Juiciness of Plant-based Meat Analogues

Yifan Zhang

Propositions

1. Juiciness of plant-based meat analogue patties is determined only by the quantity of released serum.
   (this thesis)

2. The distinction between juiciness and fattiness of plant-based meat analogue patties is driven by their fat content and the temporal profile of these attributes.
   (this thesis)

3. Quality of scientific publications increases when peer reviews and rebuttals are published with the papers.

4. Generative AI fosters education quality by simulating personalized and interactive engagement.

5. Interdisciplinary collaborations flourish more when rooted in shared curiosity than shared funding.

6. Social media promotes cultural homogenization at the expense of local traditions.

Propositions belonging to the thesis, entitled

Juiciness of plant-based meat analogues

Yifan Zhang
Wageningen, 3 July 2025

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This research was conducted under the auspices of VLAG Graduate School (Biobased, Biomolecular, Chemical, Food, and Nutrition sciences).

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr C. Kroeze,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Thursday 3 July 2025
at 1 p.m. in the Omnia Auditorium

Yifan Zhang

Juiciness of plant-based meat analogues

221 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2025)

With references, with summary in English

DOI: https://doi.org/ 10.18174/681400

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Chapter 1

General introduction

A traditional Chinese saying describes tofu as "*the meat of the poor*", highlighting its historical role as an accessible protein source in resource limited diets. Today, however, the demand for plant-based foods extends far beyond affordability. Growing concerns about environmental degradation, sustainability, animal welfare and human health require a transition from animal-derived diets toward plant protein-based diets (Aiking & de Boer, 2020; Chaudhary et al., 2018). Plant-based meat analogues (PBMAs) are one key category of food products that can potentially contribute to this transition.

1.1 What are plant-based meat analogues?

Plant-based meat analogues (PBMAs) are food products designed to mimic the taste, texture, appearance and nutritional values of meat using vegetarian or vegan ingredients (Kyriakopoulou et al., 2019; Malav et al., 2015). PBMAs can be categorized into minced-type products such as patties and meatballs, muscle-type products such as whole-cut steaks and fillets, and emulsion-type products such as sausages (Kyriakopoulou et al., 2021). This thesis focuses on PBMA patties from the minced-type product category.

A typical PBMA formulation contains water, textured and non-textured proteins, fat, binding agents, flavorings and colorings (Kyriakopoulou et al., 2019, 2021). Water, the major component of PBMAs, serves as a hydration medium for dried ingredients, facilitates matrix gelation, emulsification and texturization during manufacturing and contributes to juiciness perception during consumption (Cornet, van der Goot, et al., 2020; Kyriakopoulou et al., 2021; Peters et al., 2017). Proteins contribute not only to nutritional value, but also to water holding capacity (WHC), gelation and emulsification properties of PBMAs (Beniwal et al., 2021; Loveday, 2020; McClements & Grossmann, 2021). Part of the proteins undergo texturization and contribute to mimicking the fibrous muscle-like structure (see **section 1.2**).

Fat is an important component for the structure of PBMA patties and its sensory profile. Fat is incorporated through pre-emulsification of liquid fat with plant-based proteins and other ingredients, and through cold-mixing solid fat at the end of production (Kyriakopoulou et al., 2021; McClements & Grossmann, 2021). As many food flavors

are lipophilic, fat functions as a flavor precursor, solvent and aroma volatile release modulator, influencing flavor perception during consumption (De Roos, 2006; Mao et al., 2017). Cold-mixed solid fat not only creates a marbling appearance that mimics the appearance of animal meat (Dreher et al., 2021), it also melts upon heating contributing to a fatty and juicy mouthfeel.

Binding agents, such as methylcellulose and carrageenan, are important for structure integrity and liquid holding capacity in PBMAs by binding water, protein and fat to form a cohesive matrix (Dekkers et al., 2018; Herz et al., 2023; Kyriakopoulou et al., 2021). This function is particularly crucial in meat analogue patties, where binders help to integrate the heterogeneous TVPs enabling patties to be formed during handling and strengthening their mechanical properties after cooking (Herz et al., 2023; Kyriakopoulou et al., 2021). Moreover, binders immobilize water during processing and reduce water loss during cooking, improving the WHC of PBMAs (Herz et al., 2023; Kyriakopoulou et al., 2021; McClements & Grossmann, 2021).

To achieve a meat-like taste and appearance, PBMAs also contain flavorings and colorings. Flavoring agents mask undesirable off-flavors from plant-based ingredients, such as a beany flavor, while introducing meaty and umami-rich flavors (Leonard et al., 2023; Wang et al., 2022). Colorings, such as beetroot extract and soy leghemoglobin (heat-unstable colorants), or caramel and carotenoids (heat-stable colorants), are used to replicate the appearance of the raw and cooked appearance of meat, especially the color transformations during heating (Ryu et al., 2023; Wu et al., 2024).

1.2 Sensory quality of plant-based meat analogues

Several consumer studies highlighted that substantial improvements in the sensory properties of PBMAs are needed to improve consumer acceptability (Gómez-Luciano et al., 2019; Hoek et al., 2011; Weinrich, 2019). There are three main challenges regarding the sensory quality of meat analogues: appearance, flavor and texture/mouthfeel.

Appearance is the first visual cue consumers use to evaluate the quality of PBMAs. Ideally, PBMAs should closely resemble meat color, marbling and visual fibrous

structure before, during and after cooking. Among these aspects, color is particularly crucial as it signals freshness, doneness and overall appeal (Tomasevic et al., 2021). In meats, myoglobin, hemoglobin, the valence state of heme iron, ligands and the steric hindrance of ligands determine meat color and color changes during cooking (McClements & Grossmann, 2021; Su et al., 2024). However, plant-based proteins inherently exhibit whitish, beige, or yellowish-brown colors due to natural impurities (such as polyphenols and chlorophyll) and chemical reactions during processing (such as low-moisture extrusion of TVPs), limiting their ability to replicate meat-like colors (Wu et al., 2024). To mimic the color of meats, two main strategies are employed: incorporating plant-derived leghemoglobin in PBMAs or using plant-based pigments (Su et al., 2024). Leghemoglobin, structurally similar to myoglobin, can be produced by fermentation using genetically engineered yeast, providing a meat-like red color (Bohrer, 2019). Alternatively, due to regulatory constraints in Europe, natural plant-based pigments such as beet extract, caramel and carotene are blended to mimic meat color and changes of color during cooking. However, plant-based pigments face several formulation challenges, including sensitivity to pH, oxygen and thermal conditions, which can destabilize colorants and result in suboptimal coloration of PBMAs (Ryu et al., 2023; Silva et al., 2021; Wu et al., 2024). The need for advanced stabilization, regulatory constraints and high cost of natural pigments, require careful selection and optimization of colorants to meet consumers' expectations.

Flavor compounds in meat are formed through chemical reactions during cooking, primarily the Maillard reaction (between amino acids and reducing sugars and sulfur-containing compounds) and lipid degradation (of phospholipids and triglycerides rich in unsaturated fatty acids), generating volatile and non-volatile meat flavor (Mottram, 1998). PBMAs often carry undesirable beany, grassy or bitter notes inherent to the legume proteins, such as soy and pea protein based TVPs used in preparing the patties (Damodaran & Arora, 2013; Wang et al., 2022). To replicate meat flavors and mask off-flavors, flavor mixtures are added during production (He et al., 2021; Kyriakopoulou et al., 2019). Flavorings for PBMAs can be developed through various approaches. Yeast extracts, a widely used natural flavoring, provides meaty flavors by degrading yeast

proteins into amino acids and peptides, as well as nucleic acids into nucleotides, effectively masking off-flavors and reducing bitterness (Mittermeier-Kleßinger et al., 2021; Wang et al., 2022). Another approach is flavor generation by Maillard reactions, which generate peptides with meat-like flavors from plant-based proteins (Sun et al., 2022). Biotechnological methods such as enzymatic treatments to degrade flavor precursors, or precision fermentation to transform undesirable compounds and generate meat-like flavors are approaches to reduce off-flavors (Mittermeier-Kleßinger et al., 2021; Wang et al., 2022).

Texture and mouthfeel properties related to the muscle-like structure of meats, such as fibrousness, tenderness, chewiness and juiciness, are essential for the sensory quality of PBMAs (McClements & Grossmann, 2021; Moss et al., 2023; Su et al., 2024). However, achieving these sensations in PBMAs remains challenging due to the inherent differences in composition and structure between plant-based and animal-based proteins. The texture of meat results from the complex arrangement of muscle fibers, connective tissue and adipose tissue, which together contribute to its distinctive sensory characteristics. Myofibrillar proteins mainly influence chewiness and water-holding capacity, collagen in connective tissue affects tenderness and adipose tissue enhances juiciness and mouthfeel (Hughes et al., 2014; Purslow, 2018). To bridge the sensory texture gap between PBMAs and meat, various techniques have been developed to texturize plant-based proteins. Low-moisture extrusion is used to produce Textured Vegetable Protein (TVPs), which have a sponge-like structure that facilitates water absorption during rehydration, water retention during cooking and water release during consumption through air pockets and lamellae (Baune et al., 2022; van Esbroeck et al., 2024). TVPs are commonly used as the main ingredient in minced-type products such as patties and meatballs. High-moisture extrusion and shear cell technology are employed to create the fibrous structure of meats in whole-cut products, such as beef steaks and chicken breasts (Dekkers et al., 2018; Schmid et al., 2022; Zhang et al., 2022). More recently, emerging techniques such as 3D printing, electrospinning and wet spinning have been explored to replicate muscle fiber anisotropy, offering better control over customized structure (Dekkers et al., 2018; Su

et al., 2024; Vallikkadan et al., 2023; Wen et al., 2023). Despite the advancements in these techniques, much of the research has primarily focused on replicating the fibrous muscle-like structure of meats, and less attention has been given to improve the juiciness. Since juiciness is frequently ranked as one of the most desired sensory attributes in meats and PBMAs (Elzerman et al., 2011; O'Quinn et al., 2018; Xu & Falsafi, 2023), understanding the mechanisms underlying juiciness is a critical next step for developing guidelines to improve the sensory quality of PBMAs.

1.3 Juiciness perception of meats and meat analogues

Juiciness is a mouthfeel property and has been defined as the impression of moisture, liquid or juice released when chewing foods (Honikel & Hamm, 1994; Warner, 2017). Prior to this thesis, juiciness perception in PBMAs has been rarely explored. Cornet, Edwards, et al. (2020) suggested that in soy-based meat analogues, internal cavities (air pockets) provide pathways for water release which may contribute to juiciness perception. However, no sensory evaluation was conducted to verify this hypothesis. Another study demonstrated that juiciness could not be explained by water content, cooking loss or expressible moisture during compression for a broad range of commercially available meat analogues (Godschalk-Broers et al., 2022). This shows that at the start of the PhD project only few studies had systematically linked juiciness of meat analogues to physicochemical properties.

The composition of meat, particularly its water and fat content, is one of the most extensively investigated factors influencing juiciness perception. Many studies have shown weak correlations between juiciness and the water content of raw meats and strong positive correlations between the water content of cooked meats and juiciness (Bertram et al., 2005; Bombrun et al., 2015; Ritchey & Hostetler, 1964; Ruiz De Huidobro et al., 2005). Similar stronger positive relationships were found for the fat content of cooked meats (Choi et al., 2019; Corbin et al., 2015; Thompson, 2004; Williams et al., 1994). These findings suggest that the ability to retain water and fat during cooking is a critical factor influencing juiciness, a notion that is supported by strong negative correlations between juiciness and cooking loss across various types of meats (Aaslyng et al., 2003; Lucherk et al., 2017; Pematilleke et al., 2021; Serra et al.,

2008). Consequently, efforts to improve water holding capacity (WHC) and fat holding capacity (FHC) of meats have identified factors such as breeding, intramuscular fat content, ageing regime, and cooking methods as key contributors to juiciness (Xu & Falsafi, 2023). Most of these meat-specific ante- and post-mortem factors impacting juiciness are inapplicable for PBMAs. It is hypothesized that increasing WHC and FHC of PBMAs by strategies such as optimizing cooking methods and incorporating different ingredients may enhance juiciness of PBMAs.

While retaining water and fat during cooking is a requirement for juiciness, the release of serum during consumption has been suggested to be a key factor driving juiciness. Serum release under compression is a widely used instrumental parameter that has been linked to sensory juiciness of meats, but not meat analogues. As shown in **Figure 1 (A) and (B)**, existing setups for the quantification of serum release are relatively simple. Foods are compressed either using a Texture Analyzer to a set deformation or with a fixed weight for a certain period of time and the released serum is collected using filter papers and quantified by measuring the wet area on the paper (**Figure 1 (B)**) or gravimetrically.

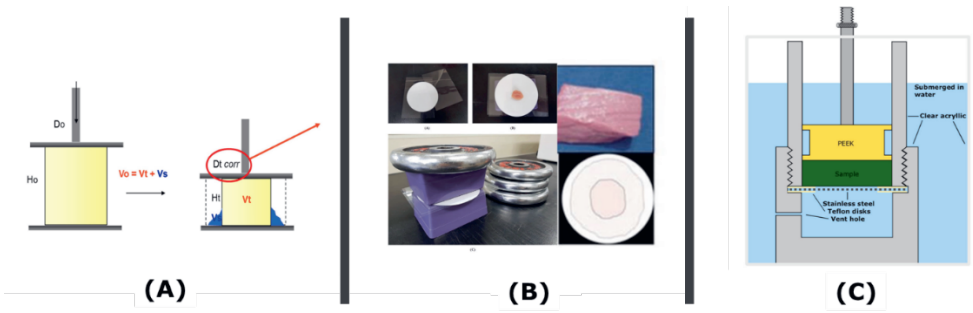

Figure 1.1. Examples of setups to quantify serum release under compression: (1) compression performed using a Texture Analyzer to certain deformation (van den Berg et al., 2007), (2) compression with a fixed weight (Joo, 2018), (3) confined compression in a submerged water environment (Cornet, Edwards, et al., 2020)

Most studies found moderate correlations between serum release under compression and sensory juiciness for various meats and fishes (Borderias et al., 1983; Lee & Patel, 1984; Lee et al., 2007; Lucherk et al., 2017; Yau & Huang, 2001). The strength of these correlations varied considerably across studies. First, all studies used filter paper for

serum collection. This method is inherently limited by the absorption capacity of the filter paper, which caps the maximum absorbable serum. Secondly, as the compression force is gradually removed after compression, released serum can be reabsorbed by the food. While reabsorption naturally occurs during mastication, uniaxial compression methods prioritize standardized assessments of maximum releasable serum rather than attempting to simulate serum release as it occurs during mastication. Recently, a confined compression method was developed to measure serum release by quantifying the volume increase caused by the serum released into the submerged water after compression (Cornet, Edwards, et al., 2020) (**Figure 1 (C)**). This method counters the reabsorption after compression and, more importantly, effectively captures serum release over time, stimulating water release kinetics. However, both confined compression and simple uniaxial compression primarily quantify the serum release, but fail to collect serum in its original liquid state, making it challenging to further analyze serum properties.

Some studies determined the nutritional composition of the released serum by drying the filter paper to determine water content and assuming the remainder to be fat. Lee et al. (2007) followed this approach and found that both water and fat content of serum released from mackerel nuggets correlated moderately with juiciness. Similar results were reported for frankfurters (Lee & Patel, 1984) and beef steaks (Lucherk et al., 2017). Developing a method to quantify serum release which mimics the serum release during mastication and takes into account reabsorption of serum during consumption would allow to further explore the relationships between serum release and juiciness and to analyze serum properties, such as viscosity and lubrication ability, deepening our understanding of the role of serum properties in juiciness perception.

As a sensory attribute, juiciness has been reported to interact with other texture attributes such as tenderness, hardness and fattiness for meats. Positive correlations between juiciness and both tenderness and fattiness for beefs have been reported (Judge et al., 2021; Liu et al., 2020; Mateescu et al., 2015; Oury et al., 2009; Thompson, 2004). These relationships have been attributed largely to intramuscular fat content.

While these interactions have been explored in meats, the interplay between juiciness and other sensory texture properties of PBMAs have been underexplored.

1.4 Role of oral structural breakdown in texture perception

Food oral processing describes the structural breakdown, transport and swallowing of food during mastication (Chen, 2009; Stieger & Van de Velde, 2013). Unlike flavor perception, which is associated with specific molecules, texture and mouthfeel are attributes assigned by consumers based on their visual and tactile interactions with food during oral processing (Pascua et al., 2013). Therefore, understanding texture and mouthfeel perception requires investigating not only the initial properties of food before consumption, but also its oral structural breakdown and bolus properties during mastication.

Devezeaux de Lavergne et al. (2015) found that in food gels, firmness, a texture property perceived early during oral processing, correlated with fracture properties, whereas melting and creaminess were perceived during later stages of mastication. Similarly, Jourdren et al. (2016) and Pu et al. (2021) showed that for breads, late-stage texture attributes, such as adhesiveness, softness, dryness and stickiness, were more influenced by bolus properties than by initial food properties. These studies highlight how the properties of the bolus are sensed during mastication and trigger texture perception.

For meats, juiciness has been hypothesized to be a dynamic perception consisting of two components, the initial juiciness, resulting from the rapid release of serum during the first chews, and sustained juiciness, influenced by fat content of the meats and uptake of saliva in the meat bolus during later stages of mastication (Warner, 2017; Winger & Hagyard, 1994). However, this hypothesis has not been experimentally verified. Some studies report the importance of bolus properties in the texture evaluation of animal meat. Mioche et al. (2003) and Yven et al. (2005) found that food bolus obtained from tough beef meats contained less fluid (mixture of juice and saliva) than the bolus obtained from tender meats, which may hint at the potential connection between bolus properties and juiciness perception, although sensory properties were

not quantified in these studies. Rizo et al. (2019) explored the relationships between texture attributes and bolus properties of different cooked hams. They suggested that fibrousness was linked to bolus's fragmentation degree (particle size distribution, number of particles and median particle area) and juiciness seemed to be related to the amount of saliva uptake. However, correlation analyses were not reported. For PBMAs, the influence of oral breakdown on bolus properties and texture perception has been rarely reported at the start of this thesis. Chen et al. (2021) found that chicken and soy-based chicken nuggets showed similar chewing behavior while chicken fragmented into more and smaller bolus particles. These differences in oral breakdown of chicken and plant-based chicken may lead to variations in texture (juiciness) perception during mastication. To summarize, how oral processing shapes bolus properties, texture and juiciness perception in PBMAs and meats is not yet understood.

1.5 Aims and outline of this thesis

This thesis aimed to understand juiciness perception of plant-based meat analogue patties by exploring the relationships between physicochemical food and bolus properties, oral structural breakdown and sensory perception.

A schematic overview of this thesis is presented in **Figure 1.2**. In **Chapter 2,** we investigated commercially available PBMA and beef patties and created patties from the same raw materials varying in juiciness by adjusting core cooking temperature. This chapter aims to understand texture and juiciness perception by linking food physicochemical properties (composition, texture and serum release of cooked patties), chewing behavior (number of bites and chews, bite size, chewing time and chewing frequency) and bolus properties at swallowing (bolus composition, texture, fragmentation and expressible liquid) to static sensory properties (Rate-All-That-Apply, RATA).

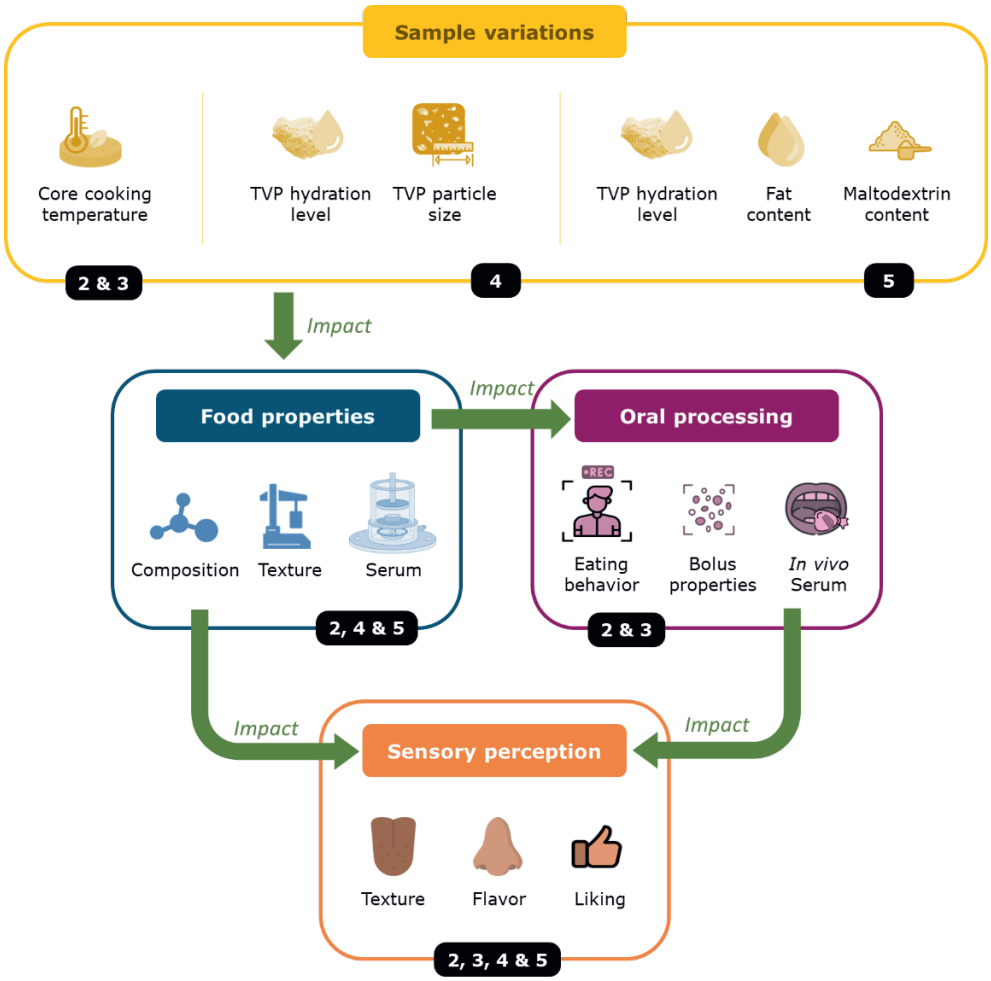

Figure 1.2 Schematic overview of the framework of this thesis. Numbers indicate the corresponding chapters.

In **Chapter 3**, we further explored the role of dynamic oral structural breakdown on temporal texture and juiciness perception by using the same patties investigated in **Chapter 2**, focusing on dynamic *in vivo* serum release, dynamic bolus properties (bolus composition, texture, fragmentation and expressible liquid) and their relationships with temporal sensory perception (Temporal Check-All-That-Apply).

As **Chapter 2 and 3** reveal limited variability in the compositional and texture properties of commercial PBMA patties with different core cooking temperature, from **Chapter 4** onward the focus shifts to lab-made PBMA patties. In **Chapter 4**, we

increased variability in composition and texture of PBMA patties by modifying the hydration level and particle size of textured vegetable proteins (TVPs). We explored correlations between sensory properties and liking using network analysis. We investigated the factors that influence juiciness by linking initial physicochemical properties of PBMA patties (composition, texture and serum release) to their sensory properties (RATA) via network analysis.

In **Chapter 5**, we specifically examined the impact of serum properties on juiciness and fattiness perception of PBMA patties, creating patties differing in amount of serum release, serum composition and viscosity by modifying TVP hydration level, fat content and maltodextrin content in raw patties. We explored how serum properties are associated with juiciness and fattiness perception by examining correlations between serum properties and their sensory properties (rank-rating evaluation) via network analysis.

Finally, in **Chapter 6** we synthesized the main findings and discussed the main drivers of juiciness perception of PBMA patties. We discussed strategies to modify juiciness, potential mechanisms of dynamic juiciness perception across product categories, methodological considerations and recommendations for future research.

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Chapter 2

Exploring relationships between juiciness perception, food and bolus properties of plant-based meat analogue and beef patties

This chapter has been published as:

Zhang, Y., Brouwer, R., Sala, G., Scholten, E., & Stieger, M. (2024). Exploring relationships between juiciness perception, food and bolus properties of plant-based meat analogue and beef patties. *Food Hydrocolloids*, 147, 109443. https://doi.org/10.1016/J.FOODHYD.2023.109443

Abstract

Plant-based meat analogues (PBMAs) often lack sensory juiciness, limiting their consumer appeal. This study aimed to better understand juiciness and texture perception of plant-based meat analogue and beef patties by linking food and bolus properties to sensory properties. Commercially available PBMA and beef patties were cooked to four core temperatures (60, 70, 80, 90°C). Juiciness intensity decreased significantly and strongly with increasing core temperature (rank-rating test), so that series of patties covering a broad range of juiciness were obtained from the same raw materials. Rate-All-That-Apply (RATA) profiling revealed that with increasing juiciness intensity PBMA patties were perceived less dry and fattier, whereas beef patties were perceived less dry, less hard, less chewy, and fattier and more tender. Juiciness intensity correlated strongly with the properties and composition of the samples. Juiciness intensity of PBMA and beef patties were correlated negatively with cooking loss and positively with serum release under compression. Serum release under compression determined instrumentally correlated strongly and positively with serum release determined in vivo during mastication. For the PBMA patties, juiciness perception was high when the patties contained more fat, while for the beef patties, juiciness perception was high when the patties contained more water. For both PBMA and beef patties varying largely in juiciness intensity, differences in food and sensory properties did not lead to significantly large differences in oral processing behavior and bolus properties at the moment of swallowing. Due to these limited variations in bolus properties, also no meaningful relationships between bolus properties at the moment of swallowing and sensory properties could be determined. We conclude that juiciness perception of plant-based meat analogue and beef patties is primarily determined by the release of serum during mastication, and is related to the sample properties rather than bolus properties at the moment of swallowing.

2.1 Introduction

Plant-based meat analogues (PBMAs) are food products designed to mimic the texture, taste and nutritional properties of meats (Kyriakopoulou et al., 2019; Malav et al., 2015). Despite recent advancements, replicating the taste and texture of meats, especially juiciness, remains difficult, as many PBMAs are still perceived as dry. Perceived juiciness and other sensory properties are critical for consumer acceptability and repeated consumption(de Boer et al., 2006; Elzerman et al., 2011; Giacalone et al., 2022). Improving the sensory properties of PBMAs, particularly their juiciness, remains a challenge for the food industry (Gómez-Luciano et al., 2019; Hoek et al., 2011; Weinrich, 2019).

Juiciness can be defined as the impression of moisture that a consumer experiences when chewing foods (Honikel & Hamm, 1994; Warner, 2017). To improve juiciness, it is important to know how it is related to the physicochemical properties of the food itself and to its structural breakdown during mastication. Studies investigating the relationships between juiciness perception and food properties of PBMAs are scarce. Research on meats has shown negative correlations between cooking loss and juiciness perception (Aaslyng et al., 2003; Lucherk et al., 2017; Pematilleke et al., 2021; Serra et al., 2008). Since cooking loss alters the composition of cooked meats, it is reasonable to speculate that such compositional changes also relate to juiciness perception. However, only weak or no correlations between composition (water and fat content) and juiciness have been found for raw meats (Hunt et al., 2014; Legako et al., 2015; Lucherk et al., 2017; Realini et al., 2021; Ripoll et al., 2008; Wilson et al., 2017), and the relationships between juiciness perception and cooked meat composition are underexplored. It has been suggested that juiciness perception is related to the amount of the serum that is released from the food matrix into the oral cavity during consumption (Warner, 2017). To quantify serum release, a common approach is to compress a fixed weight of the food for a fixed time period to a certain degree of deformation, and to measure the wet area or weight change of a filter paper placed below the sample. Although the method has been frequently used, only weak to moderate correlations between the serum release under compression and sensory

juiciness have been reported, mostly for cooked meat and fish (Borderias et al., 1983; Lee & Patel, 1984; Lee et al., 2007; Lucherk et al., 2017; Yau & Huang, 2001). The correlation strength varied considerably across these studies, probably due to differences in the compression rate and total compression degree, or due to limited absorption capacity of the filter paper, reducing the accuracy of the method. Other instrumentally measured food properties, such as drip loss (Aaslyng et al., 2003; Van Oeckel et al., 1999) and water holding capacity upon centrifugation (Lucherk et al., 2017), have been weakly correlated to sensory juiciness of various meats. To summarize, several food properties have been linked to sensory juiciness in meats, and relationships were generally weak to moderate. In contrast to meats, for PBMAs, relationships between food properties and juiciness have not been reported yet.

In addition to food properties, the structure breakdown of food during mastication is also a crucial factor to consider when examining juiciness and texture perception (Ilić et al., 2022). Food oral processing is the process of chewing foods to break down the structure and lubricate the bolus to be swallowed safely (Chen, 2009; Stieger & Van de Velde, 2013). During mastication of solid foods, the properties of the bolus change dynamically and drive the perception of texture. Bolus properties during consumption play therefore an essential role in explaining texture perception (Devezeaux de Lavergne et al., 2017; Guo, 2021; Panouillé et al., 2016). For example, meats' sensory hardness, chewiness and cohesiveness were influenced strongly by modifications of bolus properties during mastication, such as bolus particles, bolus shearing force, and saliva uptake (Djekic et al., 2021; Pematilleke et al., 2020; Yven et al., 2005). Considering bolus properties may allow to better understand the mechanisms underlying texture perception. For PBMAs, relationships between bolus properties and texture and juiciness perception are underexplored. In studies on ham and sausages, a possible correlation between bolus saliva uptake and juiciness perception has been suggested , but was not experimentally verified (Devezeaux de Lavergne, Derks, et al., 2015; Rizo et al., 2019). Due to changes in bolus properties, food texture may also considerably influence oral processing behavior (Bolhuis & Forde, 2020; Devezeaux de Lavergne, Derks, et al., 2015). Meat with tougher and dryer texture

required more mastication time, higher number of chews (Mioche et al., 2002; Pematilleke et al., 2022), and more muscular activities during mastication (Mathoniere et al., 2000). These results suggest that differences in juiciness of PBMAs and meats may influence oral processing behavior.

The aim of this study was to better understand juiciness and texture perception of plant-based meat analogues (PBMAs) and beef patties by linking sample and bolus properties to sensory characteristics. Commercially available PBMAs and minced beef were used to prepare burger patties. Raw patties were sous vide cooked to different core temperatures and grilled to obtain samples differing in juiciness. A rank-rating test was used to verify differences in juiciness intensity among samples. Rate-All-That-Apply (RATA) was then used to evaluate the texture perception of PBMA and beef patties. Relationships between food properties (total cooking loss, water and fat content of grilled patties, texture properties of grilled patties, serum release under compression, and water content of released serum), bolus properties (water and fat content of bolus, saliva uptake, texture properties of bolus, and expelled liquid in bolus), serum release during mastication and texture and juiciness perception were explored. In addition, a new instrumental method was developed to quantify serum release during compression and was related to serum release during *in vivo* mastication of PBMA and beef patties.

2.2 Materials and Methods

2.2.1 Sample preparation

Minced PBMA (Beyond Mince, Beyond Meat®, The New Plant), minced beef (AH Biologisch Rundergehakt, Albert Heijn B.V., the Netherlands), liquid whole egg (Vloeibaar Heelei Diepvries, Coco vite, Belgium) and sodium chloride (NaCl, Jozo Naturel tafelzout, Hengelo, The Netherlands) were bought in a local supermarket. To prepare PBMA patties, 110 g of minced PBMA were shaped into a patty using a burger patty shaper (diameter 80 mm). To prepare beef patties, 104.5 g minced beef, 5 g whole egg liquid and 0.5 g NaCl were first mixed by hand for 2 min, and then shaped into a patty. PBMA and beef patties were spiked with 100 ppm linalool (Symrise,

Germany) as a tracer aroma compound (citrus aroma) for another study focusing on *in vivo* aroma release and perception. The results of that study will be reported elsewhere. PBMA and beef patties were transferred to a plastic bag, 95% of air was removed, the bag was sealed (Henkovac M2, The Netherlands), and allowed to rest at 4°C for 24 h before use.

To create PBMA and beef patties differing in juiciness, patties were cooked sous vide to different core temperatures. To do so, vacuum-packed patties were placed into a water bath at temperatures of 60, 70, 80 or 90°C for 60 min, a time frame in which the core of the patties reached the target temperature. The patties were subsequently removed from the bags, transferred into foam boxes, and cooled down to a core temperature of 60°C (10 min cooling time for patties cooked at 90°C, 8 min for patties cooked at 80°C, 5 min for patties cooked at 70°C, and 0 min for patties cooked at 60°C). After cooling, the patties were grilled in a double-plate grill (DeLonghi, Italy) at 200°C for 1 min, with a distance of 2 cm between the two heating plates to ensure proper contact with both sides of patties. After grilling, the PBMA patties were allowed to rest in the foam box for 5 min, and the beef patties for 4 min, to reach a core temperature of 55°C before serving to participants or instrumental characterization.

The PBMA and beef patties were coded according to their core cooking temperature as PBMA60, PBMA70, PBMA80, PBMA90, BEEF60, BEEF70, BEEF80 and BEEF90. Sample names do not refer to the serving temperature, which was 55°C for all patties.

2.2.2 Characterization of patty properties

2.2.2.1 Composition of grilled patties

2.2.2.1.1 Total cooking loss

The total cooking loss of the PBMA and beef patties was determined by quantifying the weight difference before sous vide cooking and after grilling. Measurements were performed in 6 replicates.

2.2.2.1.2 Water content of patties

The water content of raw patties and patties after sous vide cooking and grilling was determined by analyzing the dry matter content. The patties (5 – 6 g) were placed in aluminum dishes, weighted (w_0), and dried in an air oven (Binder, Germany) for 16 – 18 h at 105°C until constant weight. After drying, samples were cooled down in desiccators and weighted again (w_1). The water content of the patties was calculated as $WC = (w_0 - w_1)/w_0 \times 100\%$. Measurements were performed in quadruplicate.

2.2.2.1.3 Fat content of patties

The fat content of raw patties and patties after sous vide cooking and grilling was determined by NutriControl (the Netherlands), using the Berntrop method (ISO 6492, International Organization for Standardization, 1999). Dried raw patties and cooked/grilled patties were weighted and suspended in 3 M HCl to hydrolyze proteins. The hydrolyzed sample was washed with water and dried in an air oven for 16 h at 60°C. The dried residue was extracted using petroleum ether with a Soxhlet apparatus (Foss-Hydrotec 8000, Denmark). After extraction, the petroleum ether was evaporated by air drying to obtain the fat as residue, which was weighted. The fat content of the patties was calculated based on total dry matter content. Measurements were performed in triplicate.

2.2.2.2 Texture properties of patties

The texture properties of patties were determined with a penetration test. A Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) equipped with a 5 kg load cell and a stainless cylinder probe (diameter 2 mm) was used. The entire patty (diameter 65 – 75

mm, height 20 mm) was used, and penetration tests were performed up to a strain of 80% of the initial height of the sample at a constant speed of 5 mm/s. Each patty was punctured at three locations, with five replicates for each type of patty. Average values and standard deviations were obtained from 15 measurements. From the obtained force-time curves, peak force, resilience and adhesiveness were calculated. Peak force was defined as the maximum peak force obtained from the puncture test at 80% strain, resilience as the ratio between the areas under the force-time curve after and before peak force, and adhesiveness as the area under the force-time curve between the start point and the time point at which the probe returned to its initial position.

2.2.3 Characterization of serum properties

2.2.3.1 Serum release under compression

Serum release under uniaxial compression was determined using a modified setup (**Figure 2.1**) of the method previously described by van den Berg et al. (2007).

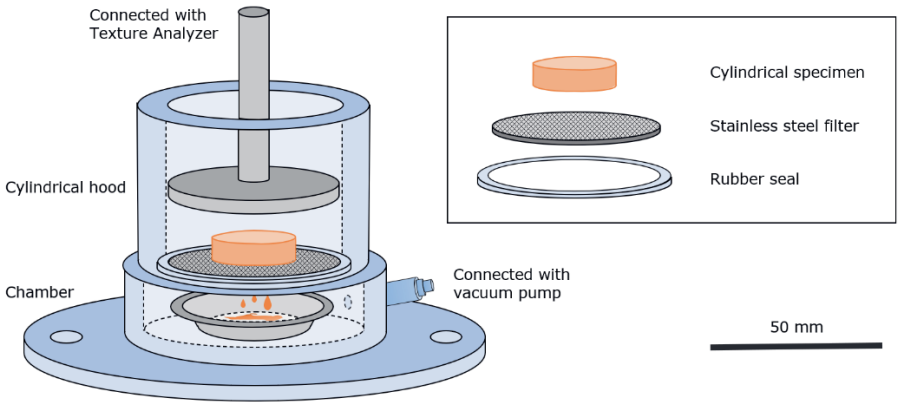

Figure 2.1. Device for determination of serum release under compression. A Texture Analyzer is connected to a probe to uniaxially compress the sample. The probe is enclosed with a cylindrical hood, which is connected to the chamber to ensure airtightness during compression. As compression occurs, serum is released from the cylindrical specimen. The released serum drips through a stainless steel filter supported by the suction of a vacuum pump, and collected in an aluminum tray placed within the chamber.

A Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) equipped with a 50 kg load cell was used. The selected stainless cylinder probe (diameter 50 mm) was equipped with a cylindrical hood slightly larger than the cylinder probe to provide a confined environment during compression. A chamber was attached to the base of the Texture Analyzer to collect the serum released during compression. A stainless steel filter (pore size 1 mm, porosity 36%) was fixed on top of the chamber with a rubber seal. An aluminum tray was placed within the chamber to collect the released serum for further analysis. A vacuum pump (pumping speed max. 2.3 m^3/h, vacuum level 7.0 mbar, vacuubrand, Germany) was connected to the chamber to accelerate the separation of the serum from the patty matrix, collecting the released serum in the tray. The samples placed on the filter were cylindrical specimens with a diameter of 30 mm and height of 20 mm, cut from the middle of the patties with an initial weight s_0. Compression was applied up to a strain of 80% of the specimens initial height (20 mm) at a constant compression speed of 5 mm/s. The probe was then held in this position (80% strain) for 90 s. After compression and removal of the released serum, the samples were removed and weighted (s_1). Serum release under compression was calculated as $SR\_compression = (s_0 - s_1)/s_0 \times 100\%$. Measurements were performed in 5 replicates.

2.2.3.2 Water content of serum

The water content of the serum (0.6 – 1.8 g) was determined by placing the serum in aluminum dishes, weighting (w_0) and drying in an air oven (Binder, Germany) for 16 – 18 h at 105°C until constant weight. After drying, the samples were cooled down in desiccators and weighted again (w_1). The water content of the serum was calculated as $WC = (w_0 - w_1)/w_0 \times 100\%$. Measurements were performed in 5 replicates.

2.2.4 Sensory evaluation

2.2.4.1 Participants

Participants were recruited from Wageningen and surroundings. Inclusion criteria were good general health (self-reported), BMI between 18.5 – 30 kg/m^2, no dental issues, no swallowing issues, normal ability to taste and smell, non-smoker, non-vegetarian/non-vegan and willing to eat both beef and PBMA patties, no allergies or

intolerances to legumes, eggs, and not pregnant. All recruited participants (n = 117) were asked to join the rank-rating and RATA evaluations for PBMA and beef patties. The rank-rating test of juiciness intensity of PBMA patties was completed by n = 97 participants (76 female and 21 male, 24.6 ± 3.4 years, BMI 23.1 ± 3.9 kg/m^2, mean ± SD), while n=100 participants (73 female and 27 male, 24.4 ± 3.6 years, BMI 23.1 ± 3.7 kg/m^2, mean ± SD) completed the rank-rating test of juiciness intensity of beef patties. The RATA evaluation of PBMA patties was completed by n = 99 participants (76 female and 23 male, 24.6 ± 3.7 years, BMI 23.2 ± 3.8 kg/m^2, mean ± SD), and n= 95 participants (68 female and 27 male, 24.3 ± 3.6 years, BMI 23.1 ± 3.8 kg/m^2, mean ± SD) completed the RATA evaluation of beef patties. The effect of gender on juiciness has been evaluated. No effect of gender on the results of the rank-rating and RATA sensory profiling were found (data not shown). The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki (2013). All participants signed informed consent forms and received financial reimbursement after completion of all sessions.

2.2.4.2 Experimental approach

The participants attended one 30 min familiarization session and two 60 min sensory evaluation sessions. During the familiarization session, the participants were introduced to the study, were familiarized with the rank-rating and RATA evaluation procedures, and were provided with the list of sensory attributes used, including attribute definitions with a special emphasis on juiciness. Familiarization was performed with PBMA70 and BEEF70 patties.

Sessions were carried out at the sensory facilities at Wageningen University & Research. The participants only evaluated one type of patty during one session (either PBMA patties or beef patties), and the order in which participants attended the two sessions was balanced. During each session, the participants first performed the rank-rating test and then the RATA test.

2.2.4.3 Rank-rating of juiciness intensity

Juiciness intensity was evaluated with a rank-rating test. The definition of juiciness (sensation of moisture/juice/liquid being released from food during consumption) was provided to the participants. Four samples (PBMA60, PBMA70, PBMA80, PBMA90 or BEEF60, BEEF70, BEEF80, BEEF90), with a size of a quarter patty, were presented simultaneously on a warm plate labelled with 3-digit codes in random order. The participants were instructed to take the samples using disposable gloves and take bites as they were accustomed to, and evaluated the samples by tasting them and placing the matching 3-digit codes on an unstructured 100 mm line scale representing juiciness intensity. The scale was anchored "not juicy" at the left end and "very juicy" at the right end. The participants were instructed to evaluate juiciness after swallowing the samples. Crackers and water were provided for cleansing the palate after evaluating all 4 samples. Data were collected in English using EyeQuestion software (Logic8, the Netherlands).

2.2.4.4 Rate-All-That-Apply (RATA)

The sensory profile of PBMA and beef patties was evaluated using the RATA method. Fourteen attributes were divided into two blocks: texture and taste/flavor, and their definitions (**Table 2.1**) were given to the participants. The order of attributes within each block was randomized over participants, but was kept constant across samples per participant per session. Four samples (PBMA60, PBMA70, PBMA80, PBMA90 or BEEF60, BEEF70, BEEF80, BEEF90), with a size of a quarter patty, were presented monadically in random order labelled with 3-digit codes, using disposable cups. For each sample, the participants were instructed to take two bites of the sample and chew as they were accustomed to. For the first bite, the participants evaluated texture attributes; for the second bite, the participants evaluated taste and flavor attributes. For each evaluation, the participants first indicated which sensory attributes were applicable to describe the perception of the sample, followed by indicating the intensity of the selected sensory attributes on a 9-point scale marked from "low intensity" to "high intensity". Crackers and water were provided for cleansing the palate

after evaluating each sample. Data were collected in English using Qualtrics software (Qualtrics, USA).

Table 2.1. Sensory attributes and definitions used to evaluate PBMA and beef patties during RATA.

Attribute	Definition
Texture	
Juiciness	Sensation of moisture/juice/liquid being released from food during consumption.
Dryness	Sensation of dryness in mouth (opposite of juiciness).
Hardness	Force applied by the (molar) teeth to bite through the food.
Chewiness	Effort required to masticate the food until it is ready to be swallowed.
Tenderness	Sensation related to how easily the food is chewed or cut and how soft it is.
Crumbliness	Extent to which the food breaks up into particles in the mouth during the first few chews.
Fibrousness	Sensation of elongated structures in the food associated with the presence of fibers.
Fattiness	Sensation of fat in the mouth.
Taste and flavor	
Saltiness	Salty taste sensation.
Beany flavor	Flavor related to beans and legumes.
Off-flavor	General sensation of unpleasant aromas or tastes.
Meat flavor	Flavor of meat, related to products like beef, chicken or pork.
Citrus flavor	Flavor associated with lemons, limes, oranges and other citrus fruits.
Umami	Savory, broth like taste sensation.

2.2.5 Chewing behavior and bolus collection

2.2.5.1 Participants

A total of 19 female participants, distinct from RATA participants, were recruited from Wageningen and surroundings. The inclusion criteria described in **section 2.2.4.1** were applied, with the addition that only females were recruited to minimize inter-individual differences in oral processing behavior. A group of n= 10 participants (25.8 ± 2.6 years, BMI 22.8 ± 2.4 kg/m^2, mean ± SD) was selected from the group of n = 19 based on their stimulated saliva flow rate (1.2 ± 0.2 g/min) assessed during screening sessions. The participants (n = 19) signed informed consent forms and received financial compensation after completion of the sessions.

2.2.5.2 Experimental approach of bolus collection

All recruited participants (n = 19) first attended a 60 min screening session. During the screening session, they were introduced to the study, familiarized with the samples

(PBMA70 and BEEF70) and bolus collection procedures, and tested for saliva flow rate and chewing behavior prior to the actual trial. The participants were instructed to consume one PBMA and one beef patty as usual, and to indicate (self-reported) number of bites by pushing a letter on a keyboard, and number of chews by pushing a different letter on a keyboard. This pre-trial aimed to estimate the average bite size and number of chews per bite during normal consumption. The results of this pre-trial was used to determine the bite size and instruct participants on the number of chews during serum release under oral conditions test (**section 2.2.5.5**).

After screening and participant selection, n = 10 participants attended two 90 min test sessions. In each session, the participants were asked to consume four different patties (either PBMA patties or beef patties) as they were accustomed to, and then expectorate the bolus at the moment of swallowing. The boli (n = 6 - 8) of each bite was individually collected in sealed containers. Patty samples were randomly presented with 3-digit codes. The participants were instructed to cleanse their palate with crackers and water after a sample was consumed, and were given a 5 min break between samples. The oral processing behavior was video recorded and analyzed as described in **2.2.5.3**. Oral processing parameters were independent of swallowing or expectorating samples (data not shown).

2.2.5.3 Characterization of oral processing behavior using video recording

The oral processing behavior of the participants was characterized using video recordings, as described by Forde et al (2017) and van Eck et al (2020). The participants (n = 10) were seated in a chair with a webcam positioned in front of them. Each participant was informed beforehand that they were video recorded while eating. The participants were instructed to eat with their heads towards the webcam, without covering their mouth or face with their hands, and were recorded for the entire consumption duration. ELAN (version 4.9.2, Max Planck Institute for Psycholinguistics, The Language Archive, Nijmegen, The Netherlands) was used to analyze videos for oral processing behavior. The parameters collected from the videos included the total number of bites and chews, bite size, total chewing time, and chewing frequency. Bite size (g) was calculated by dividing the total weight of the consumed patties (g) by the

number of bites the participant took. Total chewing time (s) was determined by cumulating the chewing time of each bite. Chewing frequency (chews/s) was calculated by dividing the total number of chews by the total chewing time. The videos were analyzed by one trained video-coder, and 10% of the randomized videos were blind-validated by a second trained video-coder with an agreement level of ≥ 80%.

2.2.5.4 Characterization of bolus properties at moment of swallowing

Depending on the number of bites taken by each participant, 6 – 8 boli were collected from one patty per participant (n = 10). One bolus was used to determine water, saliva, and fat content, two to three boli to determine texture properties, two to three boli to determine weight, water content of the expelled liquid from the bolus, and one bolus to measure the bolus particle size distribution. All boli were analyzed on the day on which they were collected.

2.2.5.4.1 Water, saliva and fat content of boli

The water content of boli was analyzed as described in **section 2.2.2.1.2** on a wet weight basis. The saliva content of boli was calculated by subtracting the water content of the patties (on a dry weight basis) and serum release under oral condition (on a dry weight basis, as described in **section 2.2.5.5**) from the water content of boli (on a dry weight basis). This can be expressed as: Saliva Uptake = $[(w_0 - w_1)/w_1 - (b_0 - b_1)/b_1 - (s_0 - s_1)/(s_0 \times w_1/w_0)] \times 100\%$, where w_0 is the weight of the cooked patty before drying, w_1 is the weight of the cooked patty after drying, b_0 is the weight of the bolus before drying, b_1 is the weight of the bolus after drying, s_0 is the weight of unchewed patties, and s_1 is the weight of patty fragments post-chewing (van Eck et al., 2020).

Due to the limited sample amount, dried boli (n = 10) (after water content determination) were pooled into three groups for fat analysis, with participant 1 to 3 in group 1, participant 4 to 6 in group 2, and participant 7 to 10 in group 3. The fat content was determined as described in **section 2.2.2.1.3**.

2.2.5.4.2 Texture properties of boli

The texture properties of the boli were determined with a penetration test. A Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) equipped with a 500 g load cell and a stainless cylindrical probe (diameter 4 mm) was used. The boli were gently transferred from sealed cups to a testing cylinder cup (diameter 35 mm, height 55 mm) until they reached a height of 30 mm; the upper surface was gently evened out with the back of a spoon to obtain a cylindrical mass with a smooth and even surface. Penetration tests were performed up to a strain of 80% of the initial height of the boli at a constant test speed of 5 mm/s. Each bolus (n = 10) was punctured at three locations to obtain a force-strain curve, from which peak force, resilience and adhesiveness were obtained as described in **section 2.2.2.1.4**.

2.2.5.4.3 Particle size distribution of boli

The particle size distribution of bolus fragments was determined using image analysis (Chen et al., 2022; van Eck et al., 2019; Zhang et al., 2021). Five g of bolus was put in a transparent acrylic tray (20.3 × 30.5 × 5.1 cm). The bolus fragments were dispersed by gently pouring 250 mL Milli-Q water into the tray, and then manually separated with a spatula. The tray was placed on a flatbed scanner (Canon CanoScan 9000F Mark II, the Netherlands) and a 600-dpi color image with a black background was captured. Images were imported into ImageJ (version 1.52, National Institute of Health, USA) to conduct image analysis. After converting images to an 8-bit format, a black-and-white threshold was used to get a binary picture. To avoid background interference, particles smaller than 0.15 mm^2 or with a circularity lower than 0.20 were excluded from data processing. For each image, the total number of particles per g of bolus and average bolus particle size (mm^2) were determined.

2.2.5.4.4 Expelled liquid from bolus

The liquid expelled from the bolus was measured by centrifugation. The expelled liquid included the remaining serum in the bolus after mastication and the uptake of saliva during consumption. Around 15 g of bolus was weighted and placed onto a cylindrical polypropylene sieve (pore size 1.1 mm) placed inside a 50 mL centrifuge

tube. The sample was centrifuged at 200 g for 10 min at 20°C (Beckman Coulter Allegra X-22R Centrifuge, United States) to allow the liquid to pass through the filter, which was collected in the bottom part of the centrifuge tube. After centrifugation, the remaining bolus present on the filter was weighted. The mass of expelled liquid was determined by subtracting the weight of the final bolus from the weight of the initial bolus. Furthermore, the expelled liquid was collected to determine its water content, as described in **section 2.2.3.2**.

2.2.5.5 Serum release under oral conditions

To determine the amount of serum released from the patties during mastication without saliva incorporation, pre-cut patties (7 – 9 g) were weighted and placed in plastic bags. Participants (n = 10) masticated the samples in the bags following the chewing protocol (**section 2.2.5.3**), with 30 chews (determined average) for PBMA patties, and 39 chews (determined average) for beef patties. After chewing, the patty fragments were carefully and manually removed from the bags to isolate them from the released serum using tweezers. These fragments were further centrifugated (**section 2.2.5.4.4**) to remove any residual serum adhering to the surfaces. The fragments were then weighed. The total serum release under oral conditions was determined by assessing the weight difference between the unchewed patty and patty fragments post-chewing.

2.2.6 Data analysis

Results are reported as mean values with standard deviation (SD). Regarding samples properties, one-way ANOVA followed by Tukey post-hoc analyses were performed for PBMA and beef patties separately. For sensory (rank-rating and RATA results) and bolus properties, linear mixed models (LMM) followed by Tukey post-hoc analyses were performed, treating samples as fixed factor and participants as random factor. LMM analysis of sensory properties and bolus properties was conducted for PBMA patties and beef patties separately. Pearson correlation coefficients were determined to assess correlations between sensory attributes for PBMA and beef patties separately. Pearson correlation coefficients and linear regressions were used to examine correlations

between food properties, sensory properties, and bolus properties for PBMA and beef patties separately. Data analysis was performed using RStudio (version 2022.07.0, PBC) with the packages emmeans (Lenth, 2022), lmerTest (Kuznetsova et al., 2017), and Hmisc (Harrell & Dupont, 2023). A significance level of $p < 0.05$ was used.

2.3 Results and Discussion

2.3.1 Rank-rating of juiciness intensity

A rank-rating test was conducted to assess juiciness intensity of PBMA and beef patties (**Figure 2.2**). The core temperature significantly affected juiciness intensity of PBMA ($F = 44.8$, $p < 0.001$) and beef patties ($F = 81.0$, $p < 0.001$). Juiciness intensity of PBMA patties increased by 56%, from 45 ± 20 for PBMA90 to 70 ± 16 for PBMA60. Similarly, juiciness intensity of beef patties increased by 128% from 26 ± 15 for BEEF90 to 58 ± 17 for BEEF60. For both PBMA and beef patties, three levels of juiciness intensity were obtained, as no significant differences were found between patties cooked at 80 and 90°C. Our results for beef patties were consistent with previous studies demonstrating that an increase in meat core temperatures led to a decrease in juiciness perception (Schwartz et al., 2022). The variability in juiciness intensity across PBMA and beef patties prepared from the same raw materials allows us to investigate how juiciness is related to food and bolus properties. Henceforth, we refer to the patties prepared at different core cooking temperatures as patties differing in juiciness.

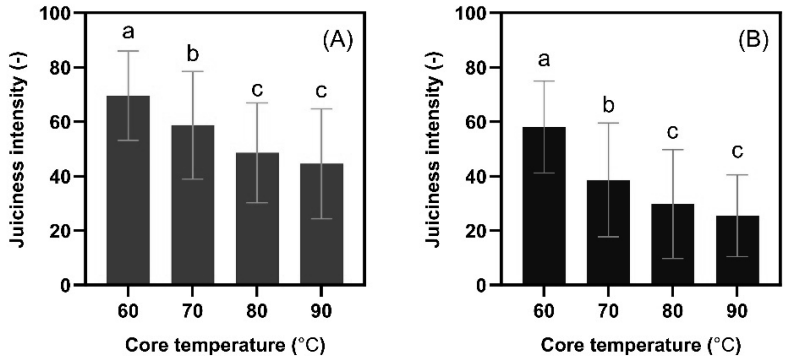

Figure 2.2. Mean scores (± SD) of rank-rating test for juiciness intensity for (A) PBMA patties (n = 97) and (B) beef patties (n = 100) cooked to different core temperatures. Different letters indicate significant differences between means ($p < 0.05$).

2.3.2 Characterization of PBMA and beef patties

The compositional, texture and serum properties of (A) PBMA and (B) beef patties are summarized in **Table 2.2**.

Table 2.2 Mean values (± SD) of compositional, texture and serum properties of (A) PBMA and (B) beef patties. Different letters indicate significant differences between samples ($p < 0.05$).

(A) PBMA patties	PBMA60	PBMA70	PBMA80	PBMA90	*F* value	*p* value
Compositional properties						
Total cooking loss (% w/w)	17.8 ± 1.3^{a}	20.1 ± 1.8^{a}	23.9 ± 1.6^{b}	27.0 ± 2.6^{c}	27.6	<0.001
Water content (% w/w)	55.6 ± 0.4^{a}	55.4 ± 0.3^{a}	55.3 ± 0.5^{a}	56.8 ± 0.5^{b}	10.5	<0.01
Fat content (% w/w)	15.0 ± 0.4^{a}	14.9 ± 0.1^{a}	13.6 ± 0.7^{a}	11.3 ± 0.7^{b}	29.8	<0.001
Texture properties						
Peak force (N)	2.1 ± 0.2	1.8 ± 0.2	1.8 ± 0.1	1.9 ± 0.2	2.8	0.071
Resilience (-)	0.8 ± 0.5	0.9 ± 0.5	1.5 ± 0.9	0.9 ± 0.8	0.9	0.463
Adhesiveness (Ns)	0.7 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	2.6	0.089
Serum properties						
Serum release under compression (% w/w)	19.8 ± 0.4^{a}	17.8 ± 1.3^{ab}	15.4 ± 2.8^{bc}	13.2 ± 1.8^{c}	12.8	<0.001
Water content of serum (% w/w)	27.3 ± 0.5^{a}	24.5 ± 2.4^{ab}	19.2 ± 6.5^{ab}	17.8 ± 7.5^{b}	3.8	<0.05
(B) Beef patties	**BEEF60**	**BEEF70**	**BEEF80**	**BEEF90**	***F* value**	***p* value**
Compositional properties						
Total cooking loss (% w/w)	32.6 ± 2.4^{a}	37.5 ± 2.5^{b}	40.2 ± 2.3^{bc}	42.0 ± 1.6^{c}	20.7	<0.001
Water content (% w/w)	61.9 ± 0.8^{a}	58.4 ± 0.9^{b}	56.8 ± 0.9^{bc}	56.2 ± 0.7^{c}	37.9	<0.001
Fat content (% w/w)	10.5 ± 0.1^{a}	11.9 ± 0.4^{ab}	12.1 ± 0.6^{b}	12.0 ± 1.0^{ab}	4.1	<0.05
Texture properties						
Peak force (N)	4.5 ± 0.4^{a}	5.0 ± 0.5^{ab}	5.6 ± 1.0^{b}	5.6 ± 0.3^{b}	3.9	<0.05
Resilience (-)	2.9 ± 0.8^{a}	5.0 ± 1.4^{b}	4.3 ± 0.8^{ab}	3.7 ± 0.7^{ab}	4.1	<0.05
Adhesiveness (N.s)	2.6 ± 0.8^{a}	3.3 ± 0.6^{ab}	3.6 ± 0.4^{ab}	4.0 ± 0.8^{b}	3.7	<0.05
Serum properties						
Serum release under compression (% w/w)	10.7 ± 2.5	8.2 ± 1.1	8.8 ± 1.5	7.6 ± 1.9	2.8	0.077
Water content of serum (% w/w)	35.7 ± 7.7^{a}	25.2 ± 6.5^{ab}	25.4 ± 4.1^{ab}	19.8 ± 6.6^{b}	5.4	<0.01

PBMA ($F = 27.6$, $p < 0.001$) and beef patties ($F = 20.7$, $p < 0.001$) differed significantly in total cooking loss. Cooking loss increased with increasing core temperature in both PBMA and beef patties, which is consistent with the findings of Vu et al., (2022). The

differences in water content between PBMA patties were significant and small (max. 1.5% w/w absolute difference), indicating that cooking at different temperatures did not lead to considerable differences in water evaporation. However, the fat content of PBMA patties decreased significantly with increasing core temperature ($F = 29.8$, $p < 0.001$), showing that fat was released more at higher temperatures. In contrast, for beef patties the fat content was similar, with significant and small differences (max. 1.6% w/w absolute difference), whereas the water content significantly decreased ($F = 37.9$, $p < 0.001$) with increasing core temperature, indicating higher water loss. Cooking temperature had a stronger effect on water loss in beef patties compared to PBMA patties since cooking at high temperatures leads to shrinking of the muscle fibers due to increased protein denaturation, leading to water expulsion (Schwartz et al., 2022). However, in PBMA patties, such muscle fiber structures do not exist and the proteins are already denatured before cooking (Janardhanan et al., 2023), leading to less water loss (water content of raw patties, 58.7 ± 0.3% for PBMA, 66.1 ± 0.1% for beef patties). A higher percentage of fat loss during cooking were found in beef patties compared to PBMA patties (fat content of raw patties, 16.4 ± 0.3% for PBMA, 16.8± 0.4% for beef patties). However, the increasing core temperature showed a stronger effect on fat loss in PBMA patties compared to beef patties.

Even though PBMA and beef patties with different juiciness levels showed significant differences in compositional properties, no large differences in texture properties were observed. For PBMA patties, there were no significant differences in peak force, resilience and adhesiveness across patties (**Table 2.2**). Beef patties showed significant but small differences in these three texture properties, with higher values for patties cooked at higher temperatures. Vu et al. (2022) also reported similar findings. The variations in texture observed in both PBMA and beef patties in response to changes in core temperatures may be related to the structure of the protein network. The denaturation of myofibrillar proteins and connective tissue proteins during cooking can result in a tougher texture, which increases at higher temperature (Schwartz et al., 2022). In addition, the differences may be related to the differences in water and accompanying dry matter content. Denatured plant-based proteins do not undergo

these additional structural changes during cooking, nor do they show differences in water content, which may explain the absence of differences in PBMA patties texture properties.

The serum released under compression significantly increased ($F = 12.8$, $p < 0.001$) with decreasing core temperature for PBMA patties. For beef patties, serum released under compression did not differ significantly ($F = 2.8$, $p = 0.077$) between patties, although a trend was found similar to that observed in PBMA patties. The water content of the released serum significantly increased with decreasing core temperature for PBMA patties ($F = 3.8$, $p < 0.05$) and beef patties ($F = 5.4$, $p < 0.01$). However, after the drying process (water content measurement), there was not sufficient sample left to perform a Soxhlet extraction to determine the fat content accurately. Small particles were expelled during compression, preventing us from calculating fat content of released serum by subtracting the weight of water from the total release. It should be noticed that only the water content of the released serum was determined whereas fat content was not quantified.

2.3.3 Sensory properties of PBMA and beef patties

Texture, taste and flavor properties of (A) PBMA and (B) beef patties are provided in **Table 2.3**. Since there were no significant differences in taste and flavor attributes between samples, except for umami in PBMA patties, and citrus flavor in beef patties (**Table 2.3**), the correlations were analyzed only among texture attributes separately for (A) PBMA and beef (B) patties, as presented in **Table 2.4**.

Table 2.3 Intensity of texture, taste and flavor attributes (mean ± SD) obtained from RATA evaluation of (A) PBMA (n = 99) and (B) beef patties (n = 95). Different letters indicate significant differences between samples in a row ($p < 0.05$) based on linear mixed model analysis. Attributes for PBMA patties were not compared with beef patties as these products were assessed in different sessions.

(A) PBMA patties	PBMA60	PBMA70	PBMA80	PBMA90	*F* value	*p* value
Texture attributes						
Juiciness	7.0 ± 1.4^{a}	6.4 ± 1.5^{b}	4.9 ± 1.7^{c}	4.5 ± 1.7^{c}	74.4	< 0.001
Dryness	1.8 ± 1.6^{a}	2.3 ± 1.7^{a}	3.3 ± 2.0^{b}	3.7 ± 1.9^{b}	35.2	< 0.001
Hardness	2.7 ± 1.6	3.0 ± 1.8	2.7 ± 1.7	2.6 ± 1.6	1.6	0.183
Chewiness	4.5 ± 1.9^{a}	3.9 ± 1.9^{b}	3.9 ± 1.8^{b}	3.9 ± 1.8^{b}	4.6	< 0.01
Tenderness	5.5 ± 1.7^{a}	5.2 ± 1.8^{ab}	5.0 ± 1.8^{b}	5.5 ± 1.7^{ac}	2.8	< 0.05
Crumbliness	4.4 ± 1.9	4.6 ± 1.8	4.5 ± 2.0	4.6 ± 1.9	0.6	0.589
Fibrousness	3.6 ± 2.1^{ab}	3.8 ± 2.0^{a}	3.3 ± 2.0^{b}	3.3 ± 1.9^{b}	3.0	< 0.05
Fattiness	5.0 ± 1.9^{a}	4.6 ± 2.0^{a}	3.7 ± 2.1^{b}	3.5 ± 1.9^{b}	33.2	< 0.001
Taste and Flavor attributes						
Saltiness	3.9 ± 1.9	3.9 ± 2.0	3.7 ± 1.8	3.6 ± 1.8	0.7	0.530
Umami	4.5 ± 1.9^{a}	4.2 ± 1.9^{ab}	3.8 ± 1.9^{b}	3.9 ± 2.0^{ab}	3.1	< 0.05
Beany flavor	3.9 ± 2.4	3.9 ± 2.3	4.1 ± 2.2	4.3 ± 2.3	0.9	0.460
Off-flavor	1.2 ± 1.8	1.2 ± 1.9	1.5 ± 2.2	1.4 ± 1.9	1.0	0.375
Meat flavor	3.4 ± 2.0	3.4 ± 1.9	3.1 ± 1.9	2.9 ± 1.8	2.5	0.061
Citrus flavor	2.6 ± 2.2	2.5 ± 2.1	2.6 ± 2.1	2.1 ± 2.0	1.5	0.220
(B) Beef patties	**BEEF60**	**BEEF70**	**BEEF80**	**BEEF90**	***F* value**	***p* value**
Texture attributes						
Juiciness	6.6 ± 1.6^{a}	4.3 ± 1.9^{b}	3.8 ± 2.3^{bc}	3.6 ± 2.0^{c}	62.2	< 0.001
Dryness	2.3 ± 1.5^{a}	4.2 ± 2.1^{b}	5.1 ± 2.5^{c}	5.4 ± 2.4^{c}	56.6	< 0.001
Hardness	2.6 ± 1.4^{a}	3.9 ± 1.9^{bc}	4.4 ± 2.0^{b}	3.7 ± 1.9^{c}	27.2	< 0.001
Chewiness	4.4 ± 1.7^{a}	4.9 ± 1.5^{ab}	5.4 ± 1.8^{b}	4.7 ± 1.7^{a}	6.7	< 0.001
Tenderness	5.4 ± 1.8^{a}	4.0 ± 1.8^{b}	3.6 ± 1.8^{b}	4.0 ± 1.9^{b}	24.6	< 0.001
Crumbliness	3.9 ± 2.0^{a}	4.2 ± 1.7^{ab}	4.7 ± 1.7^{bc}	5.3 ± 2.0^{c}	13.0	< 0.001
Fibrousness	3.6 ± 2.0^{a}	3.9 ± 2.1^{ab}	4.2 ± 2.4^{b}	3.7 ± 2.2^{ab}	3.6	< 0.05
Fattiness	5.1 ± 2.0^{a}	4.2 ± 2.0^{b}	4.0 ± 1.9^{b}	3.9 ± 1.9^{b}	15.3	< 0.001
Taste and Flavor attributes						
Saltiness	3.5 ±2.1	3.2 ± 1.7	3.2 ± 1.8	3.2 ± 1.8	2.0	0.108
Umami	4.0 ± 2.0	3.8 ± 1.9	3.7 ± 2.1	3.7 ± 1.8	1.1	0.360
Beany flavor	0.5 ± 1.1	0.5 ± 1.2	0.6 ± 1.3	0.6 ± 1.4	0.3	0.801
Off-flavor	0.9 ± 1.6	1.0 ± 1.8	1.0 ± 1.8	1.4 ± 1.8	0.3	0.842
Meat flavor	6.4 ± 1.9	6.5 ± 1.8	6.6 ± 1.7	6.5 ± 1.8	0.6	0.592
Citrus flavor	3.2 ± 2.6^{a}	2.8 ± 2.5^{a}	2.6 ± 2.5^{ab}	2.0 ± 1.8^{b}	8.0	< 0.001

Table 2.4 Pearson correlation coefficients of RATA texture attributes of (A) PBMA and (B) beef patties. Asterisks indicate that correlations are significant at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$; (n.s.) not significant.

(A) PBMA patties

	Juiciness	Dryness	Hardness	Chewiness	Tenderness	Crumbliness	Fibrousness	Fattiness
Juiciness	1	**-0.67*****	**-0.1***	n.s.	**0.26*****	n.s.	n.s.	**0.3*****
Dryness		1	**0.34*****	**0.25*****	**-0.23*****	**0.19*****	n.s.	**-0.16****
Hardness			1	**0.49*****	**-0.31*****	**0.13****	**0.28*****	**0.14****
Chewiness				1	**-0.25*****	**0.15****	**0.31*****	**0.2*****
Tenderness					1	n.s.	n.s.	n.s.
Crumbliness						1	n.s.	n.s.
Fibrousness							1	**0.15****
Fattiness								1

(B) Beef patties

	Juiciness	Dryness	Hardness	Chewiness	Tenderness	Crumbliness	Fibrousness	Fattiness
Juiciness	1	**-0.78*****	**-0.38*****	**-0.34*****	**0.54*****	**-0.11***	n.s.	**0.39*****
Dryness		1	**0.49*****	**0.41*****	**-0.37*****	**0.24*****	**0.14****	**-0.23*****
Hardness			1	**0.43*****	**-0.22*****	**0.15****	**0.26*****	n.s.
Chewiness				1	**-0.3*****	**0.14****	**0.21*****	n.s.
Tenderness					1	n.s.	n.s.	**0.35*****
Crumbliness						1	0.16**	n.s.
Fibrousness							1	n.s.
Fattiness								1

As expected, PBMA patties, which differed in juiciness intensity as determined by the rank-rating test, differed also significantly in RATA juiciness intensity ($F = 74.4$, $p < 0.001$). PBMA patties also differed significantly in dryness ($F = 35.2$, $p < 0.001$), chewiness ($F = 4.6$, $p < 0.01$), tenderness ($F = 2.8$, $p < 0.05$), fibrousness ($F = 3.0$, $p < 0.05$) and fattiness ($F = 33.2$, $p < 0.001$) (**Table 2.3**). The observed significant differences in chewiness, tenderness and fibrousness among PBMA patties were relatively small, with chewiness ranging from 3.9 to 4.5, tenderness ranging from 5.5 to 5.0, and fibrousness ranging from 3.3 to 3.8. As expected, sensory juiciness was strongly and negatively correlated with dryness ($r = -0.67$) and positively correlated with fattiness ($r = 0.30$) (**Table 2.4**). Additionally, a positive correlation was found between sensory hardness and chewiness ($r = 0.49$), which has been shown previously

in meat products (Pematilleke et al., 2022; Sasaki et al., 2013). Overall, for PBMA, juiciness was significantly and negatively correlated with dryness and fattiness, but not with other texture sensations. This finding is consistent with the instrumental texture properties (**section 2.3.2**), which showed no significant differences among PBMA patties differing in core temperatures (differing in juiciness).

In contrast to the PBMA patties, beef patties showed more variation in texture perception. All eight texture attributes differed significantly (**Table 2.3**). Decreasing core temperature led to increased juiciness, tenderness and fattiness, while dryness, hardness, chewiness and crumbliness decreased. Interestingly, despite a small decrease in fat content with decreasing core temperature (**Table 2.2**), the RATA results indicated an increased perception of fattiness, suggesting that fattiness may not solely relate to fat content, but could also be influenced by other texture attributes, such as juiciness (r = 0.39) and tenderness (r = 0.35) (**Table 2.4**). As shown in **Table 2.4**, juiciness was negatively correlated with dryness (r = -0.78), hardness (r = -0.38), chewiness (r = -0.34), and positively correlated with tenderness (r = 0.54), and fattiness (r = 0.39). Chewiness positively correlated with hardness (r = 0.43). These results are well aligned with previous studies on meat products (Damian et al., 2016; Hunt et al., 2014; Iida et al., 2015; Lang et al., 2015; O'Quinn et al., 2012).

The impact of core temperature and texture on flavor perception of PBMA and beef patties was limited. No significant differences were found for 5 out of 6 taste and flavor attributes; only umami of PBMA patties and citrus flavor of beef patties (see **section 2.2.1**, linalool was added for another study) showed significant but small differences among samples. This suggests that the impact of core temperature, which led to variations in juiciness, on taste and flavor perception may be limited.

To summarize, with decreasing core cooking temperature, PBMA patties were perceived juicier, less dry and fattier, whereas beef patties were perceived juicier, less dry, less hard, less chewy, but more tender and fattier. As discussed previously, the differences in structural changes between beef and plant-based proteins during cooking most likely explain the greater variations observed in texture perception of beef patties compared to PBMA patties.

2.3.4 Relationships between texture perception and samples properties

To understand the relationships between sample properties and texture perception, Pearson correlation coefficients between texture attributes and different properties were determined for (A) PBMA and (B) beef patties separately based on their mean values (n = 4) and are presented in **Table 2.5**.

Table 2.5 Pearson correlation coefficients between texture attributes and sample properties of (A) PBMA (n = 4) and (B) Beef patties (n =4). Asterisks indicate that correlations are significant at (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.

		Sample properties							
		Composition			**Texture**			**Serum**	
		Total cooking loss	Water content	Fat content	Peak force	Resili-ence	Adhesi-veness	Serum release under compre-ssion	Serum water content
Texture attributes	**(A) PBMA patties**								
	Juiciness	**-0.99***	-0.53	0.89	0.63	-0.52	-0.50	**0.98***	**1.00*****
	Dryness	**0.99****	0.57	-0.91	-0.61	0.47	0.54	**-0.99***	**-1.00****
	Hardness	-0.59	-0.62	0.73	-0.19	-0.25	-0.30	0.55	0.57
	Chewiness	-0.72	-0.22	0.52	0.91	-0.33	-0.45	0.76	0.74
	Tenderness	-0.15	0.64	-0.17	0.80	-0.88	0.56	0.17	0.32
	Crumbliness	0.62	0.37	-0.50	-0.71	-0.04	0.66	-0.66	-0.58
	Fibrousness	-0.83	-0.48	0.81	0.30	-0.59	-0.27	0.81	0.87
	Fattiness	**-0.98***	-0.53	0.89	0.62	-0.52	-0.49	**0.98***	**1.00****
	(B) Beef patties								
	Juiciness	**-0.97***	**0.99****	**-0.98***	-0.94	-0.63	**-0.96***	0.92	**0.96***
	Dryness	**0.99****	**-1.00*****	**0.96***	**0.97***	0.53	**0.98***	-0.90	**-0.95***
	Hardness	0.81	-0.87	0.96*	0.85	0.77	0.77	-0.73	-0.75
	Chewiness	0.54	-0.63	0.74	0.67	0.66	0.47	-0.37	-0.41
	Tenderness	-0.87	0.92	**-0.99***	-0.88	-0.77	-0.84	0.82	0.84
	Crumbliness	**0.95***	-0.91	0.75	0.90	0.17	**0.96***	-0.83	-0.92
	Fibrousness	0.46	-0.54	0.68	0.60	0.65	0.37	-0.29	-0.32
	Fattiness	**-0.98***	**0.99****	**-0.98***	-0.95	-0.60	**-0.97***	0.92	**0.96***

In both PBMA and beef patties, sensory juiciness showed strong and negative correlations with total cooking loss (r = -0.99 for PBMA and r = -0.97 for beef patties), while sensory dryness showed strong and positive correlations with total cooking loss (r = 0.99 for PBMA and r = 0.99 for beef patties). These results align with several studies that found moderate to strong correlations between juiciness perception and cooking loss in various meats (Lucherk et al., 2017; Pematilleke et al., 2020; Serra et al., 2008). In beef patties, sensory juiciness and dryness were strongly correlated with water and fat content of the patty (**Table 2.5**). However, the small and negligible differences in fat content among beef patties (**Table 2.2**) caused coincidental strong correlations between fat content and texture attributes, as the correlation analysis was performed with the mean values (**Table 2.5**). Unexpectedly, in PBMA patties, the correlation coefficients between sensory juiciness, dryness and the fat content were high but not significant, probably due to small sample size (n = 4) in the correlation analysis. To further validate our findings, we conducted linear regressions to examine the relationships between RATA juiciness, water content and fat content for PBMA and beef patties (**Figure 2.3 (A)** and **(B)**). We treated the replicate measurements of water and fat content as individual data points, because they were independent measurements. The results showed that sensory juiciness was significantly and positively correlated with water content in beef patties (r^2 = 0.89, $p < 0.001$), and with fat content in PBMA patties (r^2 = 0.72, $p < 0.001$). These findings clearly indicate that the drivers underlying juiciness perception in PBMA patties differed from those in beef patties.

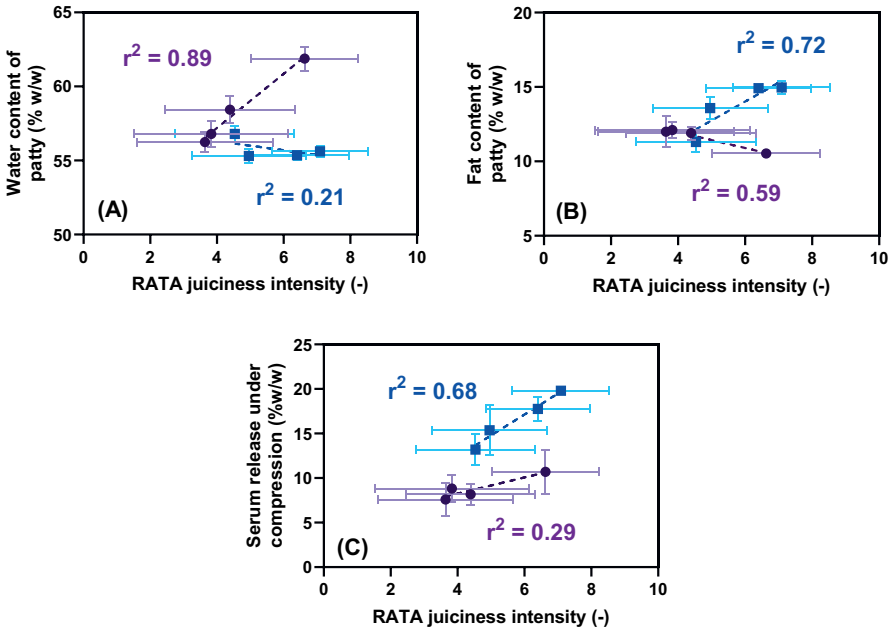

Figure 2.3 Relationships between RATA juiciness, and (A) water content, (B) fat content, (C) serum release under compression of PBMA (blue squares) and beef patties (purple circles). Error bars indicate the standard deviation of mean. Dashed lines represent linear regressions.

In PBMA patties, no significant correlations were found between sensory juiciness, dryness and instrumental measured texture properties (**Table 2.5 (A)**). In beef patties, sensory juiciness strongly and negatively correlated with adhesiveness (r = -0.96), while sensory dryness strongly and positively correlated with peak force (r = 0.97) and adhesiveness (r = 0.98) (**Table 2.5 (B)**). These results indicate that firmer beef patties were perceived as less juicy and drier, which is consistent with previous studies (Adhikari et al., 2004; Thompson, 2004).

Sensory juiciness and dryness were also significantly and strongly correlated with the amount of serum released under compression in PBMA patties (r = 0.98 for juiciness, r = -0.99 for dryness), while the same correlations in beef patties were not significant (**Table 2.5**). As described previously, replicate measurements of serum release under compression were considered as individual data points and plotted against juiciness intensity (**Figure 2.3 (C)**). Juiciness perception increased significantly with increasing serum release for PBMA (r^2 = 0.68, $p < 0.001$) and beef patties (r^2 = 0.29, $p < 0.05$). For

PBMA, the juiciness intensity thus seemed to be more dependent on the amount of serum released from the patties. Strong correlations were observed between sensory juiciness, dryness and water content of the released serum for PBMA and beef patties (absolute *r*: 0.96 – 1.00), suggesting that higher water content in the released serum leads to increased juiciness perception. However, as water content in PBMA patties did not vary much (**Figure 2.3 (A)**), this correlation may not be trustworthy.

Apart from sensory juiciness and dryness, no significant correlations were found between sensory hardness, chewiness, tenderness, crumbliness, fibrousness, and sample properties of PBMA patties. Similarly, out of 40 possible correlations (5 texture attributes × 8 sample properties) for beef patties, only 3 were significant. These results are not surprising given the limited variations in intensity of RATA texture attributes (**Table 2.3**) and instrumental measured texture properties (**Table 2.2**) of PBMA and beef patties. On the other hand, sensory fattiness in both PBMA and beef patties showed a similar correlation trend as juiciness and dryness with sample properties. This finding suggests that fattiness perception may contribute to juiciness perception, and both are related to the sensation of liquid released during mastication.

In summary, juiciness and dryness perception were correlated strongly with cooking loss, composition and serum properties of PBMA and beef patties. Limited variations in PBMA and beef texture properties resulted in no meaningful correlations with texture attributes, including juiciness. Recognizing that these relationships were not straightforward and could not be solely explained by sample properties, we decided to further investigate the oral processing behavior and bolus properties of the PBMA and beef patties, to determine whether changes in sample properties during consumption would provide a better understanding of juiciness and other texture attributes.

2.3.5 Oral processing behavior of PBMA and beef patties

We hypothesized that the differences in serum release and juiciness of PBMA and beef patties would lead to difference in oral processing behavior. The results did not confirm this hypothesis (**Table 2.6**). Core temperature had no significant impact on total number of bites, total number of chews, chewing time and chewing frequency for both PBMA and beef patties. A significant and small difference in bite size was observed for both type of patties ($F = 4.5$, $p < 0.05$; $F = 7.0$, $p < 0.01$, for PBMA and beef patties respectively). These findings suggest that (a) the differences in instrumental texture properties (**Table 2.2**) were too small (beef patties) or absent (PBMA patties) to induce differences in oral processing behavior, and (b) the increase in serum release under compression was not sufficient to reduce chewing time and number of chews by increasing lubrication.

Table 2.6. Mean (± SD) of oral processing behaviors (n = 10) of (A) PBMA and (B) beef patties. Different letters indicate significant differences between samples ($p < 0.05$) based on a linear mixed model.

(A) PBMA patties	**PBMA60**	**PBMA70**	**PBMA80**	**PBMA90**	***F* value**	***p* value**
Total number of bites (-)	8.3 ± 1.8	8.0 ± 1.9	7.9 ± 1.7	8.1 ± 1.8	0.8	0.506
Bite size (g)	11.0 ± 2.5^{ab}	11.4 ± 2.5^{a}	11.0 ± 2.3^{ab}	10.3 ± 2.2^{b}	**4.5**	**< 0.05**
Total number of chews (-)	353 ± 110	350 ± 118	372 ± 116	337 ± 85	0.9	0.431
Chewing time (s)	242 ± 54	234 ± 55	250 ± 62	233 ± 56	0.7	0.544
Chewing frequency	1.5 ± 0.2	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.2	1.0	0.400
(B) Beef patties	**BEEF60**	**BEEF70**	**BEEF80**	**BEEF90**	***F* value**	***p* value**
Total number of bites (-)	6.9 ± 1.5	6.8 ± 1.5	6.8 ± 1.3	6.8 ± 1.5	0.2	0.907
Bite size (g)	11.1 ± 2.7^{a}	10.6 ± 2.1^{ab}	10.4 ± 1.9^{b}	9.9 ± 2.1^{b}	**7.0**	**< 0.01**
Total number of chews (-)	290 ± 118	332 ± 152	310 ± 114	316 ± 113	1.7	0.196
Chewing time (s)	202 ± 62	232 ± 80	219 ± 57	224 ± 65	2.7	0.069
Chewing Frequency	1.4 ± 0.2	1.4 ± 0.3	1.4 ± 0.2	1.4 ± 0.2	0.3	0.848

2.3.6 Characterization of bolus properties at the moment of swallowing

Next to oral processing behavior, we also determined the bolus properties at the moment of swallowing, for which the results are summarized in **Table 2.7** for (A) PBMA and (B) beef patties.

Table 2.7 Mean (± SD) values of bolus properties of (A) PBMA and (B) beef patties at the moment of swallowing. Different letters indicate significant differences between samples ($p < 0.05$) based on a linear mixed model.

(A) Bolus properties of PBMA patties	**PBMA60**	**PBMA70**	**PBMA80**	**PBMA90**	***F*-value**	***p*-value**
Composition						
Water content of bolus (% w/w)	68.2 ± 2.7^{ab}	66.9 ± 2.2^{a}	68.9 ± 2.6^{b}	69.5 ± 2.1^{b}	**5.9**	**< 0.01**
Fat content of bolus (% w/w)	9.9 ± 0.9^{a}	10.7 ± 0.8^{b}	9.6 ± 0.9^{a}	7.8 ± 0.6^{c}	**55.4**	**< 0.001**
Saliva uptake (g/g dry weight)	0.6 ± 0.3^{ab}	0.5 ± 0.2^{b}	0.7 ± 0.3^{ac}	0.8 ± 0.2^{c}	**9.5**	**< 0.001**
Texture						
Bolus peak force (N)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4	0.754
Bolus resilience (-)	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.9	0.459
Bolus adhesiveness (N.s)	0.36 ± 0.05	0.36 ± 0.06	0.33 ± 0.08	0.36 ± 0.08	1.2	0.324
Fragments						
Total number of bolus particles (no./g)	349 ± 101	345 ± 92	367 ± 103	400 ± 123	1.7	0.183
Average bolus particle size (mm^2)	2.6 ± 0.6	2.5 ± 0.6	2.5 ± 0.7	2.5 ± 0.7	0.4	0.744
Expelled liquid						
Expelled liquid during centrifugation (% w/w)	24.0 ± 5.0^{a}	21.9 ± 2.3^{ab}	21.5 ± 3.8^{ab}	19.9 ± 4.5^{b}	**4.0**	**< 0.05**
Water content of expelled liquid (% w/w)	90.6 ± 1.4	90.3 ± 1.1	90.0 ± 1.2	89.8 ± 1.5	2.5	0.078
(B) Bolus properties of beef patties	**BEEF60**	**BEEF70**	**BEEF80**	**BEEF90**	***F*-value**	***p*-value**
Composition						
Water content of bolus (% w/w)	72.3 ± 2.4	71.6 ± 2.9	70.5 ± 3.8	71.4 ± 2.8	1.9	0.148
Fat content of bolus (% w/w)	7.5 ± 0.7^{a}	8.0 ± 1.0^{ab}	8.8 ± 1.3^{b}	7.1 ± 1.6^{a}	**8.6**	**< 0.001**
Saliva uptake (g/g dry weight)	0.9 ± 0.3^{a}	1.1 ± 0.4^{ab}	1.0 ± 0.5^{ab}	1.2 ± 0.4^{b}	**3.7**	**< 0.05**
Texture						
Bolus peak force (N)	1.2 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.5	0.2	0.883
Bolus resilience (-)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	1.5	0.236
Bolus adhesiveness (N.s)	0.38 ± 0.08	0.37 ± 0.11	0.35 ± 0.10	0.38 ± 0.13	0.4	0.772
Fragment						
Total number of bolus particles (no./g)	203 ± 58^{a}	271 ± 121^{ab}	317 ± 195^{b}	328 ± 116^{b}	**5.0**	**< 0.01**
Average bolus particle size (mm^2)	2.5 ± 0.6	2.4 ± 0.8	2.2 ± 0.7	2.2 ± 0.7	1.1	0.380
Expelled liquid						
Expelled liquid during centrifugation (% w/w)	18.5 ± 4.2	17.4 ± 4.0	19.8 ± 8.4	14.5 ± 4.5	2.3	0.095
Water content of expelled liquid (% w/w)	94.8 ± 0.6^{a}	95.3 ± 0.6^{b}	95.6 ± 0.6^{b}	95.3 ± 0.5^{b}	**16.9**	**< 0.001**

Significant but small differences were found in bolus water content ($F = 5.9$, $p < 0.01$) among PBMA patties differing in juiciness, while differences in bolus fat content ($F = 55.4$, $p < 0.001$) and saliva uptake ($F = 9.5$, $p < 0.001$) were slightly larger. These finding may reflect the similarity in water content and the increase in fat content with decreasing core temperature (increasing juiciness) in PBMA patties (**Table 2.2**). Even though water content of beef patties increased as core temperature decreased (**Table 2.2**), water content of the bolus did not differ significantly between beef samples ($F = 1.9$, $p = 0.148$) (**Table2.7**). This reduction in differences of water content during mastication can be attributed to a significant increase in saliva uptake when the patties had a higher core temperature (and were consequently lower in juiciness) ($F = 3.7$, $p < 0.05$). Bolus fat content showed significant but small differences between beef patties differing in core temperature (differing in juiciness) ($F = 8.6$, $p < 0.001$).

No significant differences were observed in bolus texture properties for PBMA and beef patties differing in core temperature (differing in juiciness), which again could be attributed to the limited differences in texture properties of the patties themselves before consumption. While no significant differences in oral breakdown (total number of particles and average particle size) were observed for PBMA patties differing in core temperature (differing in juiciness), beef patties displayed a significant increase in the total number of particles as juiciness decreased ($F = 5.0$, $p < 0.01$), although this was not reflected in smaller particle sizes. The increase in bolus particle number might explain why the less juicy beef patties were perceived as more crumbly (**Table 2.3**).

The similarities in bolus composition for PBMA and beef patties differing in core temperature (differing in juiciness) were also reflected in the limited differences in liquid expelled from the boli. The expelled liquid predominantly consisted of water (over 90%), primarily attributed to saliva uptake during mastication. As a result, only minor differences in water content for the expelled liquid were observed.

In summary, although differences in oral processing behavior and bolus properties were expected based on differences in sample properties and juiciness due to varied core temperature, limited differences in oral processing behavior and bolus properties

at the moment of swallowing were observed. Therefore, bolus properties at the moment of swallowing were not able to explain sensory perception in more detail.

2.3.7 Serum release under oral conditions

In the experiments discussed in the previous section, liquid expelled from the collected boli always contained saliva. To gain insights into how consumption affected the release of serum from the patties, serum release during mastication was quantified according to a method that allowed us to eliminate saliva uptake; mastication of patties were performed when the patties were present in a sealed bag, which allowed serum release from the patties while preventing saliva uptake. To examine the relationship between juiciness perception and serum release under oral conditions in PBMA and beef patties, linear regressions were performed using the replicate measurements of serum release as individual data points (**Figure 2.4 (A)**). For PBMA patties, increasing juiciness significantly correlated with higher serum released from the matrix during mastication (F = 116.8, $p < 0.001$; r^2 = 0.74, $p < 0.001$). A similar correlation was observed in beef patties (r^2 = 0.46, $p < 0.001$), but the differences in serum release were limited (F = 17.2, $p < 0.001$). Only the beef patty with the highest juiciness level (BEEF60) showed significantly higher serum release under oral conditions than other beef patties ($p < 0.05$). These results confirm the proposed hypothesis in literature that perceived juiciness is related to the serum released from the product.

To verify the consistency between serum release under oral conditions (*in vivo*) and measured serum release under compression (*in vitro*), we performed linear regressions using the means of both methods (n =4) (**Figure 2.4 (B)**). A strong correlation between serum release under oral conditions (*in vivo*) and serum release under compression (*in vitro*) was found for PBMA (r^2 = 0.95, $p < 0.05$) and beef patties (r^2 = 0.96, $p < 0.05$). This indicates that our experimental method was able to measure serum release and discriminate between samples. Although a strong correlation was obtained, the absolute values differed between methods. Serum released under compression (*in vitro*) was about two times higher than serum release under oral conditions (*in vivo*). Two reasons may explain this difference. Firstly, during mastication of the patties in the bags, the released serum may be reabsorbed by the patties,

leading to lower values for serum release. Secondly, the mechanical uniaxial compression in combination with the application of a vacuum to remove released serum from the patty may have been too severe, causing excessive serum release. A lower degree of compression could have been used to also quantitively match the *in vitro* with the *in vivo* results.

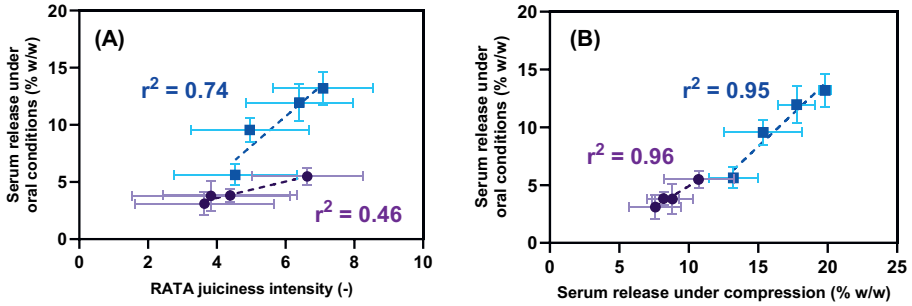

Figure 2.4 Relationships between serum release under oral conditions, and (A) RATA juiciness intensity, and (B) serum release under instrumental compression, of PBMA (blue squares) and beef patties (purple circles). Error bars indicate the standard deviation. Dashed lines represent linear regressions.

2.3.8 Relationships between sample, sensory and bolus properties

To further understand the relationships between sample properties, bolus properties, and texture perceptions, especially juiciness perception, Pearson correlations were performed separately for PBMA (n = 4) and beef patties (n = 4). (Supplementary **Table S2.1)**. Out of 80 possible correlations between 10 bolus properties and 8 sample properties, only 8 significant correlations were found in PBMA patties, and only 9 significant correlations were found in beef patties. Out of 80 possible correlations between 10 bolus properties and 8 texture attributes, only 7 correlations were significant in PBMA patties, and 6 in beef patties. These results are not surprising since the variations observed in bolus properties at the moment of swallowing for PBMA and beef patties differing in juiciness were very limited (**section 2.3.6**). Consequently, we suggest that these few significant correlations were likely coincidental and lack relevance. We suggest that bolus properties at the moment of swallowing may have had limited or negligible influence on the juiciness perception of PBMA and beef patties.

2.4. Conclusions

In this study we aimed to understand juiciness and texture perception of plant-based meat analogue and beef patties by investigating the relationships between sample properties, sensory characteristics, oral processing behavior and bolus properties. Series of patties covering a broad range of juiciness were prepared from the same raw material by varying the core temperature during cooking. With increasing juiciness, PBMA patties were perceived less dry and fattier, whereas beef patties were perceived less dry, less hard, less chewy, fattier and more tender. For both PBMA and beef patties, juiciness intensity correlated strongly with sample properties and composition. Juiciness correlated negatively with cooking loss and positively with serum release under compression, PBMA patties varied more in fat content, beef patties varied more in water content. However, these differences in sample and sensory properties between PBMA and beef patties varying largely in juiciness led to no or negligible differences in oral processing behavior and bolus properties at the moment of swallowing. No meaningful relationships between bolus properties at the moment of swallowing and sensory properties were found due to the lack of variation in bolus properties. Our results may suggest that juiciness perception is more related to properties of the food itself, then to bolus properties. To gain deeper insights into juiciness perception and the bolus properties of PBMA and beef patties underlying texture perception, further investigations into bolus properties at different stages of mastication may be necessary. The newly developed *in vitro* serum release quantification method strongly and positively correlated with juiciness perception and *in vivo* serum release during mastication, providing a potential tool to predict juiciness perception based on instrumental measures. In conclusion, in our samples, juiciness perception of plant-based meat analogue and beef patties was primarily determined by the release of serum during mastication, and was related to sample properties rather than bolus properties at the moment of swallowing.

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Supplementary material

Table S1. Pearson correlation coefficients between sample, sensory and bolus properties of (A) PBMA (n = 4) and (B) beef patties (n = 4). Asterisks indicate that correlation is significant at (*) $p < 0.05$, (**) $p < 0.01$.

		Bolus properties								
		Composition			***Texture***			***Fragments***		***Expelled liquid***	
		Bolus water content	Bolus fat content	Saliva uptake	Peak force	Resili -ence	Adhesiv -eness	Total number of particles	Average particle size	Expelled liquid during Centrifugation	Water content
Sample properties	**(A) PBMA patties**										
	Total cooking loss	0.73	-0.81	0.58	**0.96***	0.80	-0.16	0.93	-0.91	-0.95	-0.97
	Water content of patty	0.64	-0.92	0.34	0.76	0.24	0.53	0.86	-0.35	-0.60	-0.54
	Fat content of patty	-0.82	**0.95***	-0.61	**-0.98***	-0.59	-0.07	**-1.00****	0.73	0.86	0.86
	Peak force	0.01	-0.03	-0.03	-0.34	-0.92	0.46	-0.21	0.85	0.69	0.72
	Resilience	0.34	0.02	0.58	0.29	0.44	**-0.97***	0.12	-0.52	-0.25	-0.40
	Adhesiveness	0.36	-0.74	0.03	0.65	0.43	0.66	0.75	-0.45	-0.70	-0.60
	Serum release (*in vitro*)	-0.69	0.78	-0.54	-0.95	-0.83	0.15	-0.91	0.93	**0.96***	**0.98***
	Water content of serum	-0.72	0.72	-0.63	-0.93	-0.83	0.33	-0.86	0.94	0.92	**0.97***
	(B) Beef patties										
	Total cooking loss	-0.75	0.12	0.90	-0.72	-0.62	-0.25	**0.99****	-0.94	-0.43	0.88
	Water content of patty	0.80	-0.21	-0.87	0.77	0.55	0.34	**-1.00****	0.92	0.35	-0.93
	Fat content of patty	-0.83	0.38	0.80	-0.91	-0.38	-0.47	0.93	-0.77	-0.21	**0.96***
	Peak force	-0.87	0.30	0.76	-0.62	-0.45	-0.43	**0.99***	**-0.97***	-0.22	0.92
	Resilience	-0.53	0.52	0.43	-0.92	0.04	-0.50	0.46	-0.16	0.08	0.68
	Adhesiveness	-0.68	0.02	0.94	-0.73	-0.70	-0.15	**0.98***	-0.92	-0.52	0.84
	Serum release (*in vitro*)	0.51	0.05	**-0.97***	0.89	0.71	0.03	-0.86	0.70	0.60	-0.76
	Water content of serum	0.60	0.03	**-0.98***	0.82	0.73	0.08	-0.93	0.83	0.59	-0.81

Table continues on the next page

Table S1 (continued)

		Bolus properties									
		Composition			***Texture***			***Fragments***		***Expelled liquid***	
		Bolus water content	Bolus fat content	Saliva uptake	Peak force	Resili -ence	Adhesiv -eness	Total number of particles	Average particle size	Expelled liquid during Centrifugation	Water content
Sensory properties	**(A) PBMA patties**										
	Juiciness	-0.73	0.74	-0.64	-0.94	-0.81	0.32	-0.87	0.93	0.92	**0.97***
	Dryness	0.74	-0.76	0.63	**0.95***	0.81	-0.28	0.89	-0.93	-0.93	**-0.97***
	Hardness	**-0.98***	0.86	-0.93	-0.78	-0.02	0.23	-0.78	0.27	0.32	0.40
	Chewiness	-0.07	0.23	0.04	-0.52	**-0.99***	0.10	-0.46	0.92	0.88	0.86
	Tenderness	0.12	-0.39	-0.12	0.03	-0.51	0.84	0.20	0.47	0.18	0.28
	Crumbliness	-0.07	-0.24	-0.27	0.43	0.88	0.27	0.43	-0.77	-0.84	-0.75
	Fibrousness	-0.94	0.79	-0.93	-0.90	-0.45	0.49	-0.83	0.66	0.62	0.73
	Fattiness	-0.75	0.75	-0.67	-0.94	-0.80	0.34	-0.88	0.92	0.90	**0.96***
	(B) Beef Patties										
	Juiciness	0.80	-0.26	-0.87	0.85	0.50	0.37	**-0.98***	0.86	0.33	-0.94
	Dryness	-0.80	0.22	0.87	-0.78	-0.54	-0.34	**1.00****	-0.92	-0.35	0.93
	Hardness	-0.93	0.63	0.59	-0.84	-0.10	-0.71	0.86	-0.70	0.09	**0.99***
	Chewiness	-0.95	0.90	0.20	-0.59	0.31	-0.94	0.63	-0.51	0.50	0.87
	Tenderness	0.89	-0.52	-0.70	0.88	0.24	0.60	-0.91	0.75	0.06	**-0.99***
	Crumbliness	-0.56	-0.15	0.91	-0.53	-0.79	0.01	0.92	-0.95	-0.62	0.70
	Fibrousness	-0.91	0.94	0.11	-0.54	0.41	**-0.97***	0.54	-0.43	0.58	0.81
	Fattiness	0.80	-0.25	-0.87	0.83	0.52	0.36	**-0.98***	0.88	0.33	-0.94

Chapter 3

Role of bolus properties in dynamic texture perception of meat analogue and beef patties: juiciness is driven by serum release during early stages of mastication

This chapter has been published as:

Zhang, Y., Sala, G., Scholten, E., & Stieger, M. (2024). Role of bolus properties in dynamic texture perception of meat analogue and beef patties: Juiciness is driven by serum release during early stages of mastication. *Food Hydrocolloids*, *157*, 110450. https://doi.org/10.1016/j.foodhyd.2024.110450

Abstract

The sensory quality of plant-based meat analogues (PBMAs) limits wider consumer acceptance, particularly because of their lack of perceived juiciness. This study aimed to investigate the role of bolus properties at different moments of consumption in dynamic texture perception, especially juiciness, of PBMA and beef patties. Patties were cooked to three core temperatures (60, 70, 80°C) to obtain specimens differing in juiciness. For PBMA and beef patties, juiciness citation proportions (Temporal-Check-All-That-Apply) peaked within the first third of mastication, then decreased strongly until swallowing. This temporal pattern closely aligned with the serum release during mastication as 75% of serum was released from patties during the first third of mastication. Additional structural breakdown of bolus occurred until the end of mastication accompanied by less than 25% additional serum release. With increasing mastication, PBMA and beef patties showed a significant increase in saliva uptake and number of bolus particles, while bolus particle size and hardness decreased, demonstrating a progressive oral structural breakdown. No significant differences in bolus properties were observed between PBMA patties differing in juiciness, while beef patties varying in juiciness differed significantly in bolus water content and liquid expelled from bolus, as a result of the structural changes of myofibrillar protein upon heating. We conclude that, for the patties used in this study, juiciness perception of PBMA patties is driven by serum release during early stages of mastication and not effected by additional oral structural breakdown, while juiciness of beef patties is affected by initial serum release and differences in bolus properties resulting from additional oral structural breakdown.

3.1 Introduction

The transition from animal towards plant protein-based foods has been encouraged by various stakeholders to contribute to a more sustainable food system due to growing concerns regarding climate change, food supply, animal welfare, dietary restrictions and health issues (Aiking & de Boer, 2020; Chaudhary et al., 2018). Plant-based meat analogues (PBMAs) are one of the products that can contribute to this transition. However, their sensory quality, particularly their lack of juiciness, poses a challenge for wider consumer acceptance (Giacalone et al., 2022).

Sensory perception is influenced by various food properties. For example, in minced meat products, fat content has been correlated with tenderness perception, concurrently enhancing flavor and taste (Carrapiso, 2007; Cross et al., 1980; Tobin et al., 2012, 2013). For PBMAs, texture properties, including hardness and chewiness, are mainly derived from the properties of Textured Vegetable Proteins (TVPs) and binding agents used for their preparation (Kyriakopoulou et al., 2021; Schreuders et al., 2021). Sensory hardness and chewiness have been correlated to instrumental compression force and fracture force (Bakhsh et al., 2022; Younis et al., 2023). However, the relationships between juiciness perception and measurable properties in PBMAs have not been extensively studied. To gain more insights into this aspect, a recent study correlated food properties and bolus properties at the moment of swallowing with sensory perception of PBMA and beef patties (Zhang et al., 2024, **Chapter 2**). This study demonstrated that juiciness perception was primarily influenced by the serum being released from the patties into the oral cavity during mastication. Serum is defined as the liquid that is released from the food matrix during mastication or during mechanical compression. Juiciness correlated more strongly with the properties of the food, such as cooking loss, composition and serum release under uniaxial compression, than with the properties of the bolus at the moment of swallowing. In that study, juiciness was not evaluated during the entire consumption process. It has been hypothesized that juiciness perception is a dynamic process, with initial juiciness being linked to the rapid release of fluids from the food during the first few chews, and sustained juiciness being related to the stimulatory effect of fat and flavor on salivation

(Font-i-Furnols et al., 2015; Lawrie, 2006; Schwartz et al., 2022). However, these hypotheses have not yet been validated by scientific studies and its underlying mechanisms have not been reported yet. It is therefore still unclear how juiciness perception of PBMA and beef patties is related to dynamic changes during consumption.

Dynamic texture perception is related to changes in the food during oral processing, which constantly modifies bolus structure and texture. Bolus properties are characterized at different stages of mastication to quantify the oral structural breakdown during oral processing. These bolus properties have been correlated with temporal measures of sensory perception, revealing the underlying bolus properties driving specific texture perception across various foods (Chen, 2015; Devezeaux de Lavergne et al., 2017; Foster et al., 2011; Gao & Zhou, 2021; Panouillé et al., 2016). First bite and early chew-down texture attributes, such as hardness and brittleness, have been strongly correlated with fracture properties for a variety of solid foods. Texture attributes related to later chew down, such as crumbliness, creaminess and cohesiveness, have been associated with dynamic changes in bolus fluidity and other bolus properties, such as saliva incorporation, fat and water release (de Wijk et al., 2006; Devezeaux de Lavergne et al., 2017; Jourdren et al., 2016; Gao et al., 2017).

For meat, numerous studies quantified oral structural breakdown and bolus properties during mastication and/or static or dynamic sensory properties (Djekic et al., 2021; Pematilleke et al., 2020; Yven et al., 2005b). However, only few studies explored how oral structural breakdown and bolus properties drive dynamic texture perception of meats and PBMAs. For example, in cooked hams, dynamic softness and hardness perception were associated with instrumental texture properties, whereas fibrousness perception was related to the oral structural breakdown during mastication (Rizo et al., 2019). Also in another study using ham, the role of mastication was shown to be important, as juiciness was correlated with saliva uptake of the bolus (Rizo et al., 2019). Next to mastication, also individual differences in oral processing behavior have been shown to influence oral breakdown and bolus properties, resulting in differences in dynamic texture perception for sausages (Devezeaux de Lavergne, Derks, et al., 2015).

Yet, our understanding of the relationships between bolus properties and texture perception of meats and PBMAs remains limited, and the mechanisms underlying dynamic juiciness perception of these foods are underexplored.

To fill this knowledge gap, this study aimed to explore the role of bolus properties at different moments of consumption in dynamic texture perception, especially juiciness, of PBMA and beef patties. Unlike our previous study (Zhang et al., 2024, **Chapter 2**), which employed a static approach, the current study followed a dynamic approach to account for temporal changes in bolus properties and texture perception. Our previous study reported limited variations in bolus properties at the moment of swallowing, leading to no meaningful relationships between bolus properties and static sensory properties. Therefore, the current study acknowledges the temporality of oral food structural breakdown during mastication, which might lead to temporal changes in texture perception. Patties made with commercially available minced PBMA or beef were cooked sous vide to different core temperatures to obtain specimens differing in juiciness but prepared from the same raw materials. We used Temporal Check-All-That-Apply (TCATA) to quantify dynamic sensory perception of PBMA and beef patties, with an emphasis on texture perception. This study also went beyond merely static bolus measurements at the moment of swallowing. To characterize dynamic oral structure breakdown, bolus properties (water and fat content, saliva uptake, bolus texture properties, bolus particle size and number, liquid expelled from bolus) and serum release from patties into the oral cavity during mastication were determined at three stages of mastication (33, 66, 100%). Correlation analysis was performed to assess the relationships between dynamic bolus properties and dynamic sensory perception of PBMA and beef patties at the mentioned stages of mastication. This approach provided a comprehensive understanding of how bolus properties influence juiciness perception dynamically, acknowledging the temporality of food structural breakdown during mastication.

3.2 Materials and methods

3.2.1 Sample preparation

To create PBMA and beef patties differing in juiciness from the same raw materials, PBMA and beef patties were prepared following the protocol described by Zhang et al. (2024) (**Chapter 2**). In short, for PBMA patties, 110 g minced PBMA (Beyond Mince, Beyond Meat®, The New Plant) were shaped into a patty using a burger shaper (diameter 80 mm). For beef patties, 104.5 g minced beef (AH Biologisch Rundergehakt, Albert Heijn B.V., the Netherlands), 5 g egg (AH Biologisch Eieren SML, Albert Heijn B.V., the Netherlands), and 0.5 g salt (Jozo Naturel tafelzout, Hengelo, The Netherlands) were mixed by hand for 2 min, and then shaped into a patty using the burger shaper. After shaping the patties, they were vacuum-packed in plastic bags (dimension of plastic bag: 200 × 300 mm; thickness of plastic bag: 85 µm; material of plastic bag: polyamide + polypropylene; Disposable Discounter, The Netherlands) and 95% of the air was removed using a vacuum packaging machine (Henkovac M2, The Netherlands). Patties were cooked sous vide (Ilic et al., 2022) in a water bath (CHF-23, Vaive, the Netherlands) at water temperatures of 60, 70, or 80°C for 60 min to reach the respective core temperatures. The choice of the core cooking temperatures was based on a previous study (Zhang et al., 2024, **Chapter 2**) to obtain patties differing in sensory juiciness. After sous vide cooking, all patties were cooled down to 60°C core temperature, and grilled in a double-plate grill (DeLonghi, Italy) at 200°C for 1 min. This grilling step ensured browning and a pleasant crust on the patty surface to meet a familiar sensory profile of the Beyond Meat and beef patties (Schouteten et al., 2016). After grilling, patties were placed in a foam box (PBMA for 5 min, beef patties for 4 min) to reach 55°C core temperature before sensory evaluation or bolus collection. In the following, the PBMA and beef patties are referred to according to their core cooking temperature (PBMA60, PBMA70, PBMA80, BEEF60, BEEF70 and BEEF80).

3.2.2 Oral processing behavior

The oral processing behavior of PBMA and beef patties during normal consumption was quantified to define a standardized chewing protocol used for the bolus collection

(standardized bite size, standardized number of chews per bite and standardized chewing frequency) and sensory evaluation (standardized bite size). Participants (n = 19, 14 female and 5 male, 26.4 ± 2.3 years) were recruited from Wageningen and surroundings. Inclusion criteria were good general health (self-reported), BMI between 18.5 – 30 kg/m^2, no dental issues, no swallowing issues, normal ability to taste and smell, non-smoker, non-vegetarian/non-vegan and willing to eat both meat and PBMA, no allergies or intolerances to legumes, eggs, and not pregnant. Participants signed an informed consent form and received financial reimbursement after completion of the session.

Each participant joined one 60 min session between 10:00 am and 03:00 pm and was instructed not to consume any foods or drinks (except water) for 2 h prior to the session. PBMA (PBMA60, PBMA70, PBMA80) and beef patties (BEEF60, BEEF70, BEEF80) (weighing 70 – 80 g after cooking) were randomly presented to the participants and labelled with 3-digit codes. Participants were asked to take three bites of each patty, chew and swallow it as they would normally do. Patties were weighed before and after consumption. Bite size (g) was calculated by dividing the consumed weight by the number of bites (three) taken by the participant. Participants were instructed to cleanse their palate with crackers and water after a patty was consumed, and a 5 min break was given between samples. The oral processing behavior was video recorded, and annotated for number of chews per bite (-), chewing time per bite (s) and chewing frequency (chews/s) using ELAN software (version 4.9.2, Max Planck Institute for Psycholinguistics, The Language Archive, Nijmegen, The Netherlands) following the procedure previously described (Forde et al., 2017).

3.2.3 Sensory evaluation

3.2.3.1 Participants

Participants (n = 70) were recruited from Wageningen and surroundings, some of them also attended the session organized to determine the oral processing behavior (**section 3.2.2**). The same inclusion criteria described in **section 3.2.2** applied. The Temporal Check-All-That-Apply (TCATA) evaluation of PBMA and beef patties was

completed by n = 65 participants (47 female and 18 male, 25.2 ± 3.2 years, mean ± SD). All participants assessed all PBMA and beef patties in duplicate. The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki (2013). All participants signed an informed consent form and received financial reimbursement after completion of all sessions.

3.2.3.2 Temporal Check-All-That-Apply (TCATA)

Dynamic sensory perception of PBMA and beef patties was evaluated using the TCATA method, and was carried out at the sensory facilities at Wageningen University & Research. Participants attended one 60 min familiarization session, and two 60 min TCATA test sessions. During the familiarization session, participants were introduced to the study, provided with the sensory attributes (including definitions) used in TCATA, and performed two TCATA trials with PBMA70 and BEEF70 samples to get familiar with the TCATA evaluation procedure.

The two TCATA test sessions were divided into a PBMA patty and a beef patty session. Participants evaluated one type of patty (either PBMA patties or beef patties) during one session (n=65, duplicate). The order in which participants attended the two sessions (PBMA or beef patties) was randomized. Six attributes (juiciness, dryness, softness, chewiness, fattiness, meat flavor) and their definitions (**Table 3.1**) were provided to the participants. The order of attributes during the TCATA evaluation was randomized over the participants, but was kept constant across samples per participant per session. Three patties (PBMA60, PBMA70, PBMA80, or BEEF60, BEEF70, BEEF80), with a fixed bite size of 10 g (defined at **section 3.3.1**), were presented monadically to the participants in random order with 3-digit codes. For each sample, participants were instructed to click the "start" button simultaneously with putting the whole sample in the mouth, and then immediately commence tracking sensory changes. At any time between clicking start and the end of the evaluation time (80 s), participants were asked to check the attributes that applied to describe the sensory characteristics of the sample at each moment. The selected attributes automatically faded after 5 s (automatic deselection). Participants were asked to actively reselect the attributes which applied to describe the perception of the

samples. Participants were asked to indicate the main swallowing moment by clicking the button "Last main swallow". Crackers and water were provided for cleansing the palate after each sample. Data were collected in English using Compusense software (Version 23, Compusense Inc., Canada).

Each participants' TCATA mastication time (s) was normalized by the time of swallowing to obtain a relative mastication time (%). TCATA evaluation was taken until 120% of mastication time to also include the aftertaste period (100-120%). Maximum citation proportion (C_{max}), relative mastication time to reach maximum citation proportion (T_{max}), citation proportion at 33, 66 and 100% of relative mastication time ($C_{33\%}$, $C_{66\%}$ and $C_{100\%}$) and area under curve (AUC) were extracted from the TCATA profiles for each attribute.

Table 1. Sensory attributes and definitions used for TCATA evaluation of plant-based meat analogue and beef patties.

Attribute	Definition
Juiciness	Sensation of moisture/juice/liquid being released from food during consumption.
Dryness	Sensation of dryness in mouth (opposite of juiciness).
Softness	Sensation related to how easy it is to bite through the food using the (molar) teeth.
Chewiness	Effort required to masticate the food until it is ready to be swallowed.
Fattiness	Sensation of fat in the mouth.
Meat flavor	Flavor of meat, related to products like beef, chicken, or pork.

3.2.4 Characterization of bolus properties at different stages of mastication

3.2.4.1 Experimental approach

A sub-group of participants (n = 10, 8 female and 2 male, 24.7 ± 2.4 years, mean ± SD) was recruited from the participants that completed the TCATA evaluations for bolus collection. All participants signed an informed consent form and received financial reimbursement after completion of all bolus collection sessions.

Participants took part in 6 bolus collection sessions of 60 min. During each session, one patty type was masticated (PBMA60, PBMA70, PBMA80, BEEF60, BEEF70, or BEEF80). The order of sessions (patties) was randomized over participants. Within each bolus collection session, participants followed a standardized chewing protocol that was

previously determined (**section 3.3.1**). They were instructed to consume a pre-cut one-bite patty (10 g) at a chewing frequency of 1.4 chews/s, and expectorate the bolus into a sealed plastic cup after 10 chews (33% mastication; 14 s chewing time), 20 chews (66% mastication; 28 s chewing time), and 30 chews (100% mastication; 42 s chewing time). Audio signals indicated participants when to take a chew and when to expectorate the bolus. The moment of bolus expectoration was randomized and participants were not informed about the expectoration moment when taking the samples into their mouth. Participants were instructed to take a 30 s break and have a sip of water between samples. After masticating 4 samples, participants were instructed to take a 60 s break and clean the palate by taking a bite of cracker and a sip of water.

For each patty and each mastication moment (33, 66, 100%), 7 boli were collected per participant, so that in total 27 boli were collected per participant per session (60 min). To avoid moisture evaporation during bolus collection and uneven sampling after pooling, seven replicates of bolus were collected separately and used for subsequent analysis, rather than pooling boli prior to subsequent analysis. One bolus was randomly selected to determine bolus composition (water, saliva and fat content), two boli were randomly selected to assess bolus texture properties, three boli were randomly selected to measure weight and water content of the expelled liquid, and one bolus was randomly selected to analyze the number of bolus particles and their size. All boli were analyzed on the day of bolus collection.

3.2.4.2 Composition of bolus

3.2.4.2.1 Bolus water content

The water content of boli (n = 10), representing the remaining water content of the patties and the saliva uptake during mastication, was determined gravimetrically. The expectorated boli (about 10 g) were placed in aluminum dishes, weighted (w_0), and dried in an air oven (Binder, Germany) for 16 – 18 h at 105°C until constant weight. After drying, samples were cooled down in desiccators and weighted again (w_1). The water content of the bolus was calculated as $WC = (w_0 - w_1)/w_0 \times 100\%$.

3.2.4.2.2 Bolus fat content

The fat content of boli (n = 10) was determined by Soxhlet extraction. For each mastication moment (33, 66, 100%), dried boli obtained after water content determination (**section 3.2.4.2.1**) were pooled into 3 groups for fat analysis (boli of participants 1 to 3 pooled in group 1, boli of participants 4 to 6 pooled in group 2, boli of participants 7 to 10 pooled in group 3). Pooled boli were pulverized using a cryogenic grinder (6875D Freezer/Mill, Spex SamplePrep, USA). The ground dry boli (about 6 g) were weighted (F_0) and extracted with petroleum ether, using a Soxtherm extraction system (Gerhardt GmbH & Co. KG, Germany). After extraction, the petroleum ether was evaporated overnight to obtain the fat as residue, which was weighted (F_1). The bolus fat content on dry weight basis was calculated as FC = (F_0 – F_1)/F_0×100%.

3.2.4.2.3 Bolus saliva uptake

The saliva uptake of boli during mastication (n = 10) was calculated by subtracting the water content of the patties (on dry weight basis) and the serum release under oral condition (on dry weight basis, see section 2.5) from the water content of boli (on dry weight basis) as: Saliva Uptake = $[(w_0 – w_1)/w_1 - (b_0 – b_1)/b_1 - (s_0 – s_1)/ (s_0 \times w_1/w_0)]\times100\%$, where w_0 is the weight of the cooked patty before drying and w_1 is the weight of the cooked patty after drying (obtained from Zhang et al., (2024), **Chapter 2**); b_0 is the weight of the bolus before drying and b_1 is the weight of the bolus after drying; s_0 is the weight of unchewed patties and s_1 is the weight of chewed patty fragments collected from chewing bags (**section 3.2.5**).

3.2.4.3 Bolus texture properties

The texture properties of the boli (n =10) were determined with a penetration test (Zhang et al., 2024, **Chapter 2**). A Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) equipped with a 500 g load cell and a stainless steel cylindrical probe (diameter 4 mm) was used. The expectorated boli were gently transferred to a testing cylinder cup (diameter 35 mm, height 55 mm) until they reached a height of 30 mm. The upper surface was gently evened out with the back of a spoon to obtain a cylindrical bolus

mass with a smooth and even surface. Penetration tests were performed up to a strain of 80% of the initial height of the boli, and measurements continued as the cylindrical probe withdrawn until it returned to its initial position, using a constant test speed of 5 mm/s. Each bolus (n = 10) was punctured at three locations to obtain 30 force-strain curves, from which peak force, resilience and adhesiveness were obtained as averages. Peak force was defined as the maximum peak force (force at 80% strain), resilience as the ratio between the areas under the force-time curve after and before peak force, and adhesiveness as the area under the force-time curve between the start point and the end point at which the probe returned to its initial position.

3.2.4.4 Bolus particle size distribution

The particle size distribution of bolus fragments (n = 10) was determined using image analysis (van Eck, Wijne, et al., 2019; Zhang et al., 2021). One expectorated bolus (about 10 g) was placed in a transparent acrylic tray (20.3 × 30.5 × 5.1 cm). The bolus fragments were dispersed by gently pouring 250 mL Milli-Q water into the tray, horizontally shaking the tray, and then manually separating bolus particles with a spatula without fracturing bolus particles. The tray was placed on a flatbed scanner (Canon CanoScan 9000F Mark II, the Netherlands) and a 600-dpi color image with a black background was captured. Images were imported into ImageJ (version 1.52, National Institute of Health, USA) to conduct image analysis. After converting images to an 8-bit format, a black-and-white threshold was used to obtain a binary picture. To avoid background interference, particles smaller than 0.15 mm^2 or with a circularity lower than 0.10 were excluded from data processing. For each image, the total number of bolus particles per g of bolus (no./g) and average bolus particle size (mm^2) were determined.

3.2.4.5 Liquid expelled from bolus

The liquid expelled from the boli (n = 10) was measured by centrifugation (Zhang et al., 2024, **Chapter 2**). The expelled liquid included the serum remaining in the bolus after mastication and the saliva uptake during consumption. Expectorated boli (around 15 g) were weighted, and placed onto a cylindrical polypropylene sieve (pore size 1.1

mm) that was inside a 50 mL centrifugation tube. The sample was centrifuged at 200 g for 10 min at 20°C (Beckman Coulter Allegra X-22R Centrifuge, United States) to allow the liquid to pass through the filter. The expelled liquid was collected in the bottom of the centrifugation tube. After centrifugation, the bolus retained on the filter was weighted. The mass of expelled liquid was determined by subtracting the weight of the retained bolus after centrifugation from the weight of the bolus before centrifugation. Furthermore, the expelled liquid was collected to determine its water content, as described in **section 3.2.4.2.1**. Measurements were performed in duplicate for each sample for each mastication moment for each participant.

3.2.5 Serum release under oral conditions

The serum release under oral conditions was determined at three stages of mastication (33, 66, 100% of mastication time) with the same participants as described in **section 3.2.4.1**. In brief, one-bite of PBMA or beef patty (about 10 g) was weighted (s_0) and placed into a plastic bag. Participants (n =10) masticated the samples in the bags at a chewing frequency of 1.4 chews/s for 10 chews (33% mastication), 20 chews (66% mastication), and 30 chews (100% mastication). Participants were instructed to take a 30 s break and have a sip of water between each sample. After mastication, the patty fragments were manually removed from the plastic bags and isolated from the released serum using tweezers. These fragments were centrifugated (same conditions as in **section 3.2.4.5**) to remove any residual serum adhering to their surfaces. The fragments were weighted (s_1). The total serum release under oral conditions was calculated as $SR = (S_0 - S_1)/S_0 \times 100\%$. Measurements were performed in duplicate for each sample for each mastication moment for each participant.

3.2.6 Data analysis

Results are reported as mean values with standard deviation (SD). For oral processing behavior and bolus properties, linear mixed models (LMM) were applied, followed by Tukey post-hoc analyses. In the LMM analysis of oral processing behavior, core temperature (60, 70, 80°C) and sample type (PBMA, beef) were treated as fixed factors, and participants as random factor. Missing data in oral processing behavior for some participants were imputed using the mean values of the group. LMM analysis of bolus

properties treated mastication time (33, 66, 100%) and core temperature (60, 70, 80°C) as fixed factors, and participants as random factor. The interaction between mastication time and core temperature was not examined as these were independent factors, and the interaction was not of interest for the study. Both LMM analyses were conducted for PBMA and beef patties separately.

Maximum citation proportion (C_{max}), relative mastication time to reach maximum citation proportion (T_{max}) and citation proportion at 33, 66 and 100% of mastication ($C_{33\%}$, $C_{66\%}$ and $C_{100\%}$) were analyzed using logistic mixed factor analyses (LMF), followed by Tukey post-hoc analyses. Area under curve (AUC) was analyzed using LMM, followed by Tukey post-hoc analyses. Both LMF and LMM treated mastication time and core temperature as fixed factors and participants as random factor, and were conducted for PBMA and beef patties separately, since PBMA and beef patties were evaluated in different TCATA sessions, so that a direct comparison between PBMA and beef patties sensory properties is not adequate.

The relationships between bolus properties at 33, 66 and 100% of mastication and sensory perception ($C_{33\%}$, $C_{66\%}$ and $C_{100\%}$) were summarized using Principle Component Analysis and Pearson correlation coefficients for PBMA and beef patties separately. Sensory properties obtained from TCATA were replicated 10 times to match the 10 replications of bolus properties measurements to conduct the correlation analysis.

Data analysis was performed using RStudio (version 2022.07.0, PBC) with the packages emmeans (Lenth, 2022), lmerTest (Kuznetsova et al., 2017), Hmisc (Harrell & Dupont, 2023), FactoMineR (Lê et al., 2008), factoextra (Kassambara & Mundt, 2020), and GraphPad Prism (version 10.0.0, GraphPad Software, USA). A significance level of $p < 0.05$ was chosen.

3.3 Results and discussion

3.3.1 Oral processing behavior

To establish a standardized chewing protocol for bolus collection (bite size, number of chews per bite, chewing time per bite, chewing frequency) and the bite size for TCATA evaluation, the natural oral processing behavior of PBMA and beef patties was

quantified (**Table 3.2**). Core temperature had no significant impact on number of chews per bite, chewing time per bite and chewing frequency for PBMA and beef patties. A significant but small difference in bite size (relative bite size differences were smaller than 10% for PBMA and beef patties) was observed for PBMA ($F = 4.6$, $p < 0.05$) and beef patties ($F = 4.6$, $p < 0.05$) differing in core temperature. These results were consistent with our previous study that employed the same set of patties in which participants expectorated boli at the moment of swallowing (Zhang et al., 2024, **Chapter 2**). Oral processing behavior did not change depending on when the patty was consumed within the session (data not shown). Given the similarity in oral processing behavior between PBMA and beef patties, a standardized chewing protocol for all patties was used with a bite size of 10 g and 30 chews per bite until swallow at a chewing frequency of 1.4 chews/s. The standardized chewing protocol was used to mitigate inter-individual variability in bolus properties and to maximize differences in bolus properties across patties and mastication time. This approach was followed as subject-tailored mastication times might have increased inter-individual variability in bolus properties (Fontijn-Tekamp et al., 2004; Kochi et al., 2021; Maeda et al., 2020; Yven et al., 2012).

Table 3.2 Mean (± SD) of oral processing behavior parameters (averaged over n = 19 participants and three bites per sample) of (A) PBMA and (B) beef patties. Different letters indicate significant differences between samples ($p < 0.05$) based on a linear mixed model.

(A) PBMA patties	**PBMA60**	**PBMA70**	**PBMA80**	***F* value**	***P* value**
Bite size (g)	10.4 ± 4.1[a]	10.1 ± 3.9[b]	10.2 ± 3.6[b]	4.6	<0.05
Number of chews per bite (-)	29.2 ± 11.4	28.1 ± 10.9	28.2 ± 12.4	0.3	0.773
Chewing time per bite (s)	20.8 ± 8.8	18.9 ± 6.2	19.3 ± 7.6	0.9	0.414
Chewing frequency (chews/s)	1.4 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	0.9	0.418
(B) Beef patties	**BEEF60**	**BEEF70**	**BEEF80**	***F* value**	***P* value**
Bite size (g)	10.5 ± 3.7[a]	9.5 ± 3.4[b]	9.7 ± 3.8[b]	4.6	<0.05
Number of chews per bite (-)	29.1 ± 6.1	29.9 ± 9.5	32.8 ± 11.9	2.4	0.105
Chewing time per bite (s)	20.8 ± 4	21.7 ± 5.7	23.1 ± 6.3	2.0	0.144
Chewing frequency (chews/s)	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	0.8	0.442

3.3.2 Dynamic sensory perception of PBMA and beef patties

The TCATA curves of PBMA and beef patties are provided in **Figure 3.1** and the parameters extracted from the TCATA curves (C_{max}, T_{max}, $C_{33\%}$, $C_{66\%}$, $C_{100\%}$ and AUC) are summarized in **Table 3.3**.

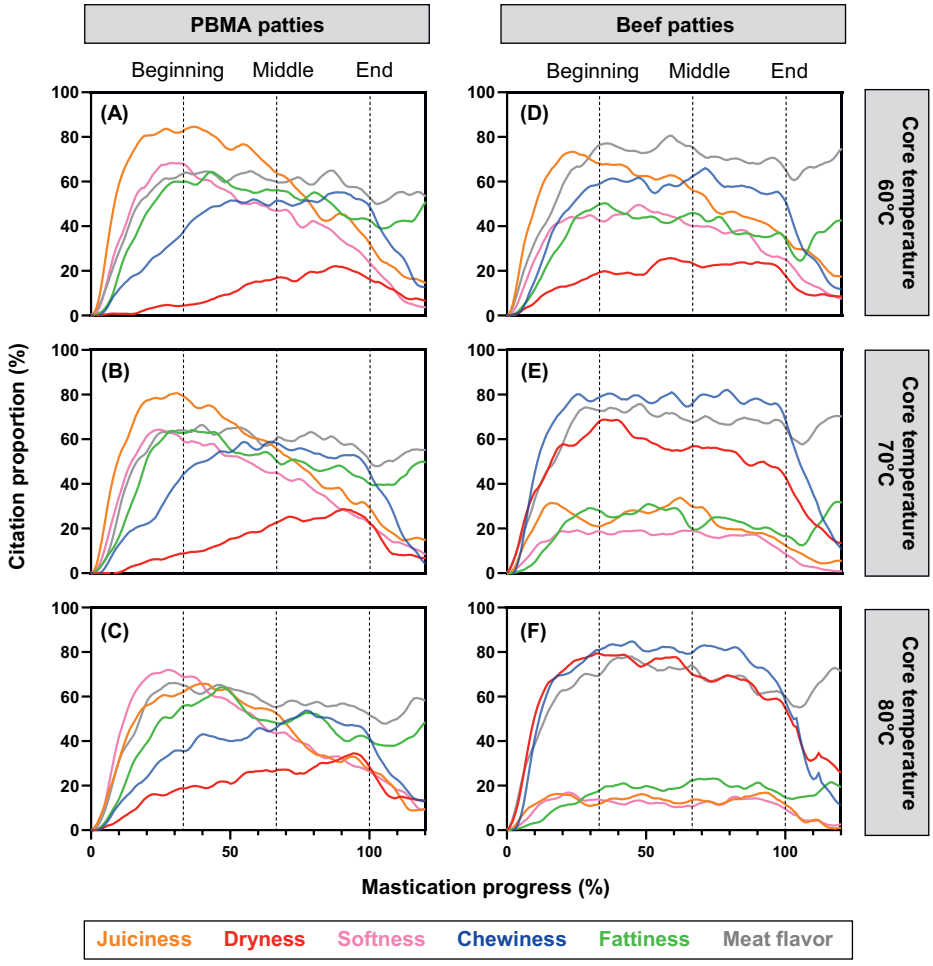

Figure 3.1. TCATA profiles of PBMA (A, B and C) and beef patties (D, E and F) prepared at core temperatures of 60°C (A, D), 70°C (B, E), and 80°C (C, F) (n = 65 participants, duplicate). Dashed lines indicate 33 (beginning), 66 (middle) and 100% (end) of mastication time.

Table 3.3. Maximum citation proportion (C_{max}), relative mastication time to reach maximum citation proportion (T_{max}), citation proportion at 33, 66 and 100% of mastication time ($C_{33\%}$, $C_{66\%}$ and $C_{100\%}$) and area under curve (AUC) (mean ± SD) obtained from TCATA profiles of (A) PBMA and (B) beef patties (n = 65 participants, duplicate) for each sensory attribute. Different letters indicate significant differences between PBMA or beef patties in a row ($p < 0.05$) based on separate logistic mixed factor analysis (Ward Chi-squares (χ^2) and p values) or linear mixed model analysis (F values and p values), * p <0.05, ** p< 0.01, *** p < 0.001.

	(A) PBMA patties						(B) Beef patties					
	PBMA 60	**PBMA 70**	**PBMA 80**	***χ2* value**	***F* value**	***p* value**	**BEEF 60**	**BEEF 70**	**BEEF 80**	***χ2* value**	***F* value**	***p* value**
Juiciness												
C_{max} (%)	85[a]	82[a]	67[b]	**17.8**	-	***	75[a]	34[b]	18[c]	**73.1**	-	***
T_{max} (%)	36	30	40	-	-	-	22	61	93	**-**	-	**-**
$C_{33\%}$ (%)	83[a]	79[a]	62[b]	**21.7**	-	***	68[a]	22[b]	12[c]	**73.4**	-	***
$C_{66\%}$ (%)	64	56	53	5.1	-	0.079	55[a]	29[b]	14[c]	**48.9**	-	***
$C_{100\%}$ (%)	33	29	26	2.6	-	0.277	35[a]	12[b]	12[b]	**32.5**	-	***
AUC (-)	67±27[a]	59±31[b]	49±32[c]	-	**24.1**	***	59±33[a]	25±26[b]	14±20[c]	-	**159.9**	***
Dryness												
C_{max} (%)	22[a]	29[ab]	35[b]	**7.9**	-	*	26[a]	69[b]	80[b]	**69.4**	-	***
T_{max} (%)	87	90	94	-	-	-	59	34	31	-	-	-
$C_{33\%}$ (%)	5[a]	9[ab]	18[b]	**13.2**	-	**	19[a]	68[b]	78[b]	**75.0**	-	***
$C_{66\%}$ (%)	15[a]	23[ab]	27[b]	**6.9**	-	*	25[a]	58[b]	70[b]	**56.0**	-	***
$C_{100\%}$ (%)	15[a]	23[ab]	29[b]	**9.7**	-	**	18[a]	42[b]	55[c]	**41.4**	-	***
AUC (-)	13±21[a]	17±24[a]	24±26[b]	-	**14.1**	***	20±27[a]	57±35[b]	73±31[c]	-	**164.6**	***
Softness												
C_{max} (%)	69	65	72	2.0	-	0.376	50[a]	20[b]	18[b]	**41.9**	-	***
T_{max} (%)	27	25	28	-	-	-	46	25	23	**-**	-	**-**
$C_{33\%}$ (%)	68	60	69	4.4	-	0.113	42[a]	19[b]	14[b]	**32.4**	-	***
$C_{66\%}$ (%)	45	45	42	0.5	-	0.769	40[a]	18[b]	11[b]	**33.9**	-	***
$C_{100\%}$ (%)	23	23	26	0.6	-	0.732	25[a]	8[b]	9[b]	**19.1**	-	***
AUC (-)	49±32	46±32	51±32	-	1.3	0.273	40±33[a]	16±23[b]	13±19[b]	-	**69.8**	***
Chewiness												
C_{max} (%)	56	59	55	0.6	-	0.723	65[a]	82[b]	85[b]	**19.8**	-	***
T_{max} (%)	85	63	77	-	-	-	69	79	44	**-**	-	**-**
$C_{33\%}$ (%)	38	44	35	3.5	-	0.171	58[a]	79[b]	81[b]	**24.4**	-	***
$C_{66\%}$ (%)	51	58	46	5.6	-	0.061	62[a]	76[b]	80[b]	**14.3**	-	***
$C_{100\%}$ (%)	48	45	42	1.4	-	0.488	51[a]	67[b]	58[ab]	**8.8**	-	**
AUC (-)	45±35	47±34	41±36	-	2.1	0.119	55±32[a]	77±25[b]	76±27[b]	-	**50.2**	***
Fattiness												
C_{max} (%)	65	65	65	0.0	-	1.000	51[a]	32[b]	24[b]	**25.7**	-	***
T_{max} (%)	41	30	46	-	-	-	35	118	75	**-**	-	**-**
$C_{33\%}$ (%)	58	62	56	1.6	-	0.455	48[a]	27[b]	18[b]	**31.1**	-	***
$C_{66\%}$ (%)	55	51	48	2.3	-	0.312	45[a]	20[b]	22[b]	**27.2**	-	***
$C_{100\%}$ (%)	42	40	41	0.3	-	0.851	34[a]	17[b]	15[b]	**19.3**	-	***
AUC (-)	56±35	55±36	52±33	-	1.4	0.239	43±33[a]	25±27[b]	19±24[b]	-	**45.4**	***
Meat flavor												
C_{max} (%)	65	68	68	0.4	-	0.836	82	76	79	1.5	-	0.465
T_{max} (%)	40	40	29	-	-	-	58	47	46	-	-	-
$C_{33\%}$ (%)	65	65	66	0.1	-	0.943	75	73	6	1.8	-	0.398
$C_{66\%}$ (%)	60	60	55	2.0	-	0.373	74	68	74	1.6	-	0.453
$C_{100\%}$ (%)	52	52	52	0.1	-	0.970	65	63	60	1.3	-	0.533
AUC (-)	65±38	64±38	63±38	-	0.4	0.679	77±30	74±31	75±32	-	0.5	0.582

For PBMA patties, with decreasing core temperature from 80 to 60°C, juiciness citation proportion significantly increased by 27% for C_{max} (χ^2 = 17.8, p < 0.001) and by 37% for AUC (F = 24.1, p < 0.001) demonstrating that, as expected, juiciness perception increased with decreasing core temperature (**Figure 3.1 (A) (B) (C) and Table 3.3 (A)**). This is in agreement with our previous study (Zhang et al., 2024, **Chapter 2**). Juiciness citation proportion peaked early during mastication (T_{max}: 30 – 40%) for all PBMA patties and then decreased rapidly during the middle and late stages of mastication. This was reflected in the higher juiciness citation proportions at 33% mastication than at 66 and 100% mastication across all three PBMAs. While juiciness citation proportion differed significantly across PBMA patties at 33% of mastication (χ^2 = 21.7, p < 0.001), this difference diminished as mastication progressed, becoming non-significant at 66% and 100% of mastication. In contrast to juiciness, dryness citation proportion significantly increased by 59% for C_{max} (χ^2 = 7.9, p < 0.05) and by 85% for AUC (χ^2 = 14.1, p < 0.001) with increasing core temperature from 60 to 80°C. Dryness, in contrast to juiciness, gradually increased throughout mastication and peaked late during mastication (T_{max}: 87 - 94%), showing significant differences at 33, 66 and 100% of mastication between PBMAs (χ^2 = 6.9 – 13.2, p < 0.05).

Remarkably, core temperature had no significant influence on the citation proportions of softness, chewiness, fattiness and meat flavor for PBMA patties. The lack of considerable structural changes during cooking in the PBMA patties made from denatured textured vegetable protein (TVP) may explain why only juiciness and dryness changed whereas other texture attributes were not affected. The TCATA results extend our previous RATA findings (Zhang et al., 2024, **Chapter 2**) by elucidating the temporality of texture perception, especially juiciness and dryness.

In contrast to PBMA patties, core temperature had a strong effect on dynamic texture perception of beef patties, with all five TCATA texture attributes differing significantly across beef patties differing in core temperature (**Table 3.3 (B)**). With decreasing core temperature, juiciness citation proportion for beef patties significantly increased by 317% for C_{max} (χ^2 = 73.1, p < 0.001), 467% for $C_{33\%}$ (χ^2 = 73.4, p < 0.001), 293% for $C_{66\%}$ (χ^2 = 48.9, p < 0.001), 192% for $C_{100\%}$ (χ^2 = 32.5, p < 0.001) and 321% for AUC (F = 159.9,

$p < 0.001$). The relative time to reach maximum citation proportion for juiciness (T_{max}) was 22, 61 and 93% of mastication time for BEEF60, BEEF70 and BEEF80, respectively. It should be noted that an early peak of juiciness citation proportions (comparable to C_{max}) appeared for BEEF70 already at 16% of mastication time and for BEEF80 at 17% of mastication time (**Figure 3.1 (E) (F)**). This demonstrates that similar to PBMA patties, juiciness was also perceived early during mastication in beef patties differing in core temperatures. For BEEF70 and BEEF80, juiciness perception was rather constant over mastication time without a clear peak.

Dryness citation proportion of beef patties significantly increased by 208% for C_{max} ($\chi^2 = 69.4$, $p < 0.001$), 310% for $C_{33\%}$ ($\chi^2 = 75.0$, $p < 0.001$), 180% for $C_{66\%}$ ($\chi^2 = 56.0$, $p < 0.001$), 206% for $C_{100\%}$ ($\chi^2 = 41.4$, $p < 0.001$) and 265% for AUC ($F = 164.6$, $p < 0.001$) with increasing core temperature (from 60 to 80), as expected.

In contrast to PBMA patties, core temperature significantly influenced the temporal perception of softness, chewiness and fattiness in beef patties. BEEF60 exhibited significantly higher citation proportions in softness and fattiness, significantly lower citation proportions in chewiness, compared to BEEF70 and BEEF80 across all parameters extracted from TCATA curves (C_{max}, $C_{33\%}$, $C_{66\%}$, $C_{100\%}$ and AUC) ($p < 0.05$). The differences in softness and chewiness can be explained by the denaturation of myofibrillar proteins and connective tissue proteins during cooking, resulting in a tougher texture at higher cooking temperatures (Schwartz et al., 2022). Although BEEF60 showed a significantly higher citation proportion for fattiness compared to BEEF70 and BEEF80, its actual fat content was slightly lower (1.6% lower fat content) (Zhang et al., 2024, **Chapter 2**). This discrepancy in fattiness perception may be related to the differences in juiciness perception. No significant effect of core temperature on meat flavor perception was observed for beef patties.

PBMA and beef patties showed similar dynamic sensory profiles after the main swallow (100% mastication). Juiciness, dryness, softness and chewiness rapidly declined whereas fattiness and meat flavor lingered or even slightly increased after swallowing. The persistence of fattiness can hypothetically be attributed to fat mouth-coating (De Wijk et al., 2011; Kupirovič et al., 2017). The lingering of the meat flavor suggests that

aroma volatiles might have been released post-swallowing from the fat mouth-coating to the nasal cavity, contributing to a persistent flavor perception (Linforth & Taylor, 2006).

3.3.3 Characterization of bolus properties at different stages of mastication

The bolus properties at different stages of mastication (mean ± SD) of PBMA and beef patties prepared at different core temperatures are summarized in **Supplementary Table S3.1.** The results of the corresponding statistical data analysis are presented in **Table 3.4** and the changes in bolus properties are visualized in **Figures 3.2 to 3.5**. These results are discussed in **sections 3.3.3.1 to 3.3.3.4**.

Table 3.4 Results of statistical data analysis describing the effects of mastication time and core temperature on bolus properties of (A) PBMA patties and (B) beef patties. *F* and *p* values are derived from linear mixed models with mastication time and core temperature as fixed factor, and participant as random effect.

	Mastication time		**Core temperature**	
(A) Bolus properties of PBMA patties	***F* value**	***P value***	***F value***	***P value***
Bolus composition				
Water content (% w/w)	142.6	<0.001	7.5	<0.01
Fat content dry basis (g/g dry weight)	2.6	0.080	3.1	0.052
Saliva uptake (g/g dry weight)	82.5	<0.001	0.5	0.623
Bolus texture				
Peak force (N)	28.3	<0.001	2.7	0.075
Resilience (-)	2.9	0.061	0.3	0.712
Adhesiveness (N·s)	4.8	<0.05	0.5	0.585
Oral structural breakdown				
Total number of bolus particles (no./g)	96.2	<0.001	2.6	0.085
Bolus particle size (mm²)	119.0	<0.001	4.2	<0.05
Liquid expelled from bolus				
Liquid expelled during centrifugation (% w/w)	18.8	<0.001	6.7	<0.01
Water content of expelled liquid (% w/w)	208.3	<0.001	11.0	<0.001

Table continues on the next page

Table 3.4 (continued)

	Mastication time		Core temperature	
(B) Bolus properties of beef patties	***F* value**	***P value***	***F value***	***P value***
Bolus composition				
Water content (% w/w)	58.8	<0.001	79.5	<0.001
Fat content dry basis (g/g dry weight)	4.1	<0.05	12.2	<0.001
Saliva uptake (g/g dry weight)	45.5	<0.001	0.4	0.643
Bolus texture				
Peak force (N)	39.3	<0.001	1.5	0.239
Resilience (-)	8.8	<0.001	0.3	0.744
Adhesiveness (N·s)	11.2	<0.001	3.2	<0.05
Oral structural breakdown				
Total number of bolus particles (no./g)	95.4	<0.001	0.02	0.978
Bolus particle size (mm^2)	37.0	<0.001	0.3	0.767
Liquid expelled from bolus				
Liquid expelled during centrifugation (% w/w)	90.4	<0.001	61.2	<0.001
Water content of expelled liquid (% w/w)	28.8	<0.001	17.0	<0.001

It should be noted that in this study oral processing behavior was standardized during bolus collection by imposing a chewing protocol to minimize inter-individual differences and maximize differences between patties (**section 3.3.1**). Bolus properties showed significant differences across mastication times and across core temperatures (**Table 3.4**). We acknowledge that a potential limitation of the approach is that inter-individual differences in oral processing behavior might have persisted leading to variability in bolus properties (Supplementary **Table S3.1**). While the mastication protocol considerably reduced inter-individual differences in mastication behavior and bolus properties, differences in oral physiology between participants, differences in the compliance of participants with the instructed chewing protocol and differences in liking of the foods may have contributed to the observed, limited variability in bolus properties despite following a standardized chewing protocol.

3.3.3.1 Compositional properties of boli

To gain insights into compositional changes of boli during oral processing, bolus water content on wet basis, bolus fat content on dry basis and bolus saliva uptake for PBMA and beef patty at different stages of mastication are shown in **Figure 3.2**.

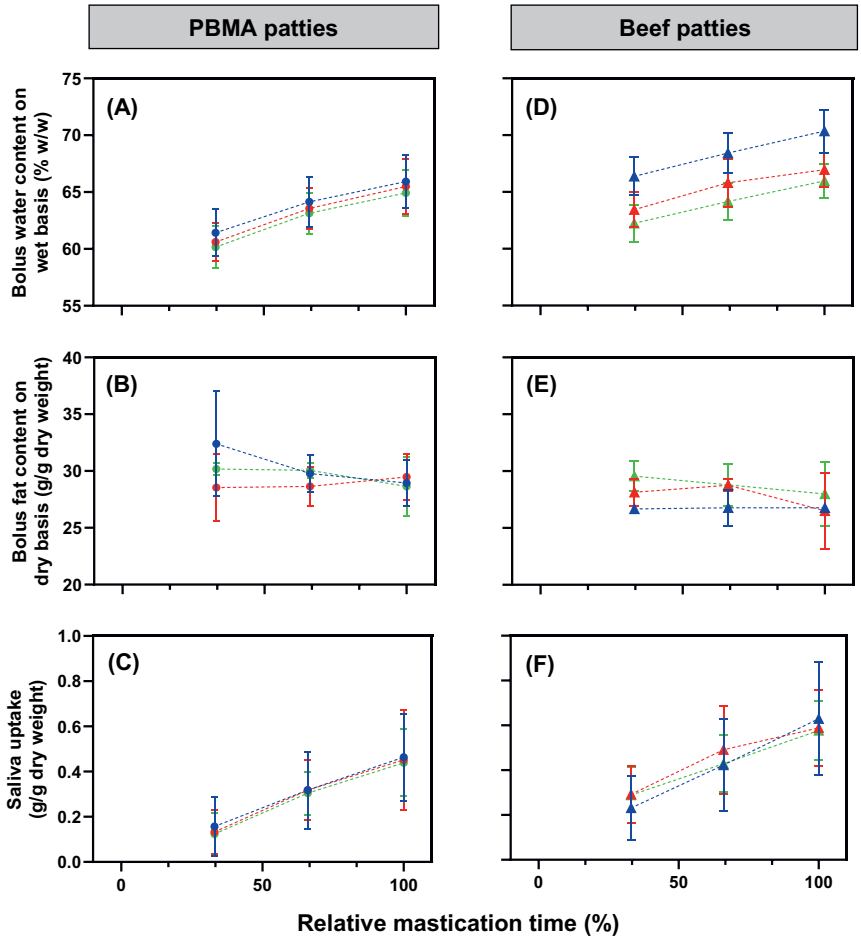

Figure 3.2 Water content on wet basis (A, D), fat content on dry basis (B, E) and saliva uptake (C, F) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) of PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60°C (blue symbols), 70°C (red symbols) and 80°C (green symbols). Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

For PBMA patties, mastication time significantly influenced bolus water content (F = 142.6, $p < 0.001$) and saliva uptake (F = 82.5, $p < 0.001$). Core temperature had a significant and small effect on PBMA bolus water content (F = 7.5, $p < 0.01$) (**Table 3.4**

(A)). The increase in bolus water content over mastication time can be explained by the uptake of saliva during mastication (**Figure 3.2 (A)** and **(C)**). This phenomenon is attributed to the stimulation of salivation by mastication followed by saliva absorption by the bolus (Rizo et al., 2019; Devezeaux de Lavergne, van de Velde, et al., 2015; van Eck, Hardeman, et al., 2019). Interestingly, our results indicated that saliva uptake was not influenced by core temperature (juiciness) of PBMA patties (F = 0.5, p = 0.623) (**Table 3.4 (A)**). We speculate that saliva uptake remained consistent across all PBMA patties since all patties contained the same amount of TVP, so that potentially the capability to absorb saliva during mastication was similar across patties independent of the core temperature.

Fat content of PBMA boli (on dry basis) was similar across PBMAs differing in core temperature (F = 3.1, p = 0.052) and did not change with mastication time (F = 2.6, p = 0.080) (**Table 3.4 (A)**), suggesting that fat was not released from the PBMA matrix into the oral cavity during mastication (**Figure 3.2 (B)**). These findings align with the TCATA results (**section 3.3.1**), which showed that the fattiness citation proportions were not significantly different across PBMA patties differing in core temperature and across different stages of mastication (**Table 3.3 (A)**).

For beef patties, mastication time (F = 58.8, p < 0.001) and core temperature (F = 79.5, p < 0.001) significantly influenced bolus water content (**Table 3.4 (B)**). While the increase in bolus water content during mastication was similar across different beef patties, the absolute values varied depending on the core temperature **(Figure 3.2 (D)**). Saliva uptake also increased similarly across patties throughout mastication (F = 45.5, p < 0.001), with no significant effect of core temperature (F = 0.4, p = 0.643) on saliva uptake (**Table 3.4 (B)**). Therefore, the observed increase in bolus water content during mastication was primarily driven by saliva uptake during mastication. The absolute difference in water content was caused by water loss during cooking, i.e. the initial water content of the beef patties (Zhang et al., 2024, **Chapter 2**).

Bolus fat content of beef patties (on dry basis) was significantly influenced by core temperature (F = 12.2, p < 0.001) and, to a lesser extent, by mastication time (F = 4.1, p < 0.05) (**Table 3.4 (B)**). These results suggest two key points: (1) boli of beef patties

prepared at lower core temperatures contained less fat (**Figure 3.2 (E)**); and (2) a limited amount of fat was released from the beef matrix into the oral cavity during mastication. The second point agrees with the TCATA results, which demonstrated that fattiness citation proportions were fairly constant over mastication time and relatively low, typically ranging between 20-50% (**Figure 3.1, Table 3.3**). Although previous research has shown that the fat content of beef patties decreased by cooking at lower temperatures (Zhang et al., 2024, **Chapter 2**), the difference in bolus fat content contrasted with the TCATA results, as an increase in fattiness citation proportion is seen with decreasing core temperature, i.e. lower fat content (**Figure 3.1, Table 3.3**). We propose that fattiness perception may not solely be determined by the fat content of the patties; it seems to be influenced by their juiciness, which is related to the amount of serum released during mastication (Zhang et al., 2024, **Chapter 2**). This highlights the intricate interplay of texture sensations contributing to fattiness perception.

3.3.3.2 Bolus texture properties

The bolus peak force, resilience and adhesiveness of PBMA and beef boli at different stages of mastication are shown in **Figure 3.3**.

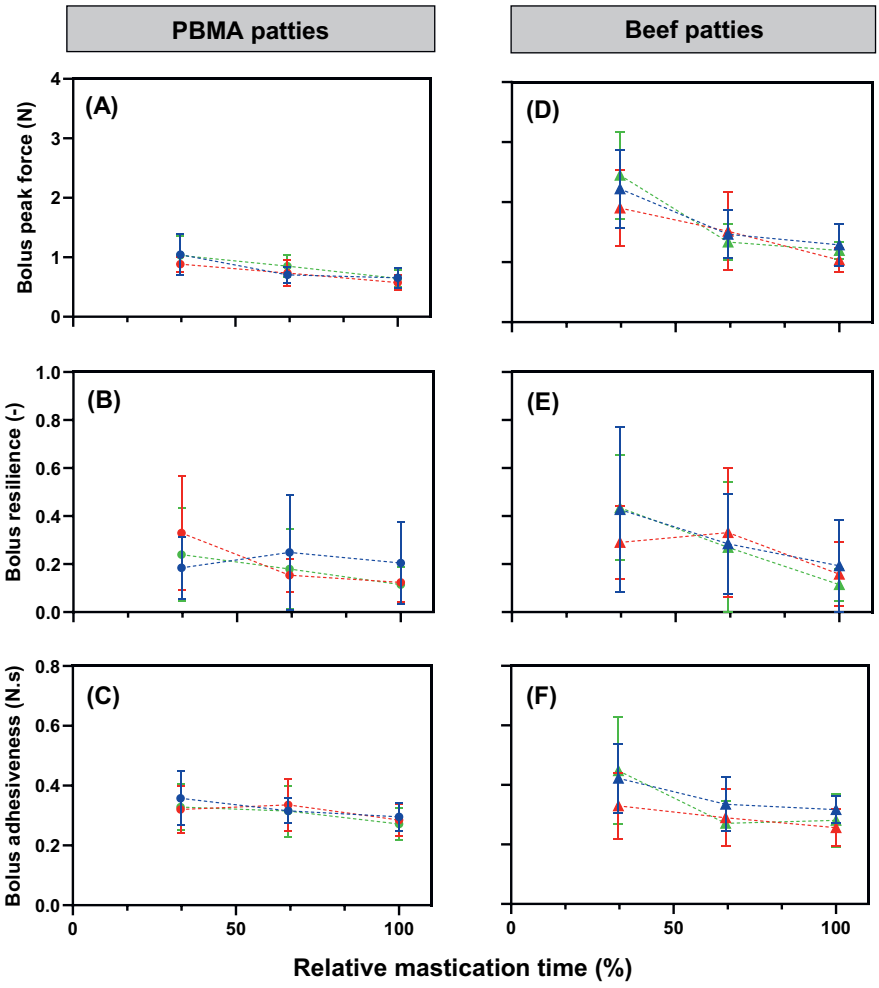

Figure 3.3 Bolus peak force (A, D), bolus resilience (B, E) and bolus adhesiveness (C, F) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) of PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60°C (blue symbols), 70°C (red symbols) and 80°C (green symbols). Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

For PBMA patties, mastication time had a significant effect on bolus peak force (F = 28.3, $p < 0.001$), no significant effect on bolus resilience (F = 2.9, p = 0.061), and a significant but small effect on bolus adhesiveness (F = 4.8, $p < 0.05$) (**Table 3.4 (A), Figure 3.3**). As mastication progressed, PBMA boli tended to become softer, although the differences in bolus peak force throughout mastication were small and might not be relevant (absolute difference in bolus peak force between 33 and 100%

mastication: 0.31 - 0.39 N) (**Figure 3.3**). Core temperature had no significant effect on any of the three bolus texture properties (**Table 3.4 (A), Figure 3.3**). This aligns with the notion that the denatured TVP reacts similarly to different cooking core temperatures, leading to limited variability in the texture of PBMA patties and the corresponding PBMA boli.

Beef patty boli showed slightly more variations in bolus texture properties but similar trends compared to PBMA boli. Mastication time significantly influenced bolus peak force ($F = 39.3$, $p < 0.001$), bolus resilience ($F = 8.8$, $p < 0.001$) and bolus adhesiveness ($F = 11.2$, $p < 0.001$) (**Table 3.4 (B), Figure 3.3**). With increasing mastication time, the peak force of the beef boli significantly decreased, while changes in bolus resilience and bolus adhesiveness were small and not consistent across the three beef patties (**Figure 3.3**). Core temperature showed no significant effect on bolus peak force ($F = 1.5$, $p = 0.239$) and bolus resilience ($F = 0.3$, $p = 0.744$), but had a small effect on bolus adhesiveness ($F = 3.2$, $p < 0.05$) (**Table 3.4 (B)**). These minor effects of core temperature on beef boli texture were expected, as cooked beef patties also exhibited small differences in texture properties when cooked at varying temperatures (maximum 1.1 N differences in peak force) (Zhang et al., 2024, **Chapter 2**). However, these subtle differences were perceivable, as evidenced by a decrease in softness citation proportions and an increase in chewiness citation proportions with increasing core temperatures in beef patties (**Figure 3.1 and Table 3.3**). The fact that those differences were not measured instrumentally may be due to the fact that a small-diameter cylindrical probe (diameter 4 mm) might not be sensitive enough to detect variations in bolus texture properties for boli with relatively high water content.

3.3.3.3 Oral structural breakdown: bolus particle number and size

To investigate the oral structural breakdown of PBMA and beef patties differing in juiciness, total number of bolus particles per gram of bolus and bolus particle size at different stages of mastication were determined (**Figure 3.4**).

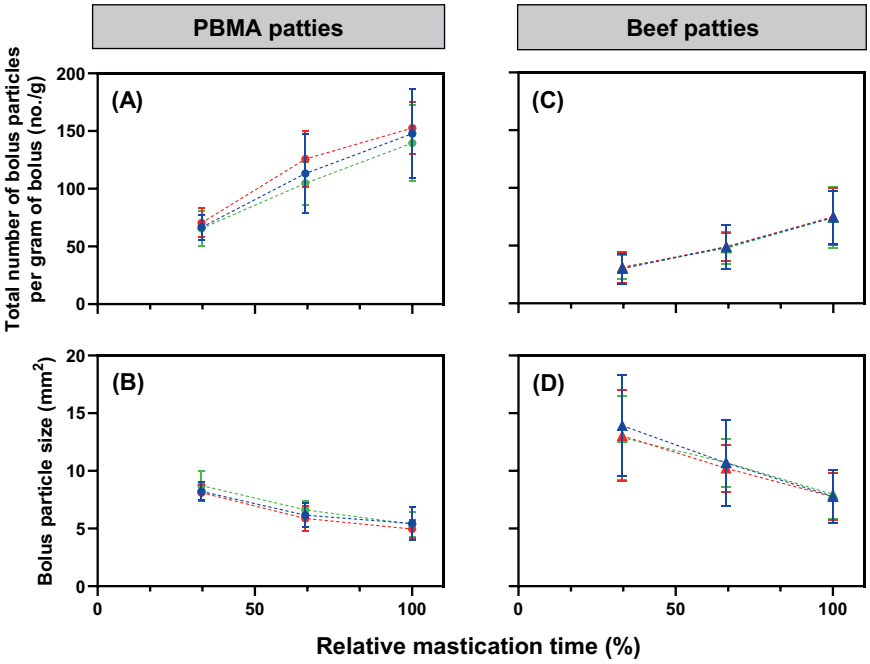

Figure 3.4 Total number of bolus particles per gram of bolus (A, C) and average bolus particle size (B, D) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) from PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60°C (blue symbols), 70°C (red symbols) and 80°C (green symbols). Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

Mastication time significantly affected the total number of bolus particles per gram of bolus and bolus particle size of PBMA (F = 96.2, p < 0.001, F = 119.0, p < 0.001, respectively) and beef bolus (F = 95.4, p < 0.001, F = 95.4, p < 0.001, respectively) (**Table 3.4**). As mastication progressed, more and smaller bolus fragments were generated (**Figure 3.4**) (Djekic et al., 2021; Lillford, 2011). Core temperature had no significant influence on total number of bolus particles of PBMA and beef boli (F = 2.6, p = 0.085, F = 0.02, p = 0.978, respectively), a significant and small influence on bolus particle size of PBMA bolus (F = 4.2, p < 0.05) and no significant influence on bolus particle size of beef bolus (F = 0.3, p = 0.767) (**Table 3.4**). The limited effect of core temperature on oral structural breakdown of PBMA and beef patties (**Figure 3.4**) can be explained based on two observations. First of all, the texture properties of PBMA

and beef patties were not strongly affected by core temperature (Zhang et al., 2024, **Chapter 2**), resulting in comparable oral processing behavior (**section 3.3.1**) and consequently in similar bolus fragment properties. Secondly, PBMA and beef patties are products that consist of particles or structural elements (texturized vegetable proteins (TVPs) for PBMA and muscle bundles for beef (Ilić et al., 2022) that are bound together using different binding agents. During mastication, the macroscopic structure is broken down, but the structural elements (TVP particles or muscle bundles) remain intact and are similar across patties prepared at different core temperatures.

3.3.3.4 Liquid expelled from bolus

The liquid expelled from the bolus and its water content may impact juiciness and fattiness perception. Therefore, the liquid expelled from bolus and its water content at different stages of mastication were quantified (**Figure 3.5)**.

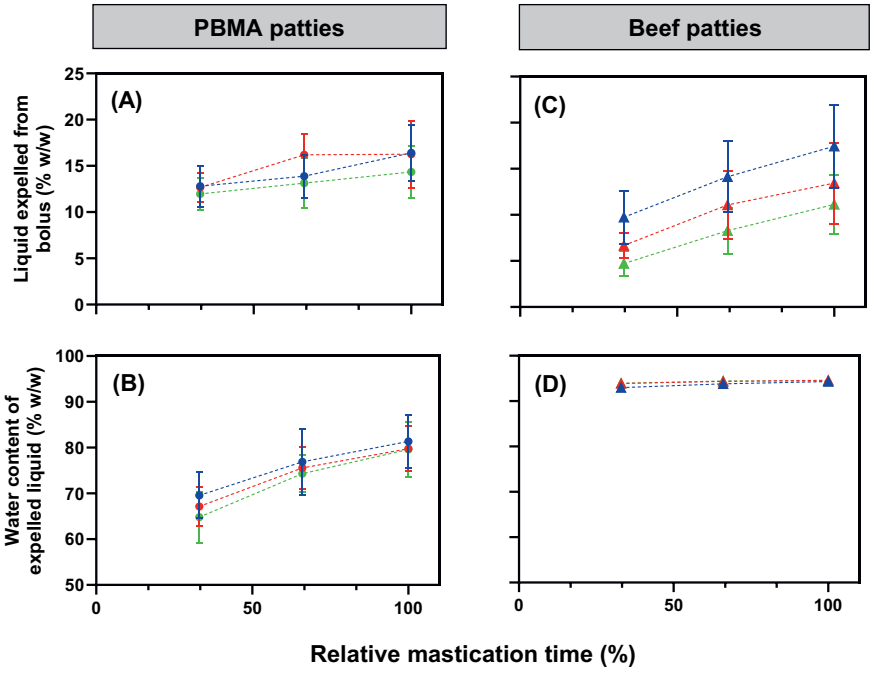

Figure 3.5 Liquid expelled (A, C) and water content of expelled liquid (B, D) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) from PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60°C (blue symbols), 70°C (red symbols) and 80°C (green symbols).

Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

Expelled liquid in PBMA boli was strongly affected by mastication time (F = 18.8, p < 0.001) and weakly by core temperature (F = 6.7, p < 0.01) (**Table 3.4 (B)**), resulting in more liquid expelled from PBMA bolus during mastication (**Figure 3.5 (A)**). In contrast, expelled liquid in beef boli was significantly affected by both mastication time (F = 90.4, p < 0.001) and core temperature (F = 61.2, p < 0.001) (**Table 3.4 (B)**), with lower core temperature or increased mastication time leading to more liquid expelled from beef boli. As discussed in **section 3.3.1**, the consistent increase in liquid expelled for PBMA and beef patties can be explained by the increase in bolus water content due to saliva uptake during mastication (**Figure 3.2 (C) (D)**). The absolute difference in expelled liquid of beef bolus was driven by the initial water content of the beef patties.

The compositions of the expelled liquid differed distinctively between PBMA and beef boli. For PBMA boli, water content in the expelled liquid increased from 70 to 80% with mastication (**Figure 3.5 (B)**) (F = 208.3, p < 0.001, **Table 3.4 (A)**), aligning with the increased water content in PBMA boli due to saliva uptake. In contrast, for beef boli, the water content in the expelled liquid remained constant during mastication (93.0 - 94.6%, **Table S3.1**), but the amount of expelled liquid changed slightly during mastication (F = 28.8, p < 0.001) and depended on the core temperature (F = 17.0, p < 0.001) (**Table 3.4 (B)**). We assume that the remaining portion of the expelled liquid was fat, indicating that beef patties contained little fat (<10%), whereas PBMA patties released slightly more fat (between 20 and 40%), depending on the mastication time. These differences in fat content of expelled liquid from PBMA and beef boli did not correspond to their differences in perceived fattiness (**Figure 3.1** and **Table 3.3**), as beef patties showed more variations in fattiness citation proportion, even though the fat content was similarly low during mastication. This suggests that fattiness perception may be influenced more by the serum release and saliva uptake during mastication than by compositional differences, i.e. fat content, of the expelled liquid.

3.3.4 Serum release under oral conditions

To understand the dynamics of serum release during mastication excluding saliva uptake, we investigated the absolute and relative serum release of PBMA and beef patties at different stages of mastication. The obtained results are shown in **Figure 3.6**.

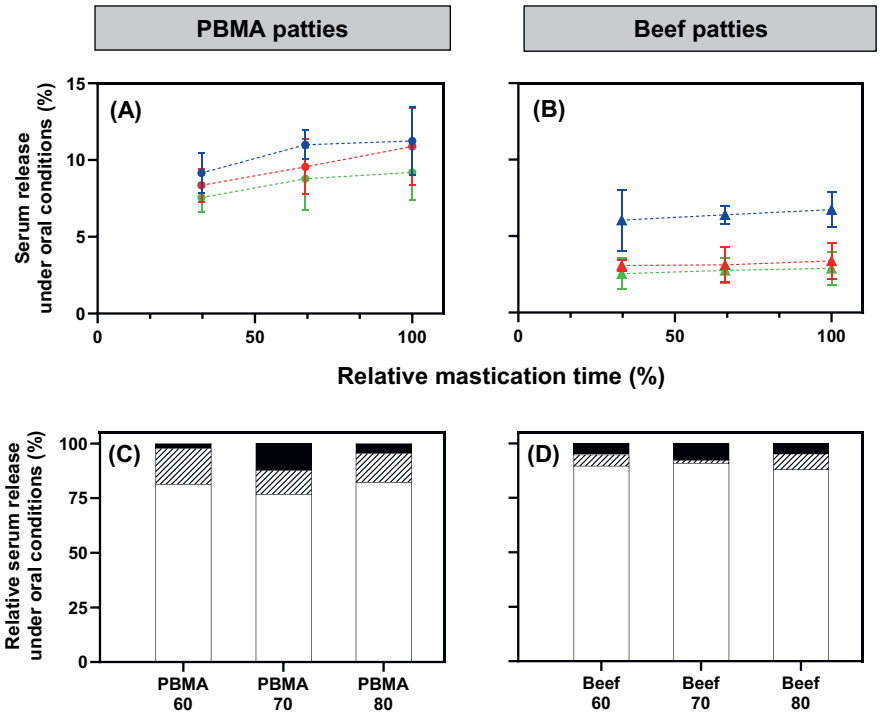

Figure 3.6 Serum release under oral conditions at 33, 66 and 100% of mastication for (A) PBMA (circles ●) and (B) beef patties (triangles ▲) prepared at core temperatures of 60°C (blue symbols), 70°C (red symbols) and 80°C (green symbols) (n = 10 participants). Dashed lines are included to guide the eye. Means are shown together with standard deviations. Figure (C) and (D) show the relative serum release under oral conditions after 33% (no fill pattern), 66% (diagonal fill pattern) and 100% (black fill pattern) of mastication for (C) PBMA and (D) beef patties prepared at different core temperatures.

For PBMA patties, serum release under oral conditions significantly increased with increasing mastication time ($F = 16.3$, $p < 0.001$) and with decreasing core temperature ($F = 13.8$, $p < 0.001$) (**Figure 3.6 (A)**). For beef patties, mastication time did not significantly influence serum release under oral conditions ($F = 1.3$, $p = 0.267$), but samples prepared at lower core temperature released significantly more serum during mastication ($F = 104.7$, $p < 0.001$) (**Figure 3.6 (B)**). The influence of core temperature

on serum release for PBMA and beef patties is in agreement with our previous findings (Zhang et al., 2024, **Chapter 2**).

Remarkably, plotting the relative amount of serum release after 33, 66 and 100% of mastication (**Figures 3.6 (C) (D)**) showed that within the first third of mastication (33%) more than 75% of serum was already released for PBMA patties and more than 85% for beef patties. Additional mastication until the moment of swallowing released less than 25% additional serum from PBMA patties and less than 15% additional serum from beef patties. The majority of serum was thus released from the patty matrix into the oral cavity at early stages of mastication, coinciding with an early peak of juiciness citation proportions (**Figure 3.1**).

These results validate the hypothesis that for PBMA and beef patties initial juiciness perception is linked to the rapid release of fluids during the first few chews. Although the oral structural breakdown of PBMA and beef patties continued until the end of mastication, the additional oral structural breakdown had a negligible effect on juiciness perception. Even though the generation of new bolus surface area could potentially enhance additional serum release, juiciness perception was not enhanced, and was even accompanied by a decline in juiciness citation proportions. Juiciness of PBMA patties is thus merely a result of initial serum release. This behavior of the PBMA patties is comparable to that of a sponge, which releases its water upon mechanical compression and does not require mechanical deconstruction for water release. It is worth noting that these conclusions are specific to the minced PBMA patties studied here. Further investigations are needed to confirm whether these findings can be generalized to other plant-based and animal-based foods.

3.3.5 Relationships between dynamic bolus properties, serum release and dynamic sensory perception

To explore the relationships between dynamic bolus properties, serum release and dynamic sensory properties, principal component analysis (PCA) was performed separately for PBMA and beef patties (**Figure 3.7**). Pearson correlation coefficients were determined at different mastication times for PBMA and beef patties separately, as presented in the **Supplementary Figure S3.2**.

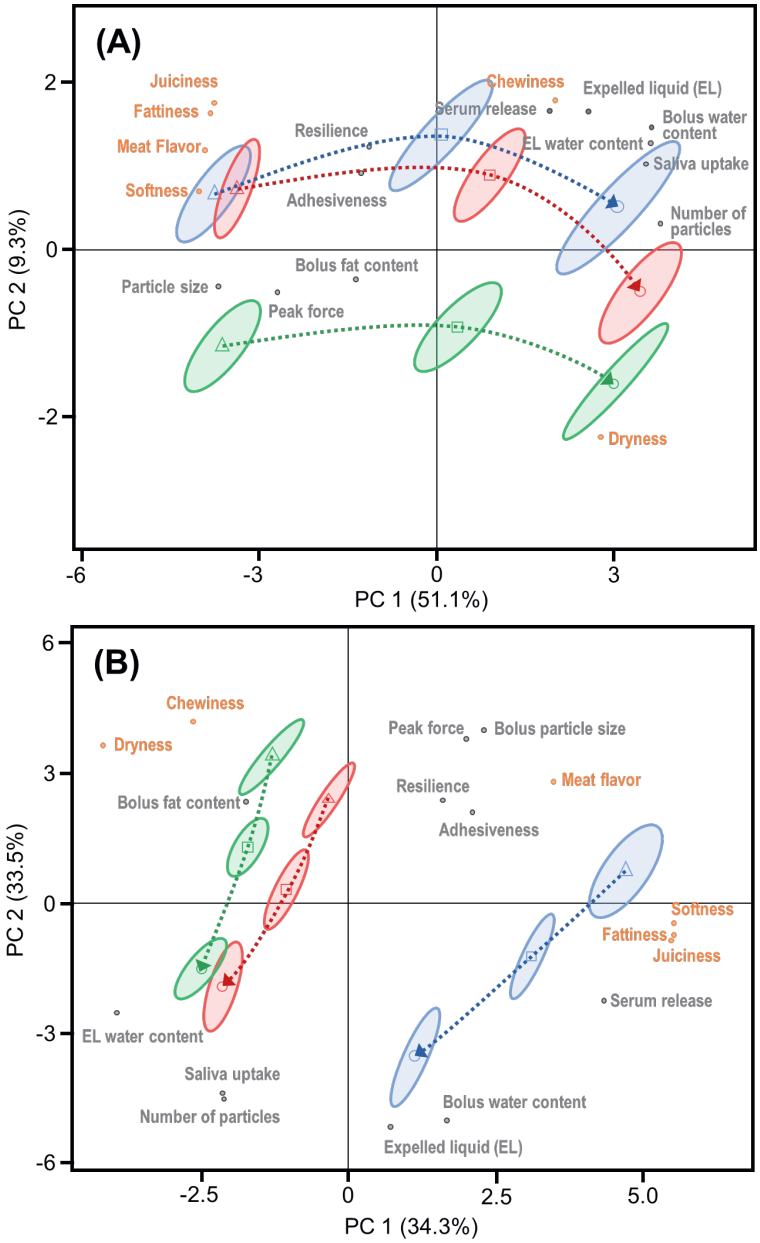

Figure 3.7 Principal component analysis (PCA) illustrating oral processing trajectories of (A) PBMA and (B) beef patties prepared at core temperatures of 60°C (blue ellipse), 70°C (red ellipse) and 80°C (green ellipse) at 33 (triangles △), 66 (squares □) and 100% (circles ○) of mastication time. Bolus properties and serum release under oral conditions (grey text) were quantified at 33, 66 and 100% of mastication time. Dynamic citation proportions (orange text) were taken at 33, 66 and 100% of mastication time from the TCATA data. The ellipses represent a confidence level of 0.95. Dashed lines are included to guide the eye, with arrows indicating the direction of oral processing.

For PBMA patties, mastication time had a more pronounced effect on the oral processing trajectory compared to that of core temperature. As illustrated in **Figure 3.7 (A),** PBMA patties varying in core temperature are positioned along the PC2 (Y axis, 9.3%). Moving from the bottom to the top of **Figure 3.7 (A)**, core temperature decreased and PBMA patties were perceived juicier and less dry, and had a higher serum release and amount of liquid expelled from the bolus (top variables contributing to PC2, **Figure S3.1 (B)**). Moreover, all PBMA patties differing in core temperature followed a similar trajectory along PC1 (X axis, 51.1%) (**Figure 3.7 (A)**), which corresponded with mastication time. The sensory trajectories for PBMA patties during mastication started with juiciness, fattiness, softness and meat flavor at the early stages of mastication, followed by chewiness perception and ending with dryness. These results confirm our hypothesis that juiciness is perceived early during mastication. Regarding the bolus properties trajectories, together with a decrease in bolus particles size and an increase in number of bolus particles, bolus peak force decreased, and saliva uptake, bolus water content and expelled liquid increased during mastication. These results align with the development of bolus properties during oral processing for other products (Devezeaux de Lavergne et al., 2017; Mosca & Chen, 2016).

A scarcity of significant correlations between bolus properties and sensory perception in PBMA patties was observed (**Figure S3.2 (A), Figure 3.7 (A)**). This may be caused by the limited influence of core temperature on PBMA bolus properties compared to the influence of mastication time (**Figures 3.2 to 3.5**) in our study, resulting in limited variability in bolus properties across samples (i.e., bolus properties were closer to coordinate origin (**Figure 3.7 (A)**). Juiciness was positively correlated with fattiness, and negatively with dryness citation proportion (**Figure 3.7 (A)**), consistently with previous studies on PBMA products (Thong et al., 2024; Zhang et al., 2024, **Chapter 2**). Similarly, meat flavor was positively correlated with softness, fattiness and juiciness (Saint-Eve et al., 2011; Weel et al., 2002). Although no correlations between juiciness citation proportion and serum release under oral conditions were found in the PCA (**Figure 3.7 (A)**), such correlations emerged when performing Pearson correlations at

different mastication times separately (**Figure S3.2 (A)**). The correlation coefficient was similar for 33 and 66% of mastication time and decreased for 100% of mastication time, suggesting that the changes in serum release drive changes in perception of juiciness. Similar results were observed for dryness, confirming the close correlations between juiciness and dryness. Mastication time thus had a great effect on serum release and juiciness citation proportion; juiciness citation proportion peaked early and then rapidly decreased, while serum release gradually increased (**Table 3.3 (A)** and **Figure 3.6 (A)**).

For beef patties, both core temperature and mastication time led to more pronounced variations in bolus properties compared to PBMA patties, reflected by PC1 explaining 34.3% of the variance in the data and PC2 explaining 33.5% of the variance (**Figure 3.7 (B)**). Moving from left to right along PC1, core temperature of beef patties decreased corresponding to higher juiciness, softness and fattiness citation proportions, increased serum release and decreased dryness and chewiness citation proportions. In contrast, the effect of mastication time was more visible on PC2, primarily influenced by bolus properties such as liquid expelled from bolus, bolus water content, number of particles, and saliva uptake (top contributors to PC2, **Figure S3.1 (D)**). Notably, sensory perception of beef patties correlated mainly with initial bolus properties and showed limited changes during mastication (**Figure 3.7 (B)**). Sensory properties therefore varied more between beef patties differing in core temperature but remained relatively consistent during mastication (**Figure 3.1**).

When linking beef bolus properties to sensory perception, more correlations were found for beef patties than for PBMA patties (**Figure 3.7 (B), Figure S3.2 (B)**). As expected, juiciness, fattiness and softness citation proportions were positively correlated with each other (**Figure 3.7 (B)**), consistently with findings from studies on sausages (Pematilleke et al., 2020; Sasaki et al., 2013). Serum release under oral conditions was also related to juiciness and dryness citation proportions, but, in contrast to PBMA patties, serum release of beef patties was additionally correlated with other texture attributes, such as softness, chewiness and fattiness in PCA (**Figure 3.7 (B)**) and Pearson correlations (**Figure S3.2 (B)**). These strong correlations between

serum release and texture attributes can be explained by the substantial variations in serum release and texture attributes when varying core temperatures compared to subtle changes during mastication. This also explains why limited correlations were observed between bolus properties and sensory properties in PCA (**Figure 3.7(B)**), whereas in Pearson correlations, where correlations were analyzed across different mastication times, bolus compositional properties and expelled liquid properties consistently correlated with various sensory attributes (**Figure S3.2 (B)**). However, the water content of expelled liquid remained constant across mastication time and core temperature (**Figure 3.5 (D)**), suggesting less reliability in significant correlations between water content of expelled liquid and texture attributes. These results suggest that water content of beef boli and liquid expelled from the boli are important factors driving dynamic texture perception of beef patties during mastication.

To summarize, although limited correlations were found between bolus properties and sensory properties at different moments of mastication in PBMA patties, juiciness perception and serum release showed an association at the beginning of mastication. This initial stage of mastication contributed to 75-85% of serum release and coincided with the peak of juiciness perception. In contrast, for beef patties, all sensory attributes were related to serum release across mastication times due to the larger variabilities in all sensory attributes when varying core temperature. Juiciness perception of beef patties was also positively correlated with water content of the bolus and the amount of expellable liquid from the bolus. These correlations can be explained by more variabilities in oral breakdown of beef patties varying in core temperature compared to PBMA patties. Therefore, we speculate that oral structural breakdown would also influence juiciness perception of PBMA products, in case these PBMA products contain greater variabilities in texture.

3.4 Conclusions

This study aimed to understand the role of bolus properties at different moments of consumption in dynamic texture perception, especially juiciness, of PBMA and beef patties. Our findings suggest that, for the patties used in this study, juiciness perception of plant-based meat analogue patties is primarily driven by the serum release during early stages of mastication. Additional oral structural breakdown did not increase serum release considerably. Conversely, juiciness perception of the beef patties used in this study was not only driven by the serum release during early stages of mastication but also influenced by additional oral structural breakdown, as juiciness correlated with bolus water content and liquid expelled from the bolus. These differences in temporal juiciness perception can be attributed to differences in the structural elements of PBMA and beef patties, particularly related to the proteins present. Patties prepared from denatured plant-based proteins (Texturized Vegetable Proteins) exhibit fairly inert behavior compared to myofibrillar animal proteins during thermal treatments. Effectively mimicking these (changes in) structural elements and the resulting dynamic texture perception remains a primary challenge in improving the sensory quality of PBMA products. Future studies should consider incorporating a wider range of texture variations of PBMAs to generalize the role of oral structural breakdown in juiciness perception across a wider product category. Examining the microstructure of boli could provide further insights into the potential influence of the microstructure of boli on juiciness and texture perception. For instance, exploring how water binds to and flows through the patty matrix could be insightful. This study highlights that employing temporal sensory methods is crucial for evaluating juiciness dynamics accurately. Our findings might offer valuable insights for industry seeking to enhance the juiciness of PBMA products. Targeting the released serum at the beginning of mastication, by either increasing its quantity or modifying its properties, may hold the key to improving the juiciness of PBMA products.

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Supplementary material

Table S1. Effect of mastication time and core temperature on bolus properties (mean ± SD) at different stages of mastication for (A) PBMA patties and (B) beef patties (n = 10 participants). F values and p values are derived from linear mixed model with mastication time and core temperature as fixed factor, and participant as random effect and are also reported in Table 4.

	33% of mastication			66% of mastication			100% of mastication			*Mastication time*		*Core temperature*	
(A) Bolus properties of PBMA patties	**PBMA60**	**PBMA70**	**PBMA80**	**PBMA60**	**PBMA70**	**PBMA80**	**PBMA60**	**PBMA70**	**PBMA80**	***F* value**	***P* value**	***F* value**	***P* value**
Bolus composition													
Water content (% w/w)	61.4±2^{a}	60.6±1.6^{ab}	60.2±1.7^{b}	64.1±2.1^{cd}	63.6±1.7^{de}	63.1±1.7^{e}	65.9±2.2^{f}	65.5±2.3^{cfg}	64.9±1.9^{cg}	142.6	**<0.001**	7.5	**<0.01**
Fat content dry basis (g/g dry weight)	32.4±4.4^{a}	28.5±2.8^{ab}	30.2±0.5^{ab}	29.8±1.5^{ab}	28.6±1.6^{ab}	30.1±0.6^{ab}	28.9±1.9^{ab}	29.5±1.9^{b}	28.6±2.5^{ab}	2.6	0.080	3.1	0.052
Saliva uptake (g/g dry weight)	0.2±0.1^{a}	0.1±0.1^{a}	0.1±0.1^{a}	0.3±0.2^{b}	0.3±0.1^{bc}	0.3±0.1^{bc}	0.5±0.2^{d}	0.5±0.2^{de}	0.4±0.1^{de}	82.5	**<0.001**	0.5	0.623
Bolus texture													
Peak force (N)	1.0±0.3^{ab}	0.9±0.1^{abc}	1.0±0.3^{a}	0.7±0.1^{cde}	0.7±0.2^{de}	0.9±0.2^{bd}	0.7±0.2^{de}	0.6±0.1^{e}	0.6±0.1^{e}	28.3	**<0.001**	2.7	0.075
Resilience (-)	0.2±0.1	0.3±0.2	0.2±0.2	0.2±0.2	0.2±0.1	0.2±0.2	0.2±0.2	0.1±0.1	0.1±0.1	2.9	0.061	0.3	0.712
Adhesiveness (N·s)	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.0	0.3±0.1	0.3±0.1	0.3±0.0	0.3±0.1	0.3±0.1	4.8	**<0.05**	0.5	0.585
Oral structural breakdown													
Total number of bolus particles (no./g)	67±10^{a}	71±12^{a}	66±14^{a}	113±33^{b}	126±23^{bcd}	105±18^{bc}	148±37^{e}	153±21^{e}	140±31^{ed}	96.2	**<0.001**	2.6	0.085
Bolus particle size (mm²)	8.2±0.8^{a}	8.1±0.7^{a}	8.7±1.2^{a}	6.2±1^{bc}	5.9±1^{cd}	6.6±0.7^{c}	5.4±1.4^{de}	5±0.7^{e}	5.4±1^{bde}	119.0	**<0.001**	4.2	**<0.05**
Liquid expelled from bolus													
Liquid expelled during centrifugation (% w/w)	12.8±2.1^{ab}	12.7±1.5^{bc}	12±1.7^{a}	13.9±2.2^{cd}	16.2±2.1^{de}	13.1±2.5^{bc}	16.4±2.9^{de}	16.3±3.5^{d}	14.3±2.7^{bce}	18.8	**<0.001**	6.7	**<0.01**
Water content of expelled liquid (% w/w)	69.6±4.8^{a}	67.1±4^{ab}	64.8±5.3^{b}	76.9±6.8^{cd}	75.6±4.3^{de}	74.3±3.8^{e}	81.3±5.5^{f}	79.8±4.6^{cfg}	79.6±5.7^{cg}	208.3	**<0.001**	11.0	**<0.001**

Table continues on the next page

Table S1 (continued)

	33% of mastication			66% of mastication			100% of mastication			*Mastication time*		*Core temperature*	
(B) Bolus properties of beef patties	**BEEF60**	**BEEF70**	**BEEF80**	**BEEF60**	**BEEF70**	**BEEF80**	**BEEF60**	**BEEF70**	**BEEF80**	***F value***	***P value***	***F value***	***P value***
Bolus composition													
Water content (% w/w)	66.4±1.6^{ab}	63.4±1.5^{c}	62.2±1.6^{d}	68.4±1.7^{e}	65.8±2.0^{b}	64.2±1.6^{c}	70.4±1.8^{f}	67±1.4^{ae}	66±1.4^{b}	58.8	**<0.001**	79.5	**<0.001**
Fat content dry basis (g/g dry weight)	26.7±0.3^{ac}	28.1±1.1^{ab}	29.6±1.3^{b}	26.8±1.5^{ac}	28.8±0.5^{ab}	28.8±1.8^{b}	26.8±0.4^{c}	26.5±3.2^{acd}	28±2.7^{abd}	4.1	**<0.05**	12.2	**<0.001**
Saliva uptake (g/g dry weight)	0.2±0.1^{a}	0.3±0.1^{ab}	0.3±0.1^{a}	0.4±0.2^{bc}	0.5±0.2^{cd}	0.4±0.1^{bd}	0.6±0.2^{de}	0.6±0.2^{e}	0.6±0.1^{ce}	45.5	**<0.001**	0.4	0.643
Bolus texture													
Peak force (N)	2.2±0.6^{a}	1.9±0.6^{a}	2.4±0.7^{a}	1.5±0.4^{b}	1.5±0.6^{b}	1.3±0.3^{b}	1.3±0.3^{b}	1.0±0.2^{b}	1.2±0.1^{b}	39.3	**<0.001**	1.5	0.239
Resilience (-)	0.4±0.3^{ab}	0.3±0.1^{bc}	0.4±0.2^{be}	0.3±0.2^{bcd}	0.3±0.3^{bcd}	0.3±0.3^{bcd}	0.2±0.2^{cde}	0.2±0.1^{de}	0.1±0.1^{cd}	8.8	**<0.001**	0.3	0.744
Adhesiveness (N·s)	0.4±0.1^{a}	0.3±0.1^{abc}	0.4±0.2^{ab}	0.3±0.1^{bd}	0.3±0.1^{d}	0.3±0.1^{cd}	0.3±0^{bd}	0.3±0.1^{d}	0.3±0.1^{cd}	11.2	**<0.001**	3.2	**<0.05**
Oral structural breakdown													
Total number of bolus particles (no./g)	30±12^{a}	31±13^{a}	32±10^{a}	49±18^{b}	49±12^{b}	48±13^{b}	75±22^{c}	75±23^{c}	74±25^{c}	95.4	**<0.001**	0.02	0.978
Bolus particle size (mm²)	13.9±4.1^{a}	13±3.7^{abc}	12.9±3.5^{ab}	10.7±3.5^{bd}	10.2±1.9^{def}	10.7±2^{cde}	7.8±2.2^{eg}	7.8±1.9^{g}	8.0±2.0^{fg}	37.0	**<0.001**	0.3	0.767
Liquid expelled from bolus													
Liquid expelled during centrifugation (% w/w)	9.7±2.8^{ab}	6.7±1.3^{c}	4.7±1.3^{d}	14.1±3.7^{e}	11.1±3.5^{a}	8.3±2.4^{bc}	17.4±4.3^{f}	13.5±4.2^{e}	11.1±3.0^{a}	90.4	**<0.001**	61.2	**<0.001**
Water content of expelled liquid (% w/w)	93±0.5^{a}	93.9±0.3^{b}	93.9±0.4^{b}	93.8±0.4^{b}	94.4±0.5^{cd}	94.4±0.3^{cd}	94.3±0.6^{bc}	94.6±0.6^{d}	94.5±0.6^{d}	28.8	**<0.001**	17.0	**<0.001**

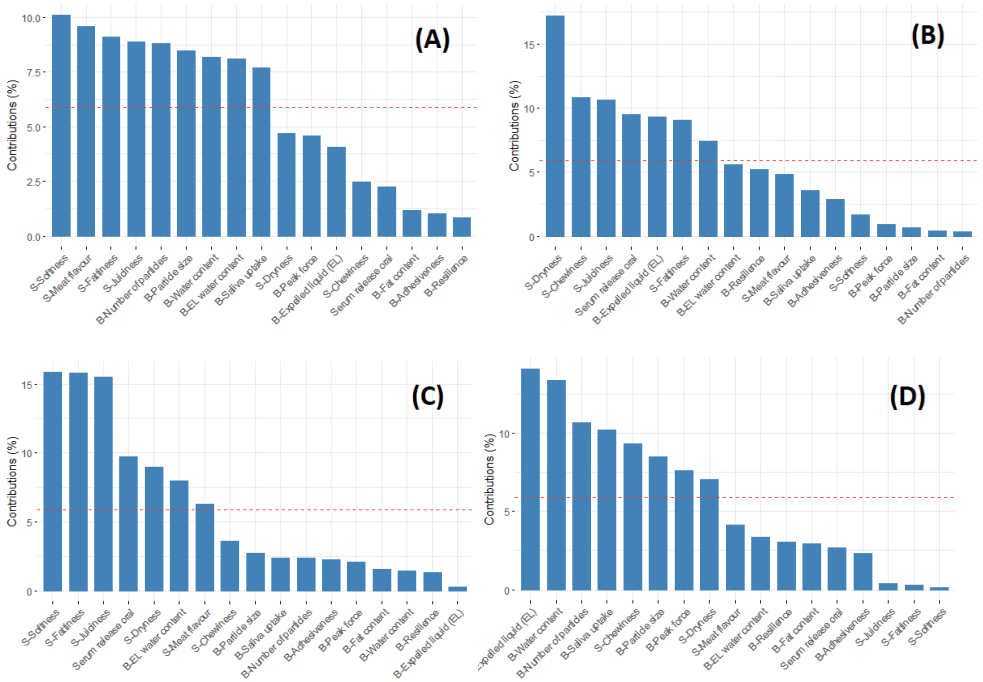

Figure S3.1 Contributions of variables to (A) PC1 and (B) PC2 of PBMA patties; contributions of variables to (C) PC1 and (D) PC2 of beef patties extracted from principal component analysis (PCA). The red dashed line indicates the expected average contribution.

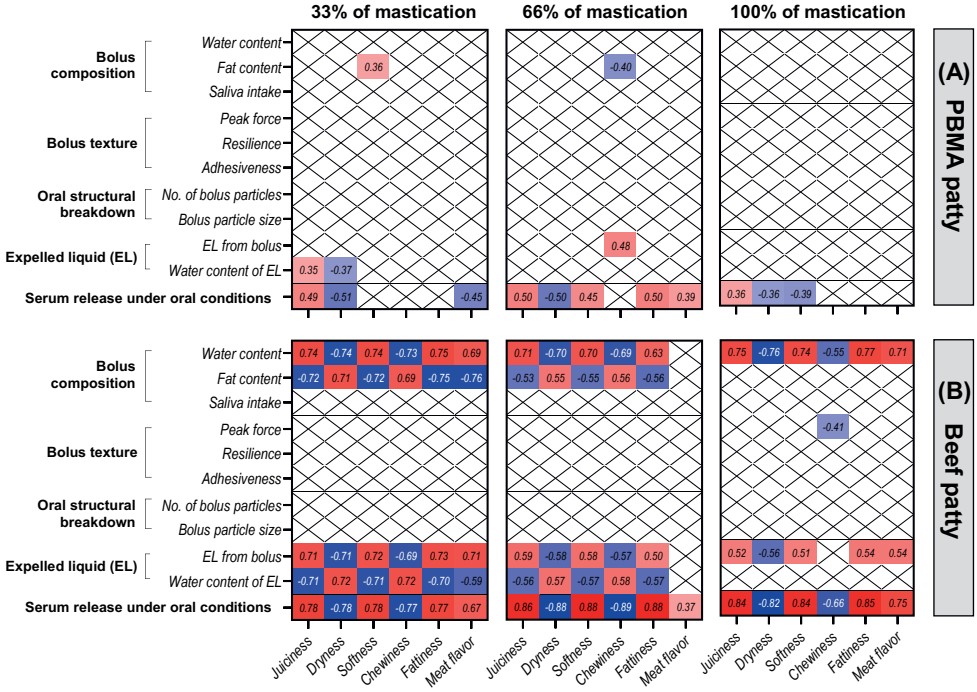

Figure S3.2 Pearson correlation coefficients between bolus properties and serum release under oral conditions at 33, 66 and 100% of mastication time and dynamic sensory properties ($C_{33\%}$, $C_{66\%}$ and $C_{100\%}$) for (A) PBMA and (B) beef patties. Significant correlations ($p < 0.05$) are indicated by color with red highlighting positive correlations and blue showing negative correlations, "X" denotes non-significant correlations.

Chapter 4

Boosting juiciness and flavor perception of meat analogue patties by altering hydration level and particle size of textured vegetable proteins

This chapter has been published as:

Zhang, Y., Király, M., Zhang, P., Navarrete Codina, R., Behrouzi, P., Sala, G., Scholten, E., & Stieger, M. Boosting juiciness and flavor perception of meat analogue patties by altering hydration level and particle size of textured vegetable proteins. *Food Hydrocolloids*, 111423. https://doi.org/10.1016/J.FOODHYD.2025.111423

Abstract

This study aimed to investigate juiciness perception of PBMA patties by exploring the relationships between physicochemical and sensory properties. Patties were designed to vary in water holding and release properties by controlling hydration level (water:TVP ratio 1:1, 1:3, 1:5) and particle size (particle surface area 0.2, 10, 20 mm^2) of Textured Vegetable Protein (TVP). Increasing TVP hydration level increased water cooking loss, while fat cooking loss remained unchanged. Despite this, higher initial water content still led to a 30% increase in water content and a 51% decrease in stiffness of cooked patties. The elevated water content significantly enhanced serum release under compression, which strongly influenced juiciness and fattiness perception. Increasing TVP hydration level increased juiciness (+204%) and fattiness (+71%), but decreased hardness (-53%) and crumbliness (-41%) intensity. Increasing TVP particle size increased cutting work during blade cutting tests, yielding harder (+62%) and chewier (+119%) patties. Network analysis revealed that increased juiciness enhanced fattiness, savory and garlic flavor and decreased hardness intensity. These variations in sensory properties influenced liking, which was positively related to juiciness, chewiness and savory flavor, and negatively to beany flavor. We conclude that increasing TVP hydration level effectively alters patty composition and texture and enhances juiciness of PBMA patties, while varying TVP particle size primarily impacts patty texture without affecting composition. Juiciness of PBMA patties is driven by retained water after cooking, serum release under compression and stiffness. Enhanced juiciness not only boosts flavor perception but also drives consumer liking.

4.1 Introduction

The increasing demand for a transition from animal-protein based to plant protein-based diets, driven by concerns for human and planetary health, has led the food industry to produce plant-based meat analogues (PBMAs), designed to mimic the sensory and nutritional properties of meats (McClements, 2024). The sensory quality of PBMAs, especially the lack of juiciness, remains a barrier to wider consumer acceptance (Godschalk-Broers et al., 2022; Gonzalez-Estanol et al., 2023; Szenderák et al., 2022).

Juiciness has been defined as the dynamic sensation of moisture and lubrication during oral processing (Warner, 2017; Winger & Hagyard, 1994; Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2 and 3**). For meats and meat products, muscle cut, intramuscular fat content and cooking method have been widely recognized as factors influencing juiciness perception. In contrast, the mechanisms behind juiciness perception of PBMAs are less explored (Ilić et al., 2022; Xu & Falsafi, 2023). Our previous studies demonstrated that juiciness of commercially available PBMA patties is determined by serum release during consumption (Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2 and 3**). Serum is defined as the fluid/juice released from the food matrix during uniaxial compression. Serum contains water, fat, proteins and other solid particles. PBMA patties differing in juiciness were prepared from the same raw material by varying the cooking temperature. Our results showed that juiciness is dominated by serum release during the early stages of mastication and not by additional changes in bolus properties, indicating that patty composition is mostly responsible for juiciness. However, the variability in texture properties across patties was limited in our previous studies as patties were prepared from the same raw material. To better understand the physicochemical properties of PBMA patties that contribute to juiciness perception, it is crucial to vary the compositional and textural properties of PBMA patties systematically over a broader range.

PBMA patties typically consist of textured and non-textured vegetable proteins, water, fat, binding agents, flavorings and colorings. To resemble the texture of ground meat, plant-based proteins are first converted into textured vegetable proteins (TVPs) through low-moisture extrusion (Kyriakopoulou et al., 2021). These low-moisture TVPs are then rehydrated with water and mixed with the other ingredients to create a patty dough. During thermal processing of these patties, the water holding properties of the TVP, and the gelling and thickening ability of non-textured protein and/or polysaccharides, such as methylcellulose, contribute to the water holding ability of the patties (Kazemi-Taskooh & Varidi, 2023). The water is distributed over both the hydrogel network (Ryu & McClements, 2024), the air pockets and the lamellae of the TVPs (van Esbroeck et al., 2024; Xu & Falsafi, 2023). Low-moisture TVPs have a sponge-like structure that enables absorption of water during rehydration, retention of water during cooking and release of water during consumption. Water retention during cooking and water release during consumption are important for the perception of juiciness of PBMA patties. By selecting TVPs with a higher water absorption capacity and hydrating the TVPs to varying levels, more water can initially be included in the raw patties, creating patties differing in composition and texture. It has not been reported how initial TVP hydration level relates to patty water content after cooking. In addition, the particle size of TVPs may influence the patty water holding and release capacities, since water is stored in the air pockets as well as the lamellae of TVPs (van Esbroeck et al., 2024). Variation in TVP particle size could also strongly affect other texture properties allowing to vary patty texture over a broader range. Variations in TVP hydration level and TVP particle size present therefore an opportunity to investigate the relationships between physicochemical properties of patties and juiciness and other texture attributes. We hypothesize that increasing TVP hydration level and particle size increase serum release and enhance juiciness perception.

The aim of this study was to understand juiciness perception of PBMA patties differing in TVP hydration level and particle size by investigating the relationships between

physiochemical, textural and sensory properties. To create PBMA patties differing in composition and texture, patties were prepared using pea-based TVP differing in hydration level and TVP particle size following a 3×3 full-factorial design. Cooked PBMA patties were characterized for compositional (water and fat content, total/water/fat cooking loss), textural (compression test, blade cutting test) and serum properties (serum release under compression, water content of released serum). Rate-All-That-Apply (RATA) was used to evaluate texture, taste and flavor perception of PBMA patties. To investigate correlations between different sensory attributes and physicochemical and textural properties, network analysis was employed (Undirected Graphical Models).

4.2 Materials and Methods

4.2.1 Materials

Textured Vegetable Proteins (TVP), NUTRALYS® TP-C, were obtained from Roquette Frères S.A, (Lestrem, France). Pea protein isolate (PPI Nutralys® S85f, Roquette Frères S.A, France), sunflower oil (Reddy®, Belgium), methylcellulose (METOLOSE® MCE-100TS, Shin Etsu, Japan), sodium chloride (Jozo Naturel tafelzout, The Netherlands), coconut oil (Symrise AG, Germany), vegetable extract red powder (GNT International B.V., the Netherlands), citrus fiber (NUTRAVA®, CP Kelco B.V., the Netherlands), caramel sugar syrup powder (EBC16000, GNT International B.V., the Netherlands), potato starch (M6, Azelis, Belgium) and garlic powder (Dalamaya, the Netherlands) were used as main ingredients of the patties. Beef flavor was kindly provided by Symrise AG (Holzminden, Germany).

4.2.2 Sample preparation

Nine PBMA patties were prepared using TVPs differing in hydration level and particle size (3 TVP hydration levels × 3 TVP particle sizes) following the formulation as outlined in **Table 4.1**. The ratio between all ingredients, except water used for TVP rehydration, was kept constant. The amount of water used for TVP rehydration was varied according to TVP hydration level but kept constant across different TVP sizes. With increasing TVP hydration level (incorporation of more water for TVP rehydration), the concentration of

TVP, vegetable extract red powder and all batter ingredients was decreased proportionally.

Table 4.1. Composition of plant-based meat analogue patties prepared for the present study. S/M/L denotes TVP particle size (Small, Medium, Large corresponding to TVP particle surface area of 0.2, 10, 20 mm^2, respectively). The ratios, 1:1; 1:3; 1:5, indicate TVP hydration level as TVP:water weight ratio. The ratio between water for TVP rehydration and TVP was adjusted according to hydration level. The weight ratio between all other ingredients remained constant. The total water content of raw patties, including water used for TVP rehydration and batter preparation, is 52.42, 65.49, 72.93 g/100 g raw patty for 1:1 S/M/L, 1:3 S/M/L and 1:5 S/M/L, respectively.

Ingredient	Content (g/100 g raw patty)		
	1:1 S/M/L	1:3 S/M/L	1:5 S/M/L
TVP preparation			
Water (TVP rehydration)	18.93	41.20	53.87
TVP	18.93	13.73	10.77
Vegetable extract red powder	0.59	0.43	0.34
Batter preparation			
Water (batter preparation)	33.49	24.29	19.06
Sunflower oil	10.41	7.55	5.93
Pea protein isolate	4.97	3.61	2.83
Coconut oil	4.73	3.43	2.69
Methylcellulose	2.60	1.89	1.48
Citrus fiber	1.18	0.86	0.67
Garlic powder	1.18	0.86	0.67
Caramel sugar syrup powder	0.83	0.60	0.47
Potato starch	0.71	0.52	0.40
Sodium chloride	0.59	0.43	0.34
Vegetable extract red powder	0.59	0.43	0.34
Beef flavor	0.24	0.17	0.13
Total	**100**	**100**	**100**

4.2.2.1 Preparation of TVPs differing in hydration level and particle size

To be able to gain a wide range of TVP hydration levels, TVP (NUTRALYS® TP-C, Roquette Frères S.A, France) with a high water absorption capacity (709 ± 5%) was chosen (van Esbroeck et al., 2024). Three rehydration levels, 1:1, 1:3 and 1:5 (ratio of TVP : water), were chosen to rehydrate the TVP to different initial water content. The

water used for TVP rehydration was mixed with vegetable extract red powder to color the TVPs (**Table 4.1**).

Three TVP particle sizes, coded as Small (S), Medium (M) and Large (L), were obtained by blending the dry or hydrated TVP with different protocols. To obtain small TVP, dry TVP (30 g) was blended in a blender (Magimix Cuisine 5200, France) for 3 min to obtain a fine-ground powder. The small TVP was then mixed with cold water (4°C) in weight ratios of 1:1, 1:3 and 1:5 and kept at 4°C for 60 min to allow for rehydration. To prepare medium and large TVPs, dry TVPs (30 g) were first mixed with cold water (4°C) in weight ratios of 1:1, 1:3 and 1:5 and kept at 4°C for 60 min to allow for rehydration. To obtain TVP particles of medium particle size, the hydrated TVPs were then blended in the blender (Magimix Cuisine 5200, France) for 45 s (1:1 hydration level), 15 s (1:3 hydration level) and 6 s (1:5 hydration level). To obtain large TVP particle size, the rehydrated TVPs were blended in the blender for 30 s (1:1 hydration level), 5 s (1:3 hydration level) and 3 s (1:5 hydration level). The particle size of the blended TVPs was characterized by image analysis of scanned pictures of particles that were manually distributed in an acrylic tray, based on earlier work (Zhang, Brouwer, et al., 2024, **Chapter 2**). The TVP particle size is expressed as an average surface area.

Three distinct TVP particle size levels - S (average particle surface area 0.2 mm^2) , M (average particle surface area 10 mm^2) and L (average particle surface area 20 mm^2) - were obtained at each hydration level (**Supplementary Figure S4.1**), confirming that adapting the blending time effectively produced TVP particles in three sizes regardless of TVP hydration level.

4.2.2.2 Batter preparation

For the batter preparation, beef flavor was first dispersed in sunflower oil and kept at 4°C before use. Pea protein isolate, methylcellulose, citrus fiber, garlic powder, caramel sugar syrup powder, potato starch, sodium chloride, and vegetable extract red powder were weighed (**Table 4.1**) and mixed in a Thermomixer (Vorwerk Thermomix® TM6, Wuppertal, Germany) at setting 3.5 (800 rotations/min) for 20 s at room temperature. The cold sunflower oil and beef flavor mixture was then added and mixed with the

Thermomixer at setting 3.5 (800 rotations/min) for 60 s, scraped and mixed with a spatula, and mixed again at setting 3.5 (800 rotations/min) for 20 s. Cold water (4°C) was added to the mixture and mixed at setting 4 (1100 rotations/min) for 30 s, followed by scraping and mixing with a spatula, and mixed at setting 5.5 (2550 rotations/min) for 45 s at room temperature. The batter was then stored at 4°C for 60 min.

4.2.2.3 Patty preparation

The cooled batter (4°C) and cooled rehydrated TVPs (4°C) were mixed together using a KitchenAid mixer (Benton Harbor, USA) at setting 2 for 30 s at room temperature. Chopped coconut oil (diameter 1 to 5 mm) was added and mixed with the KitchenAid mixer at setting 2 for another 30 s. After mixing, 110 g of the batter mixture was shaped into patties (diameter 80 mm, height 20 mm) using a burger shaper (diameter 80 mm). The patties were stored at -20°C for 2 h and then transferred into plastic bags (dimension: 200 × 300 mm; thickness: 85 µm; material: polyamide + polypropylene; Disposable Discounter, The Netherlands). After removing 95% of the air from the bags with a vacuum packaging machine (Henkovac M2, The Netherlands), the bags were sealed and the patties were stored at -20°C for a maximum of 2 weeks before use.

Patties were removed from the freezer and thawed at room temperature for 60 min. Patties were cooked sous vide in a water bath (75°C water temperature) for 60 min to ensure that they reached a core temperature of 75°C. Subsequently, they were removed from the bags and grilled using a double-plate grill (DeLonghi, Italy) at 200°C for 1 min, with a distance of 2 cm between the two heating plates to ensure proper contact with both sides of the patties. After grilling, patties were allowed to rest in foam boxes for 5 min to reach a core temperature of 55°C before undergoing instrumental characterization or sensory evaluations. Each patty was coded according to its TVP hydration level and TVP particle size as 1:1S, 1:1M, 1:1L, 1:3S, 1:3M, 1:3L, 1:5S, 1:5M, and 1:5L (**Figure 4.1**).

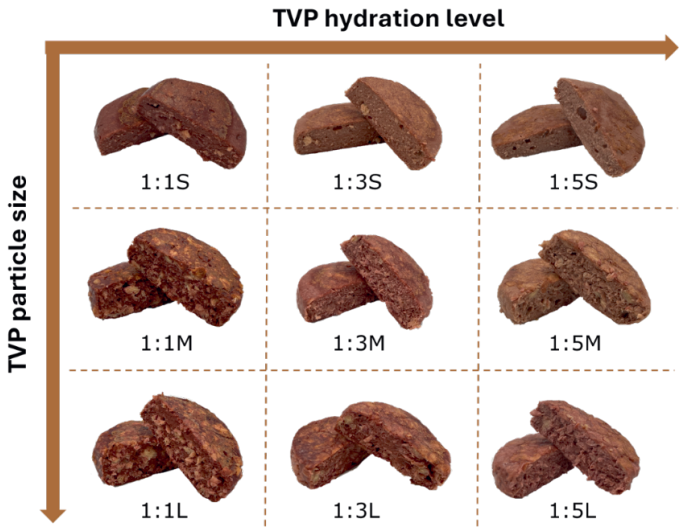

Figure 4.1. Pictures of PBMA patties with the respective sample codes. S/M/L denotes TVP particle size (Small, Medium, Large corresponding to TVP particle surface area of 0.2, 10, 20 mm^2, respectively). Ratios (1:1; 1:3; 1:5) indicate TVP hydration level as TVP:water weight ratio.

4.2.3 Characterization of patty properties

4.2.3.1 Compositional properties

4.2.3.1.1 Water content of grilled patties

The water content of grilled patties after sous vide cooking and grilling was determined by analyzing the dry matter content. Pieces of the patties, weighing 9-10 g, were placed in aluminum dishes, weighed (w_0), and dried in an air oven (Venticell, Czech) at 105°C for 16 – 18 h until constant weight. After drying, samples were cooled down in a desiccator and weighed again (w_1). The water content (WC) of patties was calculated as $WC = (w_0 - w_1)/w_0 \times 100\%$. Measurements were performed in triplicate.

4.2.3.1.2 Fat content of grilled patties

The fat content of grilled patties after sous vide cooking and grilling was determined by NutriControl (the Netherlands), using the acid hydrolysis method (ISO 6492, International Organization for Standardization, 1999). Dried grilled patties (4-5 g) were weighed (f_0) and suspended in 3 M HCl to hydrolyze proteins. The hydrolyzed samples were washed with water and dried in an air oven for 16 h at 60°C. The fat from the dried

residue was extracted using petroleum ether with a Soxhlet apparatus (Foss-Hydrotec 8000, Denmark). After fat extraction, the petroleum ether was evaporated by air drying to obtain the fat as residue, which was weighed (f_1). The fat content of patties was calculated based on total dry matter content, as FC = $f_1/f_0 \times 100\%$. Measurements were performed in duplicate.

4.2.3.1.3 Cooking loss

Three types of cooking losses were determined: total cooking loss, water cooking loss and fat cooking loss. The total cooking loss (TCL) was determined by quantifying the weight difference of patties before sous vide cooking (m_0) and after grilling (m_1), as TCL = $(m_0 - m_1)/m_0 \times 100\%$. The water cooking loss (WCL) was determined as the difference between the initial water content of raw patties before sous vide cooking (IW, including water for TVP rehydration and batter preparation) (**Table 4.1**) and the water content of grilled patties on the basis of a raw patty (**section 4.2.3.1.1**), as WCL = $[IW - WC \times (1 - TCL)] \times 100\% = [IW - (w_0 - w_1)/w_0 \times (1 - (m_0 - m_1)/m_0)] \times 100\%$. The fat cooking loss (FCL) was determined as the difference between the initial fat content of raw patties before sous vide cooking (IF, including sunflower oil and coconut oil) (**Table 4.1**) and the fat content of grilled patties on the basis of a raw patty (**section 4.2.3.1.2**), as FCL = $[IF - FC \times (1 - WC) \times (1 - TCL)] \times 100\% = [IF - f_1/f_0 \times (1 - (w_0 - w_1)/w_0) \times (1 - (m_0 - m_1)/m_0)] \times 100\%$. All measurements were performed in triplicate.

4.2.3.2 Texture properties

4.2.3.2.1 Uniaxial compression test

A Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) equipped with a 50 kg load cell and a stainless cylinder probe (diameter 50 mm) was used to perform a compression test. Cylindrical specimens with a diameter of 30 mm and height of 20 mm were cut from the center of the grilled patties using a cylindrical cutter. Compression was applied up to a strain of 80% of the specimens initial height at a constant compression speed of 5 mm/s. From the stress-strain curves, the Young's modulus was extracted as the slope in the strain range from 0 to 14%. All measurements were done in triplicate.

4.2.3.2.2 Blade cutting test

A Texture Analyzer equipped with a 10 kg load cell and a stainless blade (70 mm length, 60 mm width, 0.1 mm thickness, reversible 14° edge) was used. The blade cutting tests were performed on the entire patty (diameter 80 mm, height 20 mm) at a constant speed of 5 mm/s. The platform features a bottom hole matching the shape of the blade, allowing the blade to completely cut through the lower surface of the patties reaching 120% strain. From the obtained force-time curve (**Supplementary Figure S4.2**), cutting work was calculated. The cutting work was defined as the energy used (calculated as area under curve multiply cutting speed) for the blade to cut from the initial cutting point through the entire patty until reaching 100% strain. Cutting work was calculated as the area under the force-time-curve multiplied by the cutting speed. All measurements were performed in triplicate.

4.2.3.3 Serum release under compression

Serum release under compression was determined using the setup previously described by Zhang, Brouwer, et al. (2024) (**Chapter 2**). In brief, a texture analyzer equipped with a 50 kg load cell and a stainless cylinder probe (diameter 50 mm) was used. The cylinder probe was enclosed within a cylindrical hood slightly larger than the probe to perform a confined compression. The cylindrical hood was attached to the top of a chamber, which was fixed to the base of the Texture Analyzer and connected to a vacuum pump (vacuum level 7.0 mbar, vacuubrand, Germany). A stainless steel filter (pore size 1 mm, porosity 36%) was fixed on top of the chamber with a rubber seal to collect the serum released under compression in an aluminum tray inside the chamber. The cylindrical specimens (diameter 30 mm, height 20 mm) were cut from the center of the patties, weighed (s_0) and placed between filter and probe. The compression was performed up to a strain of 80% of the specimens initial height at a constant speed of 5 mm/s. The probe was held in this position (80% strain) for 90 s. After compression, the specimens were removed and weighed (s_1). Serum release under compression (SR) was calculated as $SR = (s_0 - s_1)/s_0 \times 100\%$. Measurements were performed in triplicate.

The water content of the released serum collected in the aluminum tray was quantified (see **section 4.2.3.1.1**). Measurements were performed in triplicates.

4.2.4 Sensory evaluation

4.2.4.1 Participants

Participants (n = 69, 50 females and 19 males, 24.3 ± 3.8 years, mean ± SD) were recruited from Wageningen and surroundings. Inclusion criteria were good general health (self-reported), BMI between 18.5 – 30 kg/m^2, no dental issues, no swallowing issues, normal ability to taste and smell, being non-smoker, no allergies or intolerances to legumes, and not being pregnant. All participants (n = 69) assessed all PBMA patties in duplicate in the sensory booths at the Human Research Facilities of Wageningen University. The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki (2013). Participants signed an informed consent form and received financial reimbursement after the completion of the session.

4.2.4.2 Rate-All-That-Apply (RATA) and liking

The sensory properties of PBMA patties were evaluated using the Rate-All-That-Apply (RATA) methodology. Participants attended one familiarization session (30 min) and two RATA test sessions (60 min each), which included the assessment of liking. During the familiarization session, participants were introduced to the study and familiarized with the RATA evaluation procedures. Participants were provided with the sensory attributes (including definitions) (**Table 4.2**), and tasted four patties to get familiar with the texture and flavor attributes. Texture familiarization was performed with 1:1S and 1:5L patties that did not contain additional flavorings (i.e., beef flavor and garlic powder). Participants were told that these two patties represented the textural extremes within the sample set. Flavor familiarization was performed with two 1:3M patties, one with added beef flavor and garlic powder, and one without. This was done to ensure that participants recognized the added flavors, namely savory/meat flavor and garlic flavor.

In each RATA test session, all nine samples (1:1S, 1:1M, 1:1L, 1:3S, 1:3M, 1:3L, 1:5S, 1:5M, and 1:5L) (**Figure 4.1**) were evaluated (n=69, duplicate). The nine sensory

attributes were divided into two blocks of five texture attributes (juiciness, hardness, chewiness, crumbliness and fattiness) and four taste/flavor attributes (saltiness, garlic flavor, beany flavor, savory/meat flavor). The definitions of attributes were provided to participants during each session (**Table 4.2**). The order of the two attribute blocks and the order of attributes within each block were randomized over participants, but were kept constant across samples per participant per session. Patties were presented monadically in random order with 3-digit codes using pre-warmed bowls. For each patty (around 15 g), participants were instructed to take two bites, with each bite focusing solely on the sensory attributes of the presented attribute block (either texture or taste/flavor), and chew as they were accustomed to. For each evaluation, participants first indicated which sensory attributes were applicable to describe their perception of the patty, followed by indicating the intensity of the selected sensory attributes on a 9-point scale marked from "1-low intensity" to "9-high intensity".

Following the RATA evaluation, participants were asked to rate overall liking of the patty (n=69, duplicate) using a 9-point hedonic scale marked with "1-dislike extremely", "2-dislike very much", "3-dislike moderately", "4-dislike slightly", "5-neither like nor dislike", "6-like slightly", "7-like moderately", "8-like very much" and "9-like extremely". Crackers and water were provided for cleansing the palate after evaluating each sample. Data were collected in English using EyeQuestion software (Logic8 B.V., the Netherlands).

Table 4.2 Sensory attributes and definitions used in RATA evaluation of PBMA patties.

Attribute	Definition
Texture	
Juiciness	Sensation of moisture/juice/liquid being released from food during consumption.
Hardness	Force applied by the (molar) teeth to bite through the food.
Chewiness	Effort required to masticate the food until it is ready to be swallowed.
Crumbliness	Extent to which the food breaks up into particles in the mouth during the first few chews.
Fattiness	Sensation of fat in the mouth.
Taste and flavor	
Saltiness	Salty taste sensation.
Garlic flavor	Garlic flavor sensation.
Beany flavor	Flavor related to beans and legumes.
Savory/meat flavor	Flavor related to savory foods and meats.

4.2.5 Data analysis

Results are reported as mean values with standard deviation (SD). To investigate the effect of TVP hydration level and TVP particle size on patties' physicochemical properties, two-way ANOVA followed by Tukey post-hoc analyses were performed. TVP hydration level, TVP particle size and the interaction between TVP hydration level and TVP particle size were set as fixed factors. To investigate the effect of TVP hydration level and TVP particle size on patties' sensory properties, linear mixed models (LMM) followed by Tukey post-hoc analyses were performed. In the LMM analysis, TVP hydration level, TVP particle size and the interaction between TVP hydration level and TVP particle size were treated as fixed factors, participants and replications as random factor.

A class of Undirected Graphical Models (UGMs) (Behrouzi et al., 2023; Behrouzi & Wit, 2019) was employed to estimate associations among sensory properties and between physicochemical and sensory properties. UGMs, referred to as network analysis, are probabilistic graphical models that represent variables as nodes, with edges between them representing conditional dependencies. These conditional dependencies are captured through partial correlations, which measure the association between two variables while controlling for the influence of all other variables in the model. This framework was used to explore complex association patterns and to distinguish direct from indirect relationships. By focusing on the conditional dependencies, a more precise understanding of how sensory properties are interconnected and how sensory properties relate to physicochemical properties was gained.

Data analysis was performed using RStudio (version 2022.07.0, PBC) with the packages emmeans (Lenth, 2022), lmerTest (Kuznetsova et al., 2017), lme4 (Bates et al., 2015), Hmisc (Harrell & Dupont, 2023), netgwas (Behrouzi et al., 2023) and GraphPad Prism (version 10.0.0, GraphPad Software, USA). A significance level of $p < 0.05$ was chosen.

4.3. Results and Discussion

4.3.1 Characterization of physicochemical properties of cooked PBMA patties

The compositional, texture and serum properties of cooked PBMA patties with the results of the statistical data analysis are summarized in **Supplementary Table S4.1**, visualized in **Figure 4.2** to **4.4**, and discussed in section 4.3.1.1 to 4.3.1.3, respectively.

4.3.1.1 Compositional properties

To gain insights into the effect of TVP hydration level and TVP particle size on composition of PBMA patties after cooking, **Figure 4.2** shows the total cooking loss, water cooking loss and fat cooking loss together with the water and fat content of cooked patties.

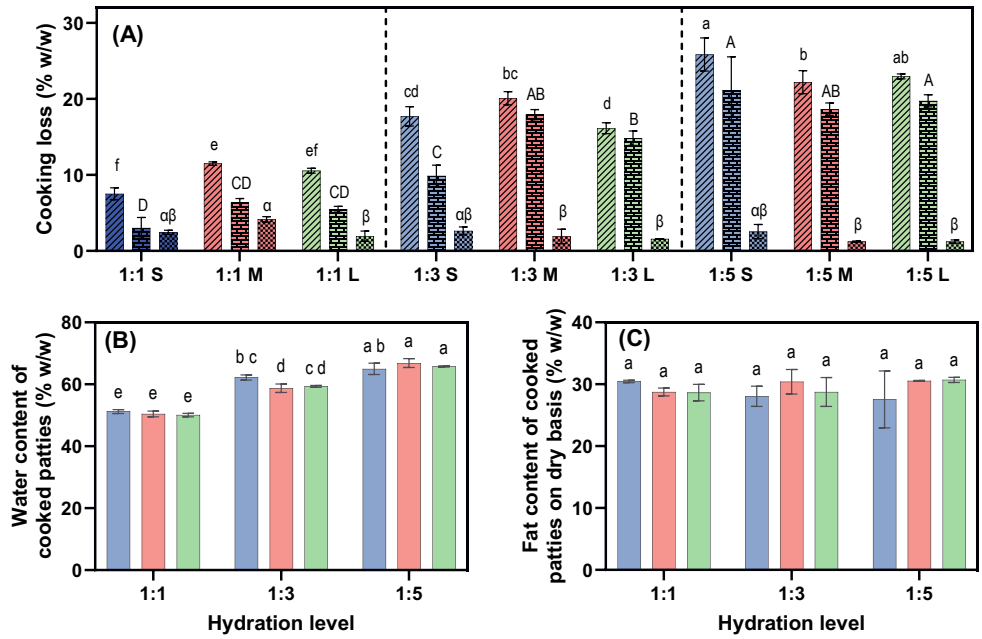

Figure 4.2 (A) Total cooking loss (stripe pattern and lower case letters), water cooking loss (brick pattern and capital letters) and fat cooking loss (check pattern and Greek letters), (B) water content on wet basis and (C) fat content on dry basis of cooked patties prepared from TVP differing in hydration level (expressed as TVP:water weight ratio, 1:1, 1:3, 1:5) and particle size (S=Small (0.2 mm^2) blue bar; M=Medium (10 mm^2) red bar; L=Large (20 mm^2) green bar). Different letters indicate significant differences between means ($p < 0.05$). Error bars represent standard deviation.

Total cooking loss and water cooking loss both significantly and strongly increased with increasing TVP hydration level ($F = 361.1$, $p < 0.001$; $F = 173.5$, $p < 0.001$, respectively) due to the increased initial water content (**Figure 4.2 (A)** and Supplementary **Table S4.1**). These findings are consistent with previous studies that reported an increase in cooking loss due to higher water incorporation in raw patties prepared with higher rehydration capacity TVPs (Hong et al., 2022; Sakai et al., 2021). Compared to the effect of TVP hydration level, the effect of TVP particle size on total cooking loss ($F = 3.7$, $p < 0.05$) and water cooking loss ($F = 7.1$, $p < 0.01$) was significant and small, with no clear trend. Since one type of TVP blended into different sizes was used in the current study, this suggests that the porous structure (air pockets) of TVPs may not be the main factor influencing cooking loss. The material itself (such as ingredients, processing conditions) seems to play a more significant role. A similar conclusion was reported in patties made from various types of intact and ground TVPs (van Esbroeck et al., 2024). Compared with water cooking loss, fat cooking loss contributed much less to total cooking loss, even though a significant and small effect was observed with respect to TVP hydration level, particle size and their interaction ($F = 7.2$, $p < 0.05$; $F = 5.4$, $p < 0.05$; $F = 4.5$, $p < 0.05$, respectively) (**Figure 4.2 (A)** and Supplementary **Table S4.1**). These small effects resulted from calculating fat cooking loss on wet basis, so that fat cooking loss was depended on water cooking loss.

While increasing TVP hydration level led to more water loss during cooking, the higher initial water content of raw patties resulted in a significantly higher water content of cooked PBMA patties ($F = 497.9$, $p < 0.001$). This was not significantly influenced by TVP particle size ($F = 2.6$, $p = 0.104$), although water content of cooked PBMA patties with 1:3 hydration level ($p < 0.05$) was significantly lower than water content of cooked PBMA patties with 1:1 hydration level. Consequently, the interaction effect hydration:particle size on water content of cooked patties ($F = 5.2$, $p < 0.01$) was significant but small (**Figure 4.2 (B)** and Supplementary **Table S4.1**). In contrast to water content of cooked patties, the fat content of cooked patties (on dry basis) was not significantly influenced by TVP hydration level ($F = 0.1$, $p = 0.898$), particle size ($F = 0.6$, $p = 0.588$) or their interaction ($F = 1.1$, $p = 0.396$) (**Figure 4.2 (B)** and

Supplementary **Table S4.1**). Since all patties had the same fat content (on dry basis) before cooking (31.8%, **Table 4.1**), this finding indicates that fat loss during cooking, and therefore fat retention, remained constant despite differences in TVP hydration level and particle size. The additional water release associated with higher TVP hydration level did not induce more fat release during cooking, suggesting that fat is well enclosed within the patty matrix.

To summarize, increasing initial water content of raw patties by adjusting TVP hydration level, provided higher water cooking loss, and total cooking loss, while keeping fat loss constant. Despite the increase in water cooking loss, higher TVP hydration level still contributed to higher water retention in the cooked/grilled patties.

4.3.1.2 Texture properties

As the composition of cooked patties varied across patties differing in TVP hydration and particle size, also differences in textural properties were expected. **Figure 4.3 (A)** and **Supplementary Table S4.1** show that the Young's modulus of patties significantly decreased with increasing TVP hydration level ($F = 44.0$, $p < 0.001$) and TVP particle size ($F = 10.8$, $p < 0.001$). The effect of TVP hydration level on Young's modulus is attributed to the higher water content retained in cooked patties due to increasing TVP hydration, in line with previous studies (Hong et al., 2022; Munialo & Vriesekoop, 2023; Wi et al., 2020). Concerning the TVP particle size, patties with small TVP particles displayed the highest Young's modulus, while those with medium and large TVP particles did not differ in Young's modulus (**Figure 4.3 (A)**). This can be explained by the denser structure of PBMA patties prepared from small TVP particles, as these were ground into powders, losing their porosity and air pockets (Godschalk-Broers et al., 2022; van Esbroeck et al., 2024).

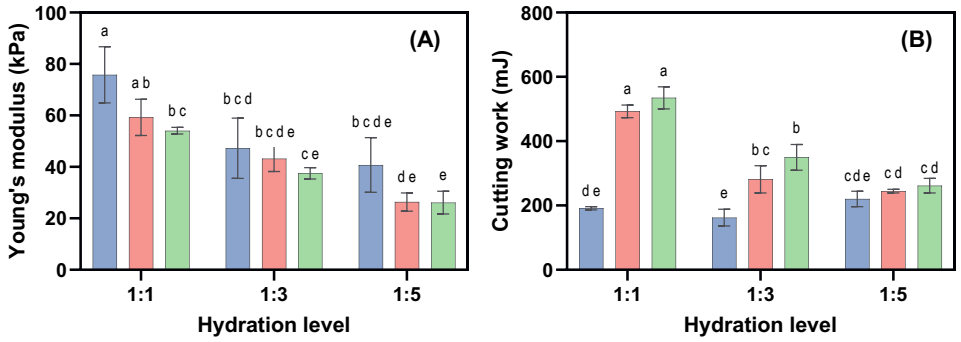

Figure 4.3 (A) Young's modulus determined from uniaxial compression tests and (B) cutting work determined from blade cutting tests of cooked PBMA patties prepared from TVP differing in hydration level (expressed as TVP:water weight ratio, 1:1, 1:3, 1:5) and particle size (S=Small (0.2 mm^2) blue bar; M=Medium (10 mm^2) red bar; L=Large (20 mm^2) green bar). Different letters indicate significant differences between means ($p < 0.05$). Error bars represent standard deviation.

The cutting work was more affected by the larger particle size, especially in patties with lower water content. Increasing TVP particle size significantly increased cutting work ($F = 118.9$, $p < 0.001$) (**Figure 4.3 (B)** and **Table S4.1**). Cutting work represents the effort required to cut through the entire patty. Patties prepared from small TVP particles required less cutting work probably because the blade could easily cut through the patty between small TVP particles without cutting through individual TVP particles. In contrast, patties prepared from medium and large TVP particles required higher cutting work probably due to resistance from individual TVP particles that have to be cut through (Giménez-Ribes et al., 2024; Oppen et al., 2023). Increasing TVP hydration level significantly decreased cutting work of PBMA patties prepared from medium and large, but not from small TVP particles ($F = 94.1$, $p < 0.001$) (**Figure 4.3 (B)** and **Table S4.1**). This can be explained by the fact that increasing TVP hydration level weakened the strength of the medium and large TVP particles itself, allowing the cutting process to become easier. As small particles did not require cutting of the TVP particles themselves, hydration level did not have a significant effect. These interdependent effects of TVP hydration level and particle size on cutting work are underpinned by a significant hydration:particle size interaction effect ($F = 28.6$, $p < 0.001$) (**Table S4.1**).

These results show that we successfully varied the texture properties of cooked PBMA patties by adjusting TVP hydration level and particle size. Increasing TVP hydration level led to a decrease in stiffness of patties, while increasing TVP particle size increased stiffness.

4.3.1.3 Serum properties

Variations in the compositional and texture properties of cooked patties, achieved by varying the TVP hydration level and particle size led to differences in serum properties under compression (**Figure 4.4** and **Supplementary Table S4.1**). A higher TVP hydration level and the related increase in water content of cooked/grilled patties logically resulted in higher serum release during compression ($F = 397.0$, $p < 0.001$) and a higher water content of the released serum ($F = 137.2$, $p < 0.001$). In addition, increasing the TVP particle size significantly reduced serum release under compression ($F = 73.6$, $p < 0.001$), indicating that larger TVP particles were able to retain more water under mechanical compression. This is possibly due to a combination of more intact fibers between air pockets and the presence of air pockets themselves (van Esbroeck et al., 2024). Although the amount of serum was affected, the water content of the released serum was not significantly affected by TVP particle size ($F = 1.0$, $p = 0.384$). This indicates that the serum contained other ingredients as a result of compression. Despite the fact that the exact composition of the released serum could not be determined in the current study due to the limited amount of serum collected, observations during serum release measurements showed that patties with smaller TVP particles released more solid fragments during compression, likely contributing to their higher serum release. In addition, small differences in fat content of the released serum may be present.

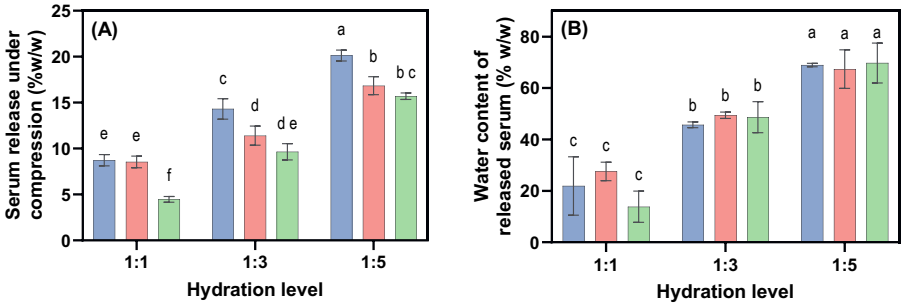

Figure 4.4 (A) Serum release under compression and (B) water content of released serum of cooked patties prepared from TVP differing in hydration level (expressed as TVP:water weight ratio, 1:1, 1:3, 1:5) and particle size (S=Small (0.2 mm^2) blue bar; M=Medium (10 mm^2) red bar; L=Large (20 mm^2) green bar). Different letters indicate significant differences between means ($p < 0.05$). Error bars represent standard deviation.

4.3.2 Sensory properties of PBMA patties

The physicochemical properties of PBMA patties (**section 4.3.1**) showed that PBMA patties with different composition, texture and serum release properties were obtained by varying the initial TVP hydration level and TVP particle size. This section discusses the sensory properties of PBMA patties (**Table 4.3**).

Table 4.3 Intensity of texture, taste and flavor attributes (mean ± SD) obtained from RATA evaluation (9-point hedonic scale), and liking (mean ± SD) from hedonic ratings (9-point hedonic scale) for PBMA patties prepared from TVPs differing in hydration level (expressed as TVP:water weight ratio, 1:1, 1:3, 1:5,) and particle size (S=Small (0.2 mm^2); M=Medium (10 mm^2); L=Large (20 mm^2)) (n = 69, duplicate). Different letters indicate significant differences between samples (p <0.05). F and p values (* p <0.05, ** p< 0.01, *** p < 0.001) are derived from linear mixed model analysis with TVP hydration level, TVP particle size and their interaction (hydration:size interaction) as fixed factors and participant and replication as random factor.

	1:1S	1:1M	1:1L	1:3S	1:3M	1:3L	1:5S	1:5M	1:5L	TVP Hydration level		TVP Particle size		Hydration: Size interaction	
										F	P	F	P	F	P
RATA - Texture attributes															
Juiciness	1.8±1.9^{a}	1.5±1.4^{a}	1.8±1.7^{a}	3.5±2.3^{b}	4.4±2.2^{cd}	4.1±2.3^{bc}	4.7±2.6^{de}	5.4±2.1^{f}	5.4±2.5^{ef}	**412.1**	***	**8.1**	***	**4.8**	***
Fattiness	2.5±2.1^{a}	2.1±2.0^{a}	2.6±2.1^{a}	3.4±2.3^{b}	3.6±2.3^{bc}	3.9±2.2^{bc}	4.1±2.5^{bc}	4.0±2.3^{bc}	4.2±2.4^{c}	**112.0**	***	**3.2**	*	1.3	0.283
Hardness	2.8±2.4^{ab}	4.8±2.3^{c}	4.6±2.3^{c}	1.9±1.7^{de}	3.0±2.1^{a}	3.1±2.2^{a}	1.5±1.4^{d}	2.0±1.8^{de}	2.2±1.9^{be}	**197.5**	***	**86.9**	***	**8.3**	***
Chewiness	2.6±2.1^{a}	5.6±2.1^{b}	5.7±1.8^{b}	2.2±1.8^{a}	4.1±2.0^{c}	5.4±1.9^{b}	2.6±1.8^{a}	4.6±2.2^{cd}	5.1±2.2^{bd}	**18.3**	***	**314.2**	***	**5.9**	***
Crumbliness	4.6±2.8^{a}	4.3±2.4^{a}	4.1±2.4^{ab}	3.8±2.8^{ac}	3.1±2.3^{cd}	3.2±2.6^{cd}	2.7±2.7^{d}	2.5±2.5^{bcd}	2.5±2.5^{cd}	**39.8**	***	1.3	0.267	**3.9**	**
RATA - Taste and flavor attributes															
Saltiness	2.9±2.1^{ab}	3.2±2.1^{a}	3.3±2.3^{a}	2.4±1.9^{bcd}	2.5±2.0^{bc}	2.7±2.1^{b}	1.5±1.5^{e}	2.1±1.8^{cd}	1.8±1.8^{de}	**93.5**	***	**8.3**	***	1.4	0.225
Garlic flavor	2.8±2.6^{a}	3.2±2.5^{ab}	3.2±2.6^{ab}	3.0±2.3^{ab}	3.6±2.6^{b}	3.2±2.4^{ab}	1.7±2.1^{c}	3.0±2.5^{ab}	3.1±2.3^{ab}	**14.7**	***	**20.6**	***	**4.7**	***
Savory/meat flavor	3.1±2.5^{ab}	3.6±2.3^{a}	3.7±2.3^{a}	3.0±2.1^{bc}	3.6±2.3^{ac}	3.6±2.3^{ac}	2.5±2.3^{b}	3.4±2.3^{ac}	3.0±2.4^{bc}	**9.7**	***	**18.4**	***	0.6	0.625
Beany flavor	4.1±2.5^{ab}	3.5±2.4^{bc}	3.5±2.4^{abc}	4.2±2.5^{a}	3.3±2.3^{c}	3.4±2.2^{bc}	3.9±2.5^{abc}	3.4±2.5^{bc}	3.3±2.3^{c}	0.6	0.531	**19.1**	***	0.5	0.706
Hedonic rating															
Liking	4.1±1.9^{a}	5.3±1.4^{b}	5.5±1.6^{bc}	4.5±1.7^{a}	6.1±1.4^{d}	5.9±1.4^{cd}	4.4±1.7^{a}	5.5±1.7^{bc}	5.4±1.6^{bc}	**16.7**	***	**114.5**	***	1.7	0.146

4.3.2.1 Texture attributes

With increasing TVP hydration level, juiciness intensity of patties increased significantly and strongly ($F = 412.1$, $p < 0.001$) (**Table 4.3**). This confirms that the increased water content of the cooked patties due to higher hydration levels was sufficient to cause an increase in juiciness perception. Although TVP particle size did impact various instrumental textural properties, its effect on juiciness perception was significant and small ($F = 8.1$, $p < 0.001$), and dependent on TVP hydration level ($F = 4.8$, $p < 0.001$). For patties made with TVPs at higher hydration levels (1:3 and 1:5), small TVP particles resulted in significantly lower juiciness perception ($p < 0.05$). These findings appear to contradict results from serum release measurements, which showed higher serum release from patties with smaller TVP particles. A possible explanation is that the increased serum release in patties with smaller TVP particles was likely due to more solid fragments being pressed out rather than more liquid serum being released. This may have contributed to higher measured serum release during compression but may not necessarily translate to a higher juiciness perception. In addition, the slightly higher Young' modulus for patties with small particles may have contributed negatively to juiciness. At a low TVP hydration level (1:1), patties with different TVP particle size showed similarly low juiciness perception. In this case, the overall low serum release appeared to dominate juiciness perception regardless of TVP particle size. Overall, these findings align with multiple studies suggesting that water content and serum release enhance juiciness perception of meat and plant-based meat products (Xu & Falsafi, 2023; Zhang, Brouwer, et al., 2024, **Chapter 2**).

Even though the fat content of cooked patties was similar and unaffected by TVP hydration level or particle size (**Figure 4.2 (B)** and **Supplementary Table S4.1**), fattiness perception significantly increased with increasing TVP hydration level ($F = 112.0$, $p < 0.001$). Fattiness was slightly affected by TVP particle size ($F = 3.2$, $p < 0.05$), and was not affected by their interaction ($F = 1.3$, $p = 0.283$) (**Table 4.3**). Similar observations have been reported previously, showing that patties had consistent fat content after cooking or during mastication, and yet their fattiness perception differed across patties (Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2 and**

3). Although we could not accurately determine the fat content of the released serum during compression (too little serum volume), the amount of fat present in the serum is most likely too little to explain these results. We hypothesize that fattiness perception in PBMA patties is mainly driven by the amount of serum released from the patty during mastication and the properties of the released serum, rather than the actual fat content of the patties.

Similar to instrumental texture measurements, changes in TVP hydration level showed a stronger effect on hardness intensity ($F = 197.5$, $p < 0.001$) than changes in TVP particle size ($F = 86.9$, $p < 0.001$), while chewiness was more strongly influenced by TVP particle size ($F = 314.2$, $p < 0.001$) rather than by hydration level ($F = 18.3$, $p < 0.001$) (**Table 4.3**). These results were expected, as hardness reflects the perceived compression force when biting. Increased water content significantly decreased the Young's modulus of cooked patties (**Figure 4.5 (A)**), decreasing stiffness and leading to lower hardness perception. On the other hand, chewiness reflects the effort required for mastication, and larger TVP particle sizes (less blended TVPs) required more effort to break patties down, resulting in a chewier perception. The influence of particle size on chewiness perception is in agreement with findings from other studies reviewing solid and semi-solid foods (Bolhuis & Forde, 2020; De Wijk & Prinz, 2006). Crumbliness significantly decreased with increasing hydration level ($F = 39.8$, $p < 0.001$) and was not affected by TVP particle size ($F = 1.3$, $p = 0.267$) (**Table 4.3**). This reduction in crumbliness may be due to more serum being released during mastication in more hydrated patties, which might increase lubrication and reduce crumbliness perception, as has previously been observed in literature (Devezeaux de Lavergne et al., 2016).

4.3.2.2 Taste and flavor attributes

With increasing TVP hydration level, the intensity of saltiness, garlic flavor and savory/meat flavor significantly decreased ($F = 93.5$, $p < 0.001$, $F = 14.7$, $p < 0.001$, $F = 9.7$, $p < 0.001$, respectively) (**Table 4.3**). These taste and flavor sensations are linked to the concentration of sodium chloride, garlic powder and beef flavor, respectively. With increasing TVP hydration level, the amount of salt and flavorings was diluted

proportionally in the raw patties (**Table 4.1**). Some of the salt and flavorings were probably removed with the cooking loss after preparation, and the lower amount in the patties was even more diluted by the higher amount of water retained in the patties after cooking. The decrease in perceived saltiness with increasing TVP hydration level was more pronounced compared to the marginal decline in garlic and savory/meat flavor perception. This difference may be due to the fact that saltiness is easier for untrained participants to assess compared to garlic and savory/meat flavor, even at relatively low intensity. In addition, the small effect of TVP hydration level on garlic and savory/meat flavors may be due to additional release of volatile compounds during mastication, which compensated the decrease in flavoring concentrations and reduced their perceived differences. The significant impact of TVP particle size on saltiness, garlic flavor and savory/meat flavor ($F = 8.3$, $p < 0.001$, $F = 20.6$, $p < 0.001$, $F = 18.4$, $p < 0.001$, respectively) was mainly caused by the significantly lower intensity of these attributes in patties with small TVP particle size (S) (**Table 4.3**). This could be attributed to the stiffer texture of patties with small TVP particles (**Figure 4.5**), which may hinder flavor release during mastication, resulting in a lower perceived flavor intensity.

Unlike other taste and flavor attributes evaluated in this study, beany flavor was not caused by added flavorings but was inherent to the pea-based TVP and pea protein isolate used to prepare the patties. Beany flavor was perceived more intensely when TVP particle size decreased ($F = 19.1$, $p < 0.001$), while it was not significantly influenced by the TVP hydration level ($F = 0.6$, $p = 0.531$) (**Table 4.3**). We speculate that decreasing TVP particle size might have increased the contact area between TVP particles and the oral surface, thereby increasing the exposure of pea protein during sensory testing, and leading to a higher intensity of beany flavor. As hydration level did not have an effect, beany flavor seems to be more related to a direct contact of the TVP material with the oral surfaces, and not related to a release of extracted compounds via compressed serum.

4.3.2.3 Liking

Compared to the significant effect of TVP hydration level on liking ($F = 16.7$, $p < 0.001$), TVP particle size showed a significant and stronger effect ($F = 114.5$, $p < 0.001$) (**Table 4.3**). This stronger effect can be attributed to the significantly lower liking for patties with small TVP particle size, potentially due to their pronounced beany taste and/or softer, less chewy and more crumbly texture. TVP hydration level and particles had an independent effect on liking (interaction effect TVP hydration level: particle size: $F = 1.7$, $p = 0.146$). Within each TVP size category (S, M or L), PBMA patties with a hydration level of 1:3 had the highest liking. Within each TVP hydration level (1:1, 1:3 or 1:5), patties with small TVP particle size had the lowest liking. Consequently, the 1:3M and 1:3L patties were the most liked samples. These results suggest that higher serum release does not necessarily lead to higher liking, an optimal level of juiciness may be more desirable. Juiciness remains an important driver of liking, but a well-balanced texture profile is crucial.

4.3.2.4 Relationships between sensory attributes via network analysis

To investigate the relationships between texture, flavor and liking of the PBMA patties, network analysis (Undirected Graphical Models) was performed and the results are summarized in **Figure 4.5** and **Supplementary Table S4.2**. Edges represent conditional dependencies between nodes (sensory attributes) from partial correlations, and the absence of edges between nodes indicates conditional independence between nodes (sensory attributes). The thickness of edges reflects the absolute value of the partial correlation coefficient and the color indicates positive (blue) and negative (red) correlations.

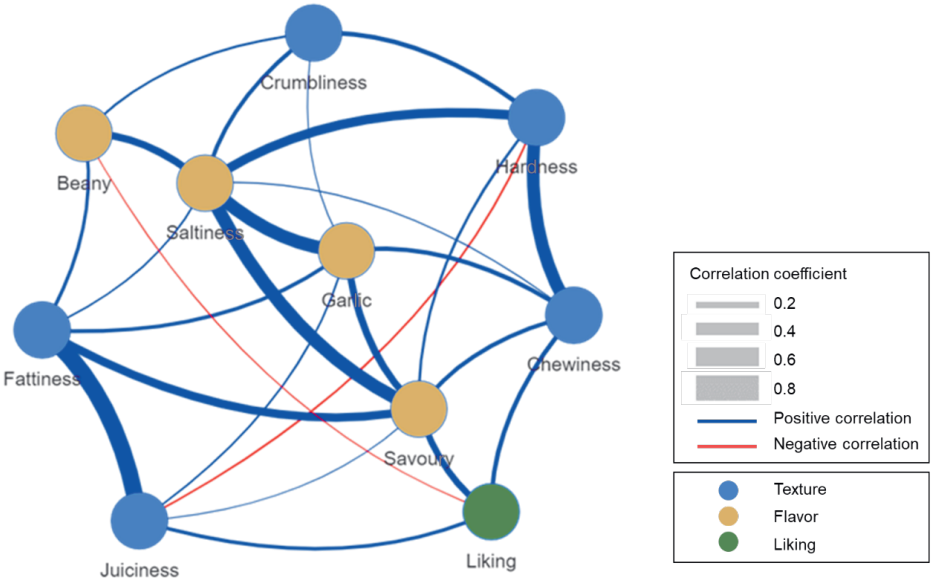

Figure 4.5. Sensory network showing simultaneous associations between texture (blue nodes) and flavor (yellow nodes) attributes obtained from RATA evaluation (n = 69, duplicate) and overall liking (green node) from hedonic rating (n = 69, duplicate).

Most partial correlation coefficients tended to be small, (**Figure 4.5** and **Table S4.2**), which can be explained by the complex interrelationships among variables. Partial correlations quantify the direct association between two variables while accounting for the influence of other variables that might confound the relationship. In systems with strong indirect associations or high multicollinearity, the direct association between any two variables often becomes weak or even close to zero due to the overlapping effects of other variables. Despite their small values, these partial correlation coefficients remain meaningful in understanding the direct relationships among these attributes.

Among the texture attributes, juiciness was positively and partially correlated with fattiness, and negatively and partially correlated with hardness (**Figure 4.5**). These correlations were expected and align with previous studies that reported strong correlations between juiciness and fattiness and weaker correlations between juiciness and hardness in both static and dynamic sensory evaluations (Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2 and 3**). Juiciness and fattiness were

independent of chewiness and crumbliness, while chewiness and crumbliness were positively and partially correlated with hardness (**Figure 4.5**). Both chewiness and crumbliness are sensations linked to the structural breakdown of the food, which is influenced by the patty's structure and hardness. Therefore, it is reasonable that these two attributes were correlated with hardness. Apparently, lack of a correlation between these attributes and juiciness indicates that structural breakdown of the patties is less relevant for juiciness. This is consistent with results obtained in our previous work, which showed that juiciness and fattiness were primarily impacted by serum release during early stages of mastication rather than the subsequent oral structural breakdown at later stages of mastication (Zhang, Sala, et al., 2024, **Chapter 3**).

Among the flavor attributes, strong, positive and partial correlations were found between saltiness, garlic flavor and savory/meat flavor (**Figure 4.5**). As discussed previously, these taste and flavor attributes were determined by the addition of flavorings. Their concentrations simultaneously decreased with increasing TVP hydration level, which explains their correlations. Beany flavor was positively and partially correlated with saltiness after controlling for the effect of other flavor attributes. As mentioned earlier, beany flavor may arise more from the increased contact area of the oral surface with the pea-based TVPs rather than compounds extracted in the serum, particularly when the TVP particle size is reduced. This increased contact area could also lead to greater exposure to sodium chloride inherently presented in the TVPs, thereby increasing saltness perception.

Texture attributes were correlated with various taste and flavor attributes. Juiciness was positively and partially associated with garlic and savory/meat flavors (**Figure 4.5**), indicating that juiciness might enhance flavor perception in PBMA patties. As mentioned previously, the concentration of added flavorings decreased proportionally with increasing TVP hydration level. So even though flavor concentration was decreased, flavor perception was increased through enhanced serum release and accompanying increase in juiciness. Flavor perception therefore seems to be driven by an absolute amount of flavorings instead of the concentration. Fattiness showed direct associations with all four taste and flavor attributes (**Figure 4.5**). Moreover, the

savory/meat flavor showed a stronger association with fattiness than with juiciness. The increased serum release during mastication might also result in a higher amount of fat being released, which could enhance the perception of the meaty flavoring used, as it contains a large proportion of hydrophobic molecules. However, this speculation should be interpreted with caution, as the actual fat content in the released serum was not quantified in the current study. Saltiness, garlic and savory/meat flavor were also positively and partially correlated with hardness and chewiness (**Figure 4.5**). Since chewiness was positively affected by TVP particle size (**Table 4.3**), larger TVP particles likely require more chewing to break down, and this prolonged mastication time may enhance flavor release. Similarly, the weak positive correlation between crumbliness and beany flavor can also be attributed to TVP particle size. As discussed earlier, reducing TVP particle size increased the number of particles and created a larger contact area between pea-proteins and oral surfaces, leading to higher crumbliness and beany flavor.

Liking was positively and partially associated with juiciness, chewiness and savory/meat flavor, and negatively associated with beany flavor (**Figure 4.5**). This is in line with literature, highlighting the crucial role of juiciness in driving liking and off-flavor in driving disliking of PBMA patties (Giezenaar et al., 2024; Godschalk-Broers et al., 2022; Sogari et al., 2023; Szenderák et al., 2022). These findings also underscore the importance of chewiness in enhancing consumer preference as a chewy texture more effectively mimics real meat.

4.3.3 Linking physicochemical and sensory properties via network analysis

To better understand the relationships between sensory and physicochemical properties of the PBMA patties, network analysis between 9 sensory properties (texture and flavor attributes) and 8 physicochemical properties of cooked patties was conducted (intermediate properties such as total, water and fat cooking loss were not included). The results are summarized in **Figure 4.6** and **Supplementary Figure S4.3**.

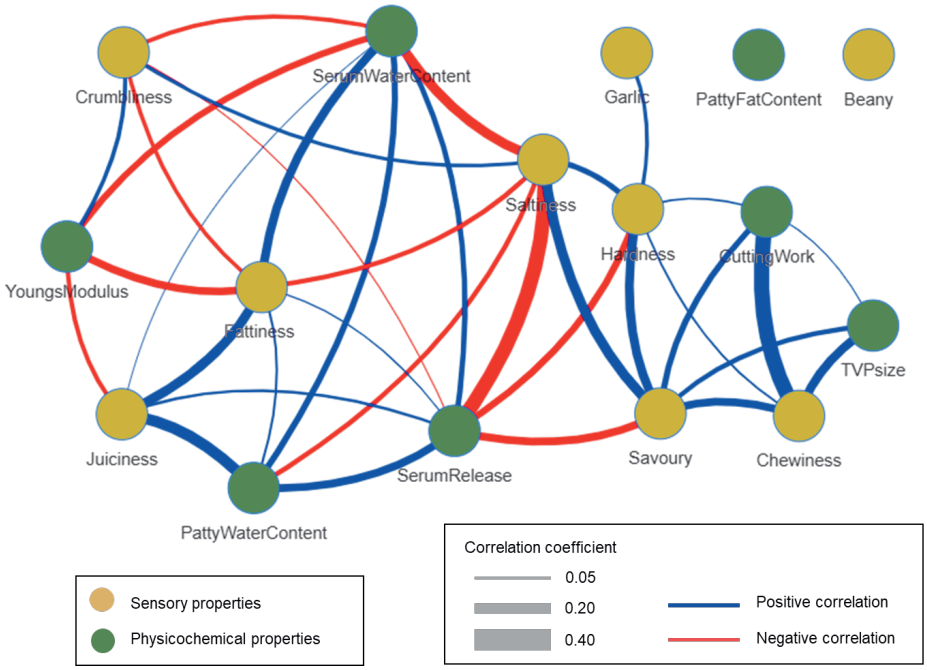

Figure 4.6 Sensory – physicochemical properties network showing simultaneous associations between sensory properties (yellow nodes) and physicochemical properties (green nodes) of PBMA patties prepared from TVPs varying in hydration level and particle size (N = 9).

Figure 4.6 reveals that the sensory – physicochemical network are divided into three clusters: (1) the left cluster represents properties that were mainly influenced by TVP hydration level, (2) the right cluster represents properties that were mainly influenced by TVP particle size, and (3) a third cluster consists two isolated nodes - beany flavor and fat content of cooked patties - which are nodes that lack direct correlations (edges) with any other nodes in the network.

In the left cluster, multiple positive and partial correlations were observed among physicochemical properties. As expected, water content of cooked patties, serum release and water content of released serum were positively and partially correlated to each other. Young's modulus was negatively and partially correlated with both water content of cooked patties and water content of released serum, but not with serum release under compression. These correlations suggest that the amount of serum

release is primarily determined by the water retained in the patty after cooking, rather than by the patty's texture.

Linking physicochemical properties with sensory perception, juiciness was positively and partially correlated with water content of cooked patties, serum release under compression and water content of released serum, and negatively correlated with Young's modulus (**Figure 4.6**). These results confirm that juiciness is primarily influenced by the compositional and texture properties of patties which are driven by changes in TVP hydration level rather than TVP particle size. Interestingly, juiciness showed a stronger correlation with Young's modulus than with serum release, indicating that juiciness is not only linked to serum release but also the initial texture deformation during chewing. Since juiciness is perceived early during mastication (Zhang, Sala, et al., 2024, **Chapter 3**), it makes sense that it relates stronger to the initial properties of patties, such as Young's modulus, which assesses the initial deformation of patties under compression.

Similar to juiciness, fattiness was correlated with Young's modulus, water content of cooked patties, serum release and water content of released serum. It was not correlated with the fat content of patties (**Figure 4.6**). These results confirmed that variations in fattiness perception were mainly influenced by the amount of serum released during compression, as well as changes in the water content of both the patty and released serum, rather than the actual fat content of cooked patties (**section 4.3.2.1**). Interestingly, fattiness showed even stronger correlations with the water content of released serum than between juiciness and water content of released serum. However, as the detailed composition of the serum was not measured in this study, the specific influence of serum composition on fattiness and juiciness perception remains unclear and requires further investigation. Crumbliness was positively correlated with Young's modulus and negatively correlated with serum release and water content of released serum. Saltiness showed negative correlations with water content of cooked patties, serum release and water content of released serum (**Figure 4.6**). These relations align with the discussions in **section 4.3.2.1** and **4.3.2.2.**

In the right cluster, sensory chewiness was positively and partially correlated with cutting work and TVP size, while sensory hardness was positively correlated with cutting work (**Figure 4.6**). These correlations were expected, as variations in TVP particle size influenced blade cutting behavior, and sensory hardness and chewiness (**Table S4.1** and **Table 4.3**). Savory/meat flavor was positively correlated with these instrumental texture properties that were mainly influenced by TVP particle size, while saltiness was related to changes in compositional properties driven by TVP hydration level (**Figure 4.6**). These different correlations between taste and flavor attributes with physicochemical properties confirmed our previous speculation in **section 4.3.2.2** that different mechanism might underlie these perceptions. In short, saltiness is determined by the NaCl concentration in the patties, while savory/meat flavor is related to the flavorings containing also volatile compounds and their release might have varied with variations in texture (which changes chewing behavior).

Unexpectedly, savory/meat flavor was negatively correlated with serum release under compression. This finding seems contradictory to the positive correlation observed between savory/meat flavor and juiciness perception (**Figure 4.5**). One possible explanation is that the differences in savory/meat favor across all patties were relatively small, showing only a slight decreasing trend when increasing TVP hydration levels (**Table 4.3**). Conversely, serum release under compression strongly increased with higher TVP hydration levels (**Table S4.1**). The use of average values when preparing the sensory – physicochemical network may have exaggerated this small trend, leading to the observed negative correlation. In contrast, the sensory network correlation between juiciness and fattiness was based on individually paired sensory evaluations, providing more robust results. This suggests that while serum release is strongly associated with juiciness, it does not fully define it.

Beany flavor, as another isolated node, showed no direct associations with TVP particle size (**Figure 4.6**), although TVP particle size showed a significant effect on beany flavor in the linear mixed model (LMM) analysis (**Table 4.3**). This contradiction can probably be explained by the fact that treating TVP particle size as a categorical variable in the LMM might better capture the categorical distinctions of TVP particle size on beany

perception than treating TVP particle size as a continuous variable in the network analysis.

In summary, the network analysis of sensory - physicochemical properties revealed distinct clusters of parameters driven by TVP hydration level and particle size. Parameters influenced by TVP hydration level, such as water content of cooked patties, serum release, serum water content and Young's modulus, were closely linked to juiciness and fattiness perception. On the other hand, TVP particle size predominantly impacted texture-related parameters, including cutting work and sensory chewiness. These findings highlight potential to modulate the sensory perception of PBMA patties by strategically adjusting TVP properties, specifically its hydration level and particle size.

4.4 Conclusions

This study aimed to investigate the role of TVP particle properties in juiciness perception of PBMA patties and gaining insights into the relationships between physicochemical and sensory properties. The composition and texture properties of PBMA patties were successfully controlled by varying TVP hydration level and particle size. TVP hydration level had a strong impact on the compositional and serum properties of cooked patties, which strongly contributed to juiciness and fattiness perception. In contrast, TVP particle size primarily affected texture properties, resulting in increased hardness and chewiness perception. Sensory tests underscore the importance of achieving a balanced texture profile, with desired juiciness and chewiness, alongside appealing flavors in driving consumer liking. In conclusion, juiciness of the PBMA patties was primarily influenced by water content of cooked patties, serum release and texture properties. Improving the water absorption capacity of TVP could offer a promising strategy to improve the juiciness of PBMA patties and further enhancing release and perception of flavors. In addition, meat analogue properties, including juiciness, can easily be altered by TVP properties related to water holding capacity and particle size.

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Supplementary material

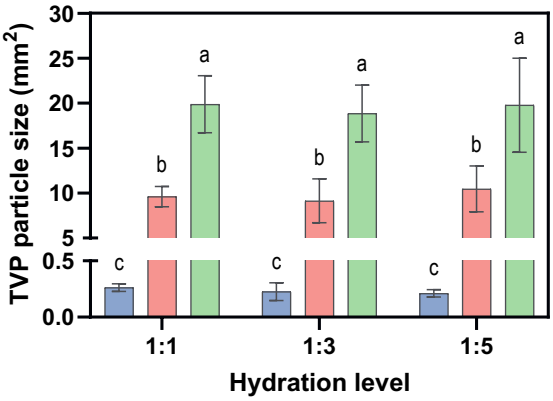

Figure S4.1 Average particle size of TVP particles rehydrated at different levels. Three distinct TVP particle size levels - small (S, average particle size 0.2 mm^2), medium (M, average particle size 10 mm^2) and large (L, average particle size 20 mm^2) - were obtained at each hydration level ($F = 123.1$, $p < 0.001$).

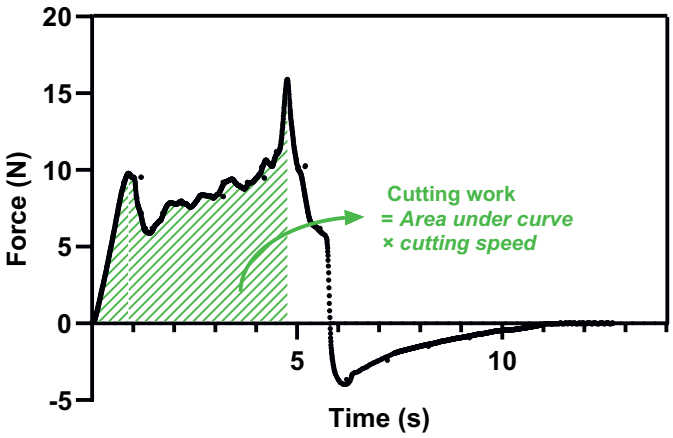

Figure S4.2 Example of force-time curve from blade cutting test. The cutting work was calculated as the energy used (green area) for the blade to cut from the initial cutting point through the entire patty until it reached 120% of the sample's initial height.

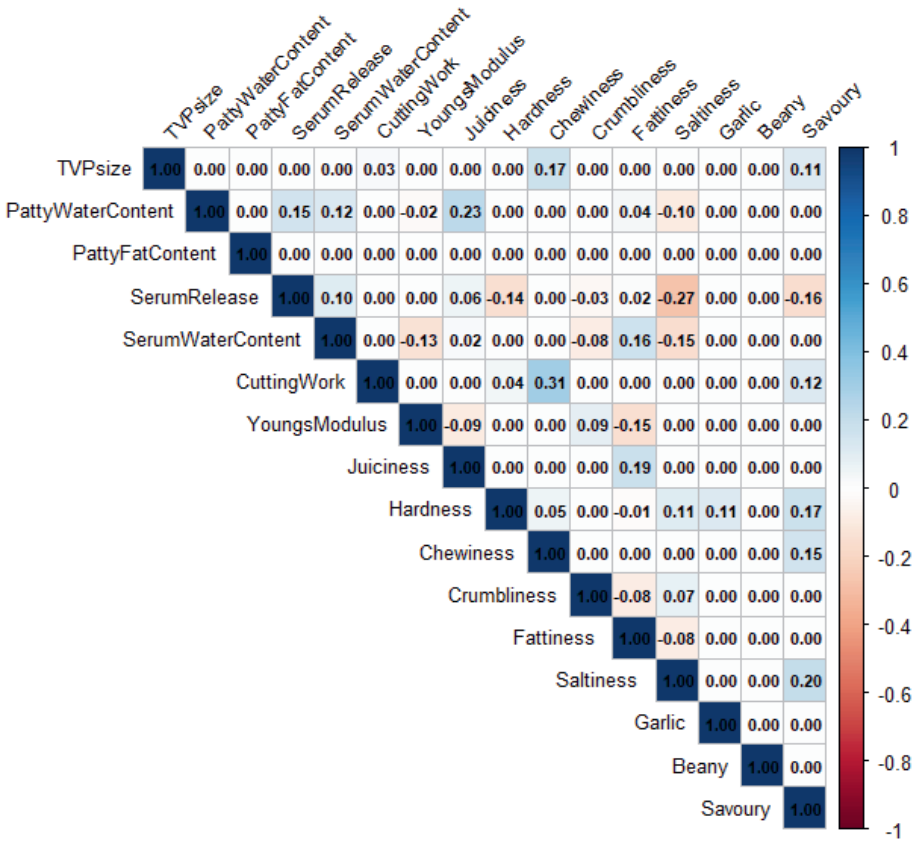

Figure S4.3. Partial correlation coefficients of relationships between physicochemical and sensory properties of PBMA patties prepared from TVPs differing in TVP hydration level (expressed as TVP:water weight ratio, 1:1, 1:3, 1:5) and particle size (S=Small (0.2 mm^2); M=Medium (10 mm^2); L=Large (20 mm^2)) (n = 9).

Table S4.1 Mean (±SD) compositional, texture and serum properties of cooked PBMA patties prepared from TVPs varying in hydration level (expressed as TVP:water weight ratio, 1:1, 1:3, 1:5) and particle size (S=Small (0.2 mm^2); M=Medium (10 mm^2); L=Large (20 mm^2)). Different letters indicate significant differences between samples (p <0.05). F and p values (* p <0.05, ** p< 0.01, *** p < 0.001) are derived from two-way ANOVA with TVP hydration level, TVP particle size and their interaction (hydration:size interaction) as fixed factors.

	1:1S	1:1M	1:1L	1:3S	1:3M	1:3L	1:5S	1:5M	1:5L	TVP Hydration level		TVP Particle size		Hydration: size interaction	
										F (2,1)	*p*	F (2,1)	*p*	F (4,1)	*p*
Compositional properties															
Total cooking loss (% w/w)	7.5±0.6^{f}	11.5±0.2^{e}	10.6±0.3^{ef}	17.7±1^{cd}	20.1±0.7^{bc}	16.1±0.6^{d}	25.8±1.8^{a}	22.2±1.2^{b}	23.0±0.3^{ab}	**361.1**	***	**3.7**	*	**13.1**	***
Water cooking loss (% w/w)	3.0±1.1^{d}	6.4±0.4^{cd}	5.5±0.3^{cd}	9.9±1.1^{c}	17.9±0.5^{ab}	14.8±0.8^{b}	21.1±3.6^{a}	18.7±0.7^{ab}	19.8±0.7^{a}	**173.5**	***	**7.1**	**	**7.2**	**
Fat cooking loss (% w/w)	2.5±0.2^{ab}	4.2±0.3^{a}	1.9±0.5^{b}	2.6±0.4^{ab}	1.9±0.7^{b}	1.6±0.0^{b}	2.5±0.7^{ab}	1.2±0.1^{b}	1.2±0.2^{b}	**7.2**	*	**5.4**	*	**4.5**	*
Water content of cooked patties (% w/w)	51.2±0.5^{e}	50.4±0.8^{e}	50.1±0.4^{e}	62.2±0.7^{bc}	58.8±1.1^{d}	59.3±0.3^{cd}	65.0±1.5^{ab}	66.9±1.2^{a}	65.8±0.2^{a}	**497.9**	***	2.6	0.104	**5.2**	**
Fat content of cooked patties (g/100 g dry weight)	30.5±0.1	28.8±0.4	28.7±1.0	28.1±1.2	30.4±1.4	28.8±1.7	27.6±3.3	30.6±0.1	30.7±0.3	0.1	0.898	0.6	0.588	1.1	0.396
Texture properties															
Young's modulus (kPa)	75.8±8.9^{a}	59.3±5.7^{ab}	54.1±1.1^{bc}	47.2±9.6^{bcd}	43.2±4.1^{bcde}	37.5±1.8^{cde}	40.7±8.7^{bcde}	26.3±2.9^{de}	26.1±3.6^{e}	**44.0**	***	**10.8**	***	0.8	0.516
Cutting work (mJ)	148.5±1.2^{cde}	502.1±33.7^{a}	568.2±68.1^{a}	102.5±11.8^{e}	244.0±16.6^{bc}	281.6±31.1^{b}	135.7±14.2^{de}	174.4±7.2^{cde}	223.6±13.3^{bcd}	**109.2**	***	**103**	***	**21.9**	***
Serum properties															
Serum release under compression (% w/w)	8.7±0.5^{e}	8.5±0.5^{e}	4.5±0.7^{f}	14.3±0.9^{c}	11.4±0.9^{d}	9.7±0.7^{de}	20.1±0.5^{a}	16.8±0.8^{b}	15.7±0.3^{bc}	**397.0**	***	**73.6**	***	**4.3**	*
Serum water content (% w/w)	21.9±9.3^{c}	27.6±3.0^{c}	13.8±5.0^{c}	45.7±0.9^{b}	49.4±1.0^{b}	48.7±4.9^{b}	69.0±0.6^{a}	67.4±6.1^{a}	69.8±6.4^{a}	**137.2**	***	1.0	0.384	1.6	0.213

Table S4.2 Partial correlation coefficients of sensory properties of PBMA patties prepared from TVPs differing in TVP hydration level (expressed as TVP:water weight ratio, 1:1, 1:3, 1:5) and particle size (S=Small (0.2 mm^2); M=Medium (10 mm^2); L=Large (20 mm^2)) (n = 69, duplicate).

	Juiciness	**Hardness**	**Chewiness**	**Crumbliness**	**Fattiness**	**Saltiness**	**Garlic flavor**	**Beany flavor**	**Savory/ meat flavor**	**Liking**
Juiciness	1	-0.029	0	0	0.529	0	0.028	0	0.001	0.086
Hardness		1	0.386	0.119	0	0.279	0	0	0.052	0
Chewiness			1	0.000	0	0.006	0.113	0	0.104	0.114
Crumbliness				1	0	0.100	0.004	0.042	0	0
Fattiness					1	0.025	0.090	0.077	0.246	0
Saltiness						1	0.406	0.207	0.408	0
Garlic flavor							1	0	0.203	0
Beany flavor								1	0	-0.003
Savory/meat flavor									1	0.181
Liking										1

Chapter 5

Impact of serum properties on juiciness and fattiness perception of plant-based meat analogues

This chapter is submitted for publication:

Zhang, Y., Mtiulishvili, L., Sala, G., Scholten, E., & Stieger, M. Juiciness of plant-based meat analogues is driven by serum release rather than serum composition and viscosity

Abstract

The aim of this study was to investigate how serum release and compositional and rheological properties of released serum (expressible fluid) affect juiciness and fattiness perception of plant-based meat analogue (PBMA) patties. PBMA patties varying in serum release under mechanical compression (8-20 %w/w), serum composition (serum water content: 29-85 %w/w; serum fat content: 12-65 %w/w) and serum viscosity (6-360 mPa·s at shear rate of 60 s^{-1}) were prepared following a 2×3×2 factorial design by altering hydration level of textured vegetable proteins (TVPs) (TVP:water weight ratio 1:2 and 1:4), fat (5, 12, 19 %w/w) and maltodextrin content (0, 3 %w/w) of raw patties. Increasing serum release increased juiciness and fattiness. Increasing fat content of raw patties increased fat content of the released serum, enhancing both fattiness and juiciness. This might have been related to an increase of serum viscosity. However, increasing serum viscosity by maltodextrin addition did not enhance juiciness and fattiness to a similar extent. Fat thus contributed to those mouthfeel attributes not only through its effect on viscosity. Network analysis revealed that juiciness was primarily determined by the amount of serum released and not by the composition and viscosity of the serum. In contrast, fattiness was influenced by both serum release and fat content of the released serum, but not by serum viscosity. We conclude that juiciness perception of plant-based meat analogue patties is primarily driven by the amount of serum that is released from the matrix upon compression rather than the composition and viscosity of the released serum.

5.1 Introduction

To address the pressing challenge of building a more sustainable global food system and minimizing environmental impact, plant-based meat analogues (PBMAs) has gained popularity as an alternative to meat. PBMAs are designed to mimic the sensory quality and nutritional profile of meats, providing a more sustainable option for consumers (Kyriakopoulou et al., 2021; McClements, 2024; Szenderák et al., 2022). Among PBMAs, burger patties have contributed substantially to the growth of the market (Caputo et al., 2024).

Burger patty PBMAs typically consist of water, textured vegetable proteins (TVPs), fat, binding agents, colorings and flavorings. TVPs, the main protein source, are often produced by low moisture extrusion, which creates a porous structure that is essential for mimicking meat texture (Kyriakopoulou et al., 2021). TVPs are rehydrated during the preparation of patties before they are mixed with the other ingredients, contributing to the patties' water holding capacity (WHC). The release of serum during consumption is important since it contributes to perception of juiciness (Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2** and **3**). Serum has been defined as "*The fluid/juice that is released from the food matrix during mastication or uniaxial compression. Serum of PBMAs may contain water, fat, proteins and solid particles* (Zhang et al., 2025, **Chapter 4**)." Plant-derived fats, such as sunflower and coconut oil, are used to provide fatty and tender mouthfeel and to enhance flavor release and perception (Jang & Lee, 2024; Kyriakopoulou et al., 2021). To improve the WHC of PBMA patties, hydrocolloids are also often added. Methylcellulose (MC) is one of the most commonly used hydrocolloids due to its strong WHC at higher temperatures, thermo-reversible gelling behavior, and ability to bind TVPs with other ingredients (Bakhsh et al., 2021; Rudge et al., 2025; Sze Wei et al., 2024).

Despite recent advances in ingredient formulation of PBMA patties, replicating the sensory quality of traditional meat, particularly juiciness and fattiness, remains a challenge (Chen et al., 2022; Godschalk-Broers et al., 2022). Juiciness perception of PBMAs contributes considerably to product liking (Godschalk-Broers et al., 2022; Sogari et al., 2023; Zhang et al., 2025, **Chapter 4**). Juiciness has been defined as the

impression of released juice and lubrication in the oral cavity during mastication (Rudge et al., 2025; Xu & Falsafi, 2023; Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2** and **3**), and is a dynamic texture sensation that varies across product types. In whole cut meats, juiciness has been suggested to depend on the rapid release of serum during early stages of mastication and the sustained lubrication provided by intramuscular fat and stimulated saliva during later stages of mastication (Smith & Carpenter, 1974; Winger & Hagyard, 1994). In PBMA patties, our previous studies have shown that juiciness is mainly determined by serum release during the early stages of mastication rather than oral structural breakdown occurring at the later stages of mastication. Consequently, the physicochemical properties of the cooked patties, such as fat content and serum release, are strongly correlated with juiciness (Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2** and **3**). Fattiness is commonly defined as the overall sensation of fat in foods (Mu et al., 2023; Pirc et al., 2023; Smith & Carpenter, 1974; Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2** and **3**). Fattiness is therefore often related to the fat content of various PBMAs and meats (Godschalk-Broers et al., 2022; Mu et al., 2023). Fat may contribute to fattiness by increasing viscosity and lubrication, which influences a variety of sensory attributes, such as thickness, smoothness and mouthcoating (Drewnowski, 1992; Mattes, 2005). Interestingly, our previous studies found that for PBMA patties, juiciness and fattiness were strongly correlated with the amount of serum released during mastication, but were not directly linked to the fat content of the patties or the fat content of the bolus formed during mastication (Zhang, Brouwer, et al., 2024; Zhang et al., 2025; Zhang, Sala, et al., 2024, **Chapter 2**, **3** and **4**). However, the variation in serum composition and physicochemical properties was limited in our previous studies, making it difficult to assess correlations. It therefore remains unclear how the composition and viscosity of released serum during mastication influence juiciness and fattiness.

The aim of this study was to investigate how serum release, serum composition and serum viscosity affect juiciness and fattiness perception of PBMA patties. We independently varied the amount of serum released from the patties under

mechanical compression, and the composition (water/fat content) and viscosity of the released serum. PBMA patties were prepared following a 2×3×2 factorial design altering the hydration level of textured vegetable proteins (TVPs) (TVP:water ratio 1:2 and 1:4), the fat (5, 12, 19 %w/w) and maltodextrin content (0, 3 %w/w) of the raw patties. Variations of the TVP hydration level were mainly used to adjust the amount of serum release, whereas fat content was used to vary the serum composition. To investigate whether the influence of fat addition on juiciness and fattiness was due to modifications of serum composition or serum viscosity (increased serum fat content is expected to increase serum viscosity), we added maltodextrin to the raw patties to create samples with similar serum viscosity but differing in serum composition (fat content). The cooked patties were characterized for composition (cooking loss, water content, fat content), instrumental texture (hardness, toughness) and serum properties (serum release, composition and viscosity). Sensory evaluation was conducted using Rank-Rating tests to assess four texture attributes (juiciness, fattiness, hardness, serum thickness). Network analysis (Undirected Graphical Models) was employed to explore correlations between serum and sensory properties.

5.2 Material and methods

5.2.1 Materials

Textured Pea Protein (NUTRALYS® TP-C, Roquette Frères S.A, France), pea protein isolate (Nutralys® S85f, Roquette Frères S.A, France), sunflower oil (Reddy®, Belgium), methylcellulose (METOLOSE® MCE-100TS, Shin Etsu, Japan), sodium chloride (Jozo Naturel tafelzout, the Netherlands), maltodextrin (dextrose equivalent: 4.0 – 7.0; CAS number: 9050-36-6; Sigma Aldrich, the Netherlands) vegetable extract red powder (GNT International B.V., the Netherlands), and caramel sugar syrup powder (EBC16000, GNT International B.V., the Netherlands) were used to prepare the studied patties. Meat flavor was kindly provided by Symrise AG (Holzminden, Germany).

5.2.2 Sample preparation

Twelve types of PBMA patties were prepared following a 2x3x2 factorial design varying textured vegetable protein (TVP) hydration level (TVP:water weight ratio 1:2 and 1:4), fat (5, 12, 19 %w/w) and maltodextrin (0, 3 %w/w) content of the raw patties (**Table 5.1**). For all patties, the weight ratio of 'hydrated TVP' to 'batter' was kept constant at 49:51. The amount of water used to rehydrate the TVP varied depending on the hydration level ratio. For batter preparation, water content was adjusted based on changes in fat and maltodextrin content to maintain a constant total weight. The proportions of all other ingredients (vegetable extract red powder, pea protein isolate, methylcellulose, sodium chloride, caramel sugar syrup, meat flavor) remained constant across patties. Each patty was coded according to its TVP hydration level (12H and 14H denoting TVP:water weight ratios of 1:2 and 1:4), fat content (5F, 12F and 19F denoting 5, 12 and 19 %w/w fat in the raw patty) and maltodextrin content (0MD and 3MD denoting 0 and 3 %w/w maltodextrin in the raw patty) as shown in **Figure 5.1**.

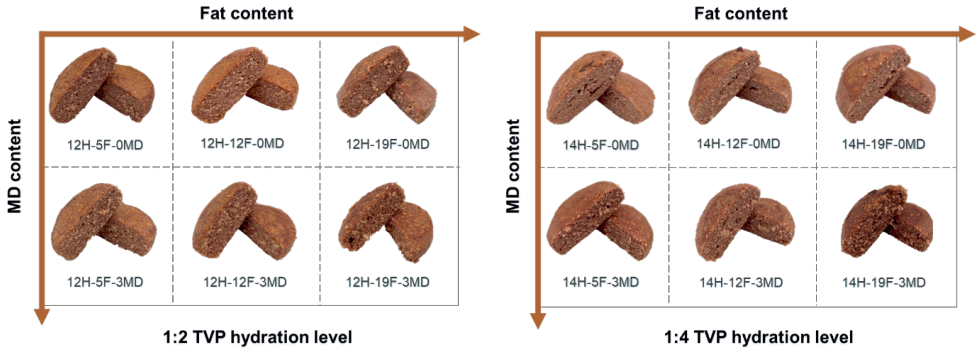

Figure 5.1 Pictures of cooked PBMA patties with the respective sample codes. Abbreviations: 12H and 14H refer to the TVP Hydration level (1:2 and 1:4 TVP:water weight ratio); 5F, 12F and 19F indicate the fat content of raw patties (5, 12 and 19% (w/w)); 0MD and 3MD represent the maltodextrin content of raw patties (0 and 3% (w/w)).

Table 5.1 Composition of twelve plant-based meat analogue patties. Abbreviations 12H and 14H refer to the TVP hydration level (1:2 and 1:4 TVP:water weight ratio); 5F, 12F and 19F indicate the fat content of the raw patties (5, 12 and 19 %w/w); 0MD and 3MD represent the maltodextrin content of the raw patties (0 and 3 %w/w).

	Content (g/100 g raw patty)											
	12H						**14H**					
Ingredient	**5F**		**12F**		**19F**		**5F**		**12F**		**19F**	
	0MD	**3MD**	**0MD**	**3MD**	**0MD**	**3MD**	**0MD**	**3MD**	**0MD**	**3MD**	**0MD**	**3MD**
TVP preparation												
Water (TVP rehydration)	32.20	32.20	32.20	32.20	32.20	32.20	38.64	38.64	38.64	38.64	38.64	38.64
TVP	16.10	16.10	16.10	16.10	16.10	16.10	9.66	9.66	9.66	9.66	9.66	9.66
Vegetable extract red powder	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Batter preparation												
Water (batter preparation)	37.75	34.75	30.75	27.75	23.75	20.75	37.75	34.75	30.75	27.75	23.75	20.75
Sunflower oil	5.00	5.00	12.00	12.00	19.00	19.00	5.00	5.00	12.00	12.00	19.00	19.00
Pea protein isolate	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72
Maltodextrin	0.00	3.00	0.00	3.00	0.00	3.00	0.00	3.00	0.00	3.00	0.00	3.00
Methylcellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sodium chloride	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Caramel sugar syrup	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53
Vegetable extract red powder	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Meat flavor	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Total water (TVP rehydration + batter preparation)	69.95	66.95	62.95	59.95	55.95	52.95	76.39	73.39	69.39	66.39	62.39	59.39

For TVP preparation, dry TVPs (30 g each batch) were first mixed with cold water (4°C) in weight ratios of 1:2 and 1:4 and kept at 4°C for 60 min to allow for full rehydration. The water used for TVP rehydration was mixed with vegetable extract red powder to color the TVPs. The rehydrated TVPs were blended in a blender (Magimix Cuisine 5200, France) for 30 s for 1:2 hydration level and for 10 s for 1:4 hydration level to obtain TVPs with similar sizes across the two hydration levels (Zhang et al., 2025, **Chapter 4**).

For batter preparation, meat flavor was first dispersed in sunflower oil and kept at 4°C before use. Pea protein isolate, maltodextrin, methylcellulose, sodium chloride, caramel sugar syrup powder and vegetable extract red powder were weighed (**Table 5.1**) and mixed in a Thermomixer (Vorwerk Thermomix® TM6, Wuppertal, Germany) at setting 3.5 (800 rpm) for 20 s at room temperature. The cold sunflower oil (pre-mixed with meat flavor) was then added and mixed with the Thermomixer at setting 3.5 (800 rpm) for 60 s, scraped and mixed with a spatula, and mixed again at setting 3.5 (800 rpm) for 20 s. Cold water (4°C) was added to the mixture and mixed at setting 4 (1100 rpm) for 30 s, followed by scraping and mixing with a spatula, and further mixed at setting 5.5 (2550 rpm) for 45 s at room temperature. The batter was then stored at 4°C for 60 min.

The cooled batter (4°C) and cooled hydrated TVPs (4°C) were mixed together using a KitchenAid mixer (Benton Harbor, USA) at setting 2 for 60 s at room temperature. After mixing, 110 g of the raw patty batter was shaped into patties (diameter 80 mm, height 20 mm) using a burger shaper (diameter 80 mm). The patties were stored at -20°C for 2 h and then transferred into plastic bags (dimension: 200 × 300 mm; thickness: 85 µm; material: polyamide + polypropylene) (Disposable Discounter, The Netherlands). After removing 95% of the air from the bags with a vacuum packaging machine (Henkovac M2, The Netherlands), the bags were sealed and the patties were stored at -20°C for a maximum of 2 weeks before use.

The frozen patties were cooked sous vide in a water bath at 75°C for 60 min to ensure that they reached a core temperature of 75°C. Subsequently, they were removed from the bags and grilled using a double-plate grill (DeLonghi, Italy) at 200°C for 1 min, with a distance of 2 cm between the two heating plates to ensure proper contact with both sides of the patties. After grilling, the patties were allowed to rest in foam boxes for 5 min to reach a core temperature of 55°C before undergoing instrumental characterization or sensory evaluation.

5.2.3 Characterization of patty properties

5.2.3.1 Compositional patty properties

5.2.3.1.1 Water content of cooked patties

The water content of the patties after sous vide cooking and grilling was determined by analyzing the dry matter content. Pieces of patties (10-15 g) were placed in aluminum dishes, weighed (w_0), and dried in an air oven (Binder, Germany) at 105°C for 16 – 18 h until constant weight. After drying, the samples were cooled down in a desiccator and weighed again (w_1). The water content (WC) of patties was calculated as $WC = (w_0–w_1)/w_0×100\%$. Measurements were performed in triplicate.

5.2.3.1.2 Fat content of cooked patties

The fat content of the patties after sous vide cooking and grilling was determined by Soxhlet extraction. Dried cooked patties were first pulverized using a cryogenic grinder (6875D Freezer/mill, SPEX, USA). The powder (5-6 g) was weighed (f_0) and fat was extracted using petroleum ether (150 mL) with a Soxhlet extraction apparatus (Soxtherm 6-place, Gerhardt, Germany). After extraction, the petroleum ether was evaporated by air drying in a fume hood at room temperature until constant weight was reached to obtain the fat as a residue, which was weighed (f_1). The fat content of patties was calculated as $FC = f_1/f_0×(1-WC)×100\% = f_1/f_0×(1-(w_0–w_1)/w_0)×100\%$. Measurements were performed in duplicate.

5.2.3.1.3 Cooking loss of cooked patties

The total cooking loss (TCL) was determined by quantifying the weight difference of the patties before sous vide cooking (m_0) and after grilling (m_1), as $TCL = (m_0–m_1)/w_0×100\%$. The water cooking loss (WCL) was determined as the difference between the initial water content of the raw patties before sous vide cooking (IW) (**Table 5.1**) and the water content of the patties after grilling on the basis of a raw patty (**section 5.2.3.1.1**), as $WCL = [IW–WC×(1–TCL)]×100\% = [IW–(w_0–w_1)/w_0×(1–(m_0–m_1)/w_0)]×100\%$. The fat cooking loss (FCL) was determined as the difference between the initial fat content of the raw patties before sous vide cooking (IF) (**Table 5.1**) and the fat content of the patties after grilling on the basis of a raw patty (**section 5.2.3.1.2**), as $FCL = [IF–FC×(1-TCL)]×100\% = [IF–f_1/f_0×(1-(w_0–w_1)/w_0)×(1-(m_0–m_1)/w_0)]×100\%$. All cooking loss measurements were performed in triplicate.

5.2.3.2 Texture properties of cooked patties

The texture properties of the patties were determined using uniaxial compression tests. A Texture Analyzer (TA.XT plus, Stable Micro Systems, UK) equipped with a 50 kg load cell and a stainless cylindrical probe (diameter 100 mm) was used to perform the compression. Cylindrical specimens with a diameter of 30 mm and height of 20 mm were cut from the center of the cooked patties using a cylindrical cutter. Compression was applied up to a strain of 80% of the specimens initial height at a constant compression speed of 5 mm/s. From the obtained force-time curve, hardness and toughness were obtained. Hardness was defined as the force needed to compress to 80% strain. Toughness was defined as the energy required to compress to 80% strain (area under force-time curve multiplied by the compression speed). All tests were performed in triplicate.

5.2.4 Characterization of serum properties

5.2.4.1 Serum release under compression

Serum release under compression was determined using the setup previously described by Zhang, Brouwer, et al. (2024) (**Chapter 2**). In brief, a Texture Analyzer equipped with a 50 kg load cell and a stainless cylindrical probe (diameter 50 mm) was used. The cylindrical probe was enclosed with a hood slightly larger than the probe to perform a confined compression. The cylindrical hood was attached to the top of a chamber fixed to the base of the Texture Analyzer and connected to a vacuum pump (vacuum level 7.0 mbar, Vacuubrand, Germany). Plastic cling foil was wrapped around the cylindrical hood and chamber to improve airtight sealing. A stainless steel filter (pore size 1 mm, porosity 36%) was fixed on top of the chamber with a rubber seal to collect the serum released under compression in an aluminum tray inside the chamber. The cylindrical specimen (diameter 30 mm, height 20 mm, around 12 g) were cut from the center of the patties, weighed (s_0) and placed between filter and probe. The compression was performed up to a strain of 80% of the specimens initial height at a constant speed of 5 mm/s. The probe was held in this position (80% strain) for 90 s. After compression, the specimens were removed and weighed (s_1). Serum release

under compression (SR) was calculated as SR = $(s_0–s_1)/s_0 \times 100\%$. Measurements were performed in triplicate.

To collect sufficient volumes of serum for viscosity measurements and compositional analysis, 2-3 patties were compressed individually and the collected released serum was pooled for one replicate. From the pooled serum, 1 mL was allocated for viscosity measurements, while the remainder (approximately 9 mL) was used for compositional analysis. Measurements were performed in triplicate.

5.2.4.2 Serum viscosity

The viscosity of the released serum was determined using a stress-controlled rheometer (MCR 302, Anton Paar, Austria) equipped with a concentric cylinder geometry (CC10/Ti) with a volume of 1 mL. Serum samples were stored in 15 mL tubes and maintained in a 55°C water bath before measurement to mimic the temperature of freshly released serum from patties during consumption. Before pipetting, the serum was vortexed for 30 s to prevent phase separation. A 1 mL sample was pipetted into the rheometer cup and the shear rate was increased from 0.1 s^{-1} to 500 s^{-1} in logarithmic steps in a time frame of 220 s, fast enough to prevent phase separation. Viscosity measurements were performed at 37°C in triplicate. The viscosity at a shear rate of 60 s^{-1} was extracted from the flow curves and used for further data analysis.

5.2.4.3 Serum composition

The composition of the released serum was analyzed using centrifugation and dry matter analysis. The serum was weighed (W_{serum}) and centrifuged at 3000 rpm for 20 min at 20°C (Beckman Coulter Allegra X–22R Centrifuge, United States). The centrifuged serum separated into two phases: the supernatant (comprising fat and water) and the sediment (comprising solid particles and water). The supernatant was pipetted into an aluminum dish, while the sediment was left in the centrifugation tube. Both supernatant and sediment were dried in an air oven (Binder, Germany) at 105°C until constant weight (approximately 5–6 h). After drying, the samples were cooled down in a desiccator and weighed again ($W_{supernatant\_dry}$ and $W_{sediment\_dry}$, respectively). Water, fat, and solids content of the serum were calculated based on a mass balance.

Water content of serum was calculated as WC_serum = (W_{serum} – $W_{supernatant\_dry}$ - $W_{sediment\_dry}$) / W_{serum} × 100%. Fat content of the serum was calculated as FC_serum = $W_{supernatant\_dry}$ / W_{serum} × 100%, and solids content of the serum was calculated as SC_serum = $W_{sediment\_dry}$ / W_{serum} × 100%. The solids content of the serum consisted of small, solid patty particles, maltodextrin and proteins that were pressed through the stainless steel filter during serum collection under compression. All measurements were performed in triplicate.

5.2.5 Descriptive rank-Rating for juiciness, fattiness, hardness and serum thickness

Participants (n = 75, 59 females and 16 males, 24.5 ± 2.5 years, BMI 22.6 ± 2.9 kg/m^2, mean ± SD) were recruited from Wageningen and surroundings. Inclusion criteria were good general health (self-reported), BMI between 18.5 and 30 kg/m^2, no dental issues, no swallowing issues, normal ability to taste and smell, being a non-smoker, no allergies or intolerances to legumes and not being pregnant. All participants (n = 75) assessed all twelve PBMA patties in the sensory booths at the Human Research Facilities of Wageningen University. The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki (2013). Participants signed an informed consent form and received financial reimbursement after completion of the session.

The sensory properties of PBMA patties were evaluated using the Rank-Rating methodology. Participants attended one familiarization session (30 min) and one test session (90 min). The test session was at least 1 day after the familiarization session. During the familiarization session, participants were familiarized with the Rank-Rating evaluation procedure and the patties. Participants were provided with the sensory attributes (including definitions) (**Table 5.2**), and tasted two patties to get familiarized with the samples. Familiarization was performed with 12H-5F-3MD and 14H-19F-0MD patties. Participants were informed that these two patties provide an impression of the variation in texture within the sample set.

In the test session, participants evaluated all 12 patties once (**Figure 5.1**). Definitions of the four attributes were provided to participants during the test session (**Table 5.2**).

The 12 patties were randomly divided into three sets of four samples. The four sensory attributes were randomly divided into two groups with two attributes per group. The order of the samples within each set and the attributes within each group were randomized over participants but remained consistent for each participant throughout the session. Participants evaluated each set of samples twice since two attributes were assessed simultaneously during each evaluation. Therefore, participants evaluated 4 sensory attributes for each patty once. For each evaluation, participants were presented with 4 random samples (one-sixth of the patty, around 12 g), labelled with 3-digit codes, and served simultaneously at 55℃. Participants were instructed to place the samples into their mouth with their hands, take bites as they were accustomed to, and rate the intensity of the two assigned attributes for each sample on unstructured 100 mm line scales. The scales were anchored with "Not juicy/fatty/hard/thick" at the left end and "Very juicy/fatty/hard/thick" at the right end. A mandatory 3-min break was given after each evaluation, during which crackers and water were provided for palate cleansing. Data were collected in English using EyeQuestion software (Logic8 B.V., the Netherlands).

Table 5.2 Sensory attributes and definitions used for Rank-Rating evaluation of plant-based meat analogue patties.

Attribute	Definition
Juiciness	Sensation of juice/liquid being released from food during consumption.
Fattiness	Sensation of fat in the mouth.
Hardness	Force applied by the (molar) teeth to bite through the food.
Serum thickness	The degree to which the juice/liquid released from the food resists flow in the mouth. *Consider for example, thickness of three fluids: water < drinking yoghurt < honey*

5.2.6 Data analysis

Results are reported as mean values with standard deviation (SD). To investigate the effect of TVP hydration level, fat content, and maltodextrin (MD) content on the physicochemical properties of the cooked patties and released serum, three-way ANOVA followed by Tukey post-hoc analyses were performed. TVP hydration level, fat content, MD content and their interactions were set as fixed factors. To investigate the effect of TVP hydration level, fat content and MD content on patties' sensory properties,

linear mixed models (LMM) followed by Tukey post-hoc analyses were performed. In the LMM analysis, TVP hydration level, fat content, MD content and their interactions were treated as fixed factors, participants and sample testing order as random factors.

Undirected Graphical Models (UGMs) (Behrouzi et al., 2023; Behrouzi & Wit, 2019), a type of network analysis method, was employed to estimate associations among sensory properties and between physicochemical and sensory properties. UGMs are probabilistic graphical models that represent variables as nodes, with edges between them representing conditional dependencies. These conditional dependencies are captured through partial correlations, which measure the association between two variables while controlling for the influence of all other variables in the model. This framework enables to explore complex association patterns and distinguish direct from indirect relationships. By focusing on the conditional dependencies, the interconnections between sensory properties and the relationships between sensory properties and physicochemical properties were analyzed.

Data analysis was performed using RStudio (version 2022.07.0, PBC) with the packages emmeans (Lenth, 2022), lmerTest (Kuznetsova et al., 2017), lme4 (Bates et al., 2015), Hmisc (Harrell & Dupont, 2023), netgwas (Behrouzi et al., 2023) and GraphPad Prism (version 10.0.0, GraphPad Software, USA). A significance level of $p < 0.05$ was chosen.

5.3. Results and discussion

5.3.1 Compositional properties of cooked patties

The compositional and texture properties of cooked patties varying in TVP hydration level, fat and maltodextrin content are shown in **Figure 5.2** and **Figure 5.3**, and the results of the related statistical data analysis are summarized in **Table 5.3**

Table 5.3. Results of statistical data analysis describing the effects of TVP hydration level, fat content and maltodextrin (MD) content on physicochemical properties of PBMA patties. F (DFn, DFd) and p values (* p <0.05, ** p< 0.01, *** p < 0.001) are derived from three-way ANOVA with TVP hydration level, fat content, MD content and their interactions as fixed factors.

	Hydration level		**Fat content**		**MD content**		**Hydration:Fat interaction**		**Hydration:MD interaction**		**Fat:MD interaction**		**Hydration: Fat:MD interaction**	
	F (1, 24)	***p***	**F (2, 24)**	***p***	**F (1, 24)**	***p***	**F (2, 24)**	***p***	**F (1, 24)**	***p***	**F (2, 24)**	***p***	**F (2, 24)**	***p***
Compositional properties														
Water cooking loss	**2696.9**	***	**505.0**	***	**192.2**	***	0.2	0.833	**107.8**	***	**38.9**	***	1.5	0.233
Fat cooking loss	0.6	0.431	**342.9**	***	**56.2**	***	**7.7**	**	**29.9**	***	**38.5**	***	**13.6**	***
Total cooking loss	**1539.7**	***	**38.9**	***	**367.9**	***	2.1	0.136	**144.1**	***	**3.9**	*	**7.3**	**
Water content of cooked patties	**427.3**	***	**1131.6**	***	**355.3**	***	**3.7**	*	0.6	0.464	**56.5**	***	2.7	0.084
Fat content of cooked patties	**37.8**	***	**321**	***	**30.2**	***	**13.1**	***	**17.4**	***	**42.4**	***	**13.7**	***
Texture properties														
Hardness	0.1	0.752	**21.8**	***	3.8	0.063	**3.8**	*	**12.7**	**	**9.5**	***	**6.8**	**
Toughness	0.3	0.572	**48.1**	***	4.0	0.058	**4.4**	*	3.0	0.094	**17.1**	***	**7.6**	**

To gain insights into how the TVP hydration level, fat and maltodextrin content of the raw patties impacted the compositional properties of the cooked patties, the total, fat and water cooking loss (**Figure 5.2 (A)**), and the water and fat content of cooked patties (**Figure 5.2 (B)**) are shown.

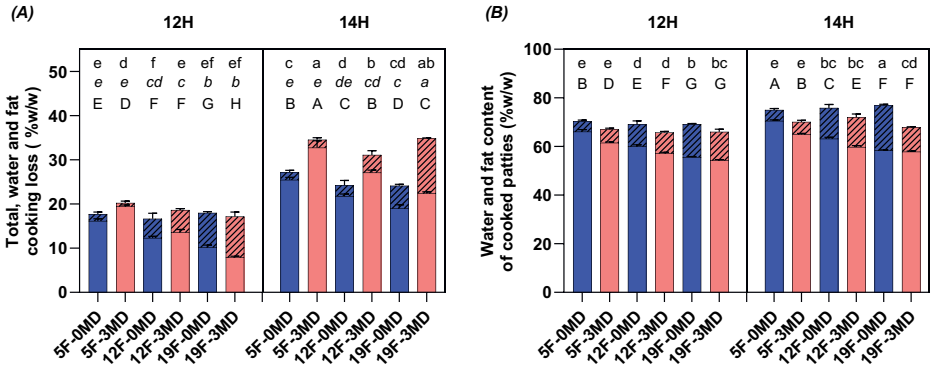

Figure 5.2 (A) Total cooking loss (entire bars and lower case letters), fat cooking loss (striped pattern and italic, lower case letters) and water cooking loss (no pattern and capital letters). (B) Fat content (striped pattern and lower case letters) and water content (no pattern and capital letters) of the cooked patties with different TVP hydration level (TVP:water weight ratio of 1:2 and 1:4, indicated as 12H and 14H), fat content (5, 12, 19 %w/w indicated as 5F, 12F, 19F) and maltodextrin content (0, 3 %w/w indicated as 0MD (blue bars) and 3MD (red bars)) (compositional variations in the raw patties). Different letters indicate significant differences between means ($p < 0.05$). Error bars represent standard deviation.

As shown in **Figures 5.2 (A)** and **Table 5.3**, with increasing TVP hydration level, total cooking loss (entire bars) and water cooking loss (no pattern) increased significantly, while fat cooking loss (stripes) was not affected, suggesting that water cooking loss was the predominant contributor to total cooking loss. This effect of TVP hydration level on water cooking loss can be explained by the patty formulation. Since the total weight of rehydrated TVP was kept constant across patties, increasing TVP hydration level from 1:2 to 1:4 required less TVPs, reducing its relative concentration from 16.0 to 9.7 %w/w (**Table 5.1**). The reduced TVP content lowered the water holding capacity of the patties and increased water cooking loss, which aligns with our previous study (Zhang et al., 2025, **Chapter 4**).

Increasing raw patties' fat content significantly decreased water cooking loss while increasing fat cooking loss (**Figure 5.2 (A)** and **Table 5.3**). These opposing effects resulted in a small but significant increase in total cooking loss with increasing raw patties' fat content. These findings aligned with previous studies on meatballs and beef patties, which showed that increasing fat content of raw meat doughs increased fat cooking loss (Serdaroğlu & Değırmencioğlu, 2004; Velioğlu et al., 2010).

When maltodextrin was added, total and water cooking loss increased, especially in patties with a higher TVP hydration level (**Figure 5.2 (A)** and **Table 5.3**). So, even though the viscosity of the released serum increased by maltodextrin addition (**section 5.3.3**), more water was released from the patties during cooking. This increase in cooking loss was not expected, as previous studies on emulsion-filled protein gels and frankfurters found that adding maltodextrin decreased cooking loss (Crehan et al., 2000; Mao et al., 2018), most likely due to slower water flow through the product matrix as a result of increased viscosity. This discrepancy may be related to the differences in the food types. In our study, the patties contained methylcellulose, which forms a thermo-reversible gel. Although it is not clear how the presence of other components alters methylcellulose gelation, maltodextrin may have changed the gel properties, inducing syneresis and subsequent release of serum during cooking. As shown in **Figure 5.2 (A)** and **Table 5.3**, maltodextrin also had a significant but small effect on fat cooking loss. For most patties, maltodextrin addition also increased fat release, although only significant for 14H-19F-0MD and 14H-19F-3MD. The simultaneous increase of water and fat release during cooking ultimately led to a significant increase in total cooking loss when adding maltodextrin (**Figure 5.2 (A)** and **Table 5.3**).

Variations in water, fat and total cooking loss also translated into significant differences in the final water and fat content of the cooked patties. Even though water cooking loss increased with increasing TVP hydration level, the 14H patties still contained a higher water content than the 12H patties after cooking (on average 6% higher) (**Figure 5.2 (B)** and **Table 5.3**), consistently with previous findings (Zhang et al., 2025, **Chapter 4**). Interestingly, raw patties' fat content had a far stronger influence on the final water content of the cooked patties than TVP hydration level. Increasing raw patties' fat

content from 5 to 12 %w/w reduced cooked patties' water content by 14% on average. Two factors might explain why fat content showed a stronger impact on water retention than TVP hydration level. First, the difference in raw patties' water content caused by changes in TVP hydration level (6.4%) was initially smaller than that caused by variation of raw patties' fat content (14.0%) (**Table 5.1**). Secondly, higher TVP hydration level strongly increased water cooking loss (**Figure 5.2 (A)**), offsetting the smaller differences in water content of raw patties caused by hydration levels. In contrast, increasing raw patties' fat content increased water cooking loss only to a limited extent (**Figure 5.2 (A)**), preserving or even enlarging the larger variation in raw patties' water content caused by different fat levels.

As expected, increasing raw patties' fat content significantly increased the total fat content of cooked patties by 97-223% (**Figure 5.2 (B)** and **Table 5.3**). In comparison, TVP hydration level had a significant and small influence on total fat content of cooked patties (7-42% increase) (**Figure 5.2 (B)** and **Table 5.3**). The effect of TVP hydration level on fat content of cooked patties can be attributed to the greater water cooking loss of 14H patties, which reduced their overall weight after cooking. The addition of maltodextrin did not significantly influence the fat content of cooked patties for five out of six TVP hydration level and fat content variations, and only the 14H-19F patties differed in fat content when maltodextrin was added (**Figure 5.2 (B)**).

To summarize, the fat content of the raw patties had the strongest influence on the composition of cooked patties. TVP hydration level mainly affected water cooking loss, which resulted in a secondary influence on composition of cooked patties. Maltodextrin introduced additional variability, particularly by increasing water cooking loss, but its effect on final composition of the cooked patties was less consistent.

5.3.2 Texture properties of cooked patties

The small effect of TVP hydration level on composition of cooked patties (**section 5.3.1**) was also reflected in the lack of significant effect of TVP hydration level on hardness and toughness (**Figure 5.3** and **Table 5.3**), likely due to the narrow range of variation in final water content among cooked patties. As variations in initial fat content lead to larger differences in fat content of the cooked patties (**Figure 5.2 (B)**), higher fat content in the cooked patties decreased hardness and toughness (**Figure 5.3** and **Table 5.3**). This finding aligned with previous studies reporting that increasing fat content of plant-based and meat patties resulted in lower instrumental hardness (Berry & Leddy, 1984; Godschalk-Broers et al., 2022; Mabrouki et al., 2023). A possible explanation is that the fat globules may act as structure breakers reducing the overall strength of the gel phase that binds the TVPs within the patty matrix. Notably, maltodextrin content did not have a significant effect on hardness and toughness, although some exceptions were found for 12H-19F-0/3MD and 14H-5F-0/3MD, but without a systematic trend (**Figure 5.3** and **Table 5.3**). So, even though maltodextrin content affected the viscosity of the serum (**section 5.3.3**), it did not affect the hardness and toughness of the patties.

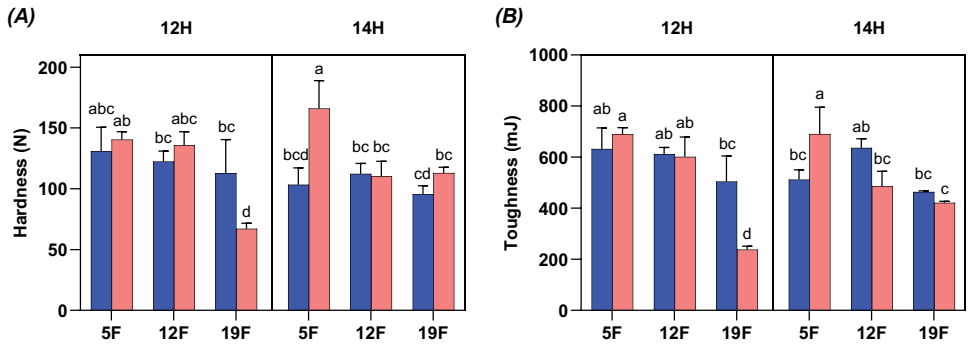

Figure 5.3 (A) Hardness and (B) toughness of cooked patties with different TVP hydration level (TVP:water weight ratio of 1:2 and 1:4, expressed as 12H and 14H), fat content (5, 12, 19 %w/w, expressed as 5F, 12F, 19F) and maltodextrin content (0 %w/w (blue bar) and 3 %w/w (red bar)) (compositional variations in the raw patties). Different letters indicate significant differences between means ($p < 0.05$). Error bars represent standard deviation.

5.3.3 Characterization of serum properties

To verify how TVP hydration level, fat and maltodextrin content of the raw patties impacted serum properties, the amount of serum released during compression, serum composition and serum viscosity were characterized. The results are shown in **Figure 5.4**, and the corresponding statistical data analysis is presented in **Table 5.4**.

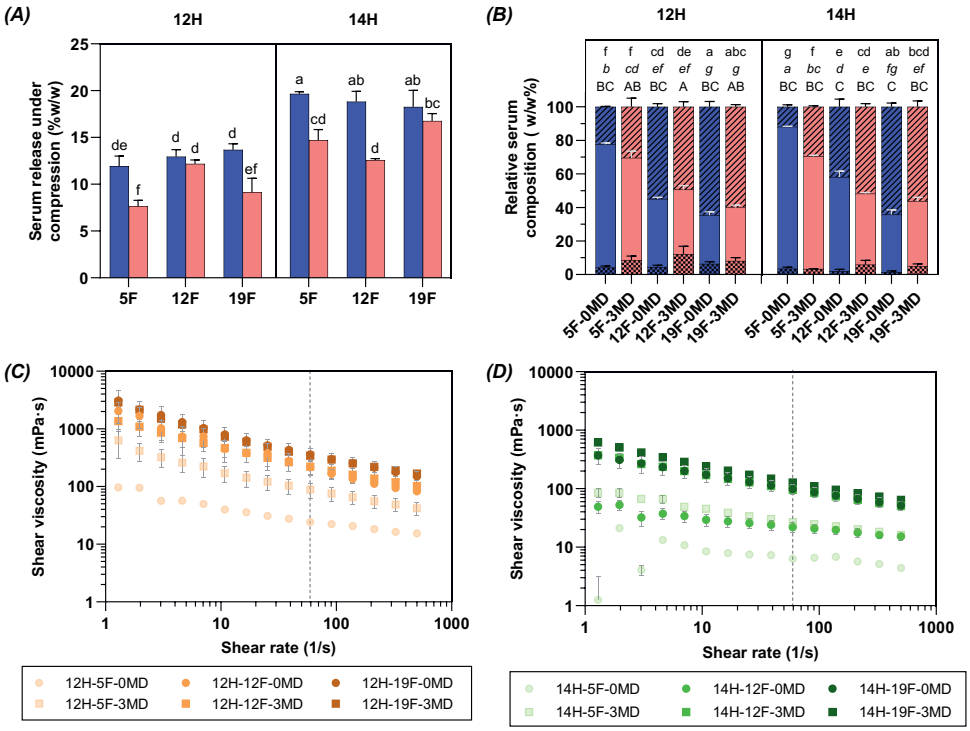

Figure 5.4 (A) Serum release under compression and (B) relative fat (striped pattern and lower case letters), water (no pattern and italic lower case letters) and solids content (dotted pattern and capital letters), and of the released serum of cooked patties with different TVP hydration level (TVP:water weight ratio of 1:2 and 1:4, expressed as 12H and 14H), fat content (5, 12, 19 %w/w, expressed as 5F, 12F, 19F) and maltodextrin content (0 and 3 %w/w, expressed as 0MD (blue bars) and 3MD (red bars)) (compositional variations in the raw patties). Different letters indicate significant differences between means ($p < 0.05$). Error bars represent standard deviation. (C) and (D) Shear viscosity of the serum released after mechanical compression from cooked patties with TVP:water weight ratio of 1:2 (C, orange colors) and TVP:water weight ratio of 1:4 (D, green colors) as a function of shear rate. Dashed lines indicate the viscosity at a shear rate of 60 s^{-1}.

Table 5.4 Results of statistical data analysis describing the effects of TVP hydration level, fat content and maltodextrin (MD) content on serum properties of PBMA patties. F (DFn, DFd) and p values (* p <0.05, ** p< 0.01, *** p < 0.001) are derived from three-way ANOVA with TVP hydration level, fat content, MD content and their interactions as fixed factors.

	Hydration level		**Fat content**		**MD content**		**Hydration:Fat interaction**		**Hydration:MD interaction**		**Fat:MD interaction**		**Hydration: Fat:MD interaction**	
	F (1, 24)	***p***	**F (2, 24)**	***p***	**F (1, 24)**	***p***	**F (2, 24)**	***p***	**F (1, 24)**	***p***	**F (2, 24)**	***p***	**F (2, 24)**	***p***
Serum release under compression	**294.3**	***	3.2	0.06	**132.0**	***	**15.4**	***	2.5	0.125	2.1	0.14	**14.5**	***
Water content of released serum	**125.5**	***	**974.7**	***	**78.9**	***	2.2	0.137	**12.4**	**	**56.4**	***	**6.8**	**
Fat content of released serum	**21.6**	***	**563.8**	***	**9.6**	**	1.5	0.253	**15.1**	***	**34.8**	***	**8.8**	**
Solids content of released serum	**32.9**	***	1.4	0.269	**29.0**	***	0.4	0.665	2.8	0.109	3.1	0.065	2.3	0.121
Serum viscosity ($\dot{\gamma}$=60 s^{-1})	**78.4**	***	**45.0**	***	2.7	0.115	**11.3**	***	0.7	0.415	0.5	0.639	0.9	0.417

Figure 5.4 (A) and **Table 5.4** show that serum release under compression significantly increased with increasing TVP hydration level, significantly decreased with addition of maltodextrin and was not significantly affected by raw patties' fat content. To explore how these results can be linked to the composition of the cooked patties, **Figure 5.5** shows the relation between serum release under compression and water (**Figure 5.5 (A)**), fat (**Figure 5.5 (B)**) and total liquid content (**Figure 5.5 (C)**) of the cooked patties. Only total liquid content showed a significant positive linear correlation with serum release under compression ($R^2 = 0.63$, $p < 0.001$, **Figure 5.5 (C)**). These results suggest that the quantity of serum release under compression was mainly determined by the total liquid content of cooked patties rather than the water or fat content of cooked patties. Neither water or oil could be easily expelled from the patties.

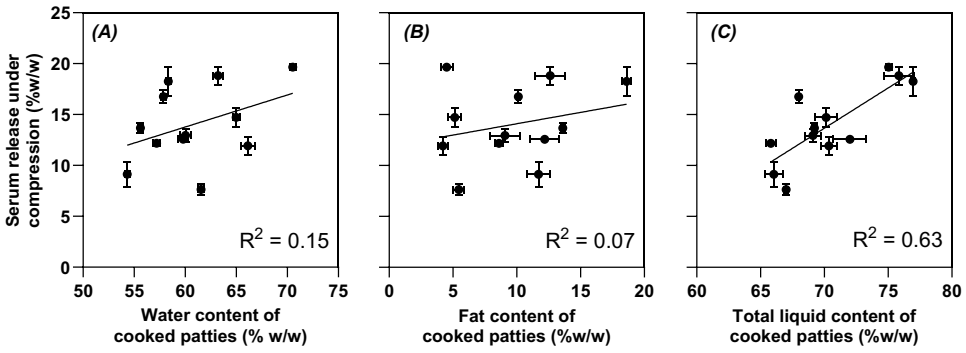

Figure 5.5 Relationships between serum release under compression and (A) water content, (B) fat content and (C) total liquid content of cooked patties. Error bars indicate the standard deviation of means. Solid lines represent linear regressions.

The liquid (water and fat) retained in the cooked patties influenced, as expected, the amount of serum release. In addition, the composition of the retained liquid affected also serum composition. As shown in **Figure 5.4 (B)** and **Table 5.4,** increasing TVP hydration level resulted in a significant 18% (on average) increase in serum water content, which led to a significant decrease in serum fat content (13%). As already mentioned in **section 5.3.1**, the effect of varying raw patties' fat content on patty composition was much larger than the effect of TVP hydration level (**Table 5.3**). Higher fat content in raw patties led to a significant 134% increase in serum fat content and a

corresponding 38% decrease in serum water content (**Figure 5.4 (B)**), consistent with the composition of the cooked patties. The presence of maltodextrin in the cooked patties also affected the composition of the released serum. **Figure 5.4 (B)** shows that the addition of maltodextrin significantly increased the solids content while decreasing the total water and fat content in the released serum, in agreement with the observed increase in total cooking loss and decrease in water and fat content in the cooked patties upon maltodextrin addition (**Figure 5.2**). The increased serum solids content could be an effect of the presence of maltodextrin itself, or an indirect effect of the release of protein, methylcellulose and TVP particles from the patty matrix.

The differences in serum fat content and solids content also affected the viscosity of the released serum. The flow curves of the serum released from all patties displayed shear-thinning behavior (**Figures 5.4 (C) (D)**). Serum with higher water content, resulting from increased TVP hydration level or low fat content, showed lower serum viscosity. Serum with higher fat content, as a result of higher initial patty fat content, showed increased serum viscosity. The addition of maltodextrin also increased serum viscosity (comparing circles with squares in **Figures 5.4 (C) (D)**), suggesting that maltodextrin was indeed released with the serum during compression. The effect of maltodextrin addition was more pronounced when patties initially contained 5% fat, and disappeared for patties with higher fat content, as these patties already contained serum with high viscosity, limiting the additional influence of maltodextrin.

To further explore the relationships between serum composition and viscosity, shear viscosity at $60s^{-1}$ was plotted against serum fat content (**Figure 5.6**). As expected, serum viscosity increased with increasing fat content. However, it is clear that two groups were obtained, which were separated based on TVP hydration level: samples with low initial TVP hydration level (12H, blue) and samples with higher initial TVP hydration level (14H, green). For the lower hydration level (12H), the lower water content may have been accompanied with a relative higher release of solids, which could be proteins, maltodextrin or other solid material, thereby contributing to the viscosity. In addition, fat may have been distributed differently within the sample, as a result of smaller droplet sizes, or increased droplet-matrix interactions. Addition of

maltodextrin (samples in triangles, **Figure 5.6**) seemed to have a limited effect, even though maltodextrin was added to increase serum viscosity. The lower contribution of maltodextrin could be related to the fact that only a small amount of maltodextrin was released with the serum, whereas a large amount might have remained in the patty. Hydration level was thus more important for the final viscosity than the addition of maltodextrin.

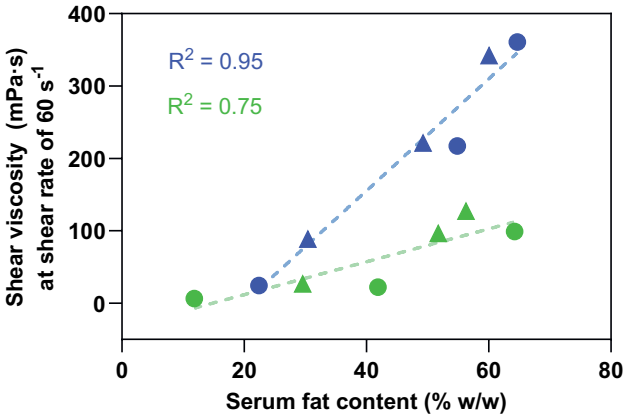

Figure 5.6. Shear viscosity at shear rate of 60 s^{-1} as a function of serum fat content. Blue (12H) and green (14H) symbols represent patties with TVP hydration levels of 1:2 and 1:4 (TVP:water weight ratio), respectively. Circles (0MD) and triangles (3MD) represent patties with 0 and 3% of maltodextrin, respectively. Dashed lines represent linear regressions.

5.3.4 Sensory properties

To assess how variations in compositional and physicochemical properties of cooked patties and released serum influenced perception, a Rank-Rating sensory analysis of the 12 patties was performed (**Table 5.5** and **Figure 5.7**).

Table 5.5 Results of statistical data analysis describing the effects of TVP hydration level, fat content and maltodextrin (MD) content on sensory properties of PBMA patties from Rank-Rating test (n = 75). F (DFn, DFd) and p values (* p <0.05, ** p< 0.01, *** p < 0.001) are derived from Linear Mixed Models with TVP hydration level, fat content, MD content and their interactions as fixed factors and participant and presentation order as random effects.

	Hydration level		**Fat content**		**MD content**		**Hydration:Fat interaction**		**Hydration:MD interaction**		**Fat:MD interaction**		**Hydration: Fat:MD interaction**	
	F (1, 888)	***p***	**F (2, 888)**	***p***	**F (1, 888)**	***p***	**F (2, 888)**	***p***	**F (1, 888)**	***p***	**F (2, 888)**	***p***	**F (2, 888)**	***p***
Juiciness	**355.1**	***	**22.6**	***	**45.0**	***	0.01	0.986	2.1	0.144	**4.3**	*	**14.4**	***
Fattiness	**148.7**	***	**55.5**	***	**39.5**	***	0.2	0.802	2.3	0.127	2.9	0.054	**4.9**	**
Hardness	**7.8**	**	**166.9**	***	**88.5**	***	**11.8**	***	**24.3**	***	**62.2**	***	**5.0**	**
Serum thickness	**5.4**	*	**3.9**	*	**6.2**	*	0.4	0.671	1.0	0.314	0.8	0.445	0.8	0.758

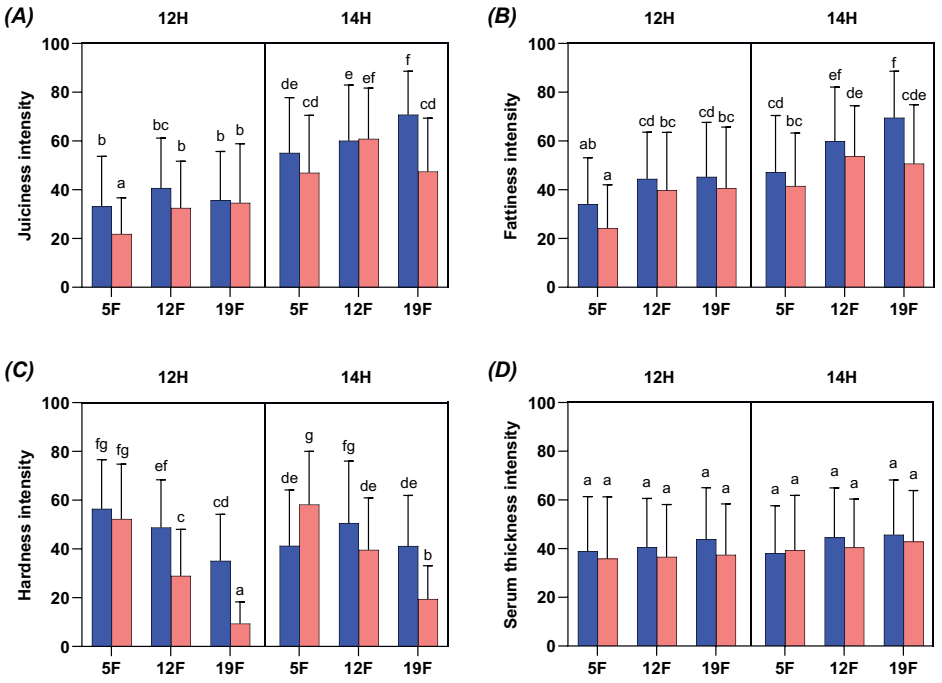

Figure 5.7 Intensity of texture attributes (mean ± SD; n=75 participants) obtained from Rank-Rating evaluation of PBMA patties with different TVP hydration level (TVP:water weight ratio of 1:2 and 1:4, expressed as 12H and 14H), fat content (5, 12, 19 %w/w, expressed as 5F, 12F, 19F) and maltodextrin content (0 %w/w (blue bar) and 3 %w/w (red bar)) (compositional variations in the raw patties). Different letters indicate significant differences between means ($p < 0.05$). Error bars represent standard deviation.

As shown in **Figure 5.7 (A)** and **(B)**, increasing TVP hydration level significantly enhanced juiciness and fattiness intensity. Increasing raw patties' fat content significantly increased juiciness and fattiness whereas the addition of maltodextrin significantly decreased juiciness and fattiness (**Table 5.5**). A higher juiciness and fattiness with higher TVP hydration level was in agreement with our previous study (Zhang et al., 2025, **Chapter 4**). Although raw patties' fat content affected the serum composition and viscosity the most (**Table 5.4**), the impact of raw patties' fat content on juiciness and fattiness were considerably smaller than the effect of TVP hydration level. TVP hydration level had the largest influence on the total amount of serum release under compression (**Table 5.4**), suggesting that juiciness and fattiness may be

mostly determined by the quantity of released serum rather than serum composition. This may also explain why maltodextrin addition decreased juiciness and fattiness since it significantly decreased total serum release (**Figure 5.4 (A)**). However, patty composition seemed to still play a role in fattiness perception, as higher raw patty's fat content led to a strong increase in fattiness (40% increase), whereas juiciness increased to a lesser extent (20% increase) (**Figure 5.7** and **Table 5.5**). Serum composition may thus be key in distinguishing between juiciness and fattiness, which will be explored further in **section 5.3.5**.

Hardness perception was mostly affected by variations in raw patties' fat content and by the addition of maltodextrin, and least affected by TVP hydration level (**Figure 5.7 (C)** and **Table 5.5**). These trends were similar with the instrumental measured hardness and toughness (**Figure 5.3 (A) (B)**), an outcome supported by multiple studies reporting strong correlations between instrumental measured and sensory hardness of PBMAs (Godschalk-Broers et al., 2022; Mabrouki et al., 2023; Sze Wei et al., 2024; Zhang, Brouwer, et al., 2024; Zhang et al., 2025, **Chapter 2 and 4**).

Serum thickness was included as a mouthfeel attribute to assess the effect of serum viscosity on perception. **Figure 5.7 (D)** and **Table 5.5** show that perceived serum thickness was similar for all 12 patties when post-hoc pairwise comparisons were performed ($p > 0.05$). A reason could be that differences in serum viscosity between samples were not large enough to be perceived as differing in serum thickness. However, the viscosity of the serum differed by a factor of 10 across all patties (**Figure 5.4 (C) (D)**), which was larger than the Weber fraction for oral thickness perception ($K = 0.26$) of model beverages (Camacho et al., 2015). Based on this, participants should have been able to discriminate serum thickness of the serum differing in viscosity when the serum would have been assessed in the absence of solid bolus fragments. Apparently, the presence of large quantities of solid bolus fragments in the mouth next to small quantities of liquid serum made it difficult for participants to evaluate serum thickness. The patties released between 8 and 20% of serum under compression (**Figure 5.4 (A)**). Assuming a bite size of 10 g (Zhang, Sala, et al., 2024, **Chapter 3**), the obtained bolus contained 8 g of solid bolus fragments (Zhang, Sala, et al., 2024,

Chapter 3)), and only 0.8 – 2 g of released liquid serum. Such low amounts of liquid in the presence of larger amounts of solid bolus fragments probably made it difficult for participants to distinguish serum thickness.

To investigate the relationships between juiciness, fattiness, hardness and serum thickness, network analysis among these sensory attributes was performed, and the results are presented in **Figure 5.8** and **Supplementary Figure S5.1**.

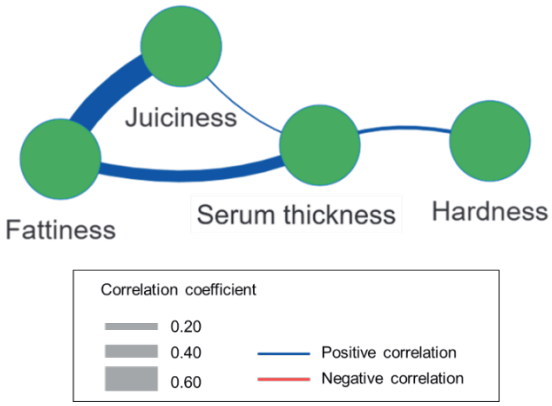

Figure 5.8. Network showing simultaneous associations between texture attributes (green nodes) obtained from Rank-Rating evaluation (n = 75). Edges represent conditional dependencies between texture attributes from partial correlations, and the absence of edges between nodes indicates conditional independence between attributes.

As expected, juiciness and fattiness were strongly positively correlated (**Figure 5.8**), confirming findings from previous studies (Zhang, Brouwer, et al., 2024; Zhang, Sala, et al., 2024, **Chapter 2, 3, 4**). Interestingly, although serum thickness showed almost no differences among patties (**Figure 5.7 (D)**), the network analysis revealed positive and partial correlations between serum thickness and fattiness and juiciness (**Figure 5.8**). These contrasting outcomes from the linear mixed models (LMM) and network analysis can be explained by the different methodologies used. LMM emphasizes the detection of inter-group differences for individual attributes while accounting for covariates, such as panelist, which can result in small effects being masked by large within-group variabilities (Bates et al., 2015; Kuznetsova et al., 2017). Network analysis examines pairwise relationships and interdependencies across all observations,

revealing meaningful correlations even when no significant differences are observed between groups (Behrouzi & Wit, 2019; Kolaczyk & Csárdi, 2020). Therefore, these positive correlations between serum thickness, juiciness and fattiness in the network analysis suggest that increasing serum thickness enhances both juiciness and fattiness perception. Such an effect of viscosity could be related to the presence of fat or maltodextrin in the serum phase. Notably, the stronger correlation between serum thickness and fattiness, compared to serum thickness and juiciness, may suggest that the effect of fat is more important, as fat content also had a larger impact on fattiness.

Serum thickness also showed a positive partial correlation with hardness perception (**Figure 5.8**) suggesting that a more viscous serum might bind bolus fragments together more effectively. Previous studies on whole-cut beef steaks have reported a negative correlation between hardness and juiciness (Judge et al., 2021; J. Liu et al., 2020; Mateescu et al., 2015; Oury et al., 2009). However, no direct correlation was found between hardness and juiciness for our PBMA patties (**Figure 5.8**). These differences may stem from initial structural differences: PBMA patties fall apart easily during mastication with their juiciness being primarily driven by rapid serum release during early breakdown (Zhang, Sala, et al., 2024, **Chapter 3**); on the other hand, juiciness perception of beef steaks involves both initial juiciness and sustained juiciness (Warner, 2017), where increased hardness requires more structural breakdown throughout mastication, prolonging serum release and contributing to sustained juiciness. Therefore, in PBMA patties, juiciness, and also fattiness, are independent of hardness.

In conclusion, juiciness and fattiness perception of PBMA patties seemed mostly affected by total amount of released serum. Serum fat content had a stronger effect on fattiness than juiciness, suggesting that fat content of the serum may be a key factor discriminating between these two attributes. The positive correlation between serum thickness, juiciness and fattiness indicated a potential effect of viscosity on juiciness and fattiness. These potential relationships will be discussed in the next section.

5.3.5 Linking serum properties to sensory perception

To gain more insight into how serum properties influence juiciness and fattiness perception, network analysis was conducted to link serum properties (amount of serum release, serum composition, serum viscosity) to juiciness and fattiness (**Figure 5.9** and **Supplementary Figure S5.2)**. Sensory hardness was excluded in the analysis as this property is related to the patty itself, and not the serum. In addition, the sensory attribute serum thickness was not included in the analysis since it did not vary across the 12 patties.

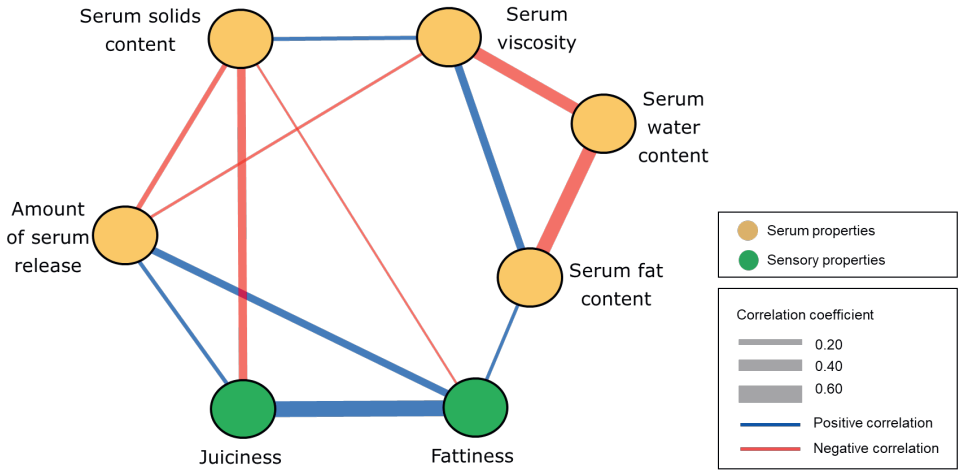

Figure 5.9 Network linking serum and sensory properties, showing simultaneous associations between serum related physicochemical properties (yellow nodes) and serum related sensory properties (green nodes) of PBMA patties differing in TVP hydration level, fat and maltodextrin content (n = 12). Edges represent conditional dependencies between texture attributes from partial correlations, and the absence of edges between nodes indicates conditional independence between attributes.

Although juiciness and fattiness were strongly correlated (**Figure 5.8** and **5.9**), the network revealed distinct factors influencing these attributes (**Figure 5.9**). Both juiciness and fattiness were positively correlated with total amount of serum release during compression, consistent with our previous findings (Zhang, Brouwer, et al., 2024; Zhang et al., 2025; Zhang, Sala, et al., 2024, **Chapter 2, 3, 4**). Juiciness and fattiness also showed a negative correlation with serum solids content (**Figure 5.9**). Serum solids content mainly represents solid material or particles that are pressed out

during serum collection. This negative relation could be an indirect effect reflecting the negative relation between amount of serum release and serum solids content. The higher amount of serum released, the lower the relative solids content, and therefore the lower juiciness perception. No direct correlation between juiciness and serum water and serum fat content was observed (**Figure 5.9**), highlighting that juiciness is determined by total serum release rather than serum composition. Unlike juiciness, fattiness did show a positive correlation with serum fat content. The differences in correlations with specific serum properties highlight a key distinction between juiciness and fattiness. Juiciness is primarily driven by the total amount of serum release, independently of serum composition, whereas fattiness is influenced by both the total serum release and serum fat content. Direct associations between serum viscosity and serum water, fat and solids content confirmed the result of **Figure 5.6** that serum composition was the main driver of serum viscosity. The absence of direct associations between serum viscosity and juiciness or fattiness (**Figure 5.9**) suggest that serum viscosity has a limited influence on these sensory attributes compared to serum composition. The influence of serum fat content on fattiness perception of PBMA patties is therefore not an effect of viscosity. The impact of fat on fattiness could be more related to enhanced lubrication of the bolus, which has been seen in other studies on different foods (de Wijk & Prinz, 2005; Fuhrmann et al., 2020; Godoi et al., 2017; K. Liu et al., 2015). However, verification of this hypothesis would require further investigation.

5.4. Conclusions

The aim of this study was to investigate how serum release and serum composition and viscosity affect juiciness and fattiness perception of PBMA patties. The amount of serum release under mechanical compression appeared to be the main driver of juiciness perception, whereas serum composition only had a limited effect on juiciness. In contrast, fattiness perception was not only influenced by the serum release under compression but also by the composition of the serum, especially its fat content. The contribution of fat was not directly related to an increase in viscosity, suggesting that fat contributes to fattiness via a different mechanism. These results suggest that

optimizing serum release can effectively modify juiciness whereas controlling serum release and serum composition (serum fat content) can impact fattiness. Future studies could explore the role of bolus properties on dynamic sensory perception to better understand juiciness and fattiness, providing possible strategies into maintaining juiciness and fattiness while reducing fat content.

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Supplementary material

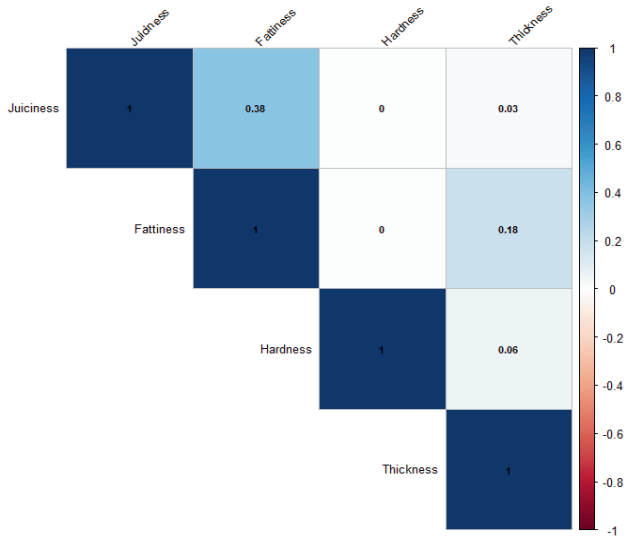

Figure S5.1 Partial correlation coefficients of sensory properties of PBMA patties prepared with different TVP hydration level, fat content and MD content (n = 75).

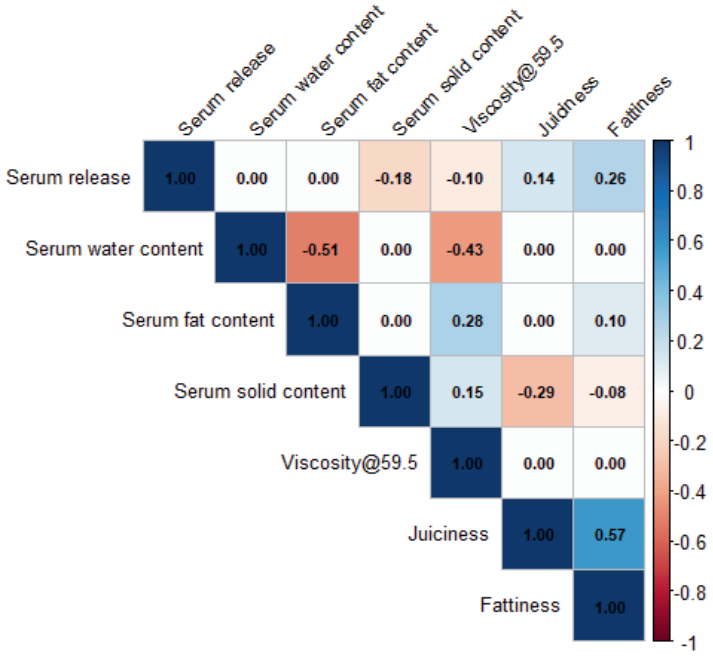

Figure S5.2. Partial correlation coefficients of relationships between physicochemical and sensory properties of PBMA patties prepared with different TVP hydration level, fat content and MD content (n = 12).

Table S1. Results of statistical data analysis describing the effects of TVP hydration level, fat content and maltodextrin (MD) content on serum viscosity of PBMA patties. F (DFn, DFd) and *p* values (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) are derived from three-way ANOVA with TVP hydration level, fat content, MD content and their interactions as fixed factor.

Shear rate	Hydration		Fat		MD		Hydration: Fat		Hydration: MD		Factor: MD		Hydration: Fat: MD	
(s^{-1})	F (1,24)	*p*	F (2,24)	*p*	F (1,24)	*p*	F (2,24)	*p*	F (1,24)	*p*	F (2,24)	*p*	F (2,24)	*p*
0.1	15.5	***	5.0	*	0.0	ns	4.1	*	0.2	ns	0.5	ns	0.5	ns
0.153	16.3	***	5.7	**	0.0	ns	4.1	*	0.2	ns	0.2	ns	0.3	ns
0.234	15.0	***	5.6	**	0.0	ns	3.5	*	0.3	ns	0.1	ns	0.2	ns
0.359	19.0	***	7.5	**	0.0	ns	4.6	*	0.2	ns	0.0	ns	0.1	ns
0.549	19.8	***	8.0	**	0.0	ns	4.4	*	0.3	ns	0.1	ns	0.2	ns
0.841	23.6	***	8.6	**	0.0	ns	4.4	*	0.6	ns	0.1	ns	0.3	ns
1.29	38.1	***	9.8	***	0.1	ns	4.3	*	1.3	ns	0.1	ns	0.2	ns
1.97	56.1	***	19.2	***	0.0	ns	8.8	**	1.4	ns	0.3	ns	0.9	ns
3.02	50.5	***	20.6	***	0.1	ns	8.3	**	1.1	ns	0.2	ns	0.6	ns
4.62	45.3	***	19.3	***	0.3	ns	7.3	**	0.7	ns	0.1	ns	0.3	ns
7.07	63.5	***	21.9	***	0.1	ns	7.4	**	1.8	ns	0.0	ns	0.5	ns
10.8	60.2	***	30.1	***	1.3	ns	9.7	**	0.5	ns	0.3	ns	0.5	ns
16.6	74.5	***	39.5	***	2.1	ns	11.9	***	0.6	ns	0.3	ns	0.8	ns
25.4	52.9	***	26.5	***	0.7	ns	7.7	**	1.0	ns	0.2	ns	1.0	ns
38.8	71.1	***	37.3	***	1.5	ns	9.9	***	1.0	ns	0.3	ns	0.9	ns
59.5	78.4	***	45.0	***	2.7	ns	11.4	***	0.7	ns	0.5	ns	0.9	ns
91	90.7	***	56.8	***	5.2	*	13.9	***	0.3	ns	0.6	ns	0.7	ns
139	86.6	***	56.7	***	5.8	*	13.6	***	0.2	ns	0.7	ns	0.6	ns
213	91.5	***	63.0	***	7.8	**	14.5	***	0.0	ns	0.7	ns	0.5	ns
327	96.6	***	68.7	***	9.9	***	15.2	***	0.0	ns	0.6	ns	0.5	ns
500	108.3	***	78.0	***	12.1	***	16.4	***	0.0	ns	0.4	ns	0.6	ns

Chapter 6

General discussion

Sensory appeal, especially texture, remains a key barrier to consumer acceptance of plant-based meat analogues (PBMAs) (Gómez-Luciano et al., 2019; Hoek et al., 2011; Weinrich, 2019). Up to now, engineering efforts have prioritized ingredient functionality and processing techniques to mimic meat-like structures (Dekkers et al., 2018; Kyriakopoulou et al., 2021; McClements, 2024; McClements & Grossmann, 2021). However, the mechanisms behind texture perception of the obtained products, particularly juiciness, are poorly understood. This thesis takes patty-type PBMAs as the research subject, aiming to explore the relationships between their physicochemical properties, oral structural breakdown and sensory characterizations, with particular focus on juiciness perception.

6.1 Main findings

Figure 6.1 summarizes the key results of this thesis. **In Chapter 2**, PBMA and beef patties with different juiciness levels were created by adjusting the core temperature of commercially available products. Core temperature had distinct effects on each patty type: in PBMA patties, lower core temperature resulted in patties with higher fat content and increased serum release without altering other texture attributes/properties, while in beef patties, lower core temperature led to patties with higher water content, increased serum release and firmer texture. For both patty types, juiciness was positively correlated with serum release and fattiness and negatively correlated with dryness. Despite these differences in food and sensory properties, neither patty type showed significant variations in oral processing behavior nor bolus properties at the moment of swallowing. These results suggest that juiciness is primarily determined by serum release during mastication and is not influenced by late-stage oral breakdown.

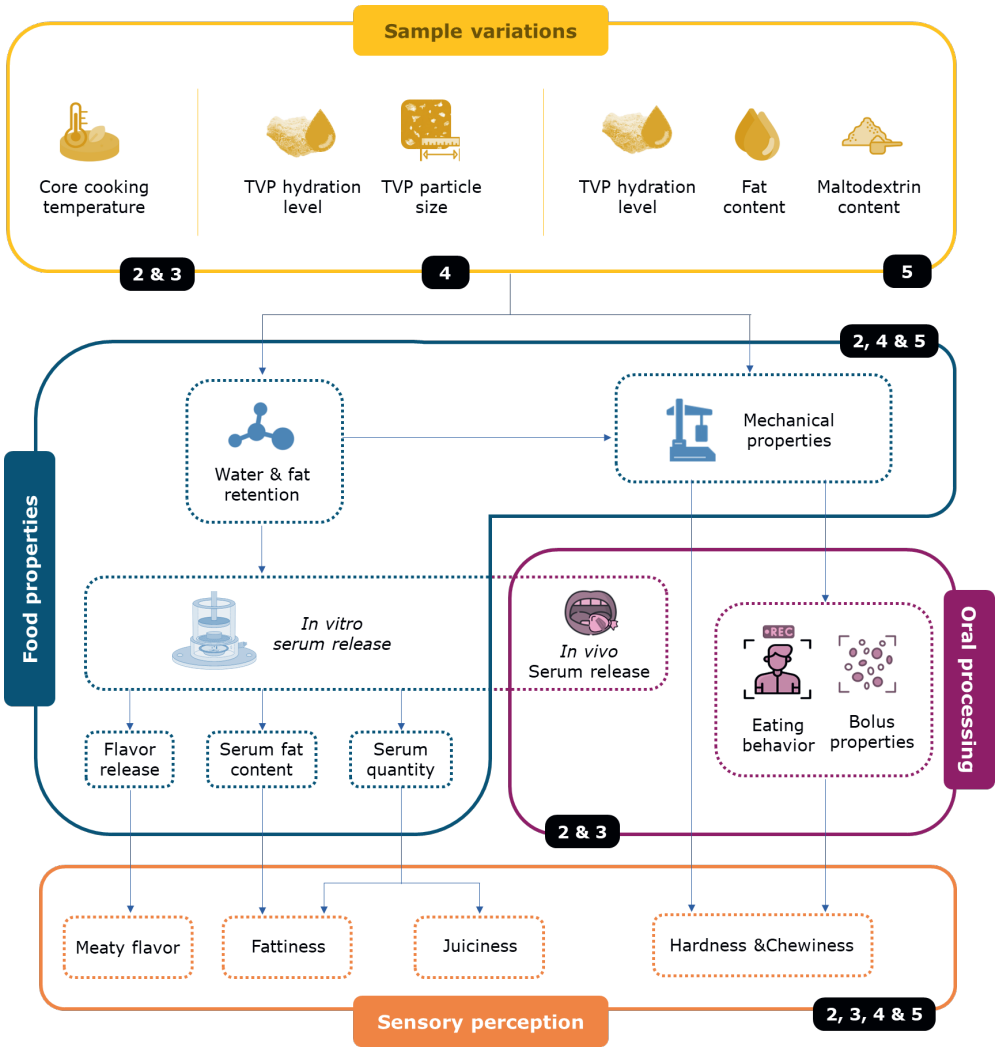

Figure 6.1 Schematic overview of the main findings in this thesis. Direction of effects is explained in section 6.1.

In **Chapter 3** we further investigated the role of oral structural breakdown on dynamic juiciness perception using the sample set of **Chapter 2**. Temporal analysis revealed that, for both PBMA and beef patties, juiciness perception peaked early during mastication, aligning closely with the rapid serum release during this early stage of mastication (75% of the total serum). While juiciness of beef patties increased with increasing water content and expellable bolus liquid, the oral breakdown of PBMA patties did not affect juiciness. These findings suggest that juiciness of PBMA patties is

primarily driven by early-stage serum release, and not affected by additional oral structural breakdown of the patty bolus. In beef patties, both early-stage serum release and sustained oral structural breakdown contributed to juiciness. The limited variability in physicochemical properties of PBMA patties in response to core temperature may explain why bolus properties had no effect on juiciness perception.

To address this, for the following experiments, PBMA patties were formulated by modifying the hydration level and particle size of textured vegetable proteins (TVPs) to introduce greater variation in compositional and mechanical properties (**Chapter 4**). Increasing TVP hydration level increased water content of the cooked patty and serum release under compression, enhancing juiciness and fattiness. Increasing TVP particle size mainly affected patty texture, increasing hardness and chewiness, which negatively correlated with juiciness. These results confirmed that serum release remains the primary driver for PBMA juiciness perception, while patties with a less stiff texture and higher water content further enhanced juiciness. Increased juiciness not only boosted flavor perception but also drove consumer liking.

Since juiciness and fattiness are highly correlated to serum release under compression (**Chapter 2-4**), **Chapter 5** explored how serum compositional and rheological properties influence juiciness and fattiness perception of PBMA patties. PBMA patties with different serum properties were prepared by adjusting TVP hydration level, fat content and maltodextrin (MD) addition. A higher TVP hydration level increased total serum release and water content in the serum; a higher raw patty fat content increased fat content in the serum and serum viscosity; MD addition reduced total serum release but increased serum viscosity. Network analysis confirmed that the amount of serum release was the primary driver of juiciness and fattiness, and serum composition and viscosity were less important. Serum composition affect fattiness, as serum fat content enhanced fattiness perception without altering juiciness.

6.2 What drives juiciness perception of PBMA patties?

6.2.1 Serum release determines juiciness perception

Serum release under compression/mastication emerged as the key determinant of juiciness across all chapters. To understand how serum release and serum composition further affect juiciness perception, **Figure 6.2** integrates data obtained from the individual studies presented in **Chapters 2, 4 and 5** to examine the correlation of total serum release and serum water content with static juiciness intensity. Rank-rating data from **Chapter 5** were proportionally transformed from a 100-point scale into a 9-point scale for direct comparison. Serum fat content was not included in the cross-chapter analysis since it was only accurately assessed in **Chapter 5** due to limited serum collection in earlier chapters.

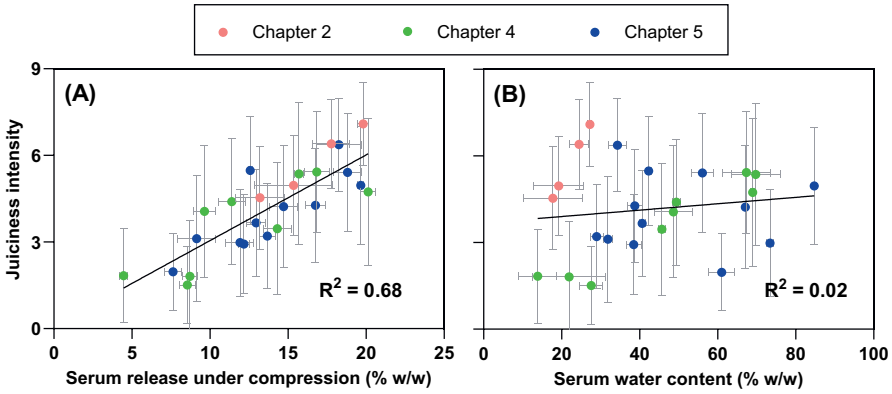

Figure 6.2 Relationship between juiciness intensity and (A) serum release under compression and (B) serum water content of PBMA patties (data from Chapter 2, 4 and 5). Error bars indicate the standard deviation of mean. Solid lines represent linear regressions.

The total amount of released serum showed a clear positive correlation with juiciness perception when combining data sets of three studies (R^2 = 0.68) (**Figure 6.2 (A)**). Notably, these studies used three different panels of untrained consumers. Despite this variability, a very consistent and robust correlation between juiciness and amount of serum release under compression was observed. In contrast, serum water content did not correlate with sensory juiciness (**Figure 6.2 (B)**), which likely stems from differences in experimental design across the three studies. A strong correlation was only found

when changes in serum water content were the primary factor driving serum release variations. For example, in **Chapter 4**, increased TVP hydration level led to higher levels of water retained in cooked patties, resulting in more water release during compression elevating total serum release. However, in **Chapter 5**, where multiple factors (such as fat content and maltodextrin addition) influenced serum release, the correlation between serum water content and juiciness disappeared. No direct correlation between juiciness and serum fat content was observed (**Chapter 5**), further supporting that composition of released serum (water and fat) play a secondary role in juiciness perception.

The relationship between serum quantity, serum composition and juiciness may be context-dependent, especially when juiciness extends beyond early mastication and is not solely driven by rapid serum release. For example, in whole-cut meat, juiciness comprises both initial juiciness and sustained juiciness (Font-i-Furnols et al., 2015; Warner, 2017). Studies on beef steaks showed that initial juiciness correlates more strongly with expressible liquid (serum release) under compression than with initial meat composition (Lucherk et al., 2016, 2017), while sustained juiciness depends on interactions between water, intramuscular fat and saliva incorporation during mastication (Aaslyng et al., 2003). In our study on beef patties (**Chapter 3**), we also found that juiciness peaked early and was primarily determined by rapid serum release. However, we did not observe the pronounced sustained juiciness in all beef patties as reported in beef steak. In patties with lower-juiciness (those with a core temperature of 70 and 80℃), increased bolus water content and expellable liquid due to saliva incorporation tended to prolong the low-intensity juiciness. This difference may be attributed to the looser structure of beef patties compared to beef steaks, which breakdowns more easily, leading to a less pronounced sustained juiciness.

Different findings have been reported for cooked sausage, a homogenous emulsion-type product with limited rapid serum release upon early mastication. In the study of Kang et al. (2020), juiciness of frankfurters seems to be correlated more strongly with the fat content of released fluid during centrifugation rather than the total fluid release (correlation analysis was not performed, but the trend can be observed). Although oral

breakdown was not quantified, the homogenous structure in which water and fat are well embedded, would likely limit rapid serum release. The increased serum fat content may enhance overall juiciness perception by improving bolus lubrication or stimulation saliva production during mastication. Therefore, if the quantity of serum release indeed governs initial juiciness, serum composition may influence sustained juiciness by shaping bolus mechanical and compositional properties.

6.2.2 Role of compositional and mechanical properties of PBMA patties

Composition and mechanical properties of PBMA patties showed inconsistent correlations with juiciness (**section 6.1**), mainly because these properties caused or coincided with changes in serum release. For example, in **Chapter 2**, juiciness increased with increasing fat content of cooked patties but was not influenced by water content and instrumental hardness of cooked patties. This is because decreasing core cooking temperature of PBMA patties enhanced fat retention during cooking while water retention and texture were less affected, ultimately leading to higher serum release. In **Chapter 4**, juiciness increased with higher water content and less stiffed cooked patties, while juiciness was not affect by patties' fat content. This was because increasing TVP hydration level (i.e., adding more water to raw patties) without increasing methylcellulose (MC) content simultaneously increased serum release and decreased patty stiffness. When both TVP hydration level and MC concentration were increased together (**Chapter 5**), only serum release increased, but patty stiffness remained unchanged, causing the correlation between juiciness, patty water content and stiffness to disappear. These findings suggest that the amount of serum release drives juiciness, while the composition and texture of cooked patties influence juiciness indirectly by modulating serum release.

In view of the role of patty composition, texture and serum release in juiciness perception, understanding which physicochemical properties of the cooked patty influence release is crucial. **Figure 6.3** summarizes how water content, fat content and total liquid content of cooked patties relate to serum release under compression across **Chapter 2, 4 and 5**. Serum release under compression showed the strongest correlation with total liquid content of cooked patties compared to the weak and no

correlation with water and fat content of cooked patties. These correlations are consistent and robust among the three studies with different PBMA patties. In some cases, a single parameter alone also strongly correlated with serum release, such as fat content (**Chapter 2)** and water content (**Chapter 4**). This is because, in these instances, either water or fat was the primary factor influencing total liquid retention after cooking. Studies on meats had already quantified expressible fluid (serum release) under compression (Aaslyng et al., 2003; Lucherk et al., 2017) and linked it to sensory juiciness. However, these works did not focus on its relationship with compositional properties to understand the factors determining serum release. This thesis therefore advances this knowledge by demonstrating that in PBMA patties total liquid retained in cooked patties is an effective indicator of serum release under compression.

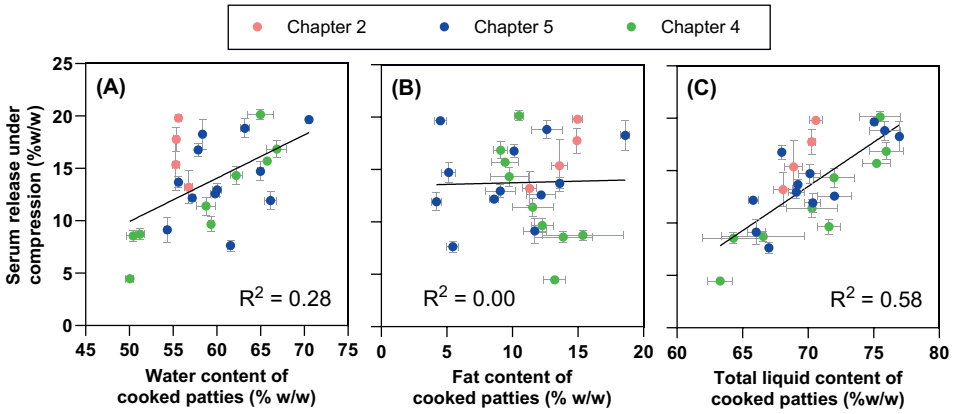

Figure 6.3 Relationships between serum release under compression and (A) water content, (B) fat content and (C) total liquid content of cooked PBMA patties (data from Chapter 2, 4 and 5). Error bars indicate the standard deviation of mean. Solid lines represent linear regressions.

The total liquid content in cooked patties does not fully explain the releasable liquid under uniaxial compression. Only the free water and fat in air pockets of TVPs or internal cavities of PBMAs (Cornet et al., 2020; van Esbroeck et al., 2024), or the fluids distributed in a homogenous gel phase (van den Berg et al., 2007b) are likely released during compression. This thesis did not investigate water/fat distribution in PBMA patties, and interactions between proteins, hydrocolloids, water and fat in the gel phase affect serum release. Future studies could address these knowledge gaps to

clarify the mechanisms underlying serum release under compression in PBMAs, providing insights to help the food industry optimize formulation strategies for effective serum release and boosting juiciness perception in PBMAs.

Beyond composition, the mechanical properties of PBMA patties may also influence serum release under compression. When examining the correlation analysis between serum release and instrumental hardness conducted separately in **Chapter 2, 4 and 5**, only **Chapter 4** showed a significant negative correlation (r = - 0.74), due to MC concentration. This result suggests that mechanical properties of cooked patty have a limited influence on serum release. Instead, the microstructure of the gel phase of PBMA patties may play a role in adjusting serum release during compression, since serum release was found to be influenced by microstructural properties, such as porosity in hydrated gels (van den Berg et al., 2007a, 2007b).

6.2.3 Role of other sensory properties

Sensory perception is a complex multimodal process involving gustation, olfaction, tactile sensation, audition and vision to measure and interpret food characteristics (Bredie & Møller, 2012; De Araujo & Rolls, 2004; Verhagen & Engelen, 2006). Among these, texture perception is particularly dynamic, as it evolves throughout oral processing and is influenced by the structural and mechanical properties of food (Devezeaux de Lavergne et al., 2017; Pascua et al., 2013). In this thesis, juiciness was not independent but was correlated with a few other texture and flavor attributes.

Fattiness was consistently found to be strongly aligned with juiciness across all studies (**Chapter 2–5**). This correlation is evident in both static sensory analysis using Rate-All-That-Apply (RATA) (**Chapter 2 and 4**) and rank-rating tests (**Chapter 2 and 5**), as well as in dynamic sensory analysis with Temporal Check-All-That-Apply (TCATA) (**Chapter 3**). Similar strong correlations between juiciness and fattiness have been reported in various meats and PBMAs (Aaslyng et al., 2003; Godschalk-Broers et al., 2022; Hong et al., 2022; Jeong et al., 2010; Lyu et al., 2024). Moreover, our findings across **Chapter 2–5** further confirm that both attributes are primarily driven by the amount of serum release during compression/mastication. Several possible mechanisms may explain the similarity in juiciness and fattiness perception. One possibility is a cognitive

association, as traditionally, consumers conflate juiciness and fattiness in meats. This learned association may bias untrained panelists to rate these two attributes similarly, even when fat content remained consistent across different PBMA patties (**Chapter 2-4**). Higher fat content can also enhance bolus lubrication and reduce resilience during oral breakdown, contributing to sustained juiciness, particularly in meats (**section 6.2.1**). Another explanation could be the temporal overlap, since both juiciness and fattiness peaked early during mastication (**Chapter 3**), the rapid release of serum may lead to the simultaneous increased intensity of both attributes.

So, what differentiates juiciness and fattiness perception? This thesis advances the understanding by examining their temporal dynamics and the influence of serum composition. We found that, compared to juiciness, which peaked early, declined sharply, and approached zero by swallowing, fattiness also peaked early but decreased more gradually and even showed a slight increase after swallowing (**Chapter 3**). This result suggests that while juiciness in PBMA patties is mainly driven by rapid serum release during early mastication, fattiness may not only influenced by the rapid serum release but also lingers longer during sustained mastication, likely due to saliva incorporation and mouthcoating effects. Serum composition further differentiates juiciness and fattiness. Juiciness is affected solely by serum quantity, whereas fattiness is also positively related to the fat content in the serum (**Chapter 5**). Although previous studies on fluid dairy and emulsion based foods have found that fat perception is largely influenced by the viscosity of the liquid or semi-solid food (Akhtar et al., 2005; Mela, 1988; Mela et al., 1994), this effect was not observed in PBMA patties (**Chapter 5**). In liquid and semi-solids foods, the bolus remains in a homogeneous liquid or semi-solid state during mastication, whereas the bolus of PBMA patties is highly heterogeneous, consisting typically of 76-88% PBMA fragments mixed with 12-24% expressible liquid (serum and saliva) (**Chapter 2 and 3**). Because only a relatively small proportion of liquid coexists with a large amount of solid bolus fragments, sensing the properties of the liquid during mastication may become more challenge for panelists and consumers. This may explain why serum viscosity had no significant effect on fattiness and juiciness perception in PBMA patties (**Chapter 5**). However,

since bolus gets more homogenous towards swallowing, varying serum viscosity might change the bolus viscosity, thereby altering dynamic sensory profile of fattiness and juiciness, especially at later stages, potentially prolonging its sensation. Future studies could further investigate how serum viscosity affects the temporal profile of fattiness and juiciness to verify this hypothesis, providing new insight into fat reduction strategies in PBMAs.

Hardness and chewiness are additional attributes that might influence juiciness. These attributes were assessed in **Chapter 2** and **4** together with juiciness, fattiness and meat flavor using RATA by different panels in two independent experimental studies. So, network analysis was performed on the combined data sets as shown in **Figure 6.4**.

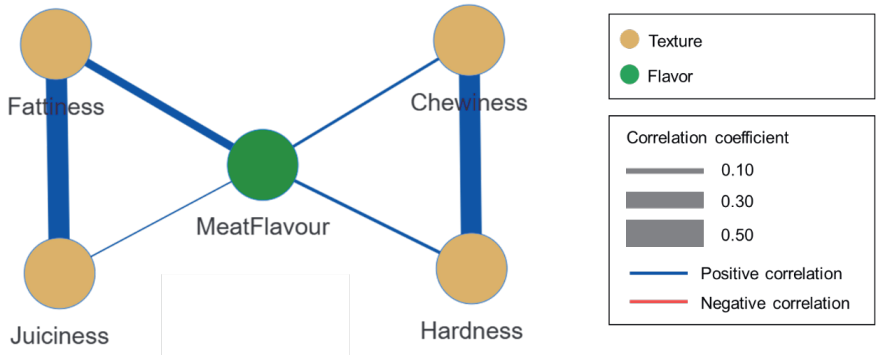

Figure 6.4 Sensory network showing simultaneous associations between texture (yellow nodes) and flavor (green nodes) attributes obtained from RATA evaluation in Chapter 2 (n = 99) and Chapter 4 (n = 69). Edges represent conditional dependencies between texture attributes from partial correlations, and the absence of edges between nodes indicates conditional independence between attributes.

No direct association was found between juiciness and hardness or chewiness (**Figure 6.4**), which was also supported by **Chapter 5**, where juiciness and hardness also showed no direct correlation. However, a negative correlation between hardness and juiciness was observed in **Chapter 4**. This contradiction can again be explained by the variation in MC concentration, as discussed in **section 6.2.2**. Therefore, we conclude that hardness perception does not directly impact juiciness in PBMA patties. The lack of correlation between juiciness and chewiness in PBMA patties can be explained by the different temporal dynamics of these two attributes during oral processing. In PBMA patties, only the initial juiciness is important, which is boosted by rapid serum

release during dramatic structural breakdown at early mastication, while chewiness persisted through and is more related to the resilience effort during mastication (**Chapter 3**). That is also why hardness and chewiness may have a stronger effect on juiciness in other type of meat and PBMAs, such as whole-cut beef steaks (Judge et al., 2021; Liu et al., 2020; Mateescu et al., 2015; Mathoniere et al., 2000; Oury et al., 2009; Thompson, 2004), where the sustained oral processing plays a larger role in juiciness perception.

Moreover, **Figure 6.4** shows that meat flavor is also directly correlated with fattiness and juiciness. This finding, based on combined data from two independent studies with different panels and PBMA patties (**Chapter 2 and 4**), further reinforces our conclusion that juiciness and fattiness enhance flavor perception in PBMA patties. The increase in serum release during mastication, which drives juiciness, might also enhance the release of hydrophobic and hydrophilic aroma compounds, thereby enhancing meat flavor perception. In other words, the released serum might facilitate the delivery of aroma compounds during mastication. This aligns with findings in the literature that increased serum release can lead to increased sweetness perception in different fruits (Harker et al., 2006; Schwieterman et al., 2014) and gels (Sala et al., 2010). Additionally, the correlation between fattiness and meat flavor was stronger than that between juiciness and meat flavor (**Figure 6.4**), likely because increased fat content in released serum enhances fattiness (but not juiciness) and also promotes the release of hydrophobic meat flavor compotes.

6.3 Towards improving the sensory quality of PBMA patties

This thesis not only explored the underlying mechanisms of juiciness perception of PBMA patties, but also advanced knowledge on how to modify juiciness perception of PBMA patties. **Table 6.1** summarizes the approaches used in this thesis to modify juiciness, fattiness and hardness and magnitude of their effect.

Table 6.1 Summary of modifications of PBMA patty properties and their effects on juiciness, fattiness and hardness perception.

Modified property	Range	Effect on juiciness		Effect on fattiness		Effect on hardness		Chapter
		Intensity variation	Effect magnitude*	Intensity variation	Effect magnitude*	Intensity variation	Effect magnitude*	
Core cooking temperature** (°C)	90, 80, 70, 60℃	**+ 55%**	Large	**+ 43%**	Medium	**+ 4%**	Very small	2
	80, 70, 60	**+ 37%**	Medium	**+ 8%**	Very small	**- 4%**	Very small	3***
TVP hydration level (TVP:Water ratio)	1:1, 1:3, 1:5	**+ 203%**	Large	**+ 71%**	Large	**- 53%**	Large	4
	1:2, 1:4	**+ 72%**	Large	**+ 41%**	Medium	**+ 8%**	Very small	5
TVP particle size (surface area, mm^2)	0.2, 10, 20	**+ 16%**	Small	**+ 7%**	Very small	**+ 60%**	Large	4
Fat content (%)	5, 12, 19	**+ 20%**	Small	**+ 40%**	Medium	**- 49%**	Medium	5
Maltodextrin content (%)	0, 3%	**- 17%**	Small	**- 16%**	Small	**- 24%**	Medium	5

***Definition of effect magnitude: very small effect: variation < 10%; small effect: variation 10-30%; medium effect: variation 30-50%, Large effect: variation > 50%. ****The effect of core cooking temperature was assessed using commercial Beyond Meat product. *****To calculate intensity variations in Chapter 3, the areas under citation proportion curves from TCATA were used.

Varying core cooking temperature is an effective way to modify juiciness and fattiness perception of PBMA patties, without affecting hardness (**Chapter 2 and 3**). Since PBMAs experience less shrinkage and structure change upon heating, core temperature mainly affects juiciness and fattiness by altering compositional properties and serum release, rather than changing mechanical properties. This explanation aligns with the study from Vu et al. (2022), who demonstrated that core temperature, rather than cooking methods, influenced PBMA patties' composition but not texture. However, this approach appears to be more effective only for PBMA patties that initially contain more liquid before cooking, such as Beyond Mince that was chosen in **Chapter 2 and 3**. Our pilot studies indicated that other commercial PBMA patties, such as the Garden Gourmer Vegan burger and De Vegentarische Slager MC2 burger, showed little variation in juiciness when varying core cooking temperatures. Therefore, although varying core cooking temperature can easily be adopted by consumers when

preparing PBMA patties, the effect will depend on the initial composition and structure of the raw patty, and may therefore not be suitable for all commercially available PBMAs. Another potential limitation of this approach is food safety. Although lower core temperatures result in juicier and fattier PBMA patties, temperature as low as 60℃ might raise concerns on safe consumption.

Among all modification approaches, varying TVP hydration level had the largest influence on both juiciness and fattiness perception (**Table 6.1**). In contrast, TVP particle size, raw patty fat and maltodextrin content only had small to moderate effects on juiciness and fattiness. Notably, increasing fat content by 3.8 times (from 5% to 19%) only increased juiciness by 20% and fattiness by 40%, whereas simply increasing initial water content of raw patty by 1.4 times (from 52% to 73%) via TVP hydration resulted in a more than 200% increase in juiciness and a similar 41% increase in fattiness. These results highlight that incorporating more water in raw patties through TVP hydration is a far more effective strategy for enhancing juiciness and fattiness perception than increasing fat content. It should also be noted that the TVPs used in **Chapter 4 and 5** were intentionally selected from commercial TVPs with high water absorption capacities (WAC), capable of absorbing up to 700% of their weight in water. This means that varying TVP hydration levels might be less feasible for PBMAs made with low-WAC TVPs. Therefore, we recommend that manufacturers select high-WAC TVPs and optimize TVP hydration levels to produce juicier PBMA patties while exploring ways to improve WAC in TVP production. This approach offers a healthier and more cost-effective way to produce juicy and fatty PBMA patties without using excessive fat.

Similar to juiciness, compositional variations also affected hardness. While modifications in TVP particle size, fat content and maltodextrin had a small effect on juiciness, it had a medium to large effects on hardness perception of PBMA patties (**Table 6.1**), As shown in **Chapter 4**, a suitable hardness and chewiness perception is also important for consumer liking. Therefore, even though modifying TVP particle size, methylcellulose content and fat content does not directly improve juiciness, these variations could still effectively improve the overall texture quality of PBMA patties. While our findings offer practical approaches for manufactures to fine-tune the texture

of PBMA patties, these approaches may not be directly applicable to other types of PBMAs and would require further research.

6.4 Proposed mechanisms underlying dynamic juiciness perception of meat analogues

Juiciness perception evolves from the first bite to the final swallow, and is shaped by the interplay between initial food properties, oral structure breakdown and other texture properties occurring during mastication. Here we speculate on the mechanisms underlying dynamic juiciness perception of minced-type, muscle-type and emulsion-type meat analogues (**Figure 6.5**).

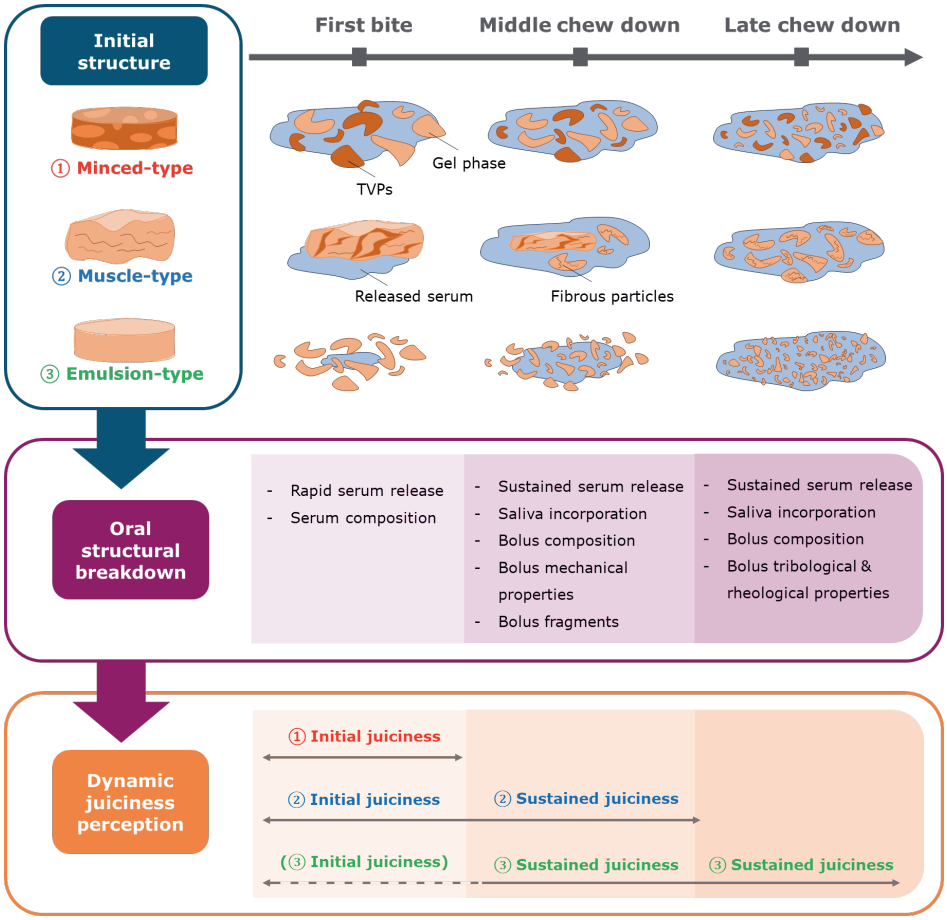

Figure 6.5 Proposed mechanisms underlying dynamic juiciness perception of different types of meat analogues.

- Minced-type PBMAs have a loose and particulate structure with weak bonds between TVP particles and high porosity, resulting in low structural integrity. During the first bite and early chews, the matrix collapses, breaking down into its original components (individual TVP chunks), and releasing free water and fat from the porous network and continuous phase. As discussed in **section 6.2**, minced type PBMAs mainly contain initial juiciness which is driven by rapid serum release during early mastication. Serum composition plays a secondary role, mainly influencing juiciness by affecting the total amount of serum release. Serum viscosity does not affect initial juiciness because its effect is overshadowed by the presence of large solid particles.

- Muscle-type PBMAs are characterized by their fibrous structure, which is replicated in PBMAs through high moisture extrusion or shear cell technology (Dekkers et al., 2018; Kyriakopoulou et al., 2021; Schreuders et al., 2021). Based on work of Font-i-Furnols et al. (2015) and Warner (2017) on meats, we propose that the structural anisotropy leads to two stages of dynamic juiciness perception: initial juiciness, driven by rapid serum release upon the first bite, and sustained juiciness, which develops during mastication. While the rapid serum release in the early stages of mastication is similar to minced-type products, the underlying mechanisms differ. In minced-type products, early serum release is primarily contributed by rapid oral structural disintegration, whereas muscle-type PBMAs rely on mechanical compression of the foods between molar teeth, with less pronounced structure breakdown. As a result, more chews are required in the middle chew down phase, during which sustained juiciness perception emerges. Juiciness perception at this stage might be influenced by sustained serum release, saliva incorporation, and bolus textural changes (such as particle size and resistance to fragmentation). For comparison, it has been shown that in meat steaks, shorter sarcomeres and lower collagen content reduce chewing resistance, promoting fragmentation, enhancing tenderness and prolonging juiciness perception (Ertbjerg & Puolanne, 2017; Listrat et al., 2016; Pematilleke et al., 2020).

- Emulsion-type PBMAs feature a homogeneous gel matrix composed of water, plant-based proteins, fat, carbohydrates, salt and spices (Dekkers et al., 2018; Kyriakopoulou et al., 2021), providing a cohesive texture that effectively minimizes free fluid and

ensures structural integrity. While emulsion-type PBMAs breakdown into small bolus fragments during the first few chews, their rapid serum release is limited compared to that of minced-type PBMAs. This difference arises due to the fact that emulsion-type PBMAs lack the sponge-like structure of minced-type PBMAs. Instead, they form hydrated gels during cooking that embed water and fat within a continuous protein matrix, restricting rapid serum release upon the first bites (**Figure 6.4**). Therefore, we speculate that initial juiciness perception may be subtle. However, exceptions exist, such as encased sausages, where cooking loss is tapped by casings, leading to more rapid serum release and more pronounced initial juiciness. As mastication progresses, the gel matrix gradually fractures, increasing particle number and surface area, promoting water and fat release (Devezeaux de Lavergne, van de Velde, et al., 2015; Sala et al., 2007) and facilitating saliva incorporation (Devezeaux de Lavergne et al., 2016; Sala & Stieger, 2013), progressively enhancing juiciness perception. Towards the end of consumption, continued fragmentation, sustained serum release and saliva incorporation increase bolus liquid content, flowability and lubrication, sustaining a moist, smooth and juicy mouthfeel until swallowing. Ultimately, dynamic juiciness perception in emulsion-type products is expected to be shaped by delayed but sustained serum release, gradual structural breakdown, saliva incorporation and enhanced bolus lubrication.

In summary, we propose that for minced-type PBMAs, juiciness perception is determined by an easy structure break down during the first bite, leading to rapid serum release and high initial juiciness, which is not sustained. When products have a higher integrity, such as muscle-type and emulsion-type PBMAs serum release during the first bite may lead to less initial juiciness, but juiciness is sustained during mastication. In this case, juiciness perception may shift from being serum-driven to saliva- and bolus-driven in later oral processing stages.

6.5 Methodological considerations

6.5.1 Physicochemical measurements

This thesis developed *in vitro* methods to quantify serum release from patties and validated the *in vitro* with *in vivo* methods (**Chapter 2**). A controlled compression setup with vacuum suction and efficient serum collection was designed for *in vitro* measurements. The rationale for this approach was to quantify the expressible serum potential under compression and enable detailed serum properties analysis. However, one might argue that this method does not fully mimic oral conditions, since sponge-like PBMA patties not only release serum upon compression but also reabsorb the serum when the force is released. Simpler methods, such as the filter paper compression (Joo, 2018; Lucherk et al., 2017; Yau & Huang, 2001) can account for reabsorption but fail to replicate the dynamic breakdown of the matrix. While these methods may better simulate reabsorption, they lack the precision and analytical capabilities of serum composition and rheology analysis. I*n vivo* serum release was assessed using an experimental chewing bag protocol, which prevented saliva incorporation while allowing for oral breakdown, serum release and serum reabsorption. Even though chewing with bags is not a fully natural chewing process, it provided a closer reproduction of oral conditions than traditional methods. The high correlation between *in vitro* and *in vivo* method, as well as their alignment with juiciness perception (**Chapter 2 and 3**), emphasizes the predictive potential of both *in vivo* and *in vitro* methods developed in this thesis. One limitation of the current *in vitro* compression method is its lack of temporal resolution. As discussed in **section 6.4,** serum release occurs in both rapid and sustained release phases, but the current *in vitro* method only quantifies total release, failing to capture the dynamic release profile as observed *in vivo* (**Chapter 3**). Future research could optimize compression parameters, such as compression time and strain, to better stimulate serum release in different mastication stages as measured by *in vivo* method. While the vacuum-based compression method presented in this thesis improves upon existing techniques by enabling serum collection and analysis, the simpler filter paper compression method may suffice for studies focused solely on quantifying serum release.

To further characterize oral structural breakdown, boli were collected and analyzed at different mastication stages (**Chapter 3**). It is well known that individual variability in oral processing behavior differs considerably across consumers (Chen et al., 2022; Devezeaux de Lavergne, Derks, et al., 2015; Devezeaux de Lavergne, van de Velde, et al., 2015; van Eck et al., 2019, 2020). The focus of this thesis was to investigate differences between food samples rather than inter-individual differences. To minimize inter-individual variability in oral processing behavior and consequently bolus properties, a mastication protocol was imposed on participants in **Chapter 2 and 3**, based on chewing behavior parameters (bite size, chewing frequency and number of chews) previously quantified using video recordings of participants consuming the foods in a natural manner. While this approach minimized inter-individual differences in bolus properties, it did not fully remove them. An alternative approach can be to generate boli using a masticator device, such as the AM^2 masticator, which has recently been used to replicate the dynamic structure breakdown of PBMA and beef patties (Giron et al., 2025). However, using such a device first requires determining *in vivo* oral breakdown to ensure the stimulated conditions reflect human mastication. Even then, validation is typically needed to confirm the simulated breakdown is representative for the oral breakdown. This process requires extensive modifications and can be highly product-specific. Given these challenges, a more practical approach to reducing inter-individual differences may be to use a single trained volunteer to produce multiple boli in a repeatable way.

6.5.2 Sensory evaluation

In this thesis, all sensory evaluations were conducted with naïve consumers, who attended only a familiarization session to become familiar with the samples and sensory methods. Despite the lack of training, these consumer panels were able to distinguish differences between samples across complex sensory attributes, such as juiciness, tenderness, chewiness and fattiness using various sensory methods (**Chapter 2-5**). Furthermore, **Figure 6.2** shows remarkable consistency in juiciness evaluation when pooling data from **Chapter 2, 4 and 5**, highlighting the capability and reliability of naïve consumer in such studies assessing these complex sensory attributes.

However, a limitation of using consumer panels is the need for a large number of participants since part of the data consists of 'noise' compared to that obtained by a trained panel. Yet, this 'noise' reflects real variability in consumer perception, which could also be seen as a strength, providing more ecologically relevant data, as their responses better represent the diversity of consumers.

Static sensory methods, Rate-All-That-Apply (RATA) (**Chapter 2 and 4**) and rank-rating (**Chapter 2 and 5**) were used to characterize attribute intensity, but they differ in efficiency and sensitivity. RATA is generally more efficient, allowing panelists to evaluate multiple attributes simultaneously (Ares et al., 2014), whereas rank-rating focuses on one attribute at a time but is more effective for detecting subtle differences between samples. This is because rank-rating serves multiple samples together, facilitating direct comparisons, while RATA follows a monadic serving approach, preventing direct comparisons (Ares et al., 2014; Kim & O'Mahony, 1998). Consequently, in **Chapter 2**, when both methods were used to evaluate juiciness, rank-rating detected larger differences between samples. Similarly, rank-rating was preferred in **Chapter 5** for identifying potential subtle differences between juiciness and fattiness. Therefore, we propose that method selection should be based on study goals, with RATA being more suitable for exploratory studies to identify potential correlations among attributes, and rank-rating is more suitable for hypothesis-driven research aiming at discriminating specific attributes.

For dynamic sensory evaluation, Temporal Check-All-That-Apply (TCATA) was chosen for its ability to track multiple co-occurring attributes over time, providing a detailed sensory profile with greater temporal resolution (Rizo et al., 2020; Visalli & Galmarini, 2024). As discussed in **section 6.2.3**, juiciness and fattiness differed slightly in temporality (**Chapter 3**). When studying how serum properties affect juiciness and fattiness in **Chapter 5**, incorporating TCATA could provide additional insights, since the PBMAs in **Chapter 5** showed more compositional and mechanical variabilities compared to the commercial products used in **Chapter 3**, potentially revealing more dynamic differences.

6.6 Concluding remarks

The growing demand for plant-based foods brings opportunities and challenges. This thesis focused on juiciness perception of meat analogue patties, concluding that juiciness is primarily driven by the amount of serum release during early mastication, and is closely linked to fattiness perception and enhances flavor perception. These results may be specific to meat analogue patties, but the methodological approach of linking physicochemical properties of food and oral structure breakdown to sensory perception used in this thesis can guide further research to explore more comprehensive mechanisms of juiciness perception across a broader range of meat and meat analogues. This thesis also provides valuable insights for the food industry on designing meat analogue patties with tailored texture perception by modifying the hydration level and particle size of textured vegetable proteins, as well as maintaining similar juiciness and fattiness perception without using excessive fat, improving nutritional profiles.

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Summary

The demand for heathy, sustainable but tasty food has driven efforts to improve the sensory quality of plant-based meat analogues (PBMAs). Juiciness, a key driver of consumer liking, remains largely underexplored. This thesis aimed to understand juiciness perception by investigating the relationships between food physicochemical properties, oral structural breakdown and sensory perception of PBMA patties.

First the relationships between food physicochemical properties, bolus properties at the moment of swallowing and sensory properties were investigated in commercially available PBMA and beef patties differing in core cooking temperature (**Chapter 2**). In PBMA patties, decreasing core cooking temperature increased fat content of the cooked patties and serum release under compression, but had no effect on instrumental texture. In contrast, beef patties with lower core cooking temperature showed increased water content of cooked patties, higher serum release, and softer texture. For both PBMA and beef patties, juiciness correlated positively with serum release (both under compression and oral conditions), positively with fattiness and negatively with dryness. Despite these differences in food and sensory properties, chewing behavior and bolus properties at swallowing remained similar across patties differing in juiciness. We concluded that juiciness in PBMA and beef patties is primarily driven by serum release during mastication, and is related to food physicochemical properties rather than bolus properties at the moment of swallowing.

In **Chapter 3**, the sample set of **Chapter 2** was used to further investigate the role of bolus properties in dynamic juiciness perception. Temporal Check-All-That-Apply demonstrated that juiciness of PBMA and beef patties increased with decreasing core temperature and peaked early during mastication, aligned with the rapid release of 75% of the total serum during early mastication. While the sustained oral breakdown had no further impact on juiciness of PBMA patties, juiciness of beef patties increased with higher bolus water content and expellable bolus liquid during mastication. We concluded that juiciness of PBMA patties is driven by serum release during early stages of mastication and not effected by additional oral breakdown, whereas juiciness of beef patties is affected by both initial serum release and bolus properties shaped by additional oral breakdown.

To investigate the role of food compositional and textural properties on juiciness perception, in **Chapter 4**, the hydration level and particle size of textured vegetable proteins (TVPs) used to prepare meat analogues patties were modified. The results demonstrated that increasing TVP hydration level increased patty water content, serum release under compression and serum water content, which elevated juiciness and fattiness perception. While TVP particle size mainly affected texture perception, with larger TVP particle sizes yielding harder and chewier PBMA patties. Network analysis showed that juiciness was positively correlated with fattiness, savory and garlic flavor and negatively correlated with hardness perception. We concluded that serum release remains the primary driver of juiciness perception of PBMA patties, while patties with softer texture and higher water content were perceived as juicier. Increased juiciness can boost flavor perception and improve consumer liking.

In **Chapter 5**, the role of serum release, composition and viscosity in juiciness and fattiness perception was investigated. PBMA patties with different serum properties were created by modifying TVP hydration level, fat content and maltodextrin content. Increasing TVP hydration level led to higher total serum release and serum water content, enhancing both juiciness and fattiness perception. Increasing raw patty fat content resulted in serum with higher fat content, boosting fattiness without affecting juiciness. Maltodextrin addition increased serum viscosity but reduced total serum release, thereby decreasing juiciness and fattiness. Network analysis confirmed that juiciness is primarily determined by the amount of serum release rather than serum composition and viscosity, while fattiness is influenced by both serum release and serum fat content.

Finally, in **Chapter 6**, the main findings of **Chapter 2 – 5** were synthesized. The discussion began with exploring the physicochemical properties, oral breakdown and sensory properties that drive juiciness. It then outlined approaches to modify juiciness, proposed potential mechanisms for juiciness across different product types, reflected on methodological considerations and recommendations for future research. We concluded that juiciness perception in plant-based meat analogue patties is primarily driven by the amount of serum release during early stages of mastication and is not

influenced by sustained oral breakdown. The composition and viscosity of the serum have limited impact on juiciness. Enhancing the liquid retention capacity of patties can increase serum release and boost juiciness perception. Juiciness is closely linked to fattiness and plays a role in enhancing flavour perception and liking.

Appendices

Acknowledgements

About the author

List of publications

Overview of completed training activities

Acknowledgements

It was never my goal as a child to become a food scientist, but my passion for talking about food and connecting with people probably helped bring me here. As much as I would like to appear effortlessly cool, being a talkative person means leaving my acknowledgements concise is simply not possible.

Dear Markus, Elke and Guido, thank you for being my PhD supervisors. I always tell people how much I have enjoyed my PhD, and you are one of the main reasons behind that. Your support, trust, kindness and fun brought me to where I am now. The story of my PhD did not start when I first contacted Markus for a position. It actually goes back to 2018, when I had the chance to work with Markus and Eva during my master. **Markus**, you have always been incredibly supportive. I enjoyed being able to just drop by your office to chat or discuss things. Your encouraging words after each small success, your problem-solving supervision style… all of that meant a lot. We have traveled to many conferences together and every time was a lot of fun (of course, with quite a few beers). You are always generous in introducing me to people and bringing us new beers, even though I am still trying to finish the old one! **Elke**, we have become closer and closer over these four years, which would not have happened without all the social activities you organized. You are direct and critical, but also very warm-hearted. I struggled at first with your comments on publications, but soon I got into the rhythm, and now carrying your 'voice' in my mind does help a lot! We have had many great conversations about academic and life during borrels. I often tried to stay until the end, because those talks and moments were what I really enjoyed. Thank you for offering me a postdoc position, I believe we will have another successful project ahead. Hi **Guido**, you are definitely the linguist in our project. Thank you for your critical insights and for helping me polish our work. After handing in my thesis, I was truly impressed by your double doctoral degrees, and I am so glad I got to witness the moment when another 'young' doctor was awarded. I have enjoyed running with you in the beautiful forest (though I am quite a slow runner) and I hope to join you more often during my postdoc.

My PhD project would not work out without the students that I supervised. I am truly grateful to the students I worked with, it was a real pleasure for me, and I hope the

same holds true for you :) Some of you are listed as co-authors on publications, which shows just how valuable your contributions were. So, thank you **Elisa**, **Nina**, **Matteo**, **Nadia**, **Sterre**, **Marci**, **Panyue**, **Rita**, **Ivo**, **Janneke**, **Jinze** and **Luka**.

I also want to thank colleagues from FBR for their support throughout the ISQA project, thank you **Monique**, **Catrienus**, **Valentina**, **Maaike**, **Marieke**, **Karen** and **Rene.**

During my PhD, I have been involved in many chair groups, FQD, FPH and SSEB. I have been lucky to take 'advantages' of the amazing facilities from different groups but more importantly, the great people. I would like to thank **Vincenzo**, **Kasper**, **Erik** and **Ciarán** for your support and for making me feel included across the groups. To our amazing secretaries, **Corine**, **Lisa**, **Kimberley**, **Maruschka** and **Els**, thank you for being so kind, supportive, efficient and patient. Your help over the last four years has been invaluable. I also want to express my gratitude to the technicians and support staffs across the three groups: **Erik**, **Frans**, **Marjanne**, **Christos**, **Jelle**, **Jochem**, **Xandra**, **Nienke** (FQD); **Floris**, **Mark**, **David**, **Roel**, **Harry** (FPH); **Corine** and **Els** (SSEB). Your professional skills and positive attitudes made my research smoother and more reliable! To **Zhen**, **Shiqi**, **Gealle** and **Rutger**, my dear office mates, I, honestly not a fan of flexible desks, so it was really great to have you around in the same office almost every week.

I joined two amazing PhD trips with FQD to Spain and Mexico with amazing people. **Didi**, **Cristina**, **Shiksha** and **Niles**, although we are all quite different, we made a good team (I mean at least we got everyone safely to Spain and back). **Luc**, **Julie**, **Rutger** and **Swantje**, after Spain, we kept the momentum going: Oktoberfest, Christmas market, 5 May… thank you for helping me experience all these truly authentic and fun moments (with a lot of drink, of course). **Tomer**, **Laura**, **Marialena** and **Didi**, my 'weird' kind of second office mates, you are the cutest bunch. I love passing by your massy room and chitchatting with you. All the beautiful memories we, together with **Andrea** and **Lucas**, had in Mexico, *boys are weak*, will stay with me for life. **Annelise**, did you know that you were more or less the first FQD colleague I ever emailed (it was before I started, about the MT meeting!) I am so happy to have met you, thank you for all your help, advice and for listening to my complaints. **Lise**, I wish we had more chances to chat, thank you for all the lovely food and coffee breaks. **Melania**, you are the sweetest person I have met in Wageningen. I really enjoyed our trip to Napoli with **Luc** and **Seren**. Thank you for your kindness and company. I hope one day I can show you

around in China. I will also miss all the nice food and stores we explored in the Netherlands and Sicily with **Yao**, **Peiheng** and **Liang**. Also, all the unique and nice teas we shared with **Bei**, **Yizhou**, **Yuqing** and **Ziqi**. **Ahmed**, thank you for all your compliments and encouragement, you also made me realize just how much I enjoy dancing! **Huifang** (caicai), **Zhihong**, **Cong** and **Bo**, thank you for helping me when I was just starting my PhD. Special thanks to caicai for asking me to be your paranymph and for that nice road trip to Spain!

Dear Rutger and Thiemo, dear my teammates, thank you for being my paranymphs. Before coming to the Netherlands, the only thing I know about you two was two tall Dutch guys :) After four years, that picture has become much richer. **Rutger**, my dear friend and brother. I never expected to find such a close friend on the other side of the word. After all this time here, you are probably the person I have the most photos with, from Belgium, Spain, Germany, Ireland, Denmark to Mexico. We are PhD trip buddies, we celebrate our 30's, stuck on German trains, you carried drunk me home, we shared so many lunches, coffees and dinners.... It just so easy to spend time with you, dankjewel. **Thiemo**, you surprised me with your talent on twerking during our PhD week and again on our trip to Hamburg. Over the years, our friendship grew stronger. You are easygoing, smart and fun, always open to help and discissions. I am especially happy that we collaborated on our last study. I really like our two boiis-trips with Rutger to celebrate our milestones, so fun! Hi, **Luc**, my dear friend. Thanks for asking me as your paranymph and being there whenever I was happy or sad. You are spontaneous (especially by Dutch standards). We had shared so many dinners and drinks together. I am truly glad to know you and to call you my friend (hmm honestly, not being friends with you would be difficult). I am so excited that we are 'scheduling' a trip to China with Rutger. This is not the end, just the start of another chapter in our friendship.

Dear baoyouer, dear **Qing H.**, **Qing R.**, **Dan**, **Xiaofang** and **Zekun**. We came together through mysterious connections. Yet, how sincere, fun and caring our little group is. We have shared so many beautiful memories. Even though we do not meet up that often now, I know there is always a place in Wageningen, where I feel safe and at home. Thank you for always being there, 友谊地久天长! A special thank you to **Qing H**., **Qing R**. and **Zekun** for your daily support in FQD.

I wish I could name every single good memory from Wageningen and thank all of you. To all those not yet mentioned, you are all part of my journey and I want to acknowledge you here: Anita, Arianne, Beatrice, Catriona, Corinna, Clara, Danielle, Dazhi, Deniz, Edoardo, Esther, Etske, Fabiola, Feilong, Fleur, Haonan, Hongwei, Jenneke H., Jiaying, Jianing, Jiawei, Jingjie, Josep, Kangni, Karen, Keqing, Laiyu, Lijiao, Lingtainxiang, Lotte, Luigi, Marielle, Martina, Maryia, Nicoletta, Nora, Obaid, Pengfei, Pien, Pieternel, Pieter D., Pieter G., Renske, Ruiwen, Ruth, Sara, Selcen, Siwei, Sjoera, Sofia, Stefan, Teresa, Trang Anh, Thisun, Wenyi, Xiangnan, Xiyu, Xin, Xuan, Yajing, Yi, Yifan L., Zhijun, Zhuojia, Zongyao from **FQD**; and Aref, Annika, Anteun, Bram, Chaya, Chen, Chon, Daisy, Dieke, Fahim, Gijs, Gerard, Huizi, Jack, Jasper, Jeta, Jingwei, Leonard, Lei, Melika, Mehdi, Mengyue, Nana, Naomi, Naoya, Nirzar, Nina, Paul, Penghui, Ployfon, Qimeng, Roos, Senna, Ting, Wanting, Wenjie, Xiaofei, Xiaohan, Xiaojia, Xiangyu, Xingfa, Xilong, Xudong, Ziyang, from **FPH**, Danni, Hongyu, Qi thank you for the nice chats, drinks or dinners. You made my Wageningen life vibrant and warm.

I would also like to take this opportunity to thank my dear master's supervisors, Prof. **Jianshe Chen** and Dr. **Hilbert van der Glas**. 陈老师，您是我心中为人师表与学者风范的最佳具象。您对学术研究的热枕和严谨、对学生的启发和人文关怀,潜移默化地影响并塑造了我对科研和学术环境的理解。感谢您多年来的教诲和支持，让我带着这份眷顾坚定前行。Thank you, **Hilbert**, not only for your support and guidance during my Master, but also the warm welcome from you and **Marleen** when I came to the Netherlands: "Your parents can be at ease, because we are your Dutch family here". Bedankt!

我还要感谢在国内一直支持着我的各位朋友：都市丽人的**志宇、青青、熊熊、阿滢、二狗、鄞哥、阿星**；铜厂董事会的**巴乔、郝伟、东东、11、施怡凡、银兰、王力、锞莹、虫虫；还有爽、志远、秀楠、汪琦、啸南**。感谢你们超越距离的陪伴和惦念，特别感谢来欧洲和我一起过十一、元旦、圣诞的**巴乔**、**郝伟**、**丹丽**，**凌迅**和**汪琦**。

Finally, I want to thank my family. 感谢**家里每一位成员**一路以来的关心、照顾和支持。这个始终充满温暖、欢乐和爱的大家庭，让我无论身处何处，都能心有所安，踏实前行。特别感谢**小姨**和**爸妈**来到荷兰见证我这段旅程的重要时刻。感谢**爸爸妈妈**一直以来的鼓励励和支持，你们给予我的无私和真挚的爱，始终是我最坚定和深远的力量。谢谢你们，我亲爱的父母和家人，山河虽远，愿四季更迭之时，皆能相伴！

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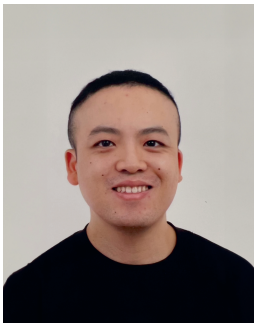
About the author



Yifan Zhang (章一帆) was born in December 1994 in Wenzhou, China. He obtained his bachelor's degree in Food Science and Technology from Zhejiang University of Science and Technology, Hangzhou, in 2017. He then pursued a master's degree at Zhejiang Gongshang University under the supervision of Prof. Jianshe Chen and Dr. Hilbert van der Glas in the Laboratory of Food Oral Processing (2017-2020). His master's thesis focused on optimizing chewing efficiency test methods and exploring the relationship between chewing efficiency, oral processing, oral physiology and sensory perception. As part of his master's research, he collaborated with Dr. Eva Ketel and Prof. Markus Stieger from Wageningen University. In 2018, he conducted a research internship at the Plant & Food Research Institute in Christchurch, New Zealand, hosted by Dr. Ester Kim and Mr. Marco Morgenstern. After completing his master's study, he worked as a research assistant in the Laboratory of Food Oral Processing (2020–2021).

In April 2021, he started his PhD at Wageningen University in Food Quality & Design and Physics & Physical Chemistry of Foods groups, under the supervision of Prof. Markus Stieger, Prof. Elke Scholten and Dr. Guido Sala. His research focused on understanding juiciness perception in plant-based meat analogues as part of the TKI project –Improving Sensory Quality of Meat Analogues.

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List of publications

This thesis:

Zhang, Y., Sala, G., Scholten, E., & Stieger, M. (2024). Role of bolus properties in dynamic texture perception of meat analogue and beef patties: Juiciness is driven by serum release during early stages of mastication. *Food Hydrocolloids*, *157*, 110450.

Zhang, Y., Brouwer, R., Sala, G., Scholten, E., & Stieger, M. (2024). Exploring relationships between juiciness perception, food and bolus properties of plant-based meat analogue and beef patties. *Food Hydrocolloids*, 147, 109443.

Zhang, Y., Király, M., Zhang, P., Navarrete Codina, R., Behrouzi, P., Sala, G., Scholten, E., & Stieger, M. Boosting juiciness and flavor perception of meat analogue patties by altering hydration level and particle size of textured vegetable proteins *Food Hydrocolloids*, 111423.

Zhang, Y., Mtiulishvili, L., Sala, G., Scholten, E., & Stieger, M. Juiciness of plant-based meat analogues is driven by serum release rather than serum composition and viscosity (submitted)

Others:

Brouwer, R., **Zhang, Y**., Scholten, E., Forde C. G., & Stieger, M. (2025). The influence of juiciness on in vivo aroma release and perception of plant-based meat analogue and beef patties. *Journal of Agricultural and Food Chemistry*, *73* (15), 9286-9296.

Zhang, Y., Jia, J., Wang, X., Chen, J., & van der Glas, H. W. (2021). Particle size distributions following chewing: Transformation of two-dimensional outcome from optical scanning to volume outcome from sieving. *Journal of Food Engineering*, *309*, 110663.

Ketel, E. C., **Zhang, Y.**, Jia, J., Wang, X., de Wijk, R. A., Chen, J., & Stieger, M. (2021). Comparison of and relationships between oral physiology, anatomy and food oral processing behavior of Chinese (Asian) and Dutch (Caucasian) consumers differing in age. *Physiology & behavior*, *232*, 113284.

Jia, J., **Zhang, Y**., Xu, Z., Wang, X., Chen, J., & van der Glas, H. W. (2021). The influence of flavor release from a solid test food, and its Time Intensity (TI) scoring, on chewing efficiency. *Food Quality and Preference*, *93*, 104247.

Zhang, Y., Liu, T., Wang, X., Chen, J., & van Der Glas, H. W. (2019). Locking up of food between posterior teeth and its influence on chewing efficiency. *Archives of Oral Biology*, *107*, 104524.

Overview of completed training activities

Activities	Country	Year
Discipline specific courses		
Sensory perception & food preference	NL	2021
Rheology: The do's and don'ts	NL	2021
Chemometrics	NL	2022
Conferences		
Virtual Summit Plant-Based Foods & Proteins	NL	2021
6th food oral processing conference	ES	2021
36th EFFoST international conference	IR	2022
9th International Symposium of Food Rheology and Structure	NL	2023
15th Pangborn Sensory Science Symposium	FR	2023
11th European conference on sensory and consumer research (EuroSense)	IR	2024
7th food oral processing conference	ES	2025
General courses		
VLAG PhD week	NL	2021
Introduction to R	NL	2021
Applied statistic	NL	2021
Research Data Management	NL	2021
Supervising BSc & MSc thesis students	NL	2021
Scientific writing	NL	2022
Assisting in teaching and supervision activities		
Food packaging and design	NL	2022
Predicting food quality	NL	2023
Thesis supervision of BSc & MSc students	NL	2022-2025
Other activities		
Preparation of research proposal	NL	2021
PhD study tour to Spain	ES	2022
PhD study tour to Mexico	MX	2024
Consortium meeting with project partners	NL, DE	2021-2025

Colophon

The research described in this thesis was financially supported by the Top Sector Agri & Food (TKI) with a consortium of partners (AAK, GoodMills Innovation, Starfield, Symrise and Vivera), and China Scholarship Council.

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover designed by studio shu | shu.artdesign@gmail.com

Printed by Proefschriftmaken.nl