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Exploring variability in detection thresholds of microparticles through participant characteristics

Marco Santagiuliana [a,b], Inés Sampedro Marigómez [b], Layla Broers [b], John E. Hayes [c,d], Betina Piqueras-Fiszman [e], Elke Scholten [b], Markus Stieger [a,f,g,*]

[a] TiFN, PO Box 557, 6700 AN Wageningen, The Netherlands

[b] Physics and Physical Chemistry of Foods, Wageningen University, PO Box 17, 6700 AA Wageningen, The Netherlands

[c] Sensory Evaluation Center, and [d] Department of Food Science, College of Agricultural Sciences, Pennsylvania State University, State College, PA, USA

[e] Marketing and Consumer Behaviour, Department of Social Sciences, Wageningen University, PO Box 8130, 6700 EW Wageningen, The Netherlands

[f] Food Quality and Design, Wageningen University, PO Box 17, 6700 AA Wageningen, The Netherlands

[g] Division of Human Nutrition and Health, Wageningen University, PO Box 17, 6700 AA Wageningen, The Netherlands

* Corresponding author. markus.stieger@wur.nl

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Abstract

This study explored how product familiarity and physiological characteristics of participants affect detectability of microparticles in viscous and semi-solid foods. Cellulose particles differing in size (50-780 μm) were added (1.5% w/w) to two dairy products, quark (viscous curd cheese) and processed cheese. Discrimination thresholds for added microparticles were determined by 47 Dutch, Caucasian and 45 Chinese, Asian women using the Method of Constant Stimuli. Particle size detection thresholds did not significantly differ between the two groups, but differed significantly between the two products. Detection threshold estimates for particle size were lower in viscous, low-fat quark than in semi-solid, high-fat processed cheese (52 μm versus 86 μm). This suggests that particle detection depends on product properties such as product consistency and composition, but not on factors linked to ethnicity and/or nationality of participants. We found no evidence to support a relationship between product familiarity and particle size detection thresholds in either product. A positive but weak correlation was found between stimulated saliva flow and particle size detection threshold in processed cheese ($r = 0.21$, $p = 0.041$), suggesting active salivation might enhance sensitivity for microparticle detection in semi-solid foods. PROP status and fungiform papillae density did not correlate with particle size detection threshold for either food. We conclude that matrix properties were the main contributors to particle size detection thresholds in young, healthy participants who differed in nationality and ethnicity. These data suggest that product characteristics are the central factor that should be considered for modifications when dealing with foods in which particles lead to negative sensations such as grittiness.

1. Introduction

Many foods contain microparticles that vary in type, origin, and properties. Particles can be either an endogenous constituent of the food (e.g., protein aggregates, starch granules, and insoluble fiber) [1–3], or an added exogenous ingredient that provides consumers with specific nutraceutical components (e.g., vitamins, bioactive peptides, minerals).[4–9] Perception and consumer acceptability of foods may be affected by the presence of such microparticles. The perception is often affected negatively, as the presence of small hard particles mainly relates to perception of grittiness [10–12] or roughness [13,14]. Such sensations negatively influence the hedonic responses of consumers to specific products. For example, Lopez et al. [5] reported that addition of spherical cellulose beads to a model liquid food resulted in a decrease of product acceptability as a function of both particle size and concentration; increasing these parameters led to higher grittiness, which decreased product acceptability. [4]

In food products, many of the negative effects associated with the presence of microparticles can be mitigated through product manipulations. Such modifications can focus on the particles themselves or the continuous phase the particles are distributed in. Physical properties of the particles (i.e., size, concentration, hardness, shape) and their effect on consumer perception have been studied extensively in model systems and common food products like soups or custards. [2,12,13,15–19] For example, Engelen and colleagues [20] showed that perception of SiO_2 and polystyrene particles varying sizes (2–230 μm) in custards was largely affected by particle properties. Generally, detectability of particles is high when particles are large, hard, irregularly shaped and/or present at high concentration. Detectability is also influenced by the properties of the surrounding matrix, as dispersed particles are more difficult to detect when the viscosity of the continuous phase is high.[5,20,21]

Most prior work has focused on the effect of the product properties on perception and detectability of microparticles, so potential variability across consumers remains under-studied. Inter-individual differences in microparticle detection may arise from different psychological and physiological factors.[13] Acceptability may depend on factors beyond physical properties of foods, such as oral tactile sensitivity or consumer expectations for the product. Product familiarity and related expectations have been shown to influence acceptability of a variety of products.[22–24] Differences in the level of familiarity for a certain product containing microparticles may result in different expectations regarding sensory properties.[25–27] Expectations may lead to opposite hedonic responses, depending on whether a smooth homogeneous (without particles) or heterogeneous (with particles) product was expected. [23,28,29] We hypothesize that presence of microparticles may be a cause for product rejection when expectations are not met. Alternatively, a product with detectable particles may still be acceptable if they anticipate the presence of microparticles, or when the consumer has no specific expectations regarding the sensory properties of the product.

Moreover, the sensitivity of the somatosensory system may also be influenced by multiple physiological parameters. Prior work suggests that oral perception of foods can be associated with fungiform papillae density (FPD), 6-n-propylthiouracil (PROP) taster status, and salivary flow rate. For example, when given milk–cream mixtures, an individual's FPD was positively correlated to creaminess perception [30,31] and had a significant influence on fat content perception.[32] While the majority of prior research on PROP has focused on taste perception, some data suggest that FPD may also be related to the perception of oral texture (e.g., [33]). When considering the effect of staling or presence of fibres in bread on perception of rough sensations, Bakke and

Vickers [14,34] found that higher FPD was not related to perceived roughness. The same study, however, found that panellists who perceived greater PROP intensity also perceived greater roughness from the bread, suggesting that PROP status may predict differences in food texture sensitivity. Elsewhere, people who reported higher bitterness from PROP (i.e., supertasters) also showed enhanced sensitivity of tactile pressure when tested with Von Frey monofilaments.[35] These monofilaments consist of nylon threads and are commonly used to measure tactile pressure sensitivity of the skin. The filaments apply a defined force to a relatively small contact area upon bending of the thread.[36–38] Here, we hypothesized that lingual tactile sensitivity, as measured with Von Frey monofilaments, might correlate with the perception of hard microparticles, as their presence in food might apply localized pressure on the tongue surface during consumption. Finally, considering the well-reported contribution of salivary lubrication during food oral manipulation [32,39–42], we also hypothesized salivary flow would influence sensitivity of microparticle detection via dilution and lubrication effects.

Variation in oral physiology affecting texture perception could also potentially arise from differences in sex, age, dental status, oral processing strategies and ethnicity. Sex differences have been previously described in terms of salivary flow, maximum bite force, and mastication frequency [43–45], with men presenting higher values for these parameters than women. Age also has the potential to affect texture perception due to either decreased eating capabilities or dental status.[46–48] Such physiological age-related changes can also affect mastication of the product, which is known to influence perceived texture of food.[49–54] Nevertheless, few studies have investigated potential variation in oral physiology and texture perception between consumers who differ in terms of ethnicity. With growing business opportunities of the Asian food market, the interest in better understanding how Western and Asian consumers differ in terms of sensory perception and oral processing behaviour is currently increasing.[55–57] To date, it has been reported that Asian subjects from China have a larger oral volume and consume foods and beverages at higher eating rate than Caucasian subjects from the Netherlands and USA, although it is not known whether such differences can lead to differences in food perception.[56,58] Here we hypothesize that consumer's ethnicity might influence texture perception of products containing microparticles.

In summary, prior work investigated the influence of endogenous microscopic constituents or exogenous microparticles added to foods, but little is known about how participant characteristics may affect the perception of foods containing microparticles. Detection of microparticles may not only be related to physical properties of the product, but may also be influenced by both product familiarity and physiological characteristics of participants. Here, we explored how consumer familiarity and physiological characteristics of participants affected oral detectability of microparticles in foods. Detection thresholds of cellulose particles were determined for a viscous (quark) and semi-solid food (processed cheese). Two groups of women (Dutch, Caucasian and Chinese, Asian) were recruited to determine whether product familiarity, consumption habits, and physiological characteristics (fungiform papillae density, PROP status, point pressure sensitivity on the tongue, and salivary flow) affect detectability of microparticles in viscous and semi-solid products.

2. Materials and Methods

2.1 Materials

Low-fat quark "Magere Milde kwark" (soft, viscous curd cheese; nutritional composition: 0.1% fat, 10.3% protein, 2.8% sugars, 0.1% salt) was provided by FrieslandCampina (Wageningen, The Netherlands). Kiri® (soft,

semi-solid, processed cream cheese; nutritional composition: 29.5% fat, 9% protein, 2% sugars, 1.4% salt) was provided by Bel Group (Fromageries Bel, Suresnes, France). K-carrageenan (GENUGEL type CHP-2) was purchased from CP Kelco (Rotterdam, The Netherlands). Microcrystalline cellulose particles (Primecel™ type PH-301; Cellets®, type Cellets 90, Cellets 127, Cellets 263, Cellets 500, Cellets 780 and Cellets 1000) were kindly provided by Harke Pharma (Mülheim an der Ruhr, Germany). A blue food colorant (Bharco Foods, NL) was purchased at a local supermarket. All ingredients were food grade, and samples were prepared under food-safe conditions.

2.2 Participants and methodology

2.2.1 Stimuli

A viscous curd cheese (quark) and a semi-solid processed cheese (Kiri®) were used as food matrices to investigate detection thresholds of added microparticles. Microcrystalline cellulose particles varying in size (average diameter of 50, 127, 263, 350, 500, 780 µm) were added at a constant concentration (1.5% w/w) to both matrices. The matrices and the embedded microparticles were both white, so any visual cues indicating the presence of microparticles were minimized. Microscopic images of the microparticles can be found in Appendix 1. As the morphology of the microparticles can also affect participant detection thresholds, spherical smooth particles (microcrystalline pellets) were used for the size range of 127-780 µm.[5] Microparticles with an average diameter of 50 µm (microcrystalline fragments) displayed a more irregular shape. The more irregular shape of the smallest microparticles might potentially enhance the detectability (larger perceived size relative to spherical microparticles), but this is not expected to influence the results (i.e., comparison of across groups of participants).

The method of particle incorporation differed between the two products. For quark, particles were added by manually mixing the cellulose particles into the matrix. For processed cheese, the method described by Santagiuliana et al.[29] was used. Briefly, a 2% (w/w) κ-carrageenan solution was first prepared using tap water. The mixture was heated in a water bath at 90°C for 30 min to obtain a gel after cooling. Next, the processed cheese was melted together with the κ-carrageenan gel (12.5% w/w) in vacuum sealed bags by placing them in a hot water bath (65°C for 20 min). Cellulose particles were added to the molten cheese, which was kept at 65°C in a vessel and manually mixed continuously. Consequently, molten cheese was poured into square petri dishes and stored at 4°C for 16-18 hours. Cheese cubes (20 x 20 x 12 mm) of ~5 g were obtained, whereas portions of 10 g were used for the viscous quark. Cellulose particles were incorporated in both matrices no more than 3 days prior to sensory evaluation.

2.2.2 Participants

Two groups of untrained participants were recruited as a part of a single-blind study investigating the perception threshold of microparticles in the two foods. The two groups were composed of 47 Dutch, Caucasian women (mean age ± SD of 21.4 ± 2.4 years; range of 18-29 years) and 45 Chinese, Asian women (mean age ± SD of 23.3 ± 1.7; range of 21-27 years). Self-reported criteria of nationality and ethnicity (Dutch Caucasians; Chinese Asians), age (between 18-35 years), health status (absence of recognized diseases), and BMI (18.5-26.5 kg/m^2) were used as inclusion criteria. Men were excluded to reduce intragroup variability in physiological parameters. Other exclusion criteria were the presence of allergies, pregnancy, smoking habit, missing teeth (except wisdom teeth) or dental implants, and self-reported deficits in taste or smell. Implementation of these criteria provided two relatively homogeneous groups of young, healthy women with different nationality and ethnicity, which were

158 expected to differ mostly in their level of product familiarity. Participants were naïve about the experimental
159 procedures and purpose of this study; they received financial compensation for their participation. Written
160 informed consent was obtained from all participants. All tests were conducted in accordance with the Declaration
161 of Helsinki.

162 The experiment was conducted at Wageningen University & Research (WUR) over three sessions: a
163 familiarization session of 20 min, and two test sessions of 45 min each. The sessions were completed by all
164 participants within 6 weeks. Participants were asked to refrain from eating 1 h before the start of the sessions. In
165 the first visit (the familiarization session), participants were instructed how to complete the sensory and
166 physiological tests. In the second session, conducted in sensory booths, participants rated their familiarity with
167 quark and processed cheese on a five point scale, where 1 = unfamiliar and 5 = very familiar, and indicated their
168 consumption frequency for these products (once or more per day, once a week, once a month, every 3 months,
169 never). Participants then assessed different samples using the methods of Constant Stimuli, which consists of a
170 balanced series of 2-Alternative Forced Choice tests.[59] Before data collection began, participants were given a
171 warm-up sample consisting of quark with added cellulose beads (average size: 1000 μm). This allowed them to
172 become acquainted with the stimulus and attribute definition (Grittiness: perception of particles in the mouth).
173 Each participant was then given a pair of samples (either two samples of quark, or two samples of processed
174 cheese) consisting of a sample without added particles and a sample with added particles. They were asked to
175 taste and swallow each sample. After tasting the pair, they were asked to indicate the grittiest sample within the
176 pair. A plastic spoon (quark) or fork (processed cheese) was provided. Small, bite-sized portions of both products
177 were served to minimize possible differences in oral processing behaviour between the two consumer groups. [56]
178 Participants rinsed their mouth with water and took a break of at least 1 min between evaluations of different
179 pairs. For each product, a total of seven pairs were evaluated by all participants: six pairs varied in the size of
180 particles added to the heterogeneous sample, and one pair contained two homogeneous samples as a control.
181 Serving order within a pair was counterbalanced, and product type (quark first or processed cheese first) was also
182 counterbalanced. Participants were requested to refrain from eating 1 h before the tasting session.

183 Two separate approaches were used to determine detection threshold estimates. In the first approach, the
184 cumulative proportion of correct identification of the grittier sample (relative to the homogeneous reference) at
185 each particle size was plotted (separately for each food matrix). The threshold value for the group was defined as
186 the particle size that corresponded to 75% correct responses (i.e., half way between chance (0.5) and perfect (1.0)
187 performance in a 2-AFC task).[59–61] In the second approach, an individual Best Estimated Threshold (BET) was
188 calculated for each participant and product type as the geometric mean of the highest concentration missed on
189 the 2-AFC test and the next higher concentration (see Lawless and Heymann, [59]). The two methods allowed to
190 calculate the overall particle detection threshold of the tested population (n=92) for each product (either quark or
191 processed cheese) via the dose-response psychometric function and the estimated thresholds of each individual
192 per product type respectively.

193 The final session (the physiological characterization session) was performed in a meeting space equipped with
194 desk dividers, with a maximum of two participants at a time. Participants followed a defined protocol which was
195 explained by the researchers ahead of time. The different physiological parameters were collected in fixed order:

salivary flow rate, determination of PROP status, point pressure sensitivity via Von Frey monofilaments, and quantification of fungiform papillae density (FPD). These are explained in detail in section 2.3.

2.3 Physiological characteristics of participants

2.3.1 Saliva flow rate

Salivary flow rates for unstimulated (USF) and stimulated (SSF) saliva were determined for each participant. They were first asked to swallow, and then to bend their heads forwards. Next, participants were instructed to spit every 30 s for a total period of 5 min into a lidded cup that had been pre-weighted. After a resting period of 3 min, they were asked to perform the same task while chewing on a piece (5x5 cm) of Parafilm® (Bemis Company, Inc., Neenah, USA). Immediately after collection, cups were placed on ice and weighted. USF and SSF (ml/min) were quantified by calculating the total mass of saliva collected within 5 min in each condition, assuming that 1 g of saliva corresponds to 1 ml.

2.3.2 PROP status determination

Responses to 6-n-propylthiouracil (PROP) were determined using the method described by Yang *et al*. [62] A 0.32 mM PROP solution (Sigma Aldrich) was prepared by dissolving the compound in demineralized water. Before evaluating the intensity, participants were instructed on how to use a general Labelled Magnitude Scale (gLMS), with "the strongest imaginable sensation of any kind" as top anchor and "barely detectable" as bottom anchor [63]. The PROP solution was provided in duplicate via saturated cotton swabs. After rinsing their mouth with demineralized water, participants were instructed to roll the cotton bud across the tongue tip for ~3 s and wait for ~20 s without swallowing before rating the perceived bitterness on a gLMS. Next, participants were instructed to rinse their mouth again and wait 3 min before proceeding with the same task for the next sample. Using means of the two ratings, participants were classified using arbitrary cut-offs.[64] Participants who rated PROP below "moderate" were classified as non-tasters (NT), participants were classified as medium-tasters (MT) when the ratings were above "moderate" but below "very strong" and participants with scores above "very strong" were classified as supertasters (ST). PROP phenotypes were used as both continuous and discrete variables (NT, MT, ST) to test possible relationships with particle size detection thresholds.

2.3.3 Point pressure detection thresholds on the tongue

Point pressure detection thresholds on the tongue were determined using Von Frey monofilaments (Baseline® Tactile™, Fabrication Enterprises, New York, USA).[65,66] For testing tactile sensitivity on the tongue, participants were instructed to rest their chin on an adjustable lab lift and to close their eyes or wear a blindfold if preferred. They were asked to extend their tongue, and two blue round dots (Ø of ~5 mm) were made by the researcher using a cotton swab saturated with food colourant. These dots were used to define a consistent region of testing on the left and right side of the tongue; these marks were placed ~0.5 cm from the tip and ~0.5 cm from the tongue midline. A temporal two alternative forced choice (2-AFC) task was used to establish the lingual tactile detection thresholds in a three-down one-up staircase procedure.[37,67,68] In practice, participants were asked to indicate in which of two sequential trials they could perceive the applied stimulus in either the left or right side of the tongue. Participants were informed that one of the trials would include no stimulus. In each test, the researcher said "trial 1" and "trial 2" and applied the stimulus in only one of the two trials; the trial containing the stimulus was randomly determined by the researcher. Participants indicated which trial of the pair contained the stimulus by using their fingers to signal one or two. After three consecutive correct detections, the force

applied was decreased by changing the Von Frey monofilament. Following a single incorrect response, the force applied was increased. No feedback was provided.

Filaments with target forces of 0.08, 0.20, 0.39, 0.68, 1.57, and 3.92 mN were used. Target forces were validated empirically using a lab balance by determining the mean force of 5 applications before and 5 applications after the completion of the entire experiment. As the values provided by the supplier differed slightly from those determined empirically, effective stress values were calculated based on the actual applied force and contact area of each filament. Contact area of filaments was quantified using a micrometre. The determined stress values were 16.08, 21.48, 36.77, 49.62, 86.79, 133.08 mN/mm^2 respectively and these will be used for the remainder of the manuscript. When testing the sensitivity of the participants, 133.08 mN/mm^2 was chosen as a starting level. Participants were asked to retract their tongue after each trial pair to keep it moistened. If participants could correctly identify the lowest stimulus (16.08 mN/mm^2) six times consecutively, the test was stopped, as the probability of hitting this floor by chance guessing is 0.0156 ($=0.5^6$). Left and right sides of the tongue were tested independently in a randomized fashion. The absolute detection threshold values were determined as five crossings or reversals of a given monofilament. After completing each individual test, the monofilaments were cleaned with a 4% Korsolex (Hartmann Group, Heidenheim an der Brenz, Germany) solution and demineralized water.

2.3.4 Fungiform papillae density

Estimates of fungiform papillae density (FPD) were determined using the Denver Papillae Protocol.[69] Briefly, after rinsing with some water, participants were asked to dry their tongue with tissue paper. With the help of a mirror, they were asked to dye the anterior part of their tongue using a cotton swab that was soaked in a 50:50 (w/w) solution of water and blue food colourant. Example pictures of the procedure and optimum colour applications were provided to the participants during this step. Participants rested their chin on a lab lift and extended their tongues, holding it steady. Pictures were taken using a 16.3-megapixel digital camera (Pentax K30). The lighting was controlled using two studio LCD lamps (Ledgo E268C), which were set to the maximum brightness. Initially, pictures of the entire anterior tongue were taken. Then, pictures were taken after application of rings of filter paper (external diameter of 2.5 cm; Macherey-Nagel GmbH & Co. KG, Düren, Germany) with a 10 mm diameter circular cut-out on the left and right side of the tongue tip (approx. 0.5 cm from the tip and 0.5 cm from the tongue midline). Two researchers independent counted the papillae manually within the 10 mm circular cut-outs (area of 78.5 mm^2) for both left and right tongue side. In the case of misplacement of the paper ring or unclear pictures, the picture of the entire anterior part of the tongue was used and a marked circle (area of 78.5 mm^2) was generated using Adobe Photoshop. Only when counts between the two researchers were the same, were the results considered valid and used further. The mean FPD for each individual was calculated from counts on the left and right side.

2.4 Sample characterization

2.4.1 Particle size characterization

The average particle size of cellulose particles was established using dynamic light scattering (Malvern MasterSizer X, Malvern, Instruments Ltd., Malvern, UK). Tests were conducted on both dry particles and particles submerged in water for different time periods (24, 96, and 120 hrs) to investigate the potential effect of water absorption over time on particle size. These results (not shown) indicate that particle sizes – expressed as

$d_{3,2}$ – were very similar to values reported by the manufacturer (i.e., 50, 127, 263, 350, 500, 780 µm). In line with the results of Lopez et al. [5], particle size was marginally influenced by water absorption, and the measured variation was <10 %. Particle size provided by the manufacturer will be used for the remainder of the manuscript for convenience.

2.4.2 Rheological properties of quark and processed cheese

The apparent viscosity of quark was determined using a Physica MCR 501 Rheometer (Anton Paar GmbH). Flow curves of quark without microparticles were obtained at 4 °C and 20 °C at shear rates ranging from 1 to 1000 s^{-1} in a total time interval of 2.50 min with a concentric cylinder geometry (beaker diameter 18.08 mm; cylinder diameter 16.66 mm; height 24.94 mm). Before the measurements were performed, a waiting time of 2 min was used. Measurements were performed in triplicates. At 4 °C and shear rates $\dot{\gamma}$ (1/s) of 10, 50, and 100, the quark had an apparent viscosity η of 4.3 ± 0.1, 2.7 ± 0.1, and 2.2 ± 0.1 Pa s, respectively. At the same shear rates of 10, 50, and 100 1/s at 20 °C, the quark had a significantly lower ($p < 0.05$) apparent viscosity η with values of 2.8 ± 0.1 , 1.8 ± 0.1 , and 1.5 ± 0.1 Pa s, respectively.

The mechanical properties of homogeneous processed cheeses (20x20x15 mm) and cheeses containing microparticles were characterized by penetration tests to determine the force needed (N) for 30% penetration. A Texture Analyser (TA.XT plus, Stable Micro Systems-SMS) equipped with a 5 kg load cell and a cylindrical flat probe (Ø: 4 mm) was used to perform this test. A crosshead velocity of 1 mm/s was used. Measurements were performed in triplicate. Homogeneous and heterogeneous processed cheeses had a maximum penetration force of approx. 0.58-0.66 N and no significant differences were found between cheeses ($p > 0.05$).

2.5 Data analysis

Data were analysed using IBM SPSS Statistics 23 (SPSS Inc., USA). Two-tailed independent sample t-tests were used to compare the two consumer groups on their familiarity with, and frequency of consumption of, the two products, and on their physiological variables (salivary flow rate, FP density, and tongue tactile sensitivity). A chi-square test was used to examine the proportions (%) of participants of each PROP status (NT, MT, ST) between Dutch and Chinese participants. Two-tailed independent sample t-tests were also used to examine the influence of participant group on perceived grittiness considering BET values as dependent variable for quark and processed cheese separately, and to compare BET values between the two matrices. To determine whether the considered psychological and physiological variables (across the two predefined groups) were related to detection threshold of grittiness, Pearson's correlation coefficients were calculated considering the whole panel (n=92).

3. Results and Discussion

3.1 Participant characteristics

Mean values of familiarity-related parameters for Dutch, Caucasian and Chinese, Asian participants are shown in Table 1.

<Table 1 roughly here>

As expected, Dutch, Caucasian participants were more familiar than Chinese, Asian participants with both products, and a larger difference in familiarity between groups was observed for the quark than for the processed cheese. This difference is also reflected in the frequency of product consumption: Chinese, Asian participants consumed quark less often than Dutch, Caucasian participants. Conversely, no differences in frequency of consumption between Chinese, Asian and Dutch, Caucasian were observed for processed cheese. The low consumption of processed cheese in both groups may be due to the fact that the specific processed cheese used in this study (Kiri) is a French product that is not traditionally part of the Dutch diet, and is not commercially available in most Dutch stores.

Across all participants, positive correlations were found between quark consumption frequency and familiarity ($r = 0.651$, $p < 0.01$), as well as between processed cheese consumption frequency and familiarity ($r = 0.529$, $p < 0.01$).

Unstimulated (USF) and stimulated (SSF) saliva flow rate did not differ significantly between the two groups, suggesting saliva flow was comparable between Dutch, Caucasian and Chinese, Asian participants (Table 2). Similar results were obtained also by Mosca et al. [57] and Pedrotti et al. [55] as no differences in saliva flow rate were observed between groups with the same ethnicity and nationality (i.e., Dutch, Caucasian and Chinese, Asian).

<Table 2 roughly here>

Overall, the group of Dutch, Caucasian participants presented a relatively high number of non-tasters (NT; 51%; mean intensity score ± SD: 7.56 ± 4.67), followed by 47 % of medium tasters (MT; mean intensity score ± SD: 31.20 ± 11.69) and only one super taster (ST; 2%; mean intensity score: 57.00). Chinese, Asian participants showed a lower proportion of NT (33%; mean intensity score ± SD: 9.10 ± 4.67), but more MT (56%; mean intensity score ± SD: 30.50 ± 8.47) and 11% ST (mean intensity score ± SD: 65.55 ± 11.90). Differences in PROP status between groups (Dutch, Caucasian vs. Chinese, Asian) were not significant. Although some studies have found differences in PROP responses when comparing subjects belonging to different ethnic groups [70,71], our results are in agreement with the more recent study of Genick et al. [72] in which differences in PROP status between subjects varying in ethnicity were not observed. For both groups, the proportions of NT was unexpectedly higher than the common ratio in an average population (approx. 20-30 %), although this could be a product of pure coincidence in the selected participants.

Using the Von Frey monofilaments, there was no evidence that tongue pressure detection thresholds differed between the Dutch, Caucasian and Chinese, Asian participants (Table 2). However, we should also note that we

observed a floor effect using the Von Frey monofilaments, as most of our participants may have point pressure detection thresholds lower than 16.08 mN/mm^2. As shown in Figure 1, the average detection thresholds were relatively low as the majority of participants (>90 %) could detect the smallest stress used (16.08 mN/mm^2). Among these high-sensitive participants, a large proportion (80%) reached the end of the test after six consecutive identifications of the weakest Von Frey monofilament (Figure 1), indicating that the individual threshold could not be quantified using a threshold definition based on five crossings (reversals) for a given monofilament. This suggests that these 74 participants (80%) would have likely required a lower amount of applied stress to estimate their thresholds. As filaments able to apply lower forces than 16.08 mN/mm^2 are not currently available, this suggests that more sensitive methods are needed to characterize tactile sensitivity of young healthy adults. We recommend that future research on the relation between tongue pressure sensitivity and texture perception should develop validated, standardized filaments able to apply lower forces than 16.08 mN/mm^2 or, alternatively, consider to use different techniques for characterization of tongue sensitivity (e.g., two point discrimination; letter identification task; grating test).[73,74] We conclude that further studies are warranted to confirm or disconfirm potential relationships between microparticle detection and tongue pressure detection thresholds or tongue threshold sensitivity.

<Figure 1 roughly here>

We failed to observe any evidence of a difference in fungiform papillae density (FPD) between the two groups (Table 2); this is in line with previous studies comparing subject with different ethnicities.[55,75,76] We conclude that physiological aspects as saliva production, PROP status, point pressure detection thresholds, and FPD did not differ significantly between the two consumer groups.

3.2 Particle size detection threshold in viscous and semi-solid foods

To quantify the effect of matrix type on detectability of microparticles, the percentages of correct answers obtained from the method of Constant Stimuli across participants (n=92) were compared. Figure 2 shows the frequency of correct answers when assessing the presence of microparticles in the two matrices. As expected, an increase in particle size resulted in an increase of frequency of correct responses. Figure 2 also shows that for the same particle size, the frequency of correct identifications was higher for the viscous quark than for the semi-solid processed cheese, meaning that particles were more perceptible in the softer, more liquid-like food. In the viscous quark, the smallest cellulose beads tested (50 µm) exceeded the a priori cutoff value of 75% correct answers. In semi-solid processed cheese, a minimum particle size of 127 µm was required before this cutoff value (75%) was reached. As smaller cellulose particles than 50 micron were not available, we are not able to precisely estimate a threshold based on the particle size for this concentration (1.5% w/w). Although feasibility tests completed before this study suggested that 1.5% w/w was an adequate concentration, a similar test with lower concentrations of particles would have provided better distinction.

Generally, the data based on the frequency of correct answers are consistent with best estimated threshold (BET) analysis. For quark, the mean BET was 52 µm, versus 86 µm for the semi-solid processed cheese, and these values differed significantly ($t(156.73) = 3.48$; $p = 0.001$). Collectively, both individual estimates and BET values

suggest that modification of matrix properties decreased perception of microparticles that may cause gritty sensations.

<Figure 2 roughly here>

To test whether the detectability of microparticles differed between the two groups (Dutch, Caucasian and Chinese, Asian), BETs were compared between groups in separate t-tests for each matrix. As we could have expected already by inspecting Table 2, no group differences were observed for microparticle detectability in viscous quark ($t(90) = 0.24$; $p = 0.814$) or semi-solid processed cheese ($t(90) = 0.78$; $p = 0.437$), suggesting that particle detectability did not differ between these groups. Such results were also confirmed by a MANOVA test (data not shown) performed considering the individual particle size threshold for quark and processed cheese as depended variables and nationality/ethnicity as an independent variable. Thus, we conclude that microparticles detection increases with an increase of particle size, it is affected by matrix properties (e.g., consistency, fat content), but it does not depend on factors related to nationality and ethnicity.

3.3 Influence of individual product familiarity on particle size detection threshold

When all participants were considered (n=92), no significant correlations were found between product familiarity and individual BETs for either quark or processed cheese. Frequency of consumption of quark and processed cheese also did not influence detection of microparticles. This suggests that product familiarity and frequency of consumption do not affect ability to detect particles (i.e., larger BET) in the same product. This finding contradicts with our initial hypothesis, where we postulated that the degree of familiarity would be inversely related to individual BET – that is, we expected the ability to detect microparticles to increase with an increase in product familiarity. Given the absence of any significant correlations, we conclude that participant awareness towards product characteristics does not influence the ability of participants to detect the presence of microparticles in each of the matrices.

3.4 Influence of participant individual oral physiology on particle size detection threshold

3.4.1 Relation between individual saliva flow rate and particle size detection threshold

When all participants were considered (n=92), no relationship was found between the individual BET of particles in quark, and either unstimulated or stimulated salivary flow rate. Conversely, individual BETs for particle size in semi-solid processed cheese were negatively correlated with stimulated salivary flow ($r = -0.213$; $p = 0.041$). This weak correlation suggests salivation induced by mastication of a semi-solid matrix might enhance sensitivity to perceive microparticles. Generally, saliva can affect food texture perception due to either its dilution effect during oral food breakdown, or lubrication properties as its presence can facilitate oral manipulation of food and swallowing by lowering in-mouth friction.[13,77] Considering this, higher salivary flows were expected to lower sensitivity (i.e., increase detection difficulty) towards microparticles present in food due to salivary lubrication. However, our results showed the opposite, as more saliva provided better detectability, suggesting that the saliva lubrication properties cannot explain the correlation between salivation and microparticles sensitivity. Such a weak correlation may be due to a dilution effect of saliva addition to the semi-solid food. That is, the incorporation of saliva into the semi-solid processed cheese may have diluted the continuous aqueous phase, and thus the processed cheese became more liquid-like during oral manipulation. As consistency plays a role in the detection

[20,21], the decrease in viscosity may have resulted in a higher sensitivity towards the microparticles. Overall, we conclude that saliva flow is not related to particle size detection threshold in a viscous product, but it might enhance detection of particles during mastication of a semi-solid solid matrix.

3.4.2 Relation between individual PROP status and particle size detection threshold

Contrary to our hypothesis that PROP status would be positively related to detectability of microparticles, there was no evidence to support a relationship between PROP intensity scores and individual particle size detection thresholds, for either quark and processed cheese, when looking across all participants. These data suggest any individual variability in microparticles perception is unrelated to PROP phenotype, when PROP intensity was treated as a continuous measure.[30] Likewise, no relationship was observed between PROP status group (NT, MT, ST) and particle size detection threshold. As sizes of the PROP groups were not equally balanced according to the categorization criterion used here, we also retested for a possible relationship using a tertile split (low sensitivity (33%), medium sensitivity (33%) and high sensitivity (33%) groups) in an exploratory analysis. We still failed to find any evidence of a relationship. Based on all three approaches, we cannot confirm the hypothesis that ability to perceive PROP is related to microparticle detection in the type of products used in our study.

3.4.3 Relation between individual tongue pressure detection thresholds and particle size detection threshold

Given the data summarized in section 3.2, the method used to determine tongue pressure sensitivity was clearly limited by a floor effect, and the point pressure detection thresholds of young healthy women likely fall below the lowest stress that can be applied with commercially available Von Frey monofilaments. Thus, we were unable to test the hypothesis that tongue pressure detection thresholds are related to perceived microparticles. In a recent study of Furukawa et al. [66], no correlation was found between particle recognition thresholds and tactile threshold tested with a comparable methodology, suggesting that this characterization method is probably not suitable to evaluate the detection threshold of microparticles.

3.4.4 Relation between individual fungiform papillae density and particle size detection threshold

Across all participants, no significant correlations were found between fungiform papillae density (FPD) and BETs for particle size detection in either quark or processed cheese. We initially hypothesized that participants' fungiform papillae density would be positively correlated to perception of microparticles in food. Based on present data, we can reject this hypothesis, and conclude that participants' fungiform papillae density does not influence microparticle detection, at least for the viscous and semi-solid products tested here.

3.5 Discussion

This work explores the influence of participants' familiarity and physiological characteristics on oral detectability of microparticles in two foods. Our data show that product properties as particle size and matrix type played a key role in determining detection of microparticles. These findings are largely in agreement with other studies, where larger particle size and lower viscosity of the dispersing phase were positively associated with microparticle detection.[2,5,11,12,15,16,20,21] Here, we demonstrated that the particle size required to determine microparticle perception increases by roughly two thirds when the particles are embedded in a soft semi-solid food rather than a thick viscous product. This suggests that the detection of microparticles in commercial food products can potentially be reduced by embedding them in products with a higher viscosity/consistency. We

anticipate that even particles larger than 86 µm could be consumed without being detected when these are added into hard, solid foods like granola/crunchy cereal or cookies.

We acknowledge, however, that the two matrices used in this study not only differed in consistency but also in fat content and microstructure. Quark did not contain fat (< 0.1 %), while processed cheese had a fat content of ~20%. The presence of fat might also have contributed to a decrease in the detectability of microparticles by increasing the lubrication properties of the processed cheese.[1,10] Additional work is needed to decouple the specific contribution of fat content and consistency of the food on detectability of microparticles. Further, additional tests should be performed using microparticle concentrations below 1.5% (w/w), as the results on individual thresholds highlighted that such particle concentration did not allow a large distinction between participants' sensitivity towards microparticles present in both matrices. Alternatively, the concentration of microparticles could be based on their number rather than on weight concentration. The strategy used in this study (i.e., % w/w) lead to a higher number of microparticles for small sizes, contributing to enhancing their detectability.

Overall, only a weak correlation was found between detection thresholds of microparticles and the tested psychological or physiological characteristics of the participants. This suggests that the individual characteristics explored here explained to a very limited extent the inter-individual variability in detection thresholds of microparticles for the participant groups we tested. From a physiological perspective, the variability in detection of microparticles for young, healthy participants may be potentially explained by other factors that were not considered in this study (e.g., differences in oral processing behaviour, tongue-palate pressure, etc.). Nonetheless, the characterization methods applied in this study might still be able to explain variability in perceived texture when other groups of participants are considered, such as elderly or subjects with decreasing eating capabilities.[46]

Considering that the two groups tested were homogenous in their age and sex, this study focused on potential differences related to nationality and ethnicity. To the best of our knowledge, minimal research has been conducted to date addressing possible differences in oral physiology between participants of different ethnicities.[55–58] Similarly, a few studies have investigated whether possible cross-cultural differences influence food texture perception. When Vietnamese and French adults evaluated soy yoghurts and jellies, only small differences were found in the perceived textural profile between the two groups, although participants differed in their degrees of products familiarity.[78,79] Correspondingly, our results also suggest that young Caucasian women from the Netherlands and young Asian women from China present very comparable physiological characteristics, while they differ primarily in terms of product familiarity. These results are in agreement with the observations from other cross-cultural studies on basic taste thresholds which fail to find differences between groups.[80–82] Thus, we conclude that the sensitivity towards oral texture appears to involve perceptual mechanisms that are unrelated to a participants' nationality and ethnicity.

Given present data, a relationship between product familiarity and particle size detection thresholds in either product could not be confirmed. However, care needs to be taken when generalizing such observation in real-life consumption conditions, as other factors could also affect the ability of participants to detect small variations in a familiar food. For instance, the discrimination ability of participants towards familiar product can be increased when subjects evaluate foods with affective (i.e., involving personal preference and emotions) rather than

analytical (i.e., pure stimulus recognition) processes.[83] We conclude that participant awareness of product characteristics was not affected by the presence of microparticles when tested in an analytically in a laboratory context. Additional work is needed to check how product familiarity might influence perception and liking of such attributes under real-life eating conditions.

The present results suggest saliva production might slightly increase perception of microparticles in a semi-solid matrix, consistent with the view that saliva influences perception of textural food properties.[32,40,41] We explain this weak relation mainly considering the dilution effect caused by the presence of saliva during mastication of soft semi-solid food. A higher amount of saliva is expected to decrease the bolus consistency [84,85], leading to a lower particle size required to trigger microparticle detection. For the quark product, no relation was observed between microparticle detection and saliva production, probably because salivation is induced mainly by mastication. The higher food consistency of the processed cheese might have had not only enhanced more saliva production, but also determined an increase in oral manipulation [86], enhancing the positive effect of saliva on the detection of microparticles. This suggests the influence of saliva on texture perception may be larger in solid foods requiring chewing versus easy-to-swallow liquid foods. To gain more insights on the role of saliva in the detection and perception of microparticles in foods, we recommend further research to investigate the incorporation of saliva into the food bolus and its influence on rheological characteristics of the bolus.

The link between the oral somatosensory system and perception of microparticles was investigated considering participants' tongue pressure detection thresholds, fungiform papillae density (FPD) and PROP status. As it is not possible to directly quantify mechanoreceptors density non-invasively, we measured FPD as a proxy instead. FPD provides a rough estimate of trigeminal fibres innervation and might be related to density of mechanoreceptors (i.e., the higher number papillae, the higher the innervation of trigeminal fibres, the higher the density of mechanoreceptors).[14,49] Correspondingly, point pressure detection thresholds were calculated using Von Frey monofilaments to indirectly evaluate consumer's mechano-sensitivity in the same areas where papillae were counted. Our data did not identify any relation between FPD and detectability of microparticles. These outcomes are in agreement with the findings of Bakke and Vickers [14,34] where no correlation was found between FPD and roughness perception of staled bread. As FPD has been positively related with other textural sensations as creaminess [30,31] and fat perception [32], we conclude that the link between textural sensations and FPD remains unclear, and further investigations are required to unravel the current inconsistent conclusions.

In this study, PROP status or scored PROP intensity were not related to individual variability in microparticle detection. Direct connections between enhanced texture discrimination (e.g., creaminess, heterogeneity, roughness) and PROP sensitivity was previously confirmed and disconfirmed by others.[14,32,35,87,88] We conclude that the relation between PROP status and texture discriminability remains unclear and might depend on the specific textural attribute being considered. Further investigations are required to confirm any possible relationships, especially for texture.

4. Conclusions

The aim of this study was to test how product familiarity and physiological characteristics affect detectability of microparticles. Our results show that particle size detection thresholds differed significantly between viscous liquid and semi-solid dairy products, but did not differ between women who differed in nationality and ethnicity

Dutch, Caucasian and Chinese, Asian women. When all participants were considered, particle size detection threshold was 52 μm for quark and 86 μm for processed cheese. Particle size detection threshold was not correlated with participants' product familiarity for neither product; still, for processed cheese but not quark, there was a positive but weak correlation with stimulated salivary flow (r = 0.21, p = 0.041). This suggests detectability of microparticles might be slightly enhanced by salivation induced by mastication, at least for a semi-solid matrix. Particle size detection threshold in both matrices did not correlate with participants PROP status, point pressure thresholds on the tongue, or fungiform papillae density. Particle size detection threshold was also not influenced by nationality and ethnicity. We conclude that variations in particle size detection thresholds in semi-solid and viscous foods are mainly explained by the product properties, while further investigations are required to identify participant characteristics responsible for differences in detection of microparticles in food.

5. Competing interest

The authors have declared that no competing interests exist.

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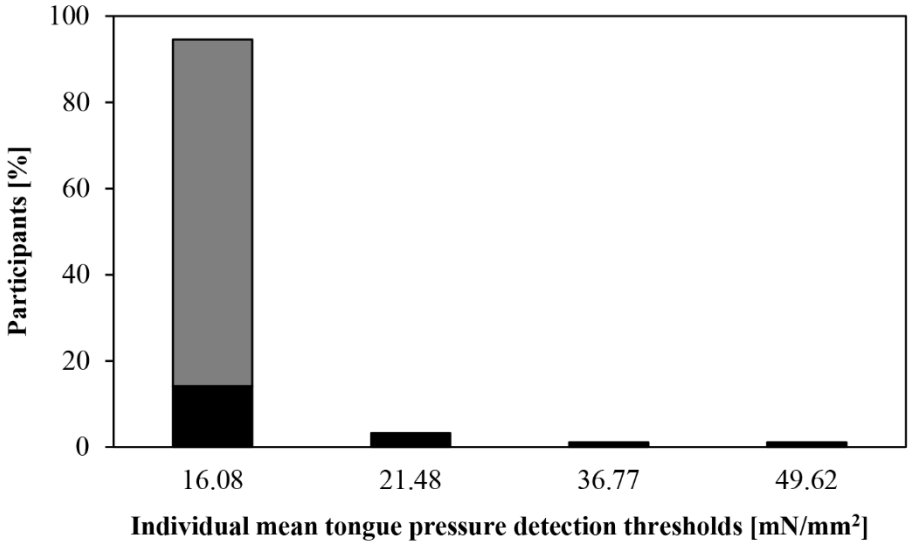
671 **Figures**

672 **Figure 1.** Individual mean tongue pressure detection thresholds tested on anterior left and right side of the tongue
673 for Dutch, Caucasian and Chinese, Asian participants (n=92) obtained using Von Frey monofilaments. Bars
674 indicate the percentages of participants for the respective pressure detection thresholds. The grey bar indicates
675 the percentages of participants whose pressure detection thresholds are expected to be possibly below 16.08
676 mN/mm^2.

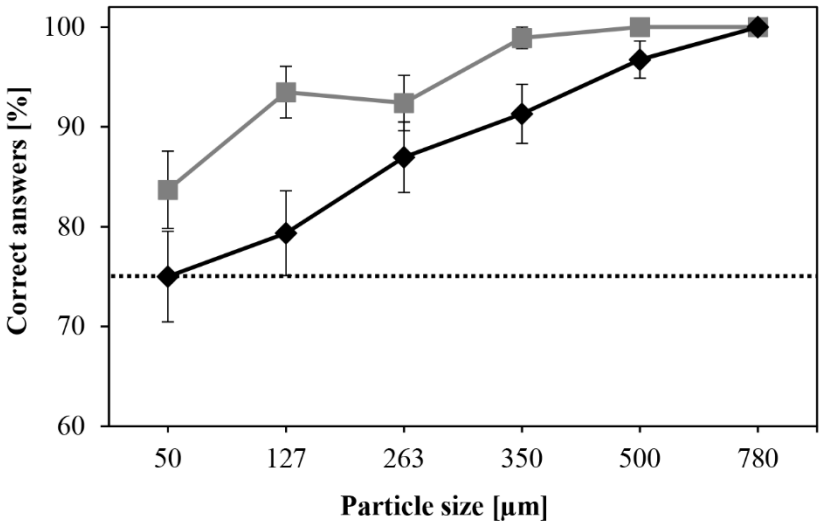
677 **Figure 2.** Perception of microparticles across all participants (n=92): cumulative frequency of correct answers as
678 a function of difference in particle size. Quark samples are represented by squares (■); processed cheese samples
679 by rhombus (♦). Error bars indicate standard error of the mean.

680 **FIGURE 1**



681

682 **FIGURE 2**



683

Tables

Table 1. Mean values and standard deviations of product familiarity (1= unfamiliar; 5= very familiar) and frequency of consumption (1= never; 5= once or more per day) considering all participants, Dutch, Caucasian and Chinese, Asian participants.

		All (n=92)	Dutch Caucasian (n=47)	Chinese Asian (n=45)	*p*-value
Product familiarity	Quark	3.77 ± 1.19	4.45 ± 0.72	3.07 ± 1.18	< 0.001
	Processed cheese	3.57 ± 1.01	3.85 ± 0.83	3.29 ± 1.12	0.008
Frequency of consumption	Quark	2.80 ± 1.18	3.15 ± 1.20	2.44 ± 1.06	0.004
	Processed cheese	2.28 ± 1.03	2.53 ± 0.91	2.93 ± 1.12	0.061

689 **Table 2.** Summary of physiological parameters across all participants (n=92), as well as for Dutch, Caucasian
690 (n=47) and Chinese, Asian (n=45) participants separately.

		All (n=92)	Dutch Caucasian (n=47)	Chinese Asian (n=45)	*p*-value
Saliva flow (g/min)	Unstimulated (USF)	0.51 ± 0.34	0.51 ± 0.34	0.51 ± 0.34	0.974
	Stimulated (SSF)	1.34 ± 0.80	1.37 ± 0.75	1.31 ± 0.86	0.728
PROP status (n participants)	Non taster (NT)	39 (42 %)	24 (51 %)	15 (33 %)	0.087
	Medium taster (MT)	47 (51 %)	22 (47 %)	25 (56 %)	
	Super taster (ST)	6 (7 %)	1 (2 %)	5 (11 %)	
Tongue pressure detection thresholds (g/mm^2)	Averaged tongue	1.66 ± 0.12	1.68 ± 0.16	1.66 ± 0.06	0.393
	Right side tongue	1.66 ± 0.08	1.67 ± 0.11	1.64 ± 0.00	0.165
	Left side tongue	1.68 ± 0.23	1.69 ± 0.31	1.67 ± 0.11	0.675
Fungiform papillae density ($count/cm^2$)	Averaged tongue	16.7 ± 9.0	16.5 ± 9.2	17.0 ± 8.8	0.825
	Right side tongue	17.2 ± 9.4	16.9 ± 9.5	17.4 ± 9.5	0.780
	Left side tongue	16.3 ± 9.0	16.2 ± 9.3	16.5 ± 8.8	0.882

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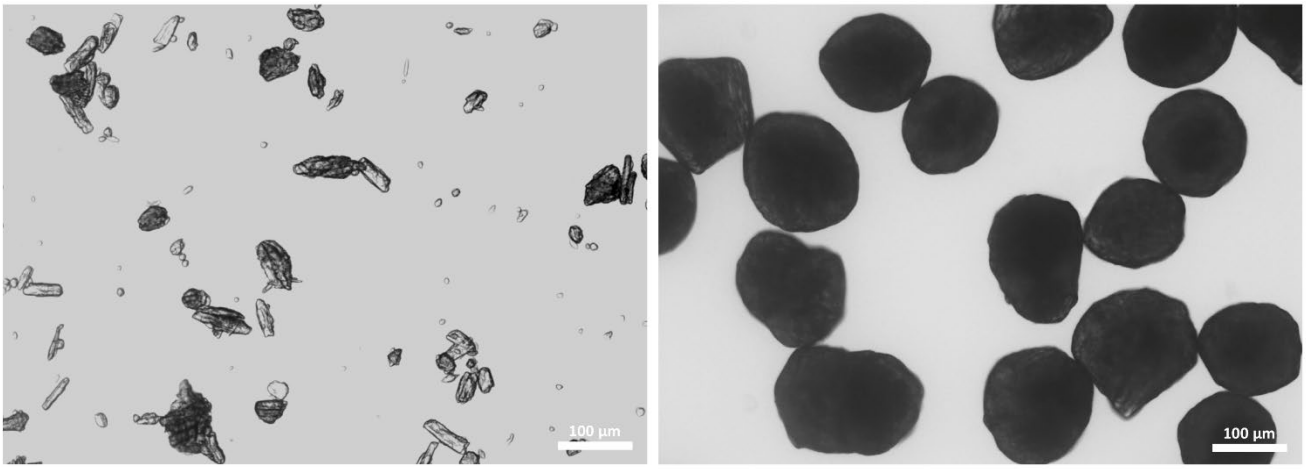
693 APPENDIX 1

694 Appearance of microcrystalline cellulose particles having an average size of 50 µm (left) and 126 µm (right).

695



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