# Changes in muscle activity and physical property of foods with different textures during chewing

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#### Abstract

This study aimed to investigate how the activity of the masseter (Mas) and suprahyoid (Hyoid) muscle are influenced by the condition of food, how changes in rheological property differ in the process of food reduction between different foods, and how different salivary flow rates affect bolus-making capability during chewing in healthy humans. Ten healthy adult males participated. Electromyographic (EMG) recordings were obtained in the Mas and Hyoid muscles, and 15 g steamed rice and rice cake were prepared as test foods. In the ingestion test, the subjects were asked to eat each food in their usual manner. The chewing duration, number of chewing cycles before the first swallow, Mas and Hyoid EMG activity, and chewing cycle time were compared between the foods. The chewing duration was divided into three substages: early, middle, and late; chewing cycle time and EMG activity per chewing cycle of each substage were compared between the foods and among the substages. In the spitting test, the rheological property of the bolus at the end of each substage was compared between the foods and among the substages. Finally, stimulated salivary flow rate was measured and the relationships between the salivary flow rate and chewing duration, EMG activity, changes in the physical properties, and EMG activity were investigated. There were significant differences in the chewing duration and number of chewing cycles between the foods with similar hardness, but not in the chewing cycle time. The Mas and Hyoid EMG activity per chewing cycle for the rice cake was significantly greater than for steamed rice throughout the recording periods. While the Mas activity did not change among the substages during chewing, the Hyoid EMG activity decreased as chewing progressed. Chewing cycle time also gradually decreased as chewing progressed. The hardness of both foods initially increased, then gradually decreased back to baseline.

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Adhesiveness of the rice cake initially increased, and did not fall throughout the recording period; adhesiveness did not significantly change for the steamed rice. Cohesiveness barely changed for the two foods during chewing, but was significantly greater for the rice cake than for steamed rice. Finally, a correlation between the stimulated salivary flow rate and chewing performance was noted only in change in Mas EMG activity. The current results demonstrate that the Mas and Hyoid muscle activity changed as chewing progressed, and was affected by hardness, adhesiveness, and cohesiveness. Salivary flow rate may affect the changes in Mas activity in the process of bolus formation.

**Keywords**: mastication, masseter muscle, suprahyoid muscles, physical property, electromyography, salivary flow rate

#### 1. Introduction

Mastication is the first step of nutrition in most mammals, and forms an important part of feeding behavior. In the masticatory sequence, bolus formation is achieved by chewing to crush food between the teeth or between the tongue surface and palate, and mixing with saliva to reach a condition appropriate for swallowing. Finally, when the swallowing reflex is initiated, the pharyngeal stage follows in rapid sequence.

Two approaches have been developed to understand how the physical condition of the food affects the process of bolus formation and how the food bolus is handled in the oral cavity during chewing: the rheological approach to food texture, and the physiological approach. The physical properties of the bolus that are important in determining the initiation of swallowing are particle-size distribution [1, 2], and lubrication due to saliva and food fluids [3]. Regarding the latter, those authors proposed a dynamic approach to characterize the perception of food texture [3]. The concept is based on the breakdown of food during oral processing from three aspects: the degree of structure, lubrication, and time. However, no quantitative attempts have been made to elucidate how the bolus properties determine the initiation of swallowing during mastication.

In contrast, the importance of intra-oral inputs to adapt masticatory movements to changing bolus conditions has been evaluated by numerous physiological studies [4-8]. There is no doubt that jaw movement trajectories and related muscle forces during mastication are highly adaptable, and food texture can modulate masticatory forces [2, 9-12], jaw movements [13, 14], chewing cycle [15, 16], and the number of cycles preceding the swallow [17]. Electromyographic (EMG) recording is the major monitoring technique for these physiological processes, and the results show a clear

relation between muscular activity and food properties [18, 19]. Moreover, the proprioceptive information on muscle activity, as recorded by EMG, could serve as the sensory basis of texture perception [19, 20].

Since steamed rice is a traditional food in Japan, many Japanese studies have focused on its mastication to evaluate masticatory and swallowing functions [21-26]. Rice cake or mochi (made by pounding glutinous rice into a paste) is also one of the most traditional Japanese foods. Although the hardness of steamed rice and rice cake is identical, rice cake exhibits a unique texture with extreme adhesiveness and tolerance to elongation [22] and is particularly difficult to chew and swallow [27]. Rice cake is very pliable, but difficult to bite into pieces and swallow safely. Our previous study showed that the masseter muscle (Mas) activity per cycle was significantly greater for rice cake than for steamed rice [25].

The presence of saliva containing amylase plays a role in early breakdown of starch components; saliva is also essential for creating a smooth surface on the food bolus to allow it to be transported through the mouth and pharynx easily. Aging has significant influences on saliva production, with elderly people having significantly reduced and altered salivary secretion [28-31]. Thus, ineffective rice cake mastication can lead to choking and rapid suffocation, and lifesaving attempts are difficult, especially among the elderly.

So far, few attempts have been made to describe the changes in EMG patterns and rheological property in the process of bolus formation during chewing of steamed rice and rice cake, and to compare the characteristics of texture and consistency of these foods. The present study was designed to investigate: 1) how steamed rice and rice cake influence the patterns and coordination of muscle activities related to chewing; 2) how

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rheological property is changed towards swallowing in the process of food reduction; and 3) how different salivary flow rates affect bolus-making capability during chewing in healthy humans.

#### 2. Materials and methods

#### 2.1. Participants

Ten normal adult males participated in this study, ranging in age from 20 to 31 years (average age 23.0 years). The participants had no reports of abnormality in number or position of teeth, no history of orthodontic treatment or temporomandibular disorders, and no occlusal abnormalities or masticating and swallowing problems, as confirmed by a dentist prior to study commencement. No participant had a history of alimentary, pulmonary or neurological disease, structural or speech disorders, or voice, mastication, or swallowing problems. Informed consent was obtained from all participants, and this study was approved by the Ethics Committee of the Niigata University Graduate School of Medical and Dental Sciences (23-R16-11-08).

#### 2.2. Physiological recordings

Paired surface electrodes with an 8-mm diameter (NT-211u, Nihon Kohden, Tokyo, Japan) were used for surface EMG recordings of both the right and left jaw-closing Mas and the jaw-opening suprahyoid muscle (Hyoid). For Mas and Hyoid recordings, the electrodes were bilaterally attached to the skin over the center of the Mas and the anterior belly of the digastric muscle with an inter-electrode distance of 2 cm. A reference electrode was affixed to the earlobe. The signals from the EMG waves were

amplified (AB611-J, Nihon Kohden, Tokyo, Japan).

Electroglottography (EGG) was recorded to monitor laryngeal elevation during swallowing using a standard laryngograph processor (Laryngograph, London, UK). In this procedure, the surface electrodes were placed at the level of the larynx, and the subject was asked to pronounce the /i/ sound. EGG was used only for detection of swallowing movement, but not for quantitative analysis of waveforms [32].

EMG and EGG signals were then converted by an analog-to-digital converter (PowerLab, ADInstruments, Colorado Springs, CO, USA) at sampling rates of 10 kHz. The data were stored on a personal computer, and data analysis was performed using the PowerLab software package (LabChart 6 Pro, ADInstruments).

### 2.3. Test foods

A 15 g portion of steamed rice and a piece of rice cake were prepared as test foods. In our previous study, 15 g was determined as the appropriate food size to be used in each trial [25].

The physical properties of the foods were measured in terms of hardness, adhesiveness, and cohesiveness using a creep meter (RE2-3305, Yamaden, Tokyo, Japan). In this procedure, food samples were placed on the plate (40 mm diameter, 15 mm height) and elevated toward a polyacetal plunger (20 mm diameter, 8 mm height) at a speed of 10 mm/s. The plunger was connected to a load cell that pressed the sample twice (compressibility 66.7%). Stress values were obtained through the load cell, and data analysis was performed using an analysis software package (Creep analysis version 2.0, Yamaden, Tokyo, Japan). This procedure was repeated five times to obtain the mean value for each parameter. The respective yield stresses (hardness) for rice and rice cake were  $9.3 \times 10^3$  and  $9.9 \times 10^3$  N/m<sup>2</sup>, adhesiveness was  $1.0 \times 10^2$  and  $2.1 \times 10^2$  J/m<sup>3</sup>, and cohesiveness was 0.39 and 0.64; this indicates that rice cake was initially more adhesive and cohesive than steamed rice.

#### 2.4. Data collection and analysis

#### 2.4.1. Ingestion test

Prior to each experiment, the subject was not allowed to eat and drink for at least one hour. The subjects were seated on a chair. The food samples were roughly hemispherical in shape and placed on a dish in front of the subjects, who were asked to put the food into their mouths using a spoon and eat in their usual manner. The order of each task was determined randomly. The time interval between trials was at least 30 sec, and subjects were able to rinse their mouths with distilled water whenever they wished between the trials.

It is reported that most food is swallowed in the first swallow of the masticatory sequence, and any residual food becomes aggregated by the tongue into a bolus and then swallowed in the last swallow [24, 25]; hence, it can be assumed that the process of bolus formation before the first swallow occurs is critical. Therefore, we analyzed EMG data recorded up to the time of the first swallow.

The EMG bursts were first full-wave rectified and smoothed (time constant 20 msec). The integrated EMG values were then obtained as the EMG activity. As the chewing side could not be detected from the recordings, the EMG activity (defined as the average of the right and left sides) was compared between the foods, as described in previous studies [23, 25]. The thresholds for the onset and offset were defined as follows: the EMGs recorded at rest were rectified for 5 sec and the mean value  $\pm$  SD

was obtained as a control. When the values exceeded the control + 3 SDs during the trials, the EMG burst was considered to be active [33].

In each individual we obtained: 1) the chewing duration defined as the time between the onset of the Mas EMG activity burst of the first chewing cycle and the onset of the Mas EMG activity burst of the first swallowing cycle; 2) the number of chewing cycles before the first swallow; 3) total Mas and Hyoid EMG activity; and 4) average chewing cycle time defined as the chewing time duration divided by the number of chewing cycles. The mean values were then compared between the foods.

The chewing duration was divided into three periods or substages: early (first one-third), middle (second one-third), and late (last one-third) according to the number of chewing cycles for each individual. Analyses were performed to ascertain the characteristics of the chewing cycle time and EMG activity per chewing cycle in each substage. One chewing cycle time was defined as the time between the onsets of two consecutive Mas EMG activity bursts on the right side during chewing. The average chewing cycle time and the average EMG activity per chewing cycle of each substage were obtained for each individual, and the mean values were compared between the foods and among the substages.

## 2.4.2. Spitting test

Prior to each experiment, the subject was not allowed to eat and drink at least for one hour. This test was conducted on the same subjects who had done the ingestion test. Using the data obtained from the ingestion test, the duration of each substage was estimated for each food and each individual. The subjects were asked to eat the food and spit it out at the end of each substage at the cue of the researcher. The rheological

property of the sample was measured using a creep meter (RE2-3305, Yamaden, Tokyo, Japan) as described above. Hardness, adhesiveness, and cohesiveness was compared between the foods and among the substages. Each subject completed three trials (first, middle, and last) for each of the two foods (rice and rice cake), and the order of each task was randomly determined.

For the ingestion test, the time interval between trials was at least 30 sec and subjects were able to rinse their mouths with distilled water whenever they wished between the trials. This experiment was performed on a separate day, but at the same time of day as the ingestion test.

#### 2.4.3. The Saxon test

The Saxon test was performed to measure the whole mouth saliva secretion during chewing. The stimulated salivary flow rate was measured in eight out of 10 subjects. This experiment was also performed on a separate day but at the same time of day as the ingestion test. The subjects were asked to chew a gauze sponge for 2 min, and the difference in the weight of the sponge before and after chewing was calculated; this weight represented the amount of stimulated saliva production and reflected the function of the parotid glands (rather than unstimulated salivation, which is predominantly from the submandibular glands) [28]. The relationship between the salivary flow rate and chewing duration, EMG activity, changes in the physical property, and EMG activity were investigated.

### 2.5. Statistical analysis

Statistical analysis was performed using Sigmaplot software (Sigmaplot 12.0, Systat

Software Inc., CA, USA). The paired *t*-test, Wilcoxon signed rank test, and repeated measures two-way analysis of variance were used for statistical evaluation of the data. Tukey's HSD test was used for further analysis (the choice of test was made by the software). *P* values < 0.05 were considered significant. All values were expressed as means  $\pm$  SD (*n* = 10 for the ingestion test and spitting test, and *n* = 8 for the Saxon test).

#### 3. Results

#### 3.1. Ingestion test

All of the participants freely ate the foods in their accustomed manner and did not report any difficulty in performing the tasks. Intake of the food bolus was represented by a large Hyoid burst of activity, followed by the start of chewing (Fig. 1). Although the chewing side could not be determined from the EMG recordings alone, the rhythmic pattern of Mas EMG activity during chewing was stable on both sides, while the pattern of Hyoid activity was variable in each cycle (Fig. 1). Swallowing was clearly distinguishable and occurred not only at the end of the sequence, but also between the chewing cycles.

Variables obtained from the sequence of mastication until the first swallow were compared between the foods. The chewing duration was significantly longer and the number of chewing cycles significantly larger for the rice cake than for steamed rice (Fig. 2). In contrast, the chewing cycle time was not different between the foods (Fig. 2). The total EMG activity of the Mas and Hyoid was significantly greater for the rice cake than for steamed rice (Fig. 3); this result was expected, as the chewing duration and cycles were significantly larger for the rice cake than for steamed rice.

Characteristics of chewing and EMG activity per cycle were then compared

between the foods and among the substages. The Mas activity was significantly larger for rice cake than for steamed rice at each substage (Fig. 4). Hyoid activity was also significantly larger for rice cake than for steamed rice. In addition, Hyoid activity significantly decreased as chewing progressed for both the steamed rice and rice cake (Fig. 4). Changes in chewing cycle time significantly decreased in the chewing process for both the steamed rice and rice cake (Fig. 5). There was no difference in chewing cycle time between the foods.

#### 3.2. Spitting test

Hardness increased at the early stage in both foods, then gradually decreased until swallowing (Fig. 6); the hardness at the late stage had returned to the pre-chewing baseline level. There was a significant difference in change in adhesiveness between the foods; adhesiveness increased greatly, and was larger until swallowing for the rice cake than for steamed rice (Fig. 6). Although adhesiveness gradually increased for steamed rice, there was no significant difference among the substages. There was no significant change in cohesiveness in each food throughout the substages, although cohesiveness was significantly larger for the rice cake than for steamed rice (Fig. 6).

### 3.3. The Saxon test

The mean value of the stimulated salivary flow rate was  $5.12 \pm 1.39$  g/2 min (range 4.13 to 8.26 g/2 min). A positive correlation was noted between the stimulated salivary flow rate and change in Mas activity for both the steamed rice and rice cake (Fig. 7). The more the Mas activity decreased in the chewing process, the greater the stimulated salivary flow. There were no other significant correlations between the salivary flow

rate and any variable.

#### 4. Discussion

The current study demonstrated the Mas and Hyoid EMG activity during chewing of steamed rice and rice cake in a natural manner. There were large differences in the chewing duration and number of chewing cycles between the foods with similar hardness, but not in the chewing cycle time. The Mas and Hyoid EMG activity per chewing cycle for the rice cake was significantly larger than for steamed rice throughout the recording periods. While the Mas activity did not change among the substages during chewing, the Hyoid activity decreased as chewing progressed. This was also the case for chewing cycle time, which gradually decreased but was not significantly different between the two foods. Time-dependent changes in hardness, adhesiveness, and cohesiveness differed between the two foods. The hardness of both foods increased at the early stage, then gradually decreased back to baseline. Adhesiveness of the rice cake increased at the early stage and did not fall throughout the recording period, while adhesiveness did not significantly change for steamed rice. Cohesiveness barely changed for the two foods during chewing, but was significantly greater for the rice cake than for steamed rice. Finally, a correlation between the stimulated salivary flow rate and chewing performance was noted only in the changes in Mas activity. Functional consideration of chewing behavior for the two different foods and the effect of salivation on food reduction is discussed below.

4.1. *Differences in chewing duration and EMG activity between the foods* In the present study, the chewing duration and number of chewing cycles were

significantly larger for the rice cake than for steamed rice. In addition, the Mas and Hyoid EMG activity were larger for the rice cake than for steamed rice; this difference in the EMG activity was expected, as the total EMG performance may be directly related to the total duration and number of chewing cycles.

It has been previously reported that chewing performance depends on the hardness [6, 12, 17, 34-38] and particle size [39-43] of foods. It is accepted that chewing behavior adapts to changes in the hardness of the bolus in such ways as the number of chewing cycles, sequence duration, and EMG activity; the harder the bolus is, the larger the number of cycles, the longer the sequence duration, and the larger the EMG activity. The ratio of the square roots of toughness and modulus of elasticity was proposed to best express the resistance to food breakdown during mastication [18, 44], and differences in masticatory parameters between several model foods have been related directly to their hardness [45, 46]. These were not observed in the present study, as the hardness of the two foods was similar. It should be noted that the initial cohesiveness and time-dependent changes in adhesiveness of the rice cake used in the present study was significantly different to those of steamed rice. The cohesiveness is the degree to which a material can be deformed before it breaks, which suggests that difference in cohesiveness has a critical effect on masticatory performance [23]. Thus, not only the hardness but also other parameters such as cohesiveness should be considered to determine the chewing behavior, including chewing duration and number of chewing cycles before the swallow.

We found no significant differences throughout the masticatory sequence in the chewing cycle time between the two foods. The chewing rhythm is centrally regulated, and is one of the parameters used for evaluating the reproducibility of the individual

performance [19, 45]. Furthermore, the rhythmicity remains constant even when the physical property of the food is changed [6, 23, 35]. These findings are basically consistent with those of the present study. However, the stability and reproducibility of chewing cycle time may be kept in a certain range of food texture; a previous study showed that chewing cycle time differed between two different types of steamed rice with different physical properties [22]. Thus it appears that chewing cycle time is the only parameter of mastication that does not adapt to a change in rheological property of the food [47].

4.2. Time-dependent changes in EMG activity and physical property of foods In the present study, we divided the chewing performance until the first swallow into three substages according to previous animal [48] and human [17, 49] studies. Hori et al. recorded tongue pressure against the palate as well as jaw movement during mastication in humans, and found that the magnitude and duration of the pressure were changeable and significantly larger in the late stage of chewing (eight strokes before initial swallowing) than in the early stage (until eight strokes after the start of mastication) [50]. During chewing, both breaking down of the food and creation of a bolus are needed for swallowing; so in the process of deformation of food, masticatory performance should adapt not only cycle by cycle but also in the process of bolus formation. However, few attempts have so far been made to describe the changes in EMG patterns in the process of bolus formation during chewing, and to compare the characteristics of bolus formation regarding texture and consistency.

In the present study, we found that not only the Mas but also the Hyoid EMG activity per cycle was larger for the rice cake than for steamed rice. For the Mas, there

was no difference among the substages. As describe above, chewing performance including jaw-closing muscle activity basically depends on the hardness of foods. Although initial hardness and its changes during chewing were almost the same between the two foods, there was a small but significant difference in the Mas EMG activity. This may be because cohesiveness was significantly larger for the rice cake than steamed rice throughout the recording period. Again, the cohesiveness is the degree to which a material can be deformed before it breaks, which suggests that difference in cohesiveness has a critical effect on not only the whole masticatory sequence but also on chewing performance per cycle [23].

Basically, Hyoid EMG activity is less modulated by changes in the condition of the bolus over a certain range during chewing, suggesting that those activities were not affected by the peripheral inputs in functions [51]. The significant difference in Hyoid EMG activity between the two foods in the present study may be explained by the fact that they have different levels of adhesiveness. Adhesiveness represents the force required to remove the food material that adheres to the mouth. As the water content increases owing to salivary secretion as chewing progresses, the adhesiveness may also increase. Since the Mas EMG activity was significantly greater for the rice cake than for steamed rice, it can be assumed that the salivary flow rate was also larger for the rice cake than steamed rice owing to the masticatory-salivary reflex [52, 53]. We suggest that the greater the adhesiveness of food, the greater the Hyoid EMG activity.

However, we also found that the Hyoid EMG activity gradually decreased, although the adhesiveness was not different among the substages. The basic pattern of Hyoid activity is determined not only by the adhesiveness, but also by the size or other parameters of the food [23]. The volume of the food used in the present study was large:

15 g of each type. Although the bolus physical conditions might not affect the muscle activity, the large bolus should at least require a large jaw opening at the beginning of chewing; hence, the Hyoid EMG activity may depend on the size but not the physical properties of the food. The particle size distribution of the food bolus is shifted toward coarser particles [3, 35]. Further studies are warranted to determine how the food bolus is broken down, and to examine the differences in the physical properties of the bolus, including not only its particle size and hardness but also such other features as fabrication, cohesiveness, and adhesiveness.

Interestingly, changes in chewing cycle time also decreased with decrease in Hyoid EMG activity. It was previously reported that masticatory rhythm is mainly controlled by the activity pattern of the jaw-opening muscles [5]. Although we did not evaluate the relationship of changes between Hyoid EMG activity and chewing cycle time for each cycle, dynamic changes in the physical properties such as adhesiveness and lubrication may determine the pattern of Hyoid EMG activity and hence chewing cycle time.

In the present study, only change in Mas EMG activity was affected by the stimulated salivary flow rate; the faster the salivation, the more the Mas EMG activity decreased as chewing progressed. Since it has been suggested that the salivary stimulus to modulate the physical conditions or surface lubrication of the bolus is also important [54], a faster salivary rate would flood the bolus earlier. The main effects of a higher salivary flow rate are to produce a smaller peak cohesive force after fewer chews, and a more rapid diminution of the cohesive force after the peak has been reached. Hutchings and Lillford described the possible factors of food perception in preparation for swallowing: texture perception is a dynamic sensory monitor of changes made to food

by processes occurring in the mouth and pharynx [3].

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# Disclosure

The authors declare that they have no conflicts of interest.

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#### **Figure legends**

# Fig. 1 Example of simultaneous electromyographic recordings during mastication of steamed rice and rice cake

The traces represent the raw data. Arrowheads indicate the swallowing event. In this case, the subject swallowed four times during steamed rice chewing and three times during rice cake chewing. Note that the chewing duration was much longer for the steamed rice than for rice cake. E: early stage; M: middle stage; L: late stage; EGG: electroglottograph; Rt-Mas: right masseter; Lt-Mas: left masseter; Rt-Hyoid: right suprahyoid; Lt-Hyoid: left suprahyoid.

#### Fig. 2 Total chewing duration, number of chewing cycles, and chewing cycle time

Total chewing duration was  $26.1 \pm 10.8$  sec for steamed rice, and  $40.6 \pm 21.8$  sec for rice cake. The number of chewing cycles was significantly lower for steamed rice (36.5  $\pm$  11.4) than for rice cake (57.6  $\pm$  26.4). Chewing cycle time for steamed rice and rice cake was  $0.77 \pm 0.16$  sec and  $0.75 \pm 0.14$  sec, respectively; there were no significant differences between the foods. \**P* < 0.01.

#### Fig. 3 EMG activity until the time of first swallow

The respective EMG activity during chewing of the steamed rice and rice cake was 0.87  $\pm 0.50 \text{ mV} \cdot \text{s}$  and  $1.49 \pm 0.75 \text{ mV} \cdot \text{s}$  for Mas, and  $0.71 \pm 0.39 \text{ mV} \cdot \text{s}$  and  $1.29 \pm 0.86$  mV  $\cdot \text{s}$  for Hyoid. They were both significantly different. \*\*\**P* < 0.001, \*\**P* < 0.01.

#### Fig. 4 EMG activity per cycle until the time of first swallow

For steamed rice, the respective Mas EMG activity per cycle in the early, middle, and late substages was  $0.026 \pm 0.009 \text{ mV} \cdot \text{s/cycle}$ ,  $0.024 \pm 0.013 \text{ mV} \cdot \text{sec/cycle}$ , and  $0.024 \pm 0.014 \text{ mV} \cdot \text{s/cycle}$ . For rice cake, the respective Mas EMG activity pre-cycle in the early, middle, and late substages was  $0.029 \pm 0.013 \text{ mV} \cdot \text{s/cycle}$ ,  $0.027 \pm 0.012 \text{ mV} \cdot \text{s/cycle}$ , and  $0.027 \pm 0.011 \text{ mV} \cdot \text{s/cycle}$ . There was a significant difference in EMG activity per cycle between the two foods, but not among the substages.

The respective Hyoid EMG activity per cycle in the early, middle, and late substages for steamed rice was  $0.023 \pm 0.009 \text{ mV} \cdot \text{s/cycle}$ ,  $0.019 \pm 0.009 \text{ mV} \cdot \text{s/cycle}$ , and  $0.018 \pm 0.009 \text{ mV} \cdot \text{s/cycle}$ . For rice cake, the respective Hyoid EMG activity per cycle in the early, middle, and late substages was  $0.026 \pm 0.008 \text{ mV} \cdot \text{s/cycle}$ ,  $0.021 \pm 0.007 \text{ mV} \cdot \text{s/cycle}$ , and  $0.020 \pm 0.007 \text{ mV} \cdot \text{s/cycle}$ . There was a significant difference between the early and middle substages, between the early and late substages, and between the two foods. \*\*\**P* < 0.001, \*\**P* < 0.01.

#### Fig. 5 Time sequence-dependent changes in chewing cycle time

The respective chewing cycle time for steamed rice in the early, middle, and late substages was  $0.77 \pm 0.16$  s,  $0.68 \pm 0.12$  s, and  $0.66 \pm 0.12$  s; there was a significant difference between the early and middle substages, and between the early and late substages. The respective chewing cycle time for rice cake in the early, middle, and late substages was  $0.75 \pm 0.14$  s,  $0.69 \pm 0.12$  s, and  $0.68 \pm 0.08$  s; there was a significant difference between the early and late substages. No significant difference was noted between the foods. \*\*\*P < 0.001, \*\*P < 0.01.

#### Fig. 6 Time sequence-dependent changes in physical property of the food

For steamed rice, the respective hardness in the pre (before chewing), early, middle and late substages was  $9,337 \pm 1,144 \text{ N/m}^2$ ,  $20,145 \pm 6,507 \text{ N/m}^2$ ,  $15,146 \pm 4,250 \text{ N/m}^2$  and  $8,941 \pm 4,986$  N/m<sup>2</sup>. For rice cake, the respective hardness in the pre (before chewing), early, middle and late substages was  $9,920 \pm 1,527 \text{ N/m}^2$ ,  $19,068 \pm 12,411 \text{ N/m}^2$ ,  $10,326 \pm$  $\pm$  3,642 N/m<sup>2</sup>, and 7,858  $\pm$  4,736 N/m<sup>2</sup>. In both cases, hardness increased at the early stage and then gradually decreased back to baseline. There was a significant difference between the early stage and the other stages in both the steamed rice and rice cake. There was also a significant difference between the foods at the middle stage. For steamed rice, the respective adhesiveness in the pre, early, middle and late substages was  $100 \pm 37 \text{ J/m}^3$ ,  $224 \pm 62 \text{ J/m}^3$ ,  $275 \pm 93 \text{ N/m}^2$ , and  $396 \pm 239 \text{ N/m}^2$ . For rice cake, the respective adhesiveness in the pre, early, middle and late substages was  $214 \pm 70$  $J/m^3$ , 1,064 ± 268  $J/m^3$ , 1,074 ± 279  $J/m^3$ , and 863 ± 342  $J/m^3$ . For rice cake, adhesiveness increased at the early stage and was significantly larger than for steamed rice throughout the recording period; while for steamed rice, the adhesiveness gradually increased, although there was no significant difference among the substages. For steamed rice, the respective cohesiveness in the pre, early, middle and late substages was  $0.39 \pm 0.06$ ,  $0.38 \pm 0.05$ ,  $0.39 \pm 0.04$ , and  $0.41 \pm 0.06$ . For rice cake, the respective cohesiveness in the pre, early, middle and late substages was  $0.64 \pm 0.07$ ,  $0.60 \pm 0.08$ ,  $0.61 \pm 0.07$ , and  $0.61 \pm 0.09$ . There was a significant difference in cohesiveness between the foods at each stage, but not among the substages for both foods.

# Fig. 7 Relationship between decrease in Mas activity and stimulated saliva flow rate

Decrease in Mas activity was defined as the difference in the Mas activity between the

early and late stages. In the case of both the steamed rice and rice cake, there was a significant correlation between decrease in Mas activity and stimulated saliva flow rate.













