

## **New image analysis of large food particles can discriminate experimentally suppressed mastication**

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## **ABSTRACT**

Objective parameters that could provide a basis for food texture selection for elderly or dysphagic patients have not been established. We therefore aimed to develop a precise method of measuring large particles ( $> 2$  mm in diameter) in a bolus and an analytical method to provide a scientific rationale for food selection under masticatory dysfunction conditions. We developed a new illumination system to evaluate the ability of twenty female participants (mean age,  $23.4 \pm 4.3$  years) to masticate carrots, peanuts and beef with full, half and one quarter of the number of masticatory strokes. We also evaluated mastication under suppressed force, regulated by 20% EMG of the masseter muscle. The intercept and inclination of the regression line for the distribution of large particles was adopted as coefficients for the discrimination of masticatory efficiency. Single set of coefficient thresholds of 0.10 for the intercept and 1.62 for the inclination showed excellent discrimination of masticatory conditions for all three test foods with high specificity and sensitivity. These results suggested that our method of analyzing the distribution of particles  $> 2$  mm in diameter might provide the basis for the appropriate selection of food texture for masticatory dysfunction patients from the standpoint of comminution.

## INTRODUCTION

The global population of elderly individuals is rapidly increasing, and the prevalence of illnesses increases with age. Such illnesses can be related to chewing and swallowing difficulties (dysphagia), as well as disease-related malnutrition (1). Furthermore, the prevalence of dysphagia is significant (3-50%) and can affect eating habits by reducing appetite and causing anxiety that reduces quality of life (2). Food texture has been regarded as an important factor for smooth deglutition (3), and food boluses with irregular mechanical properties can hamper stable deglutition. Therefore, dietary modification is widely accepted as being important to prevent aspiration (4). One basis upon which to make a clinical decision about whether to provide chopped or smooth blended food to elderly individuals would be related to their ability to masticate a food clump into a smooth and even paste. The size distribution of bolus particles is regarded as a determining factor in forming a cohesive bolus, which subsequently allows safe swallowing because food particles do not enter the airways (5). Therefore, the evaluation of food particle size distribution would be of importance when the masticatory ability is disputed from the aspects of comminution and mixing process for bolus formation.

Various methods to evaluate masticatory efficiency have been reported (6, 7). Laser diffraction is an established representative method of evaluating the size and number of natural food particles that is also used in industrial applications. However, the specifications of the equipment allow measurements only of particles < 2 mm in diameter. On the other hand, although the sieve method can be easily applied to any size of natural food particles, the sieve method of analyzing natural foods seems to have a critical limitation, which is the need for drying the sieved material.

Food particles should be well lubricated and have a diameter of 1.4-2.0 mm to achieve smooth deglutition (8). Therefore, considering the clinical importance of evaluating

masticatory efficiency among the elderly, particles  $> 2$  mm in diameter should be focused.

However, a practical methodology, which could routinely be used in daily clinics, for evaluating food particles with a large diameter and precise size distribution has not been established.

The present study aimed to develop a method of measuring natural food particles of various sizes and optical properties, and a theoretical analytical method to discriminate hampered mastication by evaluating the distribution of large particles in natural food boluses.

## **MATERIALS AND METHODS**

Written, informed consent was obtained from 20 women with healthy dentition (mean age,  $23.4 \pm 4.3$  years) who were selected according to strict dental criteria (9). Only females were selected to exclude the variability in chewing due to gender. None of them were undergoing dental treatment at the time of the recordings. They were selected as having at least 28 teeth and with molars in Angle's Class I occlusion. These individuals had never received any orthodontic treatment, and they were free of dental pathology such as caries or periodontal disease. They did not have any functional disturbance of mastication such as orofacial pain or sound in temporomandibular joint during mandibular movements as assessed by the lack of pain during palpation of masticatory muscles or during pressure of the temporomandibular joint at rest during opening or propulsion of the jaw. The subjects were not aware of tooth grinding or excessive tooth clenching. The Ethics Committee of the Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University approved the study protocol (Approval No. 915). Each woman masticated raw carrots, peanuts and beef for five sessions for each food. Portions consisted of four peanuts and cuboid ( $1 \times 1 \times 2$  cm) samples of raw

carrots and cooked beef. Peanuts were stored inside a container with silica gel to absorb moisture and the carrots and beef were prepared immediately before testing.

The women underwent four mastication trials for each test food until swallowing. The number of chewing strokes was counted and the mean number of strokes (MS) was recorded for each test food for each subject. The procedure was repeated twice more for each test food to record surface electromyographic (EMG) activity of the masseter muscle on the chewing side during usual mastication. Based on recorded activity, an EMG feedback system (MA-2000W, Oisaka Electronic Device Ltd., Fukuyama, Japan) was set to provide visual feedback of 20% EMG level to each participant. After the above preliminary sessions, one sample bolus for each test food was collected for mastication with MS, half the number of MS strokes, and quarter strokes of MS and MS with limited masseter muscle activity to 20% EMG level. The order of the experimental mastication was randomized and the bolus samples were processed immediately after the sessions.

### **Food bolus processing**

Food bolus sample was collected using a small vessel of 0.8-cm<sup>3</sup>. Five 0.8-cm<sup>3</sup> samples taken from each retrieved food bolus for digital imaging were washed with 25ml of 0.032% fatty alkanol amide solution. The samples were then washed by distilled water in a stainless steel sieve with a 0.2-mm mesh. The processed particles were then retrieved and dispersed in 0.06% benzalkonium chloride in plastic Petri dishes.

### **Digital imaging**

Dark field images of the bolus sample were obtained using a digital camera (C-2020Z, Olympus, Tokyo, Japan) and a novel double dark field illumination system consisting of two dark field lighting units positioned at the upper and lower sides of a sample dish (Fig. 1A). Digital images were then processed using image analysis software (WinROOF, Mitani Corp.,

Tokyo, Japan). As shown by the examples in Fig. 1B and C, all images were binarized using the same set of optical threshold conditions of hue, saturation and lightness (HSL) within 0 to 359, 0 to 255, and 80 to 202, respectively. No exceptional settings or manual adjustments of these optical threshold conditions were required throughout the experiment as the double dark field illumination system resulted in quality contrast images. The virtual diameter and the area of each particle was calculated by the software. To compare the area data with the results of the sieve method below, the area data were also summed over virtual diameter steps of 0.25-0.5, 0.5-0.84, 0.84-1.0, 1.0-1.68 and 1.68-2.0 mm according to the size of the mesh in the sieves.

### **Sieve analysis method**

Sample particles were returned after imaging to the source bolus to complete the sieve method. Particles obtained from the sieve were analyzed as described (10). A sample bolus was placed on a stack of six sieves (Iida Manufacturing Co. Ltd., Osaka, Japan) with apertures of 2.0, 1.68, 1.0, 0.84, 0.5 and 0.25 mm). The bolus was then washed with 1000 mL of water and the material retained in each sieve was collected on filter paper and dried (Constant Temperature Oven DN-61, Yamato Scientific Co., Tokyo, Japan) for 3 h at 100°C. The dry material was weighed using a digital scale (TE 153S-DS, Sartorius Corp., Goettingen, Germany).

### **Statistical analysis**

The relationship between ordered numbers of particles  $> 2$  mm and the digital image area was evaluated using ordinary quadratic regression. Ordinary least-square regression models were used to estimate the association between the weight of food in the sieve and the estimated digital image area of the particles. Ordinary least-square regression was also used to calculate the coefficients of inclination and Y-axis intercept for the relationship between the

ordered number of particles and natural logarithm of the digital image area of the particles. The effect of masticatory conditions on the calculated inclination and Y-axis intercept was evaluated using the Friedman test. All data were analyzed using the statistical software package SPSS Statistics, Release 17.0 (IBM Japan Ltd., Tokyo, Japan), with the probability of a type I error set at the 0.05 level.

## RESULTS

The area of peanut particles of < 2 mm in diameter summed by digital image analysis and the actual weight of the particles determined by sieve method significantly correlated ( $r = 0.833$ ,  $p < 0.05$ ). This result confirmed the relevance of the digital imaging process. However, the weight of sieved carrot and beef particles varied near the lower limit of the weighing equipment (1 mg) and seemed inappropriate for mechanical weighing after drying for 3 h at 100°C.

The scattered plot of the area of particles > 2 mm versus the serialized order showed quadratic regression for each condition (Fig. 2A). Area values were then converted to common logarithms and linear regression was achieved (Fig. 2B). The Friedman test revealed that the inclination of the regression line and the Y-axis intercept value of the regression line were significantly affected by the masticatory condition (both  $p < 0.01$ ). Based on these findings, the inclination value of the regression line and Y-axis intercept value were adopted as coefficients to represent the masticatory function of each trial. Scatter plots of these values for the median inclination for each experimental food are shown in Fig. 3. Coefficient threshold values for the inclination and intercept were sought inductively for the entire subjects, and a single set of coefficient thresholds of 0.10 and 1.62 for the inclination and intercept values was found to discriminate normal from restrained mastication. As shown in Fig. 4, the specificity and sensitivity calculated for the discrimination of overall samples were 0.90 and 0.86,

respectively. According to the coefficient threshold, all data for MS with 20% EMG could be completely discriminated from normal mastication data (Fig. 4).

## **DISCUSSION**

For the determination of masticatory performance, various food material and artificial material have been used (11, 12, 14-16). Comparing masticatory performance using a two-coloured chewing gum for mixing ability and two types of a standardized artificial test food for comminution ability, the comminution test was reported to result in better discrimination of the masticatory performance in young and elderly subject groups (13). Although the mechanical properties of artificial compounds such as silicone impression material (12) could easily be standardized, they are not edible and the mastication process might be affected by this property. Considering the purpose of evaluating masticatory efficiency among the elderly, it would be desirable that data could be derived from natural foods that patients would actually eat on a regular basis. However, the sieve method of analyzing natural foods seems to have a critical limitation, which is the need for drying the sieved material. To determine a gold standard weight of any natural food with high water content after drying would be at least difficult if not impossible. Measuring particle size would be a desirable solution in this context.

As the size of variously shaped masticatory particles ranges from  $< 0.25$  to  $> 5$  mm, the optical threshold setting for digitization usually considerably affects calculated areas. Digitization has been applied for quite some time as a simple method of picture analysis that could be applied to masticatory particle measurement (17). However, difficulties with defining the optical threshold for the digitizing process would have hampered popularization of this modality. The optical translucency of masticated carrot particles unlike nuts for example, varies at the edge of each particle and they can easily become folded when placed on a dry stage,

resulting in the difficulties in determining the optical threshold when a conventional picture is taken. A quality-controlled picture is critical to obtain reliable measurement outcomes using a reliable optical threshold. As our novel double dark field illumination system met this requirement, one fixed optical threshold could be applied to all pictures of all food particles in this study. The picture conditions necessary for the analysis comprise good contrast between particles and background, even and sufficient brightness over the entire particle surface and good particle dispersion in medium to avoid hydrophobic agglutination. The first and second conditions were fulfilled by dark illumination from the bottom and from above, respectively (Fig. 1A). The third condition was satisfied using two detergents that enabled controlled dispersion regardless of the type of food. This new methodology supported clear projection of food particles and would enable automation or semi-automation of a simple particle analysis system.

Another precise methodology for particle analysis would be laser diffraction (18) that supports precise, automated particle analysis and it is already popular for industrial applications. However, the functional capability of laser equipment limits measurable particle size to 2 mm. Because the minimum two-point discrimination distance in the oral mucosa is 1-2 mm and because it is located around the tip of the tongue and anterior hard palate (19, 20), a food particle diameter of 2 mm might be an important threshold value for mastication. Prinz and Lucas reported that good lubrication of food particles is an important condition for smooth swallowing and a particle size of 1.4-2.0 mm would be the threshold for this process (8).

The key feature of our analytical method is that a single set of coefficient thresholds for the Y-axis intercept and for the inclination of the regression line clearly discriminated normal from hampered mastication, regardless of the food texture. The inclination would be a coefficient which would largely reflect the size uniformity of particles  $> 2$  mm. Although food

particle distribution affects both coefficients, the intercept tends to reflect the maximum size of the particle in a bolus. We applied two constraints upon mastication in the present study, namely, suppression of masticatory force by EMG and suppression of the number of mastication strokes. The reported masticatory forces of peanuts on the molars of a fixed bridge and well-functioning complete denture are approximately 100 N (21) and 50 N (22), respectively. The masticatory force suppressed by 20% EMG was designed to simulate complete denture of imperfect condition. Our method of analyzing masticatory efficiency discriminated both suppressed conditions from normal mastication with high sensitivity and specificity.

These results showed that the double field illumination system provides a basis for the image analysis of natural food boluses with various optical properties. In addition, the method of analyzing a particle size distribution of  $> 2$  mm would provide the basis for food texture selection for the elderly or dysphagic patients from the standpoint of comminution. Further studies are expected to establish a database for various natural foods according to this concept.

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## **Foot Note**

This paper was read before the IADR meeting in San Diego, 2011.

## Legends for Figures

Fig. 1

A) Scheme of digital imaging system with double dark field illumination.

Petri-dish with bolus sample is placed on a platform in a dark box. Two sets of dark field illuminations are installed to provide light the dish from above and below (arrows). DC, digital camera; LS, light source; P, Petri-dish with bolus sample

B) Example of raw digital image of bolus sample taken using double dark field illumination system (raw carrot). Scale bars = 10mm

C) Processed digitized image with optical HSL threshold.

Fig. 2

A) Example of quadratic regression for relationship between ordered number of particles and area of carrot particles > 2 mm.

B) Linear regression for natural logarithm of area data shown in Fig. 2A and ordered number of particles. ♦, MS; □, ½MS; Δ, ¼MS

MS, mean number of strokes before natural swallowing; ½MS, half number of MS strokes; ¼MS, quarter number of MS strokes

Fig. 3

Scatter plot of median inclination and Y-axis intercept of regression line for particle distribution > 2 mm for three test foods. ♦, MS; Δ, ¼MS;

Thresholds of 0.10 for inclination and 1.62 for intercept clearly discriminate MS from restrained masticatory condition.

MS, mean number of strokes before natural swallowing; ¼MS, quarter number of MS strokes

Fig. 4

Summary scatter plot of median inclination and Y-axis intercept for 20 subjects including three test foods under four different masticatory conditions. ◆, MS; △,  $\frac{1}{4}$ MS; ○,  $\frac{1}{2}$ MS; ●, 20%EMG  
Note that thresholds of 0.10 for inclination and 1.62 for intercept clearly discriminate MS from other restrained masticatory conditions.

MS, mean number of strokes before natural swallowing

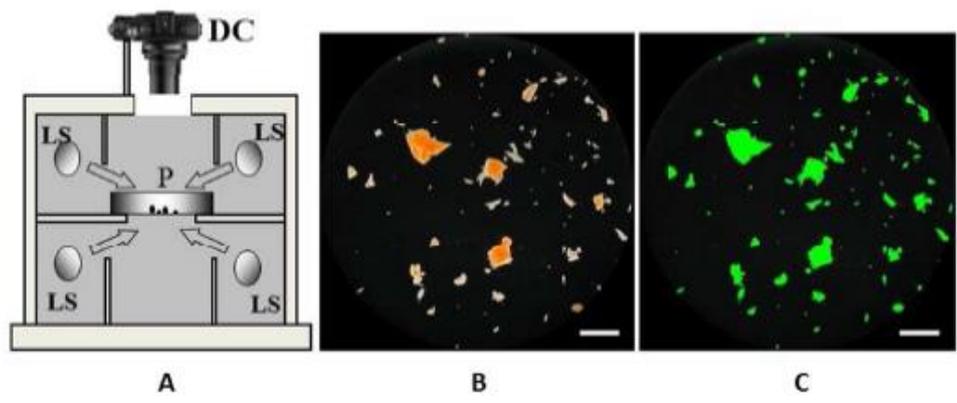


Fig. 1

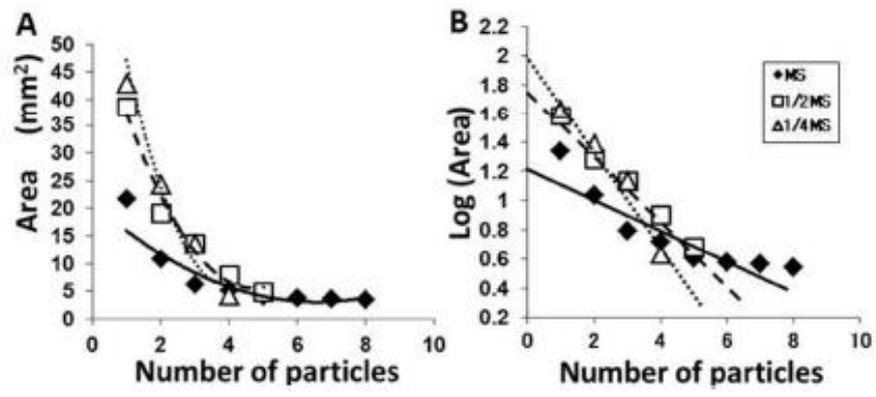


Fig. 2

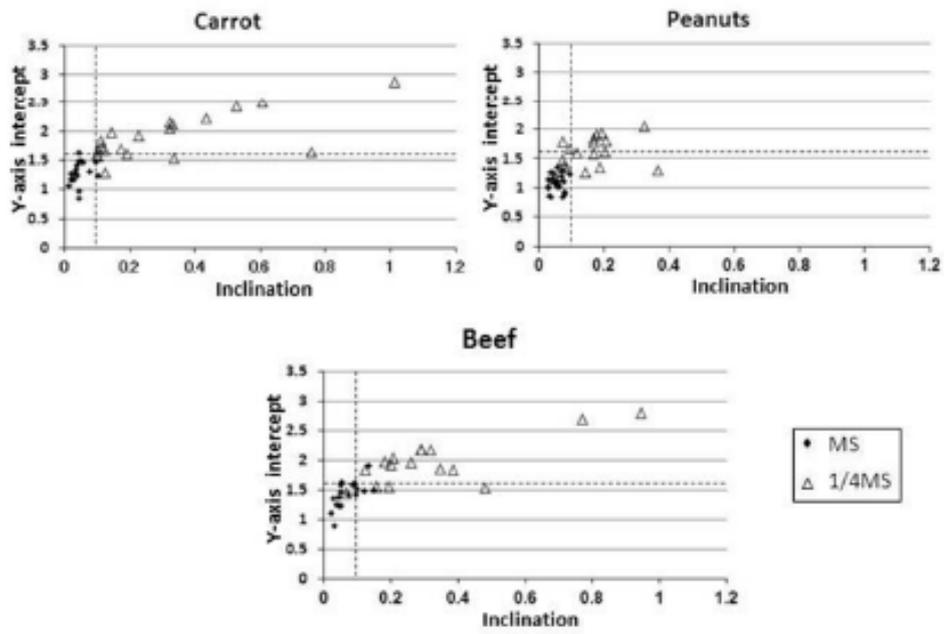


Fig. 3

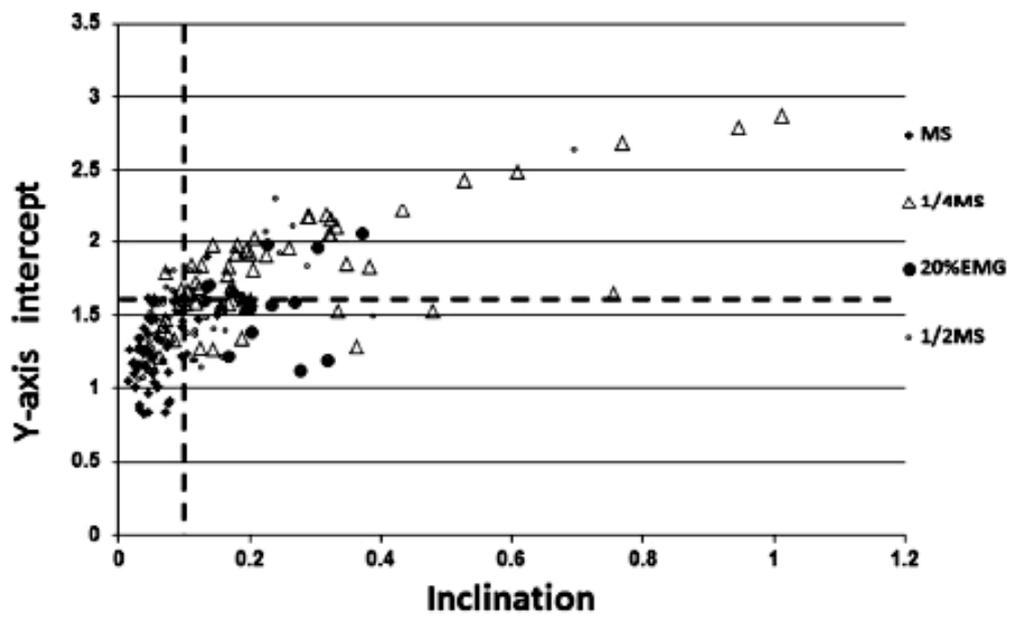


Fig. 4