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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

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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.

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, Gustafson, & Mathys, 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, Clausen, & Jaeger, 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, Berry, & Wells, 1980; Tobin, O'Sullivan, Hamill, & Kerry, 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, Ashfaq, Ahmad, Anjum, & Yousuf, 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,

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Brouwer, Sala, Scholten, & Stieger, 2024). 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, Čandek-Potokar, Maltin, & Prevolnik Povše, 2015; Lawrie, 2006; Schwartz, Marais, Strydom, & Hoffman, 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, Van de Velde, & Stieger, 2017; Foster et al., 2011; Gao & Zhou, 2021; Panouillé, Saint-Eve, & Souchon, 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, Terpstra, Janssen, & Prinz, 2006; Devezeaux de Lavergne et al., 2017; Gao, Ong, Henry, & Zhou, 2017; Jourdren et al., 2016).

For meat, numerous studies quantified oral structural breakdown and bolus properties during mastication and/or static or dynamic sensory properties (Djekic, Ilic, Lorenzo, & Tomasevic, 2021; Pematilleke, Kaur, Adhikari, & Torley, 2020; Yven, Culioli, & Mioche, 2005). 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, Peña, Alarcon-Rojo, Fiszman, & Tarrega, 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, Ketel, de Wijk, & Stieger, 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), 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.

2. Materials and methods

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). 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, Tomasevic, & Djekic, 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) 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).

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 and 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 a.m. and 03:00 p.m. 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, Leong, Chia-Ming, & McCrickerd, 2017).

2.3. Sensory evaluation

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 2.2). The same inclusion criteria described in section 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.

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 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 3.1), 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.

2.4. Characterization of bolus properties at different stages of mastication

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 3.1). They were instructed to consume a precut 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

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.

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.

2.4.2. Composition of bolus

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 $WC = (w_0 - w_1)/w_0 \times 100\%$.

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 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 $FC = (F_0 - F_1)/F_0 \times 100\%$.

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 = $[(w_0 - w_1)/w_1 - (b_0 - b_1)/b_1 - (s_0 - s_1)/(s_0 \times w_1/w_0)] \times 100\%$, 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)); 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 2.5).

2.4.3. Bolus texture properties

The texture properties of the boli (n = 10) were determined with a penetration test (Zhang et al., 2024). 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.

2.4.4. Bolus particle size distribution

The particle size distribution of bolus fragments (n = 10) was determined using image analysis (van Eck, Wijne, Fogliano, Stieger, & Scholten, 2019; Zhang, Jia, Wang, Chen, & van der Glas, 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.

2.4.5. Liquid expelled from bolus

The liquid expelled from the boli (n = 10) was measured by centrifugation (Zhang et al., 2024). 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 2.4.2.1. Measurements were performed in duplicate for each sample for each mastication moment for each participant.

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 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 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.

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, Brockhoff, & Christensen, 2017), Hmisc (Harrell & Dupont, 2023), FactoMineR (Lê, Josse, & Husson, 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. Results and discussion

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 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). 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, Van Der Bilt, Abbink, & Bosman, 2004; Kochi et al., 2021; Maeda et al., 2020; Yven et al., 2012).

3.2. Dynamic sensory perception of PBMA and beef patties

The TCATA curves of PBMA and beef patties are provided in Fig. 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.

For PBMA patties, with decreasing core temperature from 80 to 60°C, juiciness citation proportion significantly increased by 27% for C_{max} ($\chi^2 = 17.8$, $p < 0.001$) and by 37% for AUC ($F = 24.1$, $p < 0.001$) demonstrating that, as expected, juiciness perception increased with decreasing core temperature (Fig. 1(A and B and C) and Table 3 (A)). This is in agreement with our previous study (Zhang et al., 2024). 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 ($\chi^2 = 21.7$, $p < 0.001$), 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} ($\chi^2 = 7.9$, $p < 0.05$) and by 85% for AUC ($\chi^2 = 14.1$, $p < 0.001$) 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 ($\chi^2 = 6.9$–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) 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 (B)). With decreasing core temperature, juiciness citation proportion for beef patties significantly increased by 317% for C_{max} ($\chi^2 = 73.1$, $p < 0.001$), 467% for $C_{33\%}$ ($\chi^2 = 73.4$, $p < 0.001$), 293% for $C_{66\%}$ ($\chi^2 = 48.9$, $p < 0.001$), 192% for $C_{100\%}$ ($\chi^2 = 32.5$, $p < 0.001$) 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

Table 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

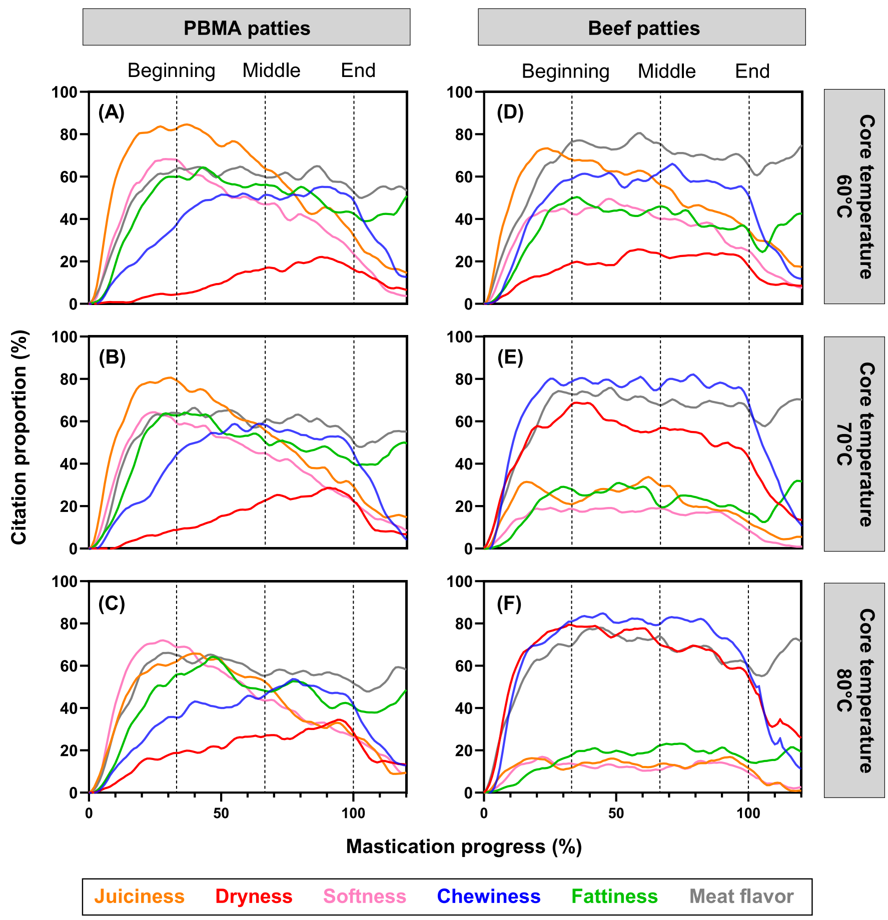

Fig. 1. TCATA profiles of PBMA (A, B and C) and beef patties (D, E and F) prepared at core temperatures of 60 °C (A, D), 70 °C (B, E), and 80 °C (C, F) (n = 65 participants, duplicate). Dashed lines indicate 33 (beginning), 66 (middle) and 100% (end) of mastication time.

mastication time and for BEEF80 at 17% of mastication time (Fig. 1(E and 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). 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, Janssen, & Prinz, 2011; Kupirovič, Elmadfa, Juillerat, & Raspor, 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. 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 S1. The results of the corresponding statistical data analysis are presented in Table 4 and the changes in bolus properties are visualized in Figs. 2–5. These results are discussed in sections 3.3.1 to 3.3.4.

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 3.1). Bolus properties showed significant differences across mastication times and across core temperatures (Table 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 S1). 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

Table 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).

	(A) PBMA patties						(B) Beef patties					
	PBMA 60	PBMA 70	PBMA 80	$\chi2$ value	F value	p value	BEEF 60	BEEF 70	BEEF 80	$\chi2$ value	F value	p value
Juiciness												
C_{max} (%)	85^{a}	82^{a}	67^{b}	**17.8**	–	**< 0.001**	75^{a}	34^{b}	18^{c}	**73.1**	–	**< 0.001**
T_{max} (%)	36	30	40	–	–	–	22	61	93	–	–	–
$C_{33\%}$ (%)	83^{a}	79^{a}	62^{b}	**21.7**	–	**< 0.001**	68^{a}	22^{b}	12^{c}	**73.4**	–	**< 0.001**
$C_{66\%}$ (%)	64	56	53	5.1	–	0.079	55^{a}	29^{b}	14^{c}	**48.9**	–	**<0.001**
$C_{100\%}$ (%)	33	29	26	2.6	–	0.277	35^{a}	12^{b}	12^{b}	**32.5**	–	**<0.001**
AUC (–)	67 ± 27^{a}	59 ± 31^{b}	49 ± 32^{c}	–	**24.1**	**< 0.001**	59 ± 33^{a}	25 ± 26^{b}	14 ± 20^{c}	–	**159.9**	**<0.001**
Dryness												
C_{max} (%)	22^{a}	29^{ab}	35^{b}	**7.9**	–	**< 0.05**	26^{a}	69^{b}	80^{b}	**69.4**	–	**<0.001**
T_{max} (%)	87	90	94	–	–	–	59	34	31	–	–	–
$C_{33\%}$ (%)	5^{a}	9^{ab}	18^{b}	**13.2**	–	**< 0.01**	19^{a}	68^{b}	78^{b}	**75.0**	–	**<0.001**
$C_{66\%}$ (%)	15^{a}	23^{ab}	27^{b}	**6.9**	–	**< 0.05**	25^{a}	58^{b}	70^{b}	**56.0**	–	**<0.001**
$C_{100\%}$ (%)	15^{a}	23^{ab}	29^{b}	**9.7**	–	**<0.01**	18^{a}	42^{b}	55^{c}	**41.4**	–	**<0.001**
AUC (–)	13 ± 21^{a}	17 ± 24^{a}	24 ± 26^{b}	–	**14.1**	**< 0.001**	20 ± 27^{a}	57 ± 35^{b}	73 ± 31^{c}	–	**164.6**	**<0.001**
Softness												
C_{max} (%)	69	65	72	2.0	–	0.376	50^{a}	20^{b}	18^{b}	**41.9**	–	**<0.001**
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**	–	**<0.001**
$C_{66\%}$ (%)	45	45	42	0.5	–	0.769	40^{a}	18^{b}	11^{b}	**33.9**	–	**<0.001**
$C_{100\%}$ (%)	23	23	26	0.6	–	0.732	25^{a}	8^{b}	9^{b}	**19.1**	–	**<0.001**
AUC (–)	49 ± 32	46 ± 32	51 ± 32	–	1.3	0.273	40 ± 33^{a}	16 ± 23^{b}	13 ± 19^{b}	–	**69.8**	**<0.001**
Chewiness												
C_{max} (%)	56	59	55	0.6	–	0.723	65^{a}	82^{b}	85^{b}	**19.8**	–	**<0.001**
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**	–	**<0.001**
$C_{66\%}$ (%)	51	58	46	5.6	–	0.061	62^{a}	76^{b}	80^{b}	**14.3**	–	**<0.001**
$C_{100\%}$ (%)	48	45	42	1.4	–	0.488	51^{a}	67^{b}	58^{ab}	**8.8**	–	**<0.05**
AUC (–)	45 ± 35	47 ± 34	41 ± 36	–	2.1	0.119	55 ± 32^{a}	77 ± 25^{b}	76 ± 27^{b}	–	**50.2**	**<0.001**
Fattiness												
C_{max} (%)	65	65	65	0.0	–	1.000	51^{a}	32^{b}	24^{b}	**25.7**	–	**<0.001**
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**	–	**<0.001**
$C_{66\%}$ (%)	55	51	48	2.3	–	0.312	45^{a}	20^{b}	22^{b}	**27.2**	–	**<0.001**
$C_{100\%}$ (%)	42	40	41	0.3	–	0.851	34^{a}	17^{b}	15^{b}	**19.3**	–	**<0.001**
AUC (–)	56 ± 35	55 ± 36	52 ± 33	–	1.4	0.239	43 ± 33^{a}	25 ± 27^{b}	19 ± 24^{b}	–	**45.4**	**<0.001**
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

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.

3.3.1. *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 Fig. 2.

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 4 (A)). The increase in bolus water content over mastication time can be explained by the uptake of saliva during mastication (Fig. 2(A)–(C)). This phenomenon is attributed to the stimulation of salivation by mastication followed by saliva absorption by the bolus (Devezeaux de Lavergne, van de Velde, van Boekel, & Stieger, 2015; Rizo et al., 2019; 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 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 4 (A)), suggesting that fat was not released from the PBMA matrix into the oral cavity during mastication (Fig. 2 (B)). These findings align with the TCATA results (section 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 (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 4 (B)). While the increase in bolus water content during mastication was similar across different beef patties, the absolute values

Table 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.

(A) Bolus properties of PBMA patties	Mastication time		Core temperature	
	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 (mm^2)	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**
(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**

varied depending on the core temperature (Fig. 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 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).

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 4 (B)). These results suggest two key points: (1) boli of beef patties prepared at lower core temperatures contained less fat (Fig. 2 (E)); and (2) a limited amount of fat was released from the beef matrix into the oral cavity during mastication. The second point is in agreement with the TCATA results, which demonstrated that fattiness citation proportions were fairly constant over mastication time and relatively low, typically ranging between 20 and 50% (Fig. 1, Table 3). Although previous research has shown that the fat content of beef patties decreased by cooking at lower temperatures (Zhang et al., 2024), 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 (Fig. 1, Table 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). This highlights the intricate interplay of texture sensations contributing to fattiness perception.

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 Fig. 3.

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

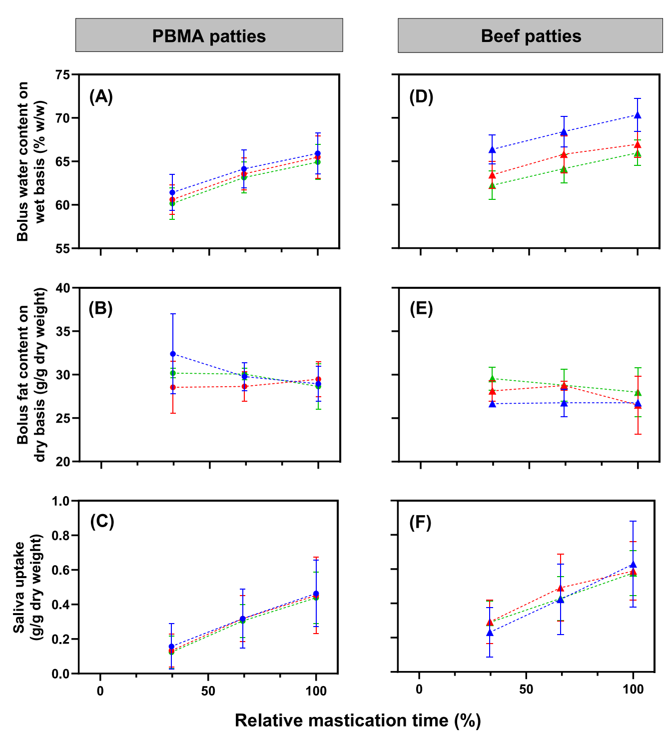

Fig. 2. Water content on wet basis (A, D), fat content on dry basis (B, E) and saliva uptake (C, F) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) of PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60 °C (blue symbols), 70 °C (red symbols) and 80 °C (green symbols). Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

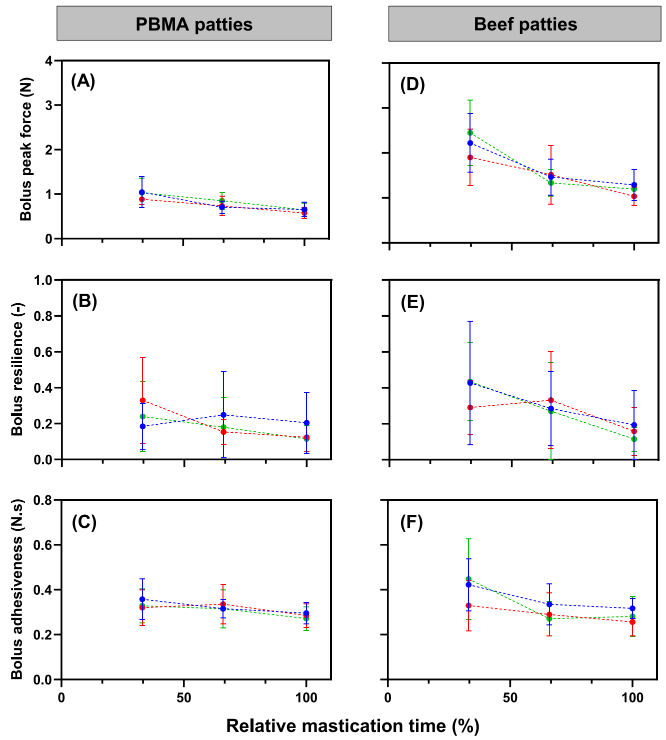

Fig. 3. Bolus peak force (A, D), bolus resilience (B, E) and bolus adhesiveness (C, F) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) of PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60 °C (blue symbols), 70 °C (red symbols) and 80 °C (green symbols). Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

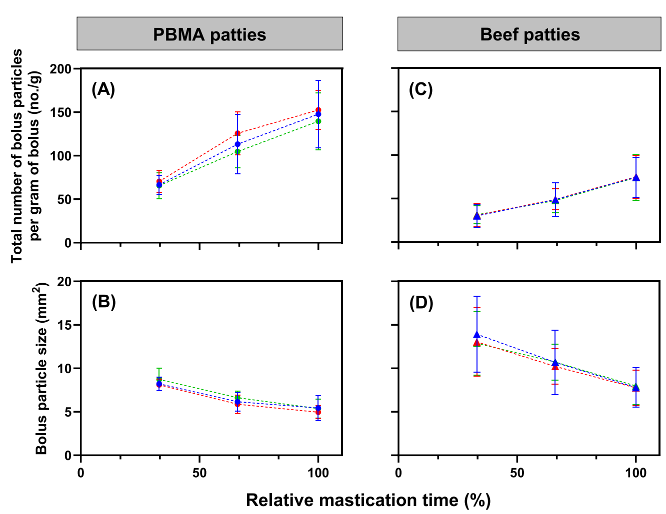

Fig. 4. Total number of bolus particles per gram of bolus (A, C) and average bolus particle size (B, D) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) from PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60 °C (blue symbols), 70 °C (red symbols) and 80 °C (green symbols). Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

($F = 2.9$, $p = 0.061$), and a significant but small effect on bolus adhesiveness ($F = 4.8$, $p < 0.05$) (Table 4 (A), Fig. 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) (Fig. 3). Core temperature had no significant

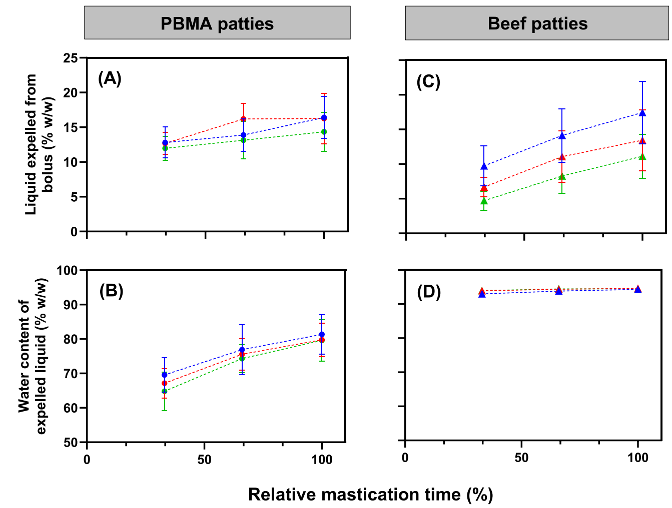

Fig. 5. Liquid expelled (A, C) and water content of expelled liquid (B, D) of boli collected at 33, 66 and 100% of mastication (n = 10 participants) from PBMA (circles ●) and beef patties (triangles ▲) prepared at core temperatures of 60 °C (blue symbols), 70 °C (red symbols) and 80 °C (green symbols). Dashed lines are included to guide the eye. Means are shown and error bars indicate standard deviations.

effect on any of the three bolus texture properties (Table 4 (A), Fig. 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 4 (B), Fig. 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 (Fig. 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 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). 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 (Fig. 1 and Table 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. 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 (Fig. 4).

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 4). As mastication progressed, more and smaller bolus fragments were generated (Fig. 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 4). The limited effect of core temperature on oral structural breakdown of PBMA and beef patties (Fig. 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), resulting in comparable oral processing behavior (section 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, Djekic, Tomasevic, Oosterlinck, & van den Berg, 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.

3.3.4. *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 (Fig. 5).

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 4 (B)), resulting in more liquid expelled from PBMA bolus during mastication (Fig. 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 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 (Fig. 2(C) and (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 (Fig. 5 (B)) ($F = 208.3$, $p < 0.001$, Table 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 A1), 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 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 (Fig. 1 and Table 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.

3.4. *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 Fig. 6.

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 ($F = 13.8$, $p < 0.001$) (Fig. 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 ($F = 104.7$, $p < 0.001$) (Fig. 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).

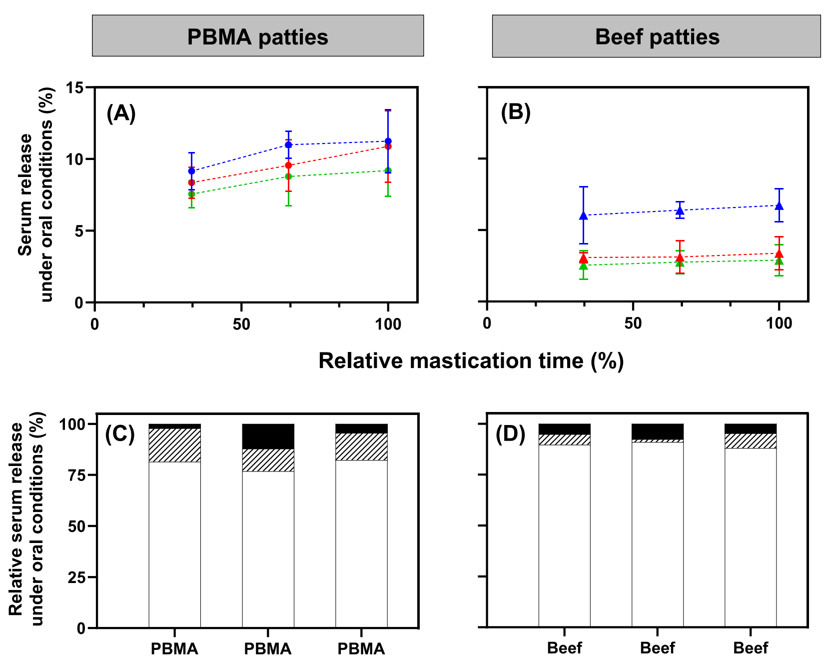

Fig. 6. Serum release under oral conditions at 33, 66 and 100% of mastication for (A) PBMA (circles ●) and (B) beef patties (triangles ▲) prepared at core temperatures of 60 °C (blue symbols), 70 °C (red symbols) and 80 °C (green symbols) (n = 10 participants). Dashed lines are included to guide the 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.

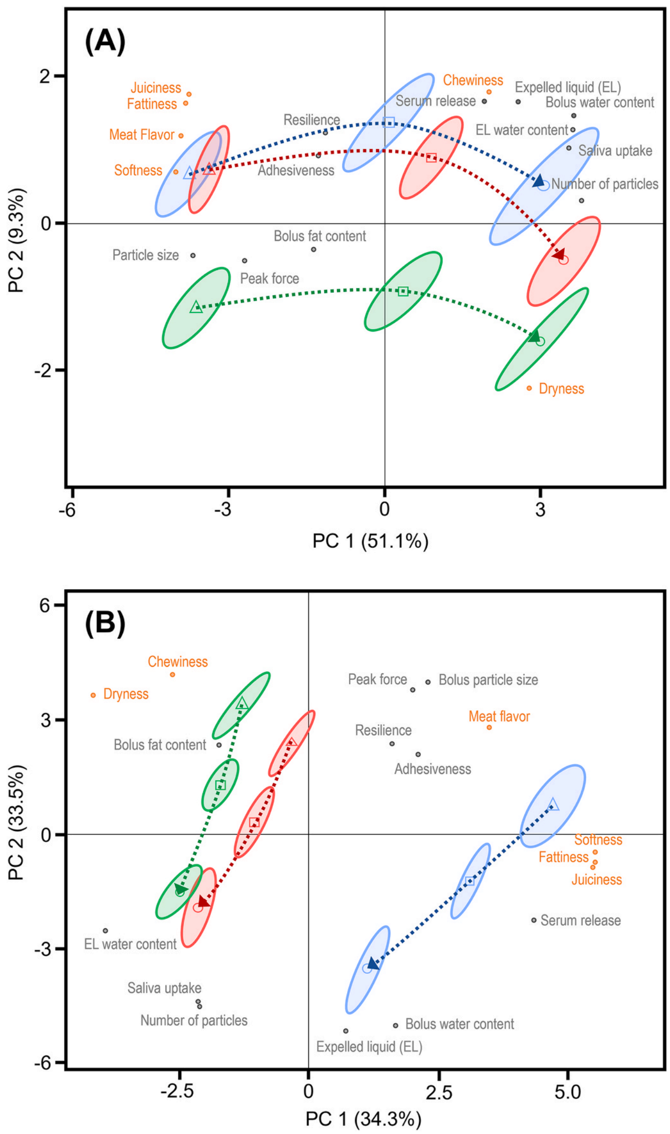

Fig. 7. Principal component analysis (PCA) illustrating oral processing trajectories of (A) PBMA and (B) beef patties prepared at core temperatures of 60 °C (blue ellipse), 70 °C (red ellipse) and 80 °C (green ellipse) at 33 (triangles △), 66 (squares □) and 100% (circles ◯) of mastication time. Bolus 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.

Remarkably, plotting the relative amount of serum release after 33, 66 and 100% of mastication (Fig. 6 (C) and (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 (Fig. 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.

3.5. 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 (Fig. 7). Pearson correlation coefficients were determined at different mastication times for PBMA and beef patties separately, as presented in the Supplementary Fig. S2.

For PBMA patties, mastication time had a more pronounced effect on the oral processing trajectory compared to that of core temperature. As illustrated in Fig. 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 Fig. 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, Fi gure S1 (B)). Moreover, all PBMA patties differing in core temperature followed a similar trajectory along PC1 (X axis, 51.1%) (Fig. 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 (Supplementary Figure S2 (A), Fig. 7 (A)). This may be caused by the limited influence of core temperature on PBMA bolus properties compared to the influence of mastication time (Figs. 2–5) in our study, resulting in limited variability in bolus properties across samples (i.e., bolus properties were closer to coordinate origin (Fig. 7 (A)). Juiciness was positively correlated with fattiness, and negatively with dryness citation proportion (Fig. 7 (A)), consistently with previous studies on PBMA products (Thong, Tan, Chan, Choy, & Forde, 2024; Zhang et al., 2024). 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 (Fig. 7 (A)), such correlations emerged when performing Pearson correlations at different mastication times separately (Supplementary Figure S2 (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 (A) and Fig. 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 (Fig. 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, Supplementary Figure S1 (D)). Notably, sensory perception of beef patties correlated mainly with initial bolus properties and showed limited changes during mastication (Fig. 7 (B)). Sensory properties therefore varied more between beef patties differing in core temperature but remained relatively consistent during mastication (Fig. 1).

When linking beef bolus properties to sensory perception, more correlations were found for beef patties than for PBMA patties (Fig. 7 (B), Supplementary Figure S2 (B)). As expected, juiciness, fattiness and softness citation proportions were positively correlated with each other (Fig. 7 (B)), consistently with findings from studies on sausages (Pematilleke et al., 2020; Sasaki, Motoyama, Narita, & Chikuni, 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 (Fig. 7(B)) and Pearson correlations (Supplementary Figure S2 (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 (Fig. 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 (Supplementary Figure S2 (B)). However, the water content of expelled liquid remained constant across mastication time and core temperature (Fig. 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.

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.

CRediT authorship contribution statement

Yifan Zhang: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Guido Sala:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Elke Scholten:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Markus Stieger:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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