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Influence of bread structure on human oral processing

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ABSTRACT

The strong interconnection between food structure and its resistance to breakdown is the rationale behind designing bread structure to control its digestion, starting from the oral phase. Three types of bread, i.e. baguette, baked bread and steamed bread, with distinct cellular structures and textures were prepared by only varying the processing conditions. Baguette with thick and dry curst required a larger chewing force and a longer chewing time than steamed bread which has a moist and soft skin. Greater chewing effort resulted in more saliva impregnated and smaller particle size in baguette bolus which might elevate starch digestion and glycaemic response. The impact of crumb structure on oral processing was more complicated which involved both the mechanical strength of the crumb and the textural perception it elicited. Strong correlation was found among bread structure, texture, and oral processing behavior. Our study demonstrated that two important factors, grain feature of bread crumb and the relative portion of bread crust, should be considered when designing bread structure.

1. Introduction

There is an increasing awareness of the relationship between food structure and human digestion. The understanding of food structure and its breakdown is critical to the design of new food for controlling the release of both macronutrients and micronutrients and increasing the satiety (Norton et al., 2007). Unfortunately, most food products are structurally complex. Their structure and mechanical properties are not well understood or easily engineered. Bread, one of the most commonly consumed staple foods, is a good example of food products with complex microstructure and a high glycaemic index (GI) in general. Physical structure of the bread was identified as one of the most important factors determining the postprandial glycaemic response (Fardet et al., 2006). However, most of the attention has been focused on reformulations using low GI ingredients (Bharath and Prabhasankar, 2014; Burton et al., 2011). Manipulating bread structure as one of the options to control bread digestion has rarely been attempted so far.

Oral processing is the first key stage of human digestion process, where food is broken down and moistened to form a bolus for safe swallowing. The level of chewing determines the degree of food disintegration which was shown to influence the glucose uptake into the blood stream. Studies on rice showed that the degree of particle size breakdown during mastication influenced both the *in vitro* digestibility and *in vivo* glycaemic response of human subjects (Ranawana et al., 2014, 2010). Similarly, Zhu et al. (2013) found that a greater number of masticatory cycle was associated with a higher postprandial plasma glucose level after eating pizza, even though it also increased satiety.

The highly porous structure of bread crumb is identified as a major contributor to its high GI value (Mishra et al., 2012). Such porous structure is developed through a series of aeration during the stage of mixing, proofing and thermal setting (Zhou and Hui, 2014). The final morphologies of bread crumb, i.e. the size, shape and distribution of cells and the thickness of cell wall, strongly influence its mechanical strength (Gibson and Ashby, 1997) and texture perception in mouth (Panouillé et al., 2014). Scanlon and Zghal (2001) provided a comprehensive review of the relationship between the cellular structure (relative density) and mechanical properties of bread crumb based on the scaling law developed by Gibson and Ashby (1997). A few studies reported the kinetics of bread destruction during oral processing in terms of saliva incorporation, particle size reduction and textural properties (Hoebler et al., 1998; Le Bleis et al., 2013; Tournier et al., 2012); however, little is known about the interconnection among bread structure, the level of oral processing required and its digestibility.

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The cellular structure of bread crumb has been characterized using 2D image analysis and 3D micro-tomography (μCT) technique (Besbes et al., 2013; Lassoued et al., 2007; Van Dyck et al., 2014). But to characterize the changes in bread structure throughout the chewing process is a challenging task. For this purpose, the adaption of chewing physiology provides us some insights on the transformation of food structure. Surface electromyography (sEMG), which measures the electric activities of jaw-closing muscles is one of the few techniques that are able to characterize the *in vivo* chewing behavior (Chen and Espinosa, 2012). Studies have reported the link between EMG results and food texture, especially the hardness and dryness of solid food, such as Melba toast, breakfast cake, and peanut (Pereira et al., 2006; Woda et al., 2006).

This study investigated the impact of bread structure on people's chewing behavior and resulting bolus properties. Variations in bread structure were created by manipulating only the processing conditions while keeping bread formulation the same. During the first stage of study, a group of panellists masticated a normal serving of bread sample that consisted of both crust and crumb. Then a single panellist was selected to participate in the second stage of study, in which bread crumb was separated from crust. This design allowed us to obtain a clear idea on the average behavior of oral processing as well as the isolated effect of bread crumb and crust. Results of this study would shed some light on the design of bread structure that could lead to prescribed levels of oral processing and digestibility.

2. Materials and methods

2.1. Bread preparation

Three types of bread were prepared using the same formulation: 1000 g flour (11.7% protein), 600 g water, 40 g sugar, 30 g vegetable shortening (Radman, Singapore), 20 g salt, and 10 g instant dry yeast (Algict Bruggeman N.V., Belgium). Bread loaves were prepared using the no-time dough method reported previously (Ananingsih et al., 2013; Wang et al., 2007) with slight modifications. The details of processing conditions are shown in Table 1.

2.2. Bread characterization

2.2.1. 2D image acquisition and analysis

Two central vertical slices (~1 cm thickness) were cut from each bread loaf using a mechanical bread slicer (Rhino CM-36, Taiwan). Each slice was scanned on both sides using a flatbed scanner (CanoScan 9000F Mark II, Canon, USA) at a resolution of 600 dpi and saved as a black and white image. A field of view (FOV) of 40 mm × 30 mm was cropped from the center of the baked and steamed bread images while a FOV of 35 mm × 30 mm was cropped from the baguette images. The cropped images were converted into binary images using Otsu thresholding method in Image J (1.46r, National Institute of Health, USA) and exported to Image Pro Plus (version 7, Media Cybernetics, UK) to quantify the porosity and mean cell size of bread. In total, 32 bread slices were analyzed for each type of bread.

2.2.2. 3D X-ray microtomography (μCT)

A cube of 1 cm × 1 cm × 1 cm was cut from the center of bread and placed in a polypropylene tube of 16 mm internal diameter. Images were obtained using a Quantum FX microCT imaging system (PerkinElmer, Hopkinton, MA) which scanned at 90 kV of peak voltage and 120 μA of current. The sample was rotated 360° which took 3 min to obtain 512 slides of 2D radiographs. The FOV was 10 mm × 10 mm which gave a resolution of 20 μm. Images were exported in DICOM format and reconstructed using Imaris (version 7.7.2, Bitpalne, Zurich, Switzerland). A volume of interest (VOI) of 6.38 mm × 6.38 mm × 6.38 mm was cropped from the center of the image to avoid the edges. CT-Analyser software (version 1.4.1, Bruker microCT, Knotich, Belgium) was used to quantify the total porosity, open porosity, mean cell diameter, cell wall thickness and the distribution of cell diameter and wall thickness. A total of 18 samples were analyzed for each type of bread.

2.2.3. Physical characterization

Specific volumes of bread were measured using a Volscan Profiler (VSP 600, Stable Micro System Ltd., Surrey, U.K.). Bread crust or skin was manually separated from the crumb and weighted to determine the ratio of crust or skin to crumb of the serving portion. Moisture contents of bread crumb and crust or skin were determined separately by drying samples of 4 g in an oven at 105 °C for 24 h.

The texture profile analysis (TPA) of the bread crumb was carried out using a TA-XT2i Texture Analyzer (Stable Micro System, Surry, UK) with a 20 mm diameter cylindrical probe. A 2 cm thick slice was cut from the center of the bread and was subjected to a double compression at 2 mm/s to 40% of its thickness. The hardness, springiness, cohesiveness and chewiness of bread crumb were quantified (Bourne, 2002). The hardness of the bread crust/skin was evaluated using a puncture test (Altamirano-Fortoul et al., 2013). The whole bread was punctured with a 2 mm diameter cylindrical probe at a speed of 40 mm/s at 5–6 different locations. This speed was chosen to simulate the biting with the front teeth (Primo-Martín et al., 2008). The peak force during the penetration was quantified as the hardness.

2.3. Masticatory performance

2.3.1. Subject selection

Fourteen healthy adults (7 females and 7 males, 22–26 years old, mean age 23.1 ± 1.5) were recruited to form a panel. The panellists were selected based on the following criteria: (i) having complete permanent dentition (excluding third molar and wisdom teeth) and normal occlusion; (ii) not having any gum or periodontal disease and major dental treatment within 6 months prior to the experiment; and (iii) not having pain or sound in their temporomandibular joints during chewing. This study had been approved by the NUS Institutional Review Board. All panellists gave informed consent to participate.

Table 1
Processing conditions of three types of bread.

	Baked bread	Steamed bread	Baguette
Mixing conditions	1 min at 45 rpm & 5 min at 100 rpm	1 min at 45 rpm & 5 min at 65 rpm	1 min at 45 rpm & 5 min at 100 rpm
Resting time (min)	15	15	15
Dough piece weight (g)	55	50	100
Proofing conditions	40 °C, 85% Relative humidity		
Proofing time (min)	70	40	90
Thermal setting	Baked at 200 °C for 10 min	Steamed at 100 °C for 10 min	Baked at 160 °C for 25 min

2.3.2. Electromyographic monitoring

A training session was conducted to allow the panellists to be familiarized with the use of a sEMG system (NTS-2000, NCC Medical co. Ltd., Shanghai, China). Three formal testing sessions were conducted at the same time on different days. Each session was separated into five sub-sessions. During each sub-session, one standard serving (5.0 ± 0.2 g) of the three different bread samples containing a fixed amount of crust/skin and crumb were given to the panellists in random order. Bread samples were freshly prepared on the day of use. The panellists were asked to swallow the first set of samples and spit out the next 4 sets. A resting time (1–3 min) was arranged between samples and sub-sessions. Collected boluses were immediately analyzed. A total of 12 boluses were collected for each type of bread over 3 sessions.

The application area of a panellist's face was cleaned carefully using 70% alcohol swab to reduce its impedance. The superficial masseter and anterior temporalis on both side of the face were located by palpation. Two surface electrodes (Red Dot™ Micropore Monitoring Electrodes, 3M Health Care, Minnesota, USA) were located 2 cm apart along the length of each muscle. An additional earth electrode was placed on the subject's forehead to minimize electrical background noise (Chen and Espinosa, 2012). The electromyograms were rectified and integrated using a root mean square method by the sEMG Version 1.0 software (NCC Medical co. Ltd., Shanghai, China). An example of the electromyogram is shown in Fig. 1. A chewing burst was defined as when muscular activity had clearly exceeded the resting activity level and lasted until it returned back to the resting level. Burst duration, chewing time, number of chewing cycle and chewing frequency were quantified. Maximum amplitude, mean amplitude, and integral area per burst were quantified respectively for all the four muscles and then their average values were taken as characteristic values for a sample. Total peak area was calculated as the sum of all the peak areas of the four muscles.

2.4. Bolus characterization

2.4.1. Saliva impregnation

The moisture content of a bolus was determined by drying it in an oven at 105 °C for 24 h (Motoi et al., 2013). The amount of saliva impregnated in the bolus was calculated as the difference between the bolus moisture mass and the bread moisture mass, based on the assumptions that all dry solids in the bolus originated from bread and saliva impregnated was mainly water.

2.4.2. Particle size distribution

Each bolus was dispersed into absolute ethanol by gentle shaking. Particles were filtered and dried in an oven at 70 °C for 30 min to ensure an adequate separation. Dried particles were measured using the combination of image analysis (Hutchings et al., 2012) and laser diffraction methods (Peyron et al., 2004), catering for the big and small particles which were separated by a 2 mm sieve, respectively. Big bolus particles were scanned and quantified to obtain the mean particle area using the 2D image analysis method that was used similarly in the crumb grain analysis (Section 2.2.1). The small bolus particles were dispersed in absolute ethanol and

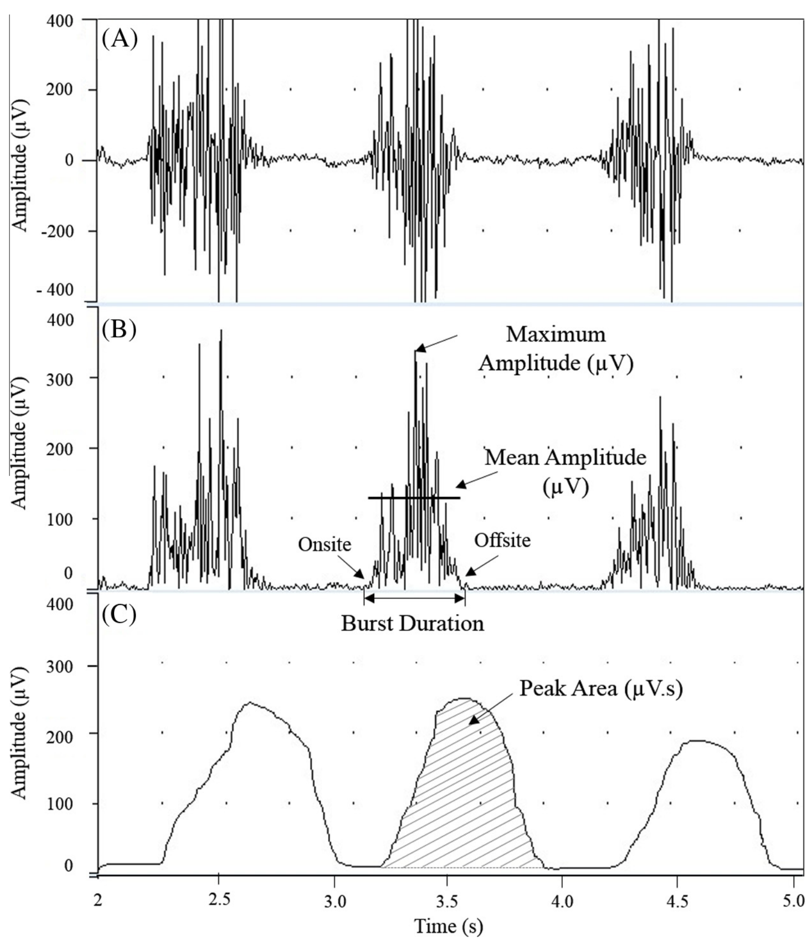

Fig. 1. An example of electromyogram: (A) raw EMG signals, (B) rectified signals, (C) root mean square (RMS) curves. Some masticatory parameters are shown in (B) and (C), including mean amplitude, maximum amplitude, burst duration and peak area. The total peak area is the sum of peak areas of all the bursts within one chewing sequence.

measured using a Laser Scattering Particle Size Analyzer (LA-950, Horiba Instrument tInc., Japan) at room temperature. The refraction index of ethanol and bolus particle were set as 1.36 and 1.54, respectively. The median diameter (d_{50}) of the small bolus particle was obtained.

2.4.3. Bolus texture

The bolus texture was measured using a back extrusion rig customized for the TA-XT2i Texture Analyzer (Stable Micro System, Surry, UK). The bolus was weighted (4.0 ± 0.2 g) and loaded in a poly(methyl methacrylate) cup with 20 mm of internal diameter and 30 mm of height. The surface of the bolus was gently levelled using a finger to remove the majority of visible bubbles in the bolus. The bolus was subjected to a plunger thrust of 60% of the bolus height at the speed of 3 mm/s using a poly(methyl methacrylate) plunger (15 mm diameter). The peak force was recorded as an indicator of the bolus hardness (Young et al., 2013).

2.5. Single panellist study

The panellist who had the highest rate of saliva impregnation and greatest reduction in the particle size of the bolus was identified to participate in the second stage of this study. Besides the serving with crust or skin, bread crumb-only samples were prepared by separating them from the crust or skin of all the three types of bread. Bread samples, both with and without crust or skin, all had similar volume (~13 ml) and shape in order to eliminate the differences in serving size. The panellist was asked to chew the six types of bread samples until the swallowing point had been reached. Chewing behavior was monitored using the sEMG and the collected boluses were analyzed using the methods described in Sections 2.3 and 2.4, respectively.

2.6. Statistical analysis

All experiments were conducted in at least triplicates. Log transformation was applied when the original data set of a property was not normally distributed. One way ANOVA and Duncan post hoc test were used to compare the means of bread properties. Repeated ANOVA with Bonferroni post hoc test was used to study the effect of bread types on chewing behavior and bolus characteristics. Paired t-test was used to compare between the same type of bread samples with and without crust or skin in the single panellist study. Pearson correlation analysis was applied to explore the correlation between bolus moisture content and hardness as well as between bolus properties and masticatory parameters. Principal component analysis (PCA) was used to study the link among bread characteristics, masticatory parameters and bolus properties in the single panellist study. Linear regression analysis was performed between bread characteristics and oral processing parameters. All statistical analyses were carried out using SPSS 20 (IBM Corporation, New York, USA) and XLSTAT (Addinsoft, New York, USA).

3. Results and discussion

3.1. Bread structure and texture

Crumb grain features of the three types of bread were revealed using the 2D and 3D image analysis techniques described in Sections 2.2.1 and 2.2.2, respectively. Typical images of bread crumb are shown in Fig. 2. Morphological parameters of the 2D and 3D images are compared in Table 2. Based on the 3D μCT analysis, all the three types of bread had a highly porous crumb structure with a total porosity of 64–79%, of which most (>99%) were open pores that were interconnected. This is in agreement with previous studies (Besbes et al., 2013; Van Dyck et al., 2014; Wang et al., 2011). The baguette crumb had the highest porosity (79.4%) and the largest mean pore size (0.857 mm) while the steamed bread had the most dense crumb structure (64.8%) and the smallest mean pore size (0.606 mm). Moreover, the steamed bread had thicker cell walls compared to the baked bread and the baguette which could be explained by its smaller degree of expansion during proofing. Differences in crumb structure were also observed in the distribution analysis of cell size and cell wall thickness (Fig. 3). The baguette had a higher percentage of cells

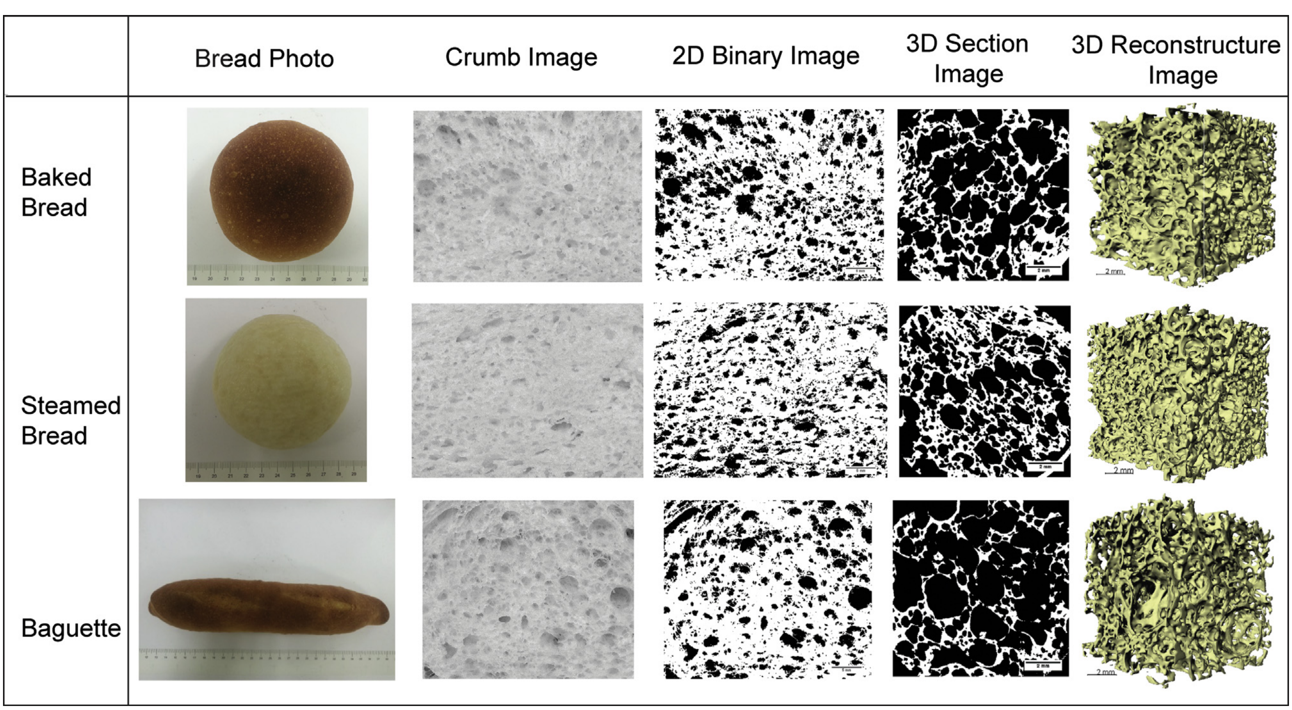

Fig. 2. Photos of bread samples and samples of scanned 2D and 3D images.

Table 2
Morphological parameters of bread crumbs based on 2D and 3D image analysis.

Parameters	Analysis	Baked bread	Steamed bread	Baguette
Total Porosity (%)	2D	21.383^{b} (2.562)	18.169^{a} (4.219)	26.666^{c} (6.573)
	3D	71.560^{b} (5.006)	64.811^{a} (5.43)	79.406^{c} (3.08)
Mean cell size (mm)	2D	0.793^{a} (0.029)	0.794^{a} (0.027)	0.872^{b} (0.101)
	3D	0.681^{a} (0.118)	0.606^{a} (0.143)	0.857^{b} (0.128)
Open porosity (%)	3D	71.534^{b} (5.024)	64.684^{a} (5.498)	79.306^{c} (3.041)
Cell wall thickness (mm)	3D	0.173^{a} (0.011)	0.187^{b} (0.013)	0.168^{a} (0.010)
Surface to volume ratio (mm^{-1})	3D	8.595^{b} (1.491)	10.135^{c} (1.608)	6.600^{a} (0.918)
Degree of anisotropy	3D	0.267^{a} (0.080)	0.328^{b} (0.086)	0.275^{a} (0.066)

$^{a-c}$ Values are mean with standard deviation in brackets ($n = 32$ or 18). Means within the same row denoted by different superscript letters differ significantly ($p < 0.05$).

with larger diameters and thinner wall thickness while the steamed bread exhibited an opposite profile.

It is noted that the crumb porosity estimated from the 2D images (18–27%) was much lower than that from the 3D µCT analysis (65–79%). This is due to the difficulties in isolating the pores when they were overlapping with the crumb solid underneath in the 2D images. The underestimation of void fraction based on 2D image analysis was also reported by Scanlon and Zghal (2001). Both the 2D and 3D imaging techniques were employed because they were applied at different scales. The cross-section of a slice of bread sample was scanned for the 2D analysis while only a small sub-volume was scanned in the 3D µCT analysis. The agreement between the rankings by these two methods reinforced the observed differences among the three different types of bread.

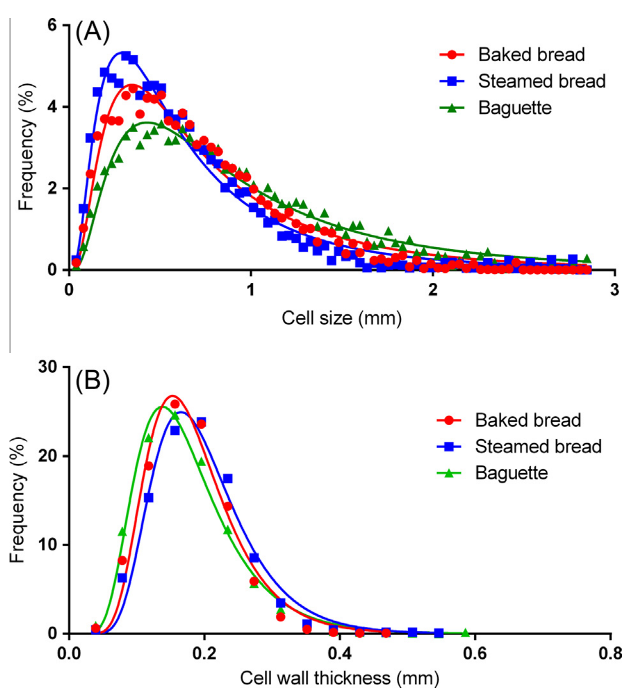

Fig. 3. Size distribution curves of (A) mean diameter of crumb cell and (B) cell wall thickness obtained from 3D µCT analysis.

The three types of bread were significantly different in their physicochemical properties, especially the moisture content and texture (Table 3). Among the three types of bread, the baguette had the lowest amount of moisture, the softest crumb, and the hardest crust while the steamed bread had the highest moisture, the hardest crumb, and the softest skin. The variations in the mixing, proofing, and thermal setting steps of processing were responsible for the differences in the bread structures and the resulting texture. Intensive mixing of the high-protein flour developed a strong gluten network in the baked bread and the baguette (Cauvain, 2003). The long fermentation time of baguette allowed a greater gas generation and consequently, the creation of highly porous crumb with large pores embedded. Low-temperature and long-time baking helped to form the thick and dry crust of baguette. High humidity and low temperature resulted in the formation of unique thin and smooth skin of steamed bread.

In summary, the bread loaves constructed in this study were clearly heterogeneous food items, especially the baguette, which consisted of rigid crust on the surface and soft crumb within. Combination of the two parts was expected to cause complex texture perceptions and chewing behavior by consumers (Tournier et al., 2012).

3.2. Oral processing behavior

3.2.1. Group panellist: bread with crust

The average chewing behaviors for the three types of bread were significantly different among each other (Table 4). Four parameters that characterize muscular activities, i.e. mean amplitude, maximum amplitude, peak area, and total peak area, all showed the same trend that mastication of the baguette elicited the greatest muscular contraction followed by chewing of the baked bread and the steamed bread. Moreover, chewing the

Table 3
Physicochemical characteristics of three types of bread.

	Baked bread	Steamed bread	Baguette
Specific volume of the whole bread (ml/g)	3.98^{b} (0.06)	3.19^{a} (0.15)	4.80^{c} (0.11)
Moisture content (% wet basis)			
Crust	18.16^{b} (1.91)	45.56^{c} (2.26)	10.53^{a} (1.62)
Crumb	43.48^{a} (0.37)	43.29^{a} (0.34)	43.13^{a} (0.48)
Crumb texture			
Hardness (N)	1.40^{b} (0.11)	2.24^{c} (0.20)	1.16^{a} (0.11)
Springiness	2.07^{b} (0.12)	1.20^{a} (0.20)	2.00^{b} (0.18)
Cohesiveness	0.86^{a} (0.01)	0.87^{a} (0.01)	0.86^{a} (0.02)
Chewiness	253.82^{b} (13.96)	237.25^{b} (37.08)	202.41^{a} (29.42)
Crust/skin hardness (N)	1.61^{b} (0.32)	0.49^{a} (0.11)	5.98^{c} (2.08)
Bread serving (5 g)			
Volume (ml)	18.33^{b} (1.53)	12.33^{a} (1.15)	21.33^{c} (2.52)
Crust portion (%wt)	24.4^{a} (3.2)	24.6^{a} (3.9)	47.6^{b} (7.6)
Moisture content (%)	36.40^{b} (0.82)	43.74^{c} (0.390)	33.19^{a} (5.615)
Bread serving (13 ml, with crust)			
Weight (g)	3.60^{a} (0.26)	5.61^{b} (0.52)	3.54^{a} (0.30)
Crust portion (%wt)	27.43^{b} (7.71)	20.81^{a} (3.43)	31.32^{b} (5.24)
Moisture content (%)	35.45^{b} (1.52)	44.81^{c} (0.88)	30.45^{a} (1.23)
Bread serving (13 ml, without crust)			
Weight (g)	3.99^{b} (0.70)	4.89^{c} (0.30)	3.39^{a} (0.32)

$^{a-c}$ Values are mean with standard deviation in brackets ($n = 9$ or 18). Means within the same row denoted by different superscript letters differ significantly ($p < 0.05$).

Table 4
Average oral processing parameters of group panellists.

	Baked bread	Steamed bread	Baguette
Muscular activity			
Ln(mean amplitude (μV))	4.82^{b} (0.28)	4.66^{a} (0.36)	4.90^{c} (0.24)
Ln(max amplitude (μV))	5.32^{b} (0.28)	5.14^{a} (0.37)	5.41^{c} (0.24)
Ln(peak area (μV s))	4.16^{b} (0.26)	3.96^{a} (0.35)	4.25^{c} (0.23)
Ln(Total peak area (μV s))	9.55^{b} (0.40)	9.24^{a} (0.47)	9.70^{c} (0.40)
Temporal parameters			
Burst duration (s)	0.52^{b} (0.05)	0.50^{a} (0.001)	0.53^{b} (0.05)
Chewing time (s)	41.49^{b} (12.93)	35.80^{a} (12.26)	44.06^{c} (14.67)
Number of chewing cycle	59.31^{b} (19.70)	52.03^{a} (17.85)	62.66^{c} (21.74)
Chewing frequency (s^{-1})	1.43^{a} (0.17)	1.46^{a} (0.19)	1.42^{c} (0.18)

$^{a–c}$ Values are mean with standard deviation in brackets ($n = 14$). Means within the same row denoted by different superscript letters differ significantly ($p < 0.05$). Mean amplitude, max amplitude and peak area were log transformed in order to obtain normal distribution for statistical analysis.

baguette required the longest duration and the largest number of masticatory cycles as compared to the baked bread and the steamed bread. These findings suggested that the baguette required the greatest amount of chewing effort to form a safe-to-swallow bolus while the steamed bread required the least.

Increase in the number of chewing cycle and muscular activity of masseters and temporalis were known to be associated with an increase in food hardness (de Wijk et al., 2008; Woda et al., 2006). The largest muscular activity used to chew the baguette suggested that it was perceived as the hardest bread item among the three. Besides hardness, decrease in moisture content in some foods such as bread (Motoi et al., 2013) and toast (Pereira et al., 2006) can also cause an increase in chewing time. Hence, the low moisture content of the baguette, due to the presence of its drier crust, was another contributor to its prolonged oral processing time. Similarly, the moisture content of the steamed bread being the highest might be the reason for its lowest masticatory effort required.

It seems that the crust played a more dominant role in affecting one's chewing behavior since the crust was the major part that contributed to greater hardness and lower moisture content in the baguette and the baked bread as compared to the steamed bread. However, the impact of bread crumb could not be ignored and it could only be assessed by isolating the crumb from the crust, which was the objective of conducting the single panellist study.

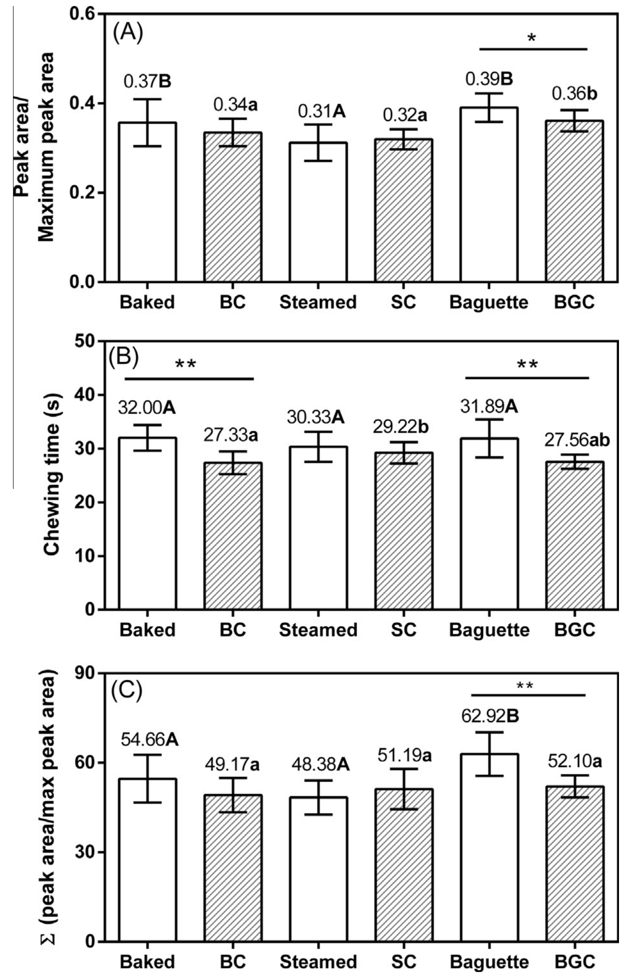

Fig. 4. Chewing behavior of single panellist: (A) mean amplitude, (B) number of chewing cycles, and (C) total peak area. BC = crumb of baked bread, SC = crumb of steamed bread, and BGC = crumb of baguette. Numbers above the histogram are mean values. $^{A–B}$ Means of bread with crust or skin with the same superscript letters are not significantly different ($p < 0.05$). $^{a–b}$ Means of bread crumb with the same superscript letters are not significantly different ($p < 0.05$). $*$ and $**$ denote significant difference between the same type of bread with and without crust or skin according to paired t-test at $p < 0.05$ and $p < 0.01$, respectively.

3.2.2. Single panellist: bread crumb

The single panellist used significantly higher muscle activities in chewing the baguette crumb than chewing the baked bread crumb and the steamed bread crumb (Fig. 4A). A significant difference between chewing the baguette crumb and the steamed crumb was observed right from the beginning until 50% of the chewing time (Fig. 5). This means that the difference in chewing force was attributed to the difference in the initial crumb features and was disappearing when the structure was significantly disintegrated. Therefore, the higher muscular activity used to chew the baguette was due to not only the superior mechanical strength of its crust but also its crumb. However, this was not shown in the results of TPA analysis which suggested that the baguette crumb was the softest and easiest to be compressed. Thus the standard TPA analysis using compressive force may not be a good enough to simulate the deformation force experienced during eating which includes shearing and tearing (Chen, 2014).

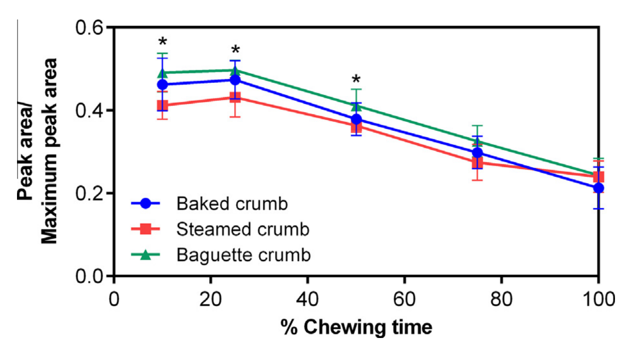

Fig. 5. Stage analysis of the muscular activity of single panellist during chewing of bread crumb. $*$ Denotes significant difference between the baguette crumb and the steamed crumb at $p < 0.05$.

Pairwise comparison results showed that bread crust significantly increased the chewing time of the baked bread and the baguette (Fig. 4B). This conclusion is in agreement to that from the group panellist study and confirms the importance of crust moisture in determining the chewing duration. Interestingly, the steamed crumb required a longer chewing time than the baked crumb and the baguette crumb. This was unlikely due to the need for salivation since the three types of bread crumb were similar in their moisture content before and after chewing. As described by the panellist, the unique texture perceived during eating the steamed bread and the steamed bread crumb was "stickiness", which is defined as the sensation of food sticking to the palate or teeth (Panouillé et al., 2014). So the difficulties in moving the bolus and cleaning the palate and teeth surface during chewing could be the reason for the prolonged chewing of the steamed bread crumb.

The total chewing effort was quantified by the total peak area (Fig. 4C). All the three types of bread crumb required similar total efforts due to the balance between muscle activities and chewing duration, i.e. the baguette crumb elicited a higher muscular force but required a shorter chewing time while the steamed bread crumb required a longer chewing duration but with a lower muscular force.

It is evident that the impact of bread structure on chewing behavior is complex due to the dynamic change in mechanical properties and multi-sensations involved. Studies of other food products, such as cheese, also showed that standard mechanical tests failed to predict textural properties evaluated during the masticatory sequence, including adhesiveness, cohesiveness and smoothness (Brown et al., 2003). The existing imitative texture analysis can only, to its best, predict part of the texture perceived at the very beginning of chewing (Stokes et al., 2013). After a few chews, especially with the impregnation of saliva, the bread matrix would be evolved dramatically from its original state and hardly be assessable by the standard texture analysis. Hence, sEMG would be an indispensable a tool for the understanding of the change in food structure throughout the oral processing.

3.3. Bolus characterization

3.3.1. Group panellists: bread with crust

Results of bolus characterization are shown in Table 5. The moisture content of bread bolus varied in a small range of 61–64%, which serves as a threshold value of moistness to initiate swallowing of bread (Le Bleis et al., 2013). The baguette had a higher level of saliva impregnation (0.791 g saliva/g bread) compared to the baked bread (0.631 g saliva/g bread) and the steamed bread (0.591 g saliva/g bread) because of its thick and dry crust which required more saliva to moisten and soften. This inverse relationship between food moisture content and saliva secretion was previously reported by Gavião et al. (2004) in the study of chewing toast, breakfast cake and cheese.

The bolus of baguette had a smaller fraction of large particles and smaller sizes of small particles compared to those of the baked bread and the steamed bread, indicating a higher level of structure breakdown. This can be explained by its prolonged chewing duration and the larger muscular force used. It also suggests that for harder bread, the swallowing threshold of particle size is smaller.

Results from the back extrusion analysis showed that the bolus of the steamed bread was softer in texture compared to those of the baked bread and the baguette. The softer bolus texture was largely due to its higher moisture content. A significant negative correlation ($r = -0.954$, $p < 0.001$) was found between the bolus moisture content and the bolus texture. Linear correlation analysis showed that the bolus texture could well be predicted by its moisture content alone ($R^2 = 0.911$, RMSE = 0.314 (5.6% of mean)) regardless of bread type. This further illustrates the importance of saliva in softening dry bakery product in order to ensure a comfortable and safe passage of the bolus through the esophagus.

Table 5
Characteristics of ready-to-swallow boluses of the three types of bread.

	Baked bread	Steamed bread	Baguette
Saliva to bread ratio (g saliva/g bread)	0.63^{a} (0.22)	0.59^{a} (0.24)	0.79^{b} (0.27)
Moisture content (%, wet basis)	61.13^{a} (5.63)	63.48^{b} (5.82)	61.29^{a} (5.98)
Mass fraction of large particle (%)	61.67^{b} (11.88)	62.24^{b} (13.38)	52.14^{a} (8.51)
Large particle median area (A50) (mm^2)	8.24^{a} (1.40)	7.93^{a} (1.15)	8.14^{a} (0.89)
Small particle median diameter (d_{50}) (μm)	1230.01^{b} (135.67)	1285.71^{b} (202.93)	1002.43^{a} (165.82)
Ln (Peak area (10^{-3} N sec))	7.94^{b} (1.03)	7.70^{a} (1.05)	8.00^{b} (1.07)

$^{a–b}$ Values are mean with standard deviation in brackets ($n = 14$). Means within the same row denoted by different superscript letters differs significantly ($p < 0.05$). Bolus texture was log transformed in order to obtain normal distribution for statistical analysis.

3.3.2. Single panellist: bread crumb

Fig. 6 shows the bolus characteristics of the six types of bread samples. The most important finding is that the boluses of the three types of bread crumb were similar in moisture content as well as mass fraction of large particles and texture, suggesting a uniform swallowing threshold for bread crumb. On the contrary, the swallowing thresholds for bread samples with crust or skin were significantly different from each other and this was in agreement with the results from the group panellist study. The bolus of the steamed bread had higher moisture content and contained a larger percentage of big particles due to the absence of dry and hard crust. The bolus of the baguette was harder than those of the baked bread and the steamed bread because of its hard crust, which could not be softened completely during the short course of chewing. This was confirmed by the pairwise comparison which showed that the baguette bolus was significantly harder than the baguette crumb bolus. On the whole, the level of destruction was similar among bread crumb samples but varied significantly with the presence of bread crust. A larger portion of hard and dry crust would most likely lead to an extensive disintegration of bread structure.

3.4. Correlation between bread structure and oral processing

Bread crust was identified as an important factor in determining one's chewing behavior in the group panellists study. The correlations between bread structure and oral processing, including both chewing behaviors and bolus characteristics, are illustrated in Fig. 7. Firstly, the hardness of bread crust was positively correlated with the total peak area while negatively correlated with the mass fraction of large particles (Fig. 7A). Moreover, the moisture content of bread crust was positively correlated with both the chewing time and the amount of saliva impregnated (Fig. 7B). This is because a larger force was required to breakdown the hard crust while a longer time was required to impregnate a sufficient amount of saliva to soften the dry crust. The larger force and longer time, in turn, resulted in an extensive breakdown of bread structure, which may not be favorable for slowing down the digestion in the gastrointestinal tract. This hypothesis is supported by the results from our recent study of the *in vivo* digestibility of bread, which showed that the glycaemic response of bread was reduced when the processing method was changed from baking to steaming (Lau et al., 2015). The extensive breakdown and salivation required for bread with hard and dry crust during oral processing could be one of the contributors to the higher digestibility of baked bread compared to steamed bread in that study. The relationships among bread properties, masticatory parameters and bolus properties of the single panellist study can be illustrated using the PCA plot (Fig. 8). The three types of bread with crust or skin were differentiated from each other based on the principal component 1 (PC1), which accounted for 56.19% of the total variance. Moisture content of the bread was negatively correlated with the masticatory effort and positively correlated with the bolus moisture content and particle size, which was in agreement with the group panellist study. The removal of bread crust had a significant effect on the chewing behavior and bolus properties of the baked bread and the baguette but not the steamed bread. The three

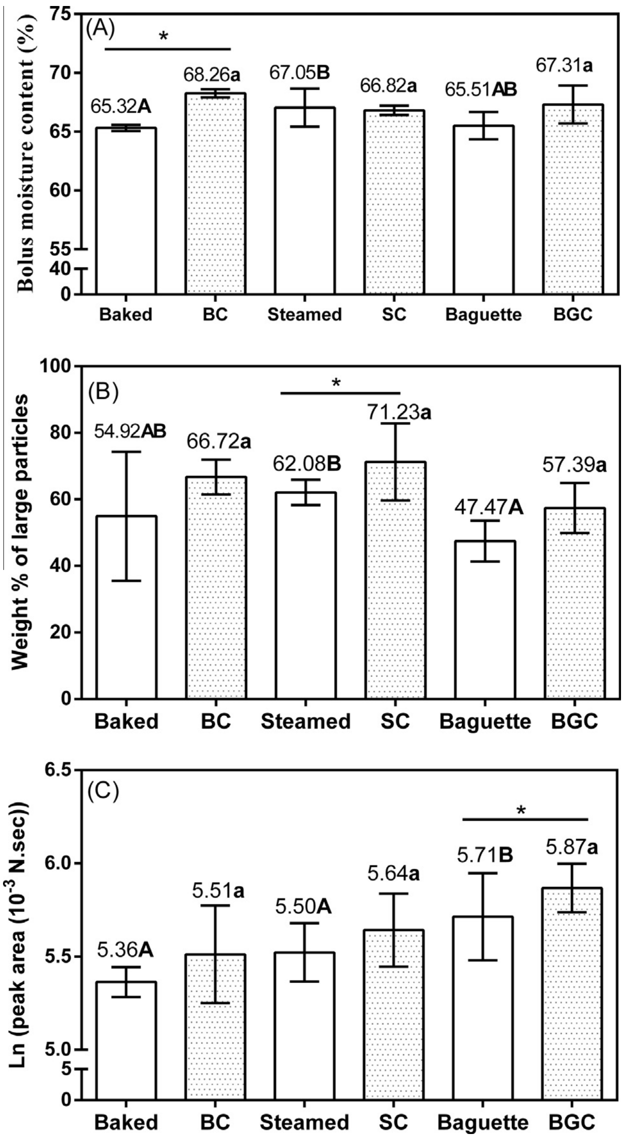

Fig. 6. Bolus characterization at the swallowing point: (A) moisture content, (B) mass fraction large particles, and (C) peak area in the bolus texture. BC = crumb of baked bread, SC = crumb of steamed bread, and BGC = crumb of baguette. Numbers above the histogram are mean values. [A–B] Means of bread with crust or skin with the same superscript letters are not significantly different ($p < 0.05$). [a–b] Means of bread crumb with the same superscript letters are not significantly different ($p < 0.05$).⁎ and ⁎⁎ denote significant difference between the same type of bread with and without crust or skin according to paired t-test at $p < 0.05$ and $p < 0.01$, respectively.

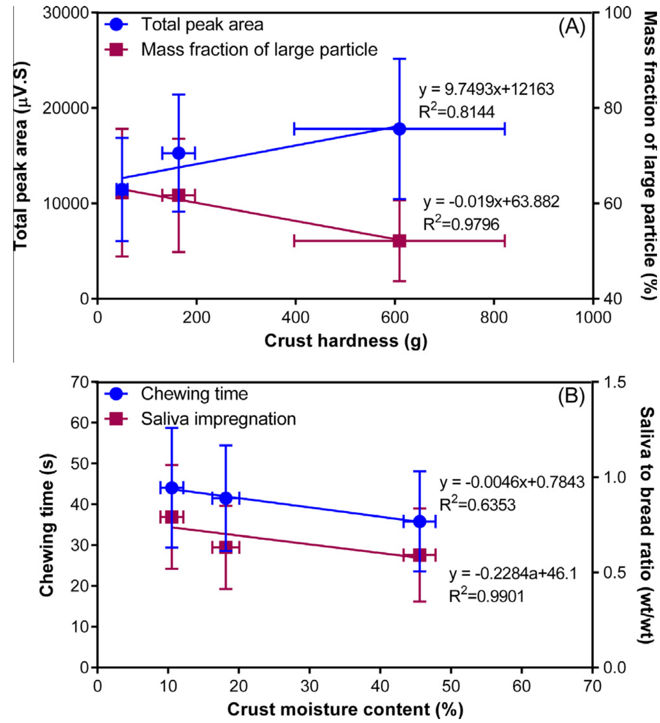

Fig. 7. Correlation plots for group panellists study: (A) between total peak area, mass fraction of large particles, and crust hardness, and (B) between chewing time, saliva impregnation, and crust moisture content.

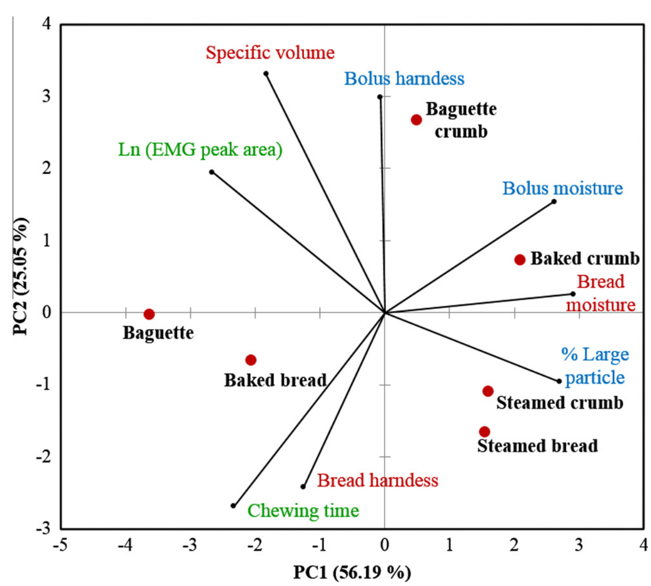

Fig. 8. PCA biplot of bread characteristics, chewing behavior and bolus properties of six types of bread samples in the single panellist study.

types of bread crumb were scattered along the principal component 2 (PC2), which accounted for 25.05% of the total variance. Specific volume of bread crumb was positively correlated with the masticatory effort and bolus hardness. This correlation between the structure of bread crumb and one's chewing behavior is more difficult to be interpreted. Conventional power law predicts that mechanical strength decreases with an increase in the porosity of open-cell solid foams, both within and beyond the linear elastic range (Gibson and Ashby, 1997). According to this law, the baguette crumb should have the lowest resistance to deformation since it had the largest porosity. However, opposite findings in this study suggested that the impact of bread structure on its mechanical properties is far beyond the impact of its bulk density. The material properties of cell wall and the structure of both cell and cell wall, especially their shape, uniformity and dimension, would play more influential roles. The strength of the cell wall material of bread crumb was known to be related to its moisture content, starch content and strain hardening behavior of the protein (Scanlon and Zghal, 2001). Since all the three types of bread had the same formulation and similar moisture content, the difference in their material strength might be attributed to the different levels of gluten development. The superior strength of baguette crumb might be due to the better development of its gluten network through intensive mixing and the additional molding steps. Moreover, how the cellular structure would respond to multiple forces together with the effect of salivation is beyond the coverage of conventional mechanical properties discussed by Gibson–Ashby power law. An important question for future studies is to design a mechanical test that can closely resemble the deformation process happening in the mouth.

4. Conclusion

This study demonstrated the possibility to produce bread with various structures and textures by only adjusting the processing conditions. People's masticatory behavior and swallowing threshold were greatly influenced by the properties of both bread crust and crumb. The presence of dry and thick crust was identified to be a major factor in increasing the masticatory effort, which led to an extensive breakdown of bread structure. The cellular structure of bread crumb not only determined its resistance to mechanical breakdown but also affected the panellists' texture perception during chewing. Most interestingly, the most porous bread crumb (i.e. the baguette crumb) required the largest masticatory force while the densest bread crumb (i.e. the steamed crumb) required the longest chewing time, which could not be explained by conventional power law. The observed discrepancy between the results from standard mechanical tests and oral processing behavior studies suggests that a more advanced, perhaps also more complicated, mechanical test is required to be able to better predict the deformation behavior of food in mouth.

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