astringency in the human oral cavity. For example, the majority of these studies (Kurogi et al., 2012, 2015) have utilized *in vitro* animal models. Kurogi et al., (2012) showed that EGCG activated TRPA1 in mouse intestinal enteroendocrine cell lines, however these cells are not reflective of human oral epithelial cells. In a follow up study (Kurogi et al., 2015), the same authors showed that only the auto-oxidized product of EGCG (oxiEGCG) activates TRPA1 in the oral epithelial cells lines of rodents, whereas the original forms of EC and EGCG did not.

Most recently, Takahashi et al., (2021) showed that TA and oxiEGCG at 20 μM can activate TRPA1 in human cell lines. Although *in vitro* results indicate the possibility of ligand-receptor binding, the interaction of these compounds within the human oral cavity *in vivo* may be different. TRPA1 receptors are expressed in deeper layers of the oral epithelium (B. Wang et al., 2011) or in nociceptive cells that reside beneath oral epithelial cells with receptor ending that rarely reach the surface (Kichko et al., 2018). Thus, its unlikely that relatively large astringent compounds can penetrate the multilayered epithelium like mustard oil can (Kichko et al., 2018). Furthermore, catechins like EC and EGCG have a high affinity for lipid bilayers, trapping them there (Huang & Xu, 2021; Sirk et al., 2009). Therefore, TRPA1 likely does not play a significant role in astringency perception in the human oral cavity.

5.5.2 Role of TRPV1 in Astringency Perception

When capsaicin was administered to desensitize TRPV1 channels in the lingual epithelium, subjects chose the desensitized side of the tongue to be more astringent compared to the control for only TA. However, a similar trend was shown for EC, EGCG, and Alum with a majority of subjects indicating the desensitized side to be more astringent compared to the control side, although these did not reach significance. These findings are in contrast to what is expected if TRPV1 activation underpinned astringency perception. One possible explanation for the increased perceived astringency following TRPV1 desensitization may be due to tissue warming resulting from capsaicin induced vasodilation (Jancsó-Gábor & Szolcsányi, 1972; Nielsen et al., 2013). Prior psychophysical studies have indicated an increase in mechanosensitivity following tissue warming (Jia et al., 2012; Lv et al., 2020; Stevens, 1982). However, the 2-AFC results

were not consistent with astringency intensity ratings. This discrepancy may be attributed to the cognitive nature of the two tasks, where forced choice methods are more sensitive, and able to pick up smaller differences compared to rating methodologies (O'Mahony, 1992; O'Mahony & Rousseau, 2003).

Similar to TRPA1, prior literature has shown *in vitro* that rodent and human cell lines expressing TRPV1 can be activated by oxiEGCG (Kurogi et al., 2015; Takahashi et al., 2021) and TA (Takahashi et al., 2021). Like TRPA1, TRPV1 receptors are expressed in deeper layers of the oral (Wang et al., 2011) or in nociceptive cells that reside beneath oral epithelial cells, where large polyphenolic compounds are unlikely to gain access due to their size. The only astringent that is comparable in size to capsaicin (293.40 g/mol) is EC (290.26 g/mol), whereas EGCG (458.37 g/mol), Alum (474.37 g/mol), and TA (1701.19 g/mol) are much larger. Therefore, when combined with these prior findings, our results suggest that TRPV1 likely does not play a significant role in astringency perception in the human oral cavity.

5.6. Conclusion

Astringency is a complex oral sensation associated with polyphenol rich foods. While compounds eliciting astringency are known, the mechanism behind its perception remains debated. Presently, we investigated the role of TRP A1 and V1 in astringency perception.

We found that desensitizing TRP A1 and V1 receptors did not reduce perceived astringency, suggesting that these receptors do not have a significant role in human astringency perception. Overall, this study underscores the complexity of astringency

perception, suggesting that it may be mediated predominantly via mechanosensitive pathways. Future studies should focus on other potential mechanisms, including loss of salivary lubricity due to astringent compounds binding to PRP, and/or the disruption of the mucosal pellicle.

Chapter 6. Conclusion

The growing consumer demand for plant-based beverages presents a significant challenge in replicating the desirable textural and mouthfeel properties of their animal-based counterparts. The current research aims to bridge this gap by identifying nuanced textural and mouthfeel differences between these beverages and identifying potential oral mechanosensory mechanisms subserving these differences. This study successfully developed a comprehensive sensory lexicon focusing on texture and mouthfeel attributes, characterizing nuanced differences between plant and animal-based milk beverages. Results from Chapter 3 showed that textural and mouthfeel difference were primarily influenced the type of protein used rather than the protein concentration (8 vs 13 g of protein/8fl. oz). These findings provide valuable insights for product developers aiming to improve the sensory characteristics of plant-based beverages to better mimic those of animal-based products.

To better understand how humans perceive these differences, Chapter 4 explored the relationship between oral tactile sensitivity and texture and mouthfeel perception. Significant correlations were observed between specific suprathreshold oral tactile sensitivities and astringency, mouth coating, and smoothness perception. These results underscore the need for further research to explore the complex interactions between oral tactile sensitivity and food texture perception.

Lastly, Chapter 5 investigated the role of transient receptor potential (TRP) channels in astringency perception in the human oral cavity. The desensitization of TRPA1 and TRPV1 receptors did not significantly alter the perceived intensity of astringency, and suggests that other mechanisms may be involved in oral astringency

perception. Thus, further research is needed to elucidate the specific mechanisms which may then enable more fruitful approaches to enhancing the palatability of polyphenol-rich plant-based foods.

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