Cross-cultural differences in lingual tactile acuity, taste sensitivity phenotypical markers, and preferred oral processing behaviors

Camilla Cattaneo¹ §, Jing Liu² §, Anne C. Bech³, Ella Pagliarini¹, Wender L.P. Bredie² *

1Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Milan 20133, Italy
2Department of Food Science, Faculty of Science, Section for Food Design and Consumer Behaviour, University of Copenhagen, Frederiksberg C, 1958, Denmark
3Arla Innovation Centre, Arla Foods AMBA, Aarhus N, 8200, Denmark

§These authors contributed equally to this work.

Correspondence to be sent to:
Wender L.P. Bredie
Department of Food Science
Section for Food Design and Consumer Behaviour
University of Copenhagen
Rolighedsvej 26
1958 Frederiksberg C, Denmark
E-mail: wb@food.ku.dk
Telephone: +45 35 33 32 42

Keywords: Cross-cultural differences; preferred mouth behavior; PROP; fungiform papillae; tactile acuity
Abstract

Cultural and genetic differences in consumer populations across the world are important determinants for food preferences. The present study investigated differences in preferred oral processing behaviors between Chinese Asian and Danish Caucasian consumers and the possible relationship to lingual tactile acuity and the two most well-researched phenotypic markers of taste sensitivity, such as 6-n-propylthiouracil (PROP) responsiveness and Fungiform Papillae Density (FPD).

A total of 152 consumers (75 Chinese, 77 Danish) were enrolled in the study and categorized by their preferred oral processing behaviors. Lingual tactile acuity was assessed according to responses to stimulation with Von Frey filaments. The responsiveness to PROP and the FPD were also determined.

Cross-population differences were found in preferred food oral processing behaviors in these two cohorts, as Chinese consumers were characterized by a larger number of ‘Soft processing likers’ (77% of the population) who preferred soft food processing in the mouth. Contrarily, Danish consumers mostly belonged to the ‘Firm processing likers’ group (73% of the population) who had preferences for foods that needed firm processing on biting and chewing. Moreover, the group of ‘Firm processing likers’ were shown to be more sensitive than the ‘Soft processing likers’ in both population cohorts.

Cross-population differences in lingual tactile acuity were not significant. Differences in FPD and PROP responsiveness were found between these two population cohorts, with Chinese consumers generally characterized by greater FPD and PROP responsiveness compared to the Danish subjects.

This study provides evidence on cross-cultural differences in preferred oral processing behaviors and in the two phenotypic marker of taste sensitivity. However, further studies are needed to draw conclusive relationships between preferred oral processing behavior and oral tactile acuity, PROP responsiveness and tongue anatomy.
1. Introduction

The variation in oral texture perception of foods across consumer populations is supposed to depend on individual differences in tactile acuity and processing behaviors in the mouth. Tactile acuity has been widely studied at the surface of the skin (for a review: Abraira & Ginty, 2013) and four mechanoceptors have been identified. These specialized nerve endings convey specific sensations such as light pressure and touch as well as stretch and high-frequency vibration. In the anterior tongue, neuroanatomical studies have shown that somatosensory trigeminal neurons terminate as a network of fibers in the peri-gemmal tissue (des Gachons et al., 2011; Suemune et al. 1992; Whitehead, Beeman, & Kinsella, 1985). Gairs and Garven (1952) were the first to find anatomical evidence in humans that somatosensory endings from the trigeminal nerve (V) innervate Fungiform Papillae (FP). Later research confirmed these findings and showed that twenty-five percent of FP innervation arise from the chorda tympani nerve (taste), and seventy-five percent from the trigeminal nerve (pain, touch and temperature) (Silver & Finger, 1991). Mechanical stimuli are likely to activate some receptors of the trigeminal nerve endings, which surround taste buds in the FP and terminate in the papilla apex (des Gachons et al., 2011). Considering FP as a common anatomical unit of the sense of taste and the somatosensory system, these anatomical structures are assumed to act as an ‘array of sensors for detecting oral touch sensations’ (Bartoshuk et al. 1994; Prescott, Soo, Campbell, & Roberts, 2004; Prutkin et al., 2000) and predict tactile acuity and discrimination (Bangcuyo & Simons, 2017; Engelen & Van der Bilt, 2008; Prescott, Soo, Campbell, & Roberts, 2004). This anatomical colocation can explain the positive correlations between FP and trigeminally mediated oral somatosensations such as the tongue spatial resolution acuity (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone, 2003), the textural aspects of creaminess (Hayes & Duffy, 2007; 2008; Nachtsheim & Schlich, 2013; Proserpio et al., 2016) and roughness perception (Bakke & Vickers, 2008).

In addition to the relationship between FP and lingual tactile acuity the responsiveness to the bitter tastant 6-n-propylthiouracil (PROP) has also been proposed has another factor involved in texture perception. Indeed, several studies have associated PROP taster status with the perceived intensity and the ability to discriminate trigeminal sensations and textures (Bakke & Vickers, 2011; Bartoshuk et al., 1994; de Wijk et al., 2007; Pickering, Simunkova, & Di Battista, 2004; Pickering & Robert, 2006; Tepper & Nurse, 1997; Yackinous & Guinard, 2001). However, results on association between PROP status and texture are still contradictory and some studies could not find associations between PROP status and oral texture perception (Drewnowski et al. 1998; Lim, Urban, & Green, 2008).

There are some indications that Asian and Caucasian consumers have different oral chemosensory abilities (Guo & Reed, 2001; Tepper, 2008). In Caucasian populations, 20 to 25% is estimated to be PROP non-taster (less responsive to PROP). Whereas the estimated proportion of non-tasters in Asian
populations in China and Japan is between 10 and 20% (Guo & Reed, 2001). Additionally, Essick and colleagues (2003) found that PROP sensitivity seems to covary among Asian and Caucasian females, reflecting individual differences in the density and diameter of FP on the anterior tongue. However, very few studies investigated possible ethnicity differences in food texture perception and no differences in oral tactile acuity between Caucasian and Asian subjects could be detected by the letter recognition method (Essick, Chopra, Guest, & McGlone, 2003).

This letter recognition method has been popular for measuring oral touch sensitivity across subjects (Essick, Chen, & Kelly, 1999; Essick, Chopra, Guest, & McGlone 2003; Lukasewycz & Mennella 2012; Steele, Hill, Stokely, & Peladeau-Pigeon, 2014). In this test subjects are asked to use their tongues to identify letters of the alphabet of varying sizes embossed onto Teflon strips (Essick et al., 1999). A challenge with the oral letter test is that subjects across cultures may differ in their recognition ability due to different alphabets and symbols in their languages, thus making this method possibly less suitable for cross-cultural studies. Other methods for oral touch acuity include a two-point discrimination task (Engelen, Van der Bilt, & Bosman, 2004), grating orientation discrimination (Van Boven & Johnson, 1994), and other physiological measures (Bangcuyo & Simons, 2017; Linne & Simons, 2017). The majority of studies on oral tactile acuity utilized static or moving two-point discrimination or grating recognition tasks, which may have limited reliability as tools for determining touch detection and punctate pressure (Miles et al., 2018). An alternative method concerns a localized one point touch testing with von Frey fibers (Semmes-Weinstein monofilaments). This method concerns a touch detection task, where subjects report presence or absence of the stimulus. This method is reported to be repeatable, accurate, and most reliable for measuring light touch–deep pressure sensibility of the tongue and the hard palate (Bell-Krotoski & Tomancik, 1987; Bodin, Jäghagen, & Isberg, 2004; Cordeiro, Schwartz, Neves, & Tuma, 1997; Henkin & Banks, 1967). When studying cross-cultural differences in oral touch acuity, methods aiming at point touch detection may be more suitable as they provide localized absolute detection thresholds. Thus preventing biases from cultural differences in object recognition.

Besides the phenotypic markers of taste sensitivity and lingual tactile acuity, there is a growing body of research on oral processing behavior, i.e. the way to manipulate and manage a food in the mouth (de Wijk, van Gemert, Terpstra, & Wilkinson, 2003; Jeltema, Beckley, & Vahalik 2014; 2015; 2016; Yackinous & Guinard, 2001). It has been suggested that differences in food manipulation and mastication could affect sensory sensations (Lassauzay et al., 2000; Po et al., 2011). Brown and Braxton (2000) identified four different groups of people based on their efficiency in reducing the size of foods (i.e. almonds and chewing gum) and suggested that individual differences in the mouth ability to manipulate and handle the product may be an important driver of liking and preferences.
More recently, Jeltema and collaborators (2014) suggested the existence of Mouth Behavior (MB) groups and showed that consumers can be typified by the way they manipulate food in their mouths. Their scheme categorized consumers into so-called (a) Smooshers, (b) Suckers, (c) Chewers, and (d) Crunchers. These groups fell into two major mouth processing styles. The first one, represented by Suckers and Smooshers, preferred to process food between the mouth’s roof and tongue. They diverged principally in the hardness of preferred foods. Suckers preferred harder foods that could be sucked on for a long time, such hard candies and foods that they could hold in their mouths. Smooshers preferred soft foods, such as puddings or creamy candies that would spread throughout the mouth and could be held in for a longer time, not requiring much mouth activity. The second one, represented by Crunchers and Chewers, preferred to use their teeth to break down foods. In particular, Crunchers were more forceful in their bite, preferring foods that broke up on biting. Chewers liked foods that did not fracture on biting and could be chewed. If such consumer MB groups exist, one would expect a possible cultural dimension, as different populations have different habits on how to prepare and consume foods. However, such differences in oral MB may also be related to fundamental differences in mouth anatomy and texture perception. Moreover, other factors such as salivary flow, mouth size, dental bite, dental status and health could play a role in defining subjects’ MB, affecting chewing and mastication performance (Chen, 2009; Jeltema, Beckley, & Vahalik, 2016).

Thus, there are evidences to suggest that PROP phenotypical and population cultural factors may play a role in texture perception and preferences. PROP tasters appear to have a better lingual tactile acuity than other taster groups and may more readily detect small particles and granularity in foods. It seems plausible that PROP tasters will be more sensitive to gritty contaminants in foods and may more readily reject such foods (Essick, Chopra, Guest, & McGlone, 2003). Thus, a high responsiveness to PROP and variation in FPD are possibly involved in choices of some food textures. Likewise, other influences such as personality traits, cultural habits and societal factors are most certainly important in texture preferences. As reported above, it has well been established that Asian and Caucasian populations differ in PROP responsiveness, which is most definitely seen in the higher proportion of supertaster-tasters in the Asian population. It is less certain that Asian and Caucasian populations differ in lingual tactile acuity beyond differences in their PROP status and FPD counts. The two populations have not been shown to differ in the letter recognition task on the anterior dorsal part of the tongue, but no studies have reported differences on touch detection ability on this part of the tongue.

The present study aimed at investigating whether differences exist in PROP responsiveness, FPD and touch detection ability on the anterior dorsal part of the tongue among Asian and Caucasian adults. Furthermore, the importance of such sensory differences for preferred oral food processing behaviors
in these populations has been explored. The objectives were to (i) find relationships between PROP taste sensitivity, FPD and touch detection ability among Asian and Caucasian population cohorts, (ii) classify Asian and Caucasian according to their preferred food oral processing behaviors, and (iii) explore lingual touch detection ability in relation to oral texture preferences.

2. Material and methods

2.1 Subjects

One hundred and fifty-two healthy, non-smoking subjects between the ages of 18 and 55 years were recruited to attend the consumer test. Two cohorts were recruited from the greater Copenhagen area and included seventy-five of the subjects, the Asian cohort (56 F, 19 M; mean age= 26.9 ±2.9; age distribution: 56% aged 18–30 years and 44% aged 31–55 years; BMI= 21.6 ±3.6) and another seventy-seven subjects, the Caucasian cohort (52 F, 15 M; mean age= 29.8 ±9.1; age distribution: 61% aged 18–30 years and 39% aged 31–55 years; BMI= 24.1 ±5.0). Seventy-two percentage of Chinese subjects had been living in Denmark for less than two years at the moment of the test.

Informed, written consent was obtained from all subjects on the first test day. The present study was performed according to the principles established by the Declaration of Helsinki and the protocol was approved by the Institutional Ethics Committee of the University of Copenhagen.

2.2 General procedure

Participants attended one study session lasting 1 h and completed 4 different tasks: 1) a first questionnaire to collect general demographic information followed by a second questionnaire to identify subjects’ mouth behavior; 2) Lingual tactile acuity task using three von Frey filaments; 3) tongue pictures for the estimation of FPD; 4) a screening procedure for PROP responsiveness.

2.3 Questionnaire to assess mouth behavior

Participants’ mouth behavior was assessed through a questionnaire, which was derived from the work of Jeltema and collaborators (Jeltema, Beckley, & Vahalik 2014; 2015; 2016). There were 20 text-based questions where subjects were asked to respond to a variety of statements aimed at understanding how they preferred to manipulate food in their mouths. Additionally, 4 picture-based questions were used to further evaluate the subjects’ liking of a group of products from a mouth processing perspective. These products were carefully chosen to represent those that would best differentiate between groups. A Likert 6-point agree/disagree scale anchored ‘strongly disagree’ (1)
to ‘strongly agree’ (6) was used for all statements. The reader is referred to Table S1 (Supplementary material) for the presentation of questions that were used to type individuals for MB.

2.4 Lingual tactile acuity evaluation

During the lingual tactile acuity evaluation blindfolded subjects were seated in an upright position and their tongue stimulated or not with three von Frey filaments (no. 1.65, 2.36 and 2.44), one at a time. The number of the filaments corresponds to a logarithmic function of the equivalent forces of 0.008, 0.02 and 0.04 g, respectively, according to the manufacturer (Aesthesio®: Precise Tactile Sensory Evaluator, DanMic Global, LLC, San Jose, California, USA). For each filament, the subjects were given 5 true and 5 mock touch exposures on the tongue’s apex. The stimulation order was counterbalanced for the three filaments. The true touch with a filament was defined as ‘signal’ and the corresponding response as either ‘hit’ or ‘not detected’. The responses from the mock exposures were defined as ‘correct rejection’ or ‘false positive’. The subjects also rated their degree of certainty in their response (either signal sure, signal not sure, no signal not sure, or no signal sure). From the subjects' responses and certainty ratings R-index values (%) were calculated (O'Mahony, 1992). As reported by Lee and Van Hout (2009), ‘the R-Index is an estimated probability of correctly identifying a target stimulus (the signal) when presented pairwise with a 2nd stimulus (the noise). As frequently happens with difference test, the R-Index values could range from 50% to 100%. If the subject cannot discriminate between the 2 stimuli, the judge will have to guess and the chances of correctly identifying the signal the R-Index will be 50%, otherwise the R-Index will be 100% if the judge can discriminate perfectly between the 2 stimuli. Thus, the better the discrimination, the higher the value will be’.

2.5 Fungiform Papillae Density (FPD)

The area to count individual FPD was selected following the procedure adapted from Bakke and Vickers (2011) and previously described by Proserpio and colleagues (2016). Tongue pictures were collected with blue staining, which was obtained by swabbing with blue food coloring, using a cotton-tipped applicator. This fungiform papillae easily visible on the anterior portion of the dorsal surface of the tongue. Digital pictures were recorded using a Canon digital camera (Canon EOS 700D) in a brightly light room using the camera’s macro mode with no flash. The best photograph of each blue stained tongue was selected to measure the FPD, and ImageJ software was used to mark the area in which papillae were to be counted. A set of three 0.6 cm diameter circles was drawn on the front of the anterior tongue, according to Bakke and Vickers, (2011). One operator, blind to any data
concerning subjects and with 3-year experience, counted FP in two different moment (at least 3 weeks between the first and second count). The counts were submitted to 1-way fixed ANOVA. Counts were considered valid if the operator effect was not significant (p > 0.05). FP were counted inside the three marked circles and the average count over the three circles was used for each subject (Proserpio et al., 2016). The Denver Papillae Protocol (Nuessle, Garneau, Sloan, & Santorico, 2015) was followed to determinate FP according to shape, color, size and recession. The individual FPD was then calculated by reporting the number of FP to a common unit area of 1 cm².

2.6 Taste responsiveness to PROP

A method proposed by Prescott and colleagues (2004) was used for evaluating participants’ PROP status. The intensity of bitterness of a supra-threshold 0.0032 M solution of PROP (European Pharmacopoeia Reference Standard, Sigma-Aldrich) was rated using the Generalized Labeled Magnitude Scale, gLMS (Bartoshuk et al., 2004), anchored at the top with the descriptor ‘strongest imaginable sensation of any kind’, which was defined in the context of all sensations, including painful ones. Practice on the use of the gLMS was provided to ensure that participants understood the scale and examples of the intensities of an array of ordinary sensory experiences (e.g., loudness of whispers, brightness of the sun) were provided. Then, subjects were presented with two identical samples (10 ml) and were instructed to hold each sample in their mouth for 10 s, then to expectorate the solution. After 20 s they were asked to evaluate the bitterness intensity. To control for carry-over effect, a 90s break was given to the subjects to rinse their mouths with water after the first sample evaluation (Laureati et al., 2018). The average of bitterness scores was used for each subject and respondents were grouped according to their PROP status based on arbitrary cut-offs. Non-tasters (NT) were 17.8% of total sample (arbitrary cut-off gLMS ≤17, moderate), whereas Super-tasters (ST) were 36.2% (arbitrary cut-off gLMS ≥53, very strong). The remainder of the respondents were considered as Medium-tasters (MTs) (Fischer et al., 2013; Hayes et al., 2010).

2.7 Data Analysis

In order to identify the two major different Mouth Behavior styles among consumers, a latent class analysis with two classes was performed on the scores (6-point scale) for the 24 evaluated items of the questionnaire. The differences across clusters are identified by Wald test ($\chi^2$) along with p-values and $R^2$.

The association between population cohort, Mouth Behavior and tactile acuity (expressed as R-index value) was analysed by a Generalized Linear Model considering Population cohort (Danish and Chinese), Mouth Behavior groups (‘Firm processing likers’ and ‘Soft processing likers’) and
Filament thickness (no. 1.65, 2.36, 2.44) and their 2-way interactions as independent variables. Data were further analyzed separately for Danish and Chinese considering Mouth Behavior groups (‘Firm processing likers’ and ‘Soft processing likers’) and Filament thickness (no. 1.65, 2.36, 2.44) and the respective interaction as independent variables in order to have better insights on the relative contribution of these factors on dependent variables. Post-hoc comparisons using the Bonferroni test adjusted for multiple comparison were conducted when appropriate.

The relationship between tactile acuity (R-index for the thinner filament no. 1.65), PROP responsiveness and FPD was evaluated graphically and with Pearson's correlation r.

To determine the cross-cultural relationship between Mouth Behaviors and FPD, a Generalized Linear Model was constructed with the FPD as dependent factor, and Population cohort (Danish and Chinese), Mouth Behavior groups (‘Firm processing likers’ and ‘Soft processing likers’) and the respective interaction as independent factors. To check for possible confounding or modulating effects, the analysis was performed by adding R-index values, as covariate to the model. Additionally, the same model was run using the mean intensity ratings of PROP responsiveness as dependent factor.

Both models have been run separately on Chinese and Danish subjects.

For all the analyses, a p-value of 0.05 was considered as threshold for statistical significance. Data are presented as means with standard errors (SEM). Statistical analysis was performed using IBM SPSS statistical software version 25 (SPSS Inc, Chicago, IL, USA). Latent class analysis was performed in Latent Gold 5.1 (Statistical Innovations, Belmont, USA).

3 Results

3.1 Mouth Behavior mapping

Two distinct clusters were identified: Cluster 1 ‘Soft processing likers’ with 79 participants and ‘Firm processing likers’ composed of 73 participants. The number of participants in each cluster is listed in Table 1.

Table 1. Numbers of participants in each MB group by population cohort.

<table>
<thead>
<tr>
<th>MB Group</th>
<th>Description</th>
<th>Chinese</th>
<th>Danish</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firm processing likers (FPL)</td>
<td>Prefer foods that require to use the incisors and/or molars to break down rapidly or deform the food matrix</td>
<td>17 (23%)</td>
<td>56 (73%)</td>
<td>73 (48%)</td>
</tr>
</tbody>
</table>
Soft processing likers (SPL) Prefer foods that could be held in the mouth for a longer time and manipulate them between the tongue and roof of the mouth 58 (77%) 21 (27%) 79 (52%) 75 77 152

For the two clusters significant differences were identified for 14 of the 24 questions used for the classification, results shown in Figures 1, with the questions sorted according to size of the difference. The most discriminating questions are Q8, Q18 and Q22 (p<0.0001) and regards soft smooth (banana/ripe peaches) versus more crunchy fruits (apples/pears), yogurt with crunchy versus softened muesli and finding or joy and pleasure consuming crunchy food as raw carrots, apples, peanuts coated with chocolate, crunchy granola or not. Questions that are best explained by the cluster are Q4 and Q18 (R² = 0.462 and 0.427) followed by Q22, Q8 and Q24 (Table 2). The 10 non-significant questions may be either not discriminating, were difficult to understand, or be more relevant for further subgrouping in larger population samples.

Table 2. Cluster means and statistical Wald, p-values and R² for the 24 indicators (questions).

<table>
<thead>
<tr>
<th>Items</th>
<th>Cluster 1 - Soft processing likers</th>
<th>Cluster 2 - Firm processing likers</th>
<th>Wald</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 I usually prefer a chewy piece of candy like e.g. wine gum over a hard piece of candy.</td>
<td>3.79</td>
<td>3.88</td>
<td>0.13</td>
<td>0.72</td>
<td>0.001</td>
</tr>
<tr>
<td>Q2 I usually prefer chocolate with crunchy fillings like nuts over chocolate that easily melts in my mouth</td>
<td>3.87</td>
<td>4.44</td>
<td>4.80</td>
<td>0.03</td>
<td>0.038</td>
</tr>
<tr>
<td>Q3 When I eat oranges I enjoy to put the slices into my mouth and suck the orange juice out of the slices instead of just chewing the slices right away</td>
<td>2.96</td>
<td>2.47</td>
<td>2.88</td>
<td>0.09</td>
<td>0.024</td>
</tr>
<tr>
<td>Q4 When I eat breakfast cereals I usually let them soften quite a bit in the milk before I eat them, as opposed to eating them straight away</td>
<td>3.95</td>
<td>1.48</td>
<td>13.11</td>
<td>0.001</td>
<td>0.462</td>
</tr>
<tr>
<td>Q5</td>
<td>When I eat chocolate I usually prefer chocolate with a good chewing texture over chocolate that easily melts in the mouth</td>
<td>3.65</td>
<td>3.72</td>
<td>0.07</td>
<td>0.78</td>
</tr>
<tr>
<td>Q6</td>
<td>When I eat fruits I usually prefer crunchy fruits like fresh apples over more chewy fruits that I can chew on like pineapple or strawberries</td>
<td>3.10</td>
<td>3.47</td>
<td>2.32</td>
<td>0.13</td>
</tr>
<tr>
<td>Q7</td>
<td>When it comes to chocolate I usually prefer chocolate that is hard enough to suck on over chocolate that quickly melts in my mouth</td>
<td>3.35</td>
<td>3.44</td>
<td>0.17</td>
<td>0.68</td>
</tr>
<tr>
<td>Q8</td>
<td>I usually prefer soft and smooth fruits like ready to eat bananas and ripe peaches over hard and crunchy fruits like fresh apples and pears</td>
<td>4.09</td>
<td>3.01</td>
<td>16.77</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Q9</td>
<td>I usually prefer the texture of soft whole grain bread over the texture of crispbread and crackers</td>
<td>4.27</td>
<td>3.43</td>
<td>11.37</td>
<td>0.001</td>
</tr>
<tr>
<td>Q10</td>
<td>When I eat ice cream I eat it right out of the freezer instead of letting it thaw a little</td>
<td>3.43</td>
<td>3.71</td>
<td>0.90</td>
<td>0.34</td>
</tr>
<tr>
<td>Q11</td>
<td>I usually prefer to suck on hard candy until they are paper thin instead of crunching them after a short while</td>
<td>4.07</td>
<td>3.21</td>
<td>7.79</td>
<td>0.005</td>
</tr>
<tr>
<td>Q12</td>
<td>I enjoy to eat foods that are smooth and easily spreads in my mouth like puddings and ice cream</td>
<td>4.86</td>
<td>4.52</td>
<td>3.63</td>
<td>0.06</td>
</tr>
<tr>
<td>Q13</td>
<td>When I eat cake I usually prefer a chewy cake like brownie instead of a crunchy cake like biscuits</td>
<td>4.40</td>
<td>4.33</td>
<td>0.10</td>
<td>0.75</td>
</tr>
<tr>
<td>Q14</td>
<td>When I eat snacks I usually prefer snacks that makes a crunchy sound when I chew them like potato chips</td>
<td>3.73</td>
<td>4.49</td>
<td>10.27</td>
<td>0.002</td>
</tr>
<tr>
<td>Q15</td>
<td>I usually prefer carbonated soft drinks like Coca-Cola over non-carbonated soft drinks like lemonade</td>
<td>2.84</td>
<td>3.66</td>
<td>7.21</td>
<td>0.007</td>
</tr>
<tr>
<td>Q16</td>
<td>When I eat sweets I usually prefer chocolate that easily melts in my mouth over hard candy that I would need to suck on</td>
<td>4.20</td>
<td>3.88</td>
<td>1.83</td>
<td>0.18</td>
</tr>
<tr>
<td>Q17</td>
<td>When I compare myself to my friends and family I often find myself chewing my foods at a faster pace than them</td>
<td>3.00</td>
<td>3.66</td>
<td>5.82</td>
<td>0.02</td>
</tr>
<tr>
<td>Q18</td>
<td>When I eat toppings on yoghurt products, I always prefer crunchy muesli and I avoid eating yoghurts where the muesli has softened</td>
<td>3.53</td>
<td>5.33</td>
<td>18.11</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Question</td>
<td>Description</td>
<td>Mean 1</td>
<td>Mean 2</td>
<td>Mean 3</td>
<td>Mean 4</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Q19</td>
<td>I often find myself chewing foods on one side of the mouth only</td>
<td>3.89</td>
<td>3.49</td>
<td>2.72</td>
<td>0.09</td>
</tr>
<tr>
<td>Q20</td>
<td>I often experience difficulties in chewing when I eat tough foods like e.g. tough meat or wine gum</td>
<td>3.25</td>
<td>2.66</td>
<td>5.43</td>
<td>0.02</td>
</tr>
<tr>
<td>Q21</td>
<td>I find great joy and pleasure in consuming products like these that have a good chew (examples are chewy strawberry, jelly gums, whole grain bread, chewy biscuits, illustrated with photos)</td>
<td>4.25</td>
<td>4.75</td>
<td>6.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Q22</td>
<td>I find great joy and pleasure in consuming products like these that have a good crunch (examples are raw carrots, apples, peanuts coated with chocolate, crunchy granola, illustrated with photos)</td>
<td>4.08</td>
<td>5.09</td>
<td>17.20</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Q23</td>
<td>I find great joy and pleasure in consuming products like these that I can suck on until they dissolve (examples are oat meal, banana, chocolate pudding, ice cream, illustrated with photos)</td>
<td>3.76</td>
<td>3.09</td>
<td>9.54</td>
<td>0.002</td>
</tr>
<tr>
<td>Q24</td>
<td>I find great joy and pleasure in consuming products like these that I can smoosh and I even smoosh foods that I could chew (examples are orange slices, hard candy, mints, chocolates without nuts and pieces, illustrated with photos)</td>
<td>4.75</td>
<td>3.83</td>
<td>13.58</td>
<td>0.001</td>
</tr>
</tbody>
</table>

287

288

a) ![Graph showing the comparison between Soft processing likers and Firm processing likers](image)
Figure 1a-b. Latent Class Analysis output regarding (a) significant text-based questions and (b) significant picture-based questions for Soft and Firm processing likers.

3.2 Relationship between tactile acuity, population cohort and Mouth Behavior

The Generalized linear model for tactile acuity showed that the main factors Filament thickness and Mouth Behavior were highly significant sources of variation (Wald $\chi^2=172.50$, p <0.0001; Mouth Behavior: Wald $\chi^2=12.02$, p <0.001, respectively). The main factor Population cohort presented a tendency toward significance (Wald $\chi^2=3.01$, p =0.08). Post-hoc tests revealed significant higher R-index values when the tongue was stimulated with the thicker filament no. 2.44 ($R$-index = 91.7$^a$ ± 1.8), as compared to stimulation with the filament no. 2.36 ($R$-index= 86.8$^b$ ± 1.8), and the thinnest filament no. 1.65 ($R$-index= 71.6$^c$ ± 1.8).

The R-index values were higher, although not significant, in Chinese (84.6 ± 1.1) compared to the Danish population cohort (82.0 ± 1.0). Similar results were found when analyzing the data according to signal detection theory (d-prime values).

Subjects characterized as ‘Firm processing likers’ obtained significant higher R-index values (85.9 ± 1.1) compared to subjects characterized as ‘Soft processing likers’ (80.7 ± 1.0).

The same model, conducted separately on Chinese and Danish subjects, indicated that the main factor Filament Thickness was a significant source of variation in both populations (Chinese: Wald $\chi^2$
=69.40, p <0.0001; Danish: Wald \( \chi^2 = 71.38, p < 0.0001 \). The main factor Mouth Behavior was a significant source of variation for both Chinese (Wald \( \chi^2 = 5.22, p <0.05 \)) and Danish consumers (Wald \( \chi^2 = 7.17, p <0.01 \)). Indeed, post-hoc tests revealed that ‘Soft processing likers’ obtained significantly lower \( R \)-index values compared to ‘Firm processing likers’ in both population cohorts (Chinese: SPL: 82.2 ± 1.9 vs. HPL: 87.1 ± 1.0 and Danish: SPL: 79.3 ± 1.7 vs. HPL: 84.7 ± 1.1).

None of the 2-way interactions were significant.

3.3 Relationship between tactile acuity, PROP status and Fungiform Papillae Density

The characteristics of participants in each population cohort are listed in Table 2.

Table 2. Subjects’ characteristics according to PROP status and FPD in Chinese and Danish population cohorts.

<table>
<thead>
<tr>
<th>PROP status</th>
<th>Chinese</th>
<th></th>
<th>Danish</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>FP/cm(^2) (mean ±SEM)</td>
<td>n</td>
<td>FP/cm(^2) (mean ±SEM)</td>
<td>n</td>
<td>FP/cm(^2) (mean ±SEM)</td>
</tr>
<tr>
<td>Supertaster (ST)</td>
<td>36</td>
<td>69.7 ±2.9</td>
<td>19</td>
<td>62.5 ±3.8</td>
<td>55</td>
<td>67.2 ±2.4</td>
</tr>
<tr>
<td>Medium taster (MT)</td>
<td>29</td>
<td>57.4 ±3.9</td>
<td>41</td>
<td>57.7 ±2.6</td>
<td>70</td>
<td>57.6 ±2.1</td>
</tr>
<tr>
<td>Non taster (NT)</td>
<td>10</td>
<td>66.1 ±5.4</td>
<td>17</td>
<td>46.1 ±4.1</td>
<td>27</td>
<td>53.5 ±3.4</td>
</tr>
</tbody>
</table>

No correlations were found between the tactile acuity (\( R \)-index) and the other two variables considered. Additionally, the subject's ratings of the bitterness of the PROP solutions was positively correlated with the FPD (\( r = 0.28; p < 0.001 \); \( R^2 = 0.08 \)), although with a very low Pearson’s correlation. Notably, no significant correlation in the Chinese population cohort was found (\( r = 0.17, p = 0.15 \); \( R^2 = 0.03 \)).

3.4 Cross-cultural differences in Mouth Behaviors in relation to FPD and to PROP status

A summary of the main results obtained through the Generalized Linear Model to determine the cross-cultural relationship between Mouth Behaviors and FPD and PROP has been reported in Table 3.

Table 3. Summary of the main results obtained through the Generalized Linear Model to determine the cross-cultural relationship between Mouth Behaviors and FPD and PROP.

<p>| Phenotypical marker | Chinese | Danish |</p>
<table>
<thead>
<tr>
<th></th>
<th>FP/cm² (mean ±SEM)</th>
<th>FP/cm² (mean ±SEM)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPD</td>
<td>62.9 ±1.4</td>
<td>56.6 ±1.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PROP responsiveness</td>
<td>51.4 ±2.0</td>
<td>38.3 ±1.9</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

A significant effect of Population cohort on subjects’ FPD (Wald $\chi^2 = 10.67; p < 0.001$) and PROP responsiveness (Wald $\chi^2 = 21.78; p < 0.0001$) was found, with Chinese population cohort characterized by a greater FPD and a greater responsiveness to PROP compared to Danish consumers. The main factor Mouth Behavior and the interaction factor Population cohort x Mouth Behavior were not a significant source of variation on FPD and PROP responsiveness.

Interestingly, when the relationship between Mouth Behaviors and FPD was analyzed separately on Chinese and Danish subjects, the main factor Mouth Behavior have been found as significant source of variation only in Chinese population cohort (Wald $\chi^2 =4.25, p <0.05$), with Chinese ‘Soft processing likers’ characterized by a greater FPD (65.8 ±1.4 FP/cm²) than ‘Firm processing likers’ (59.9 ±2.5 FP/cm²). No significant effect has been highlighted running the same model considering the main factor Mouth Behavior in relation to PROP responsiveness.

4 Discussion

With focus on the two major groups of MB, quantitative latent class analyses (McCutcheon, 1987; Magidson & Vermunt, 2004; Vermunt & Magidson, 2005) was used for the classification of preferred mouth behavior. This approach deviated from that proposed by Jeltema and colleagues (2014), in which subjects were forced to choose the type of mouth behavior most desirable to them based on a pictorial presentation of different foods and, at the same time, to indicate what mouth behavior they rejected most. This approach was reported to be more accurate in separating behavioral groups than the standard surveys (Jeltema, Beckley, & Vahalik, 2015). However, it is important to note that just because a person claims to prefer a specific mouth behavior, does not mean that the others are rejected. Indeed, even though a person may generally like soft textures, and often chose foods that could be smooshed or sucked, it could be possible that (s)he may also prefer hard texture foods for other reasons, making the classification in the subgroups of Jeltema and colleagues difficult.

Moreover, texture preferences could also be potentially affected by characteristics related to food product itself, such as flavor, particle size, matrix type, fat content and microstructure, and/or related to consumers psychological and physiological factors, such as consumer’s familiarity, expectations and sensitivity.
To segment our subjects in relation to their preferred mouth behavior, the present study used a quantitative alternative, the latent class analysis which has several advantages: it is a statistical method and probability based, all relevant information about the respondents perception of mouth behavior can be taken into account (in this case the scoring of the 24 items on a 6 point ordinal scale), the output includes for each respondent the probability to belong to each of the clusters and the classification is based on the highest probability. Furthermore, the relationship between the items and the clusters is also based on probabilities and the level of significance for each of the 24 items is included.

In the present study, with two clusters the broad categorization of preferred oral food processing into ‘Firm processing likers’ and ‘Soft processing likers’ was confirmed. Besides, the two classes of preferred MB showed, that the Danish Caucasian population with 73% ‘Firm processing likers’ had similarity to the North American population reported by Jeltema and colleagues (2014). In their study among 500 participants 76% had preferences for foods that needed firm processing on biting and chewing (33% Crunchers and 43% Chewers). The two populations of Chinese and Danish subjects showed distinct differences in preferred food oral processing behaviors. The Chinese population was characterized by a larger number of ‘Soft processing likers’ (77% of the population), who preferred foods that could be held in the mouth for a longer time and manipulated between the tongue and roof of the mouth. On the contrary, Danish consumers mostly belonged to the ‘Firm processing likers’ group, who preferred foods that require using the incisors and/or molars to break down or deform the food in the mouth. A further classification of participants in subgroups of respectively ‘smoothers’ and ‘suckers’ and ‘chewers’ and ‘crunchers’, as suggested by Jeltema and colleagues (2014), was not feasible due to the limited sample size.

Chinese population in the present study deviated significantly from the Caucasian with much higher preferences for soft food processing in the mouth. These results may indicate that cultural and dietary habits in food consumption (e.g. cooked, refined (noodles) foods vs less cooked, less refined (rye bread) foods) may influence the preferred oral texture processing between Asians and Caucasians. The large differences also suggest that Chinese participants, living in Denmark for less than two years, did not adapt towards similar texture preferences as the Danish consumers (who had been resident since birth). Other studies have indeed shown that Chinese beliefs and food preferences persist up to twenty years after moving to a foreign country and continue even in subsequent generations (Murray, Easton, & Best, 2001).

It should be noted that preferred MB does not necessarily relate to preferred oral texture perception. Differences in tactile acuity as measured with the von Frey filaments showed that Chinese subjects are equally sensitive across the range of fibers as Danish subjects, confirming previous results
reported by Essick and colleagues (2003). Moreover, the group of ‘Firm processing likers’ were shown to be more sensitive than the ‘Soft processing likers’ in both population cohorts. Thus results, suggested that lingual acuity did not play a role in subjects’ decreased preferences of foods rich in texture. Perhaps other aspects than tactile acuity, such as culturally-driven experience and familiarity with foods as discussed above, have a more influencing role in establishing food texture preferences. Previous studies found that lingual tactile thresholds were significantly associated with FPD, such that higher densities resulted in greater tactile acuity (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone, 2003). Moreover, several studies have found correlations between PROP intensity and texture perception (Bakke & Vickers, 2011; de Wijk et al., 2007; Hayes & Duffy, 2007; Pickering, Simunkova, & Di Battista, 2004; Pickering & Robert, 2006). A reasonable explanation has been that PROP intensity is related to FPD, which in turn is related to trigeminal innervation. However, to our knowledge, no direct association has been shown to exist between the density of trigeminal (tactile) innervation and the density of taste buds and/or fungiform papillae. Indeed, the areas between fungiform papillae are also innervated and could conceivably be more densely innervated in subjects with a lower density of papillae. Nevertheless, our study failed to establish a direct correlation between tactile acuity and PROP responsiveness or FPD, as previously suggested (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone; 2003). This could be due to the different tasks used to measure tactile acuity in this study (von Frey Filaments) compared to the previous ones (letter-recognition tasks). Moreover, in the present study we only measured mechanical stimulation and not orientation, which perhaps more likely could highlight differences related to morphological variables. As previously reported, the relationship between FPD and PROP has been extensively studied, since both measures have been used as indices of taste sensitivity in general. Many studies have reported a positive relationship between these two measures, but the magnitude of this association has shown considerable variation, ranging from relatively high Pearson’s r values > 0.8, to moderate (r ≤ 0.5) and low (r ≤ 0.3) (see Piochi, Dinnella, Prescott, & Monteleone, 2018 for a review). Consistent with some of these studies, we found that FPD and perceived PROP intensity were correlated with each other, but presented a low Pearson’s r value. No association was found in the Chinese population cohort, with some overlap among the three PROP status groups. Moreover, looking at the subjects’ characteristics according to PROP status and FPD in Chinese and Danish population cohorts, it is possible to observe that Chinese presented a great variability in FPD, with NT subjects characterized by a similar density as the STs. A possible explanation for these unexpected findings is that populations with different genetic admixtures were studied, and the presence of more extreme phenotypes in some populations relative to others (e.g. a greater number of ST in Asians compared to the Caucasians) may be driving the observed effects (Barbarossa et al., 2015; Tepper,
Moreover, several recent studies have failed to find a significant relationship between FPD and PROP phenotype (Dinnella et al., 2018; Fisher et al. 2013; Garneau et al. 2014), and other factors than polymorphism of TAS2R38 have been hypothesized as possible variables involved in FPD variation (e.g. polymorphisms of gene controlling for gustin functionality) (Barbarossa et al., 2015; Calò et al., 2011; Melis et al., 2013; Padiglia et al., 2010).

As far as we know, this is the first study to investigate the relationship between morphological and phenotypical data (FPD and PROP responsiveness) and preferred oral processing behavior. Chinese consumers generally presented a greater responsiveness to PROP and were characterized by a greater FPD compared to Danish consumers. However, our results suggest that the cross-cultural differences found in preferred Mouth Behaviors seem not to be associated with FPD and PROP responsiveness.

Thus, we conclude that oral processing behaviors appear to involve other perceptual mechanisms that are unrelated to morphological or phenotypical subject characteristics. For this reason, due to the complex nature of texture perception and preferences, it is essential to identify other relevant factors and define characteristics that govern the processes involved.

While research findings from this work are significant, limitations of the study should also be noted. The first limitation of our study is the relatively small sample size, not balance for gender. While there were several statistically significant observations, the findings may not be generalized to the entire Chinese and Danish populations, and it is recommended to extend the number of subjects involved in the experiment in order to avoid ‘false positive’ associations and obtain a more robust and generalizable outcome. Another issue to be noted is that the evaluation of taste sensitivity is limited to PROP responsiveness and FPD evaluation and these two general markers for taste sensitivity still present contradictory relationship between each other and between perception of basic tastes (see Piochi, Dinnella, Prescott, & Monteleone, 2018 for a review). Thus, a combination of taste perception measurements, such as data related to fundamental taste thresholds, should be included to better characterize the overall subjects’ perception. Moreover, the use of the von-Frey filaments for detection threshold could be insufficient for this task due to the fact that the lowest available force (0.008 g) could not be sensible enough to establish the real detection threshold of the subjects. Thus, different tongue sensitivity methods (e.g. Luneau Cochet-Bonnet aesthesiometers) (Miles et al., 2018) could be employed to measure touch detection threshold since these aesthesiometers had the benefit of providing an increased number of extremely lower-force stimuli than the filaments. Additionally, two-point discrimination task and stereognostic letter-recognition task could be used to evaluate the roughness sensitivity and the point-and-edge sensitivity, respectively, in order to include discrimination of size and orientation. However, it should be noted that the latter method could not
be suitable for cross-cultural studies among populations with very different handwritten characters (e.g. Latin vs Chinese alphabet characters).

It would be necessary in future researches to investigate whether lingual tactile acuity is related to sensitivity toward, rather than preference for, textural aspects of foods. For example, particle detection could be associated to lingual tactile acuity and, therefore, could highlight more evidence about how subjects perceive and prefer foods.

**Conclusion**

Cross-cultural differences in preferred oral processing behavior were found, as Chinese subjects predominantly preferred to manipulate foods between the tongue and roof of the mouth. On the contrary, Danish subjects mostly preferred to use the teeth to break down foods in the mouth. Chinese subjects presented differences in oral tongue anatomy as shown by greater FPD and PROP responsiveness compared to Caucasian Danish subjects.

A significant but low correlation was found between PROP status and FPD, while no direct correlation between tactile acuity and PROP responsiveness or FPD were found. The reason of having no direct correlation between subjects’ ability in touch detection and morphological and phenotypical data (FPD and PROP status) is still not certain. These observations suggest that thoughtfulness should be applied in studying texture perception, since focusing solely on PROP and FPD evaluation may be not sufficient to understand the variability and complexities of phenomena raised from the interaction between food and mechanoreceptors in the oral cavity. Thus, the physiological parameters that we investigated should be examined in more detail (e.g. PF size and relevant distributions, more sensitive method to valuate touch detection). Moreover, this study provides evidence that cultural background could represent a strong influence in the oral process preference for texture.

**Acknowledgement**

The authors wish to thank Selma Amimi, Belinda Lange, Charlotte Dandanell and Lisbeth Pii Nielsen for their help in the data collection. Lisbet Bjerre Knudsen and Kevin Kantono are thanked for proof reading the manuscript.

**Funding sources**

This work was financially supported by Arla Foods amba, Viby, Denmark as part of a postdoctoral grant.

**Competing interests**
The authors have declared that no competing interests exist.

References


### Table S1. Types of questions used in the survey to place individuals into Mouth Behavior groups.

<table>
<thead>
<tr>
<th>Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I usually prefer a chewy piece of candy like e.g. wine gum over a hard piece of candy</td>
</tr>
<tr>
<td>2. I usually prefer chocolate with crunchy fillings like nuts over chocolate that easily melts in my mouth</td>
</tr>
<tr>
<td>3. When I eat oranges I enjoy to put the slices into my mouth and suck the orange juice out of the slices instead of just chewing the slices right away</td>
</tr>
<tr>
<td>4. When I eat breakfast cereals I usually let them soften quite a bit in the milk before I eat them, as opposed to eating them straight away</td>
</tr>
<tr>
<td>5. When I eat chocolate I usually prefer chocolate with a good chewing texture over chocolate that easily melts in the mouth</td>
</tr>
<tr>
<td>6. When I eat fruits I usually prefer crunchy fruits like fresh apples over more chewy fruits that I can chew on like pineapple or strawberries</td>
</tr>
<tr>
<td>7. When it comes to chocolate I usually prefer chocolate that is hard enough to suck on over chocolate that quickly melts in my mouth</td>
</tr>
<tr>
<td>8. I usually prefer soft and smooth fruits like ready to eat bananas and ripe peaches over hard and crunchy fruits like fresh apples and pears</td>
</tr>
<tr>
<td>9. I usually prefer the texture of soft whole grain bread over the texture of crispbread and crackers</td>
</tr>
<tr>
<td>10. When I eat ice cream I eat it right out of the freezer instead of letting it thaw a little</td>
</tr>
<tr>
<td>11. I usually prefer to suck on hard candy until they are paper thin instead of crunching them after a short while</td>
</tr>
<tr>
<td>12. I enjoy to eat foods that are smooth and easily spreads in my mouth like puddings and ice cream</td>
</tr>
<tr>
<td>13. When I eat cake I usually prefer a chewy cake like brownie instead of a crunchy cake like biscuits</td>
</tr>
<tr>
<td>14. When I eat snacks I usually prefer snacks that makes a crunchy sound when I chew them like potato chips</td>
</tr>
<tr>
<td>15. I usually prefer carbonated soft drinks like Coca-Cola over non-carbonated soft drinks like lemonade</td>
</tr>
<tr>
<td>16. When I eat sweets I usually prefer chocolate that easily melts in my mouth over hard candy that I would need to suck on</td>
</tr>
<tr>
<td>17. When I compare myself to my friends and family I often find myself chewing my foods at a faster pace than them</td>
</tr>
<tr>
<td>18. When I eat toppings on yoghurt products, I always prefer crunchy muesli and I avoid eating yoghurts where the muesli has softened</td>
</tr>
</tbody>
</table>
19 I often find myself chewing foods on one side of the mouth only

20 I often experience difficulties in chewing when I eat tough foods like e.g. tough meat or wine gum

   Please look at the picture and state to what extent you agree with the following statement:

21 I find great joy and pleasure in consuming products like these that have a good chew
   (Please consider all four products as a group, and consider your liking as an average for all four products)

22 Please look at the picture and state to what extent you agree with the following statement:

23 I find great joy and pleasure in consuming products like these that have a good crunch
   (Please consider all four products as a group, and consider your liking as an average for all four products)

24 Please look at the picture and state to what extent you agree with the following statement:

25 I find great joy and pleasure in consuming products like these that I can suck on until they dissolve
   (Please consider all four products as a group, and consider your liking as an average for all four products)

26 Please look at the picture and state to what extent you agree with the following statement:

27 I find great joy and pleasure in consuming products like these that I can smoosh and I even smoosh foods that I could chew
   (Please consider all four products as a group, and consider your liking as an average for all four products)