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Effect of texture on *in vivo* oral mastication kinetics of foods

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Abstract
Food oral mastication is a dynamic bio-mechano-chemical process in which the food is chewed with the incorporation of saliva in order to form the moistened bolus with swallow able consistency. In this study, *in vivo* oral mastication attributes have been evaluated for the foods having different texture, and the effect of oral mastication on the texture and particle size distribution. Four sample foods (wheat bread, cake, cheese wafer, and high fibre cookies) are selected to analyse the *in vivo* oral mastication kinetics. The experiments reveal that the texture of the food product has a significant influence on the oral mastication attributes such as total consumption time, saliva incorporation rate, solid loss and chewing rate. The results of this study provide insights for the development of a dynamic *in vitro* oral mastication system and the parameters to be taken into consideration in the system design.

Keywords: Oral processing, *in vivo*, texture, granulometry, mastication.

Introduction

Understanding the food DE structuring kinetics during food digestion has been identified to be an important phenomenon of determining the nutrient absorption in humans (Capuano & Pellegrini, 2019) [4]. Food DE structuring in oral cavity is an important process during which the food is broken down to finer fraction where a significant amount of macronutrient digestion also take place. This indicates that, the oral mastication can modulate the nutrient release from the complex food matrices (Chen *et al*., 2022) [5]. The oral processing behaviour of different foods can be typically defined based on two opposing mechanical influences namely forces that fracture the food particles and the forces that make the fragmented particles adhere together for the formation of agglomerated mass. Thus, the mechanical properties of the foods have a major influence on the DE structuring of foods in the oral cavity as reported by (Lucas, Prinz, Agrawal, & Bruce, 2004) [13]. The mechanics of food fracture are used in the rational design of initial structure of foods (hard/firm/soft) which is measured through textural measurements (Witt & Stokes, 2015) [20]. It is also important to note that the textural perception after the first bite has a major influence on consumer acceptability.

In vivo oral mastication of foods is an important field of research, as the *in vivo* mastication dynamics needs to be explored for the different food samples. *In vivo* oral mastication kinetics of foods have been studied in human subjects for various purposes such as determining the mechanism of salivary amylase incorporation (Mosca & Chen, 2017) [14] on the complex carbohydrate structures, salivary responses to various stimulants and to estimate the masticatory performance (Hama *et al*., 2017) [6]. It also needs to be noted that the inter-individual differences in the oral mastication dynamics also needs to be considered while an *in vitro* simulation system is developed to study the oral mastication dynamics (Ranawana Viren, Henry, & Pratt Megan, 2010) [19].

Chewing in the oral cavity is primarily influenced by various properties of food especially the texture (Horio & Kawamura, 1989) [7], internal microstructure (Bellisle, 2020) [3], initial particle size (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007) [10], moisture content (Hutchings *et al*., 2011) [8], water uptake (Le Bleis, Chaunier, Montigaud, & Della Valle, 2016) [12], cohesiveness, adhesiveness (Iguchi *et al*., 2015) [9]. The mechanical properties of the foods play a major role in the oral disintegration of foods, as the required mechanical force applied through teeth is mainly based on the mechanical properties of foods (Pascua, Koç, & Foegeding, 2013) [17]. Thus, it is essential to understand the texture dependent disintegration behaviour of foods.

This study focuses on investigating the *in vivo* oral processing behavior of foods with different Texture, in order to understand the bolus forming kinetics and mastication dependent particle break down kinetics. 5 model foods such as cooked rice, bread, cake, cheese wafer and high fibre cookies were chosen to study the properties of food and bolus during *in vivo* oral processing. This study can provide insights into the feasibility and challenges associated in conducting the *in vivo* experiments to establish the food disintegration dynamics and the breakdown assisted changes in food when it forms a bolus with swallow able consistency.

Materials and Methods

Materials

Parboiled rice (variety: White ponni), wheat bread, cake, cheese wafer and high fibre cookies were purchased from local supermarket at Thanjavur. The samples were selected based on their levels of firmness which is expected to influence the bolus forming kinetics. All the samples were used without any further processing.

Sample preparation

The parboiled rice grains were washed using the water for the removal of foreign matter if any. The rice was then soaked for a period of 30 min and then the rice was cooked for a period of 15 min with 3:1 (v/v) of water to rice ratio. Induction stove (Prestige, PIC 6.1 V3 1600 W) was used to cook the sample at the temperature of 110-115 °C. The remaining water post cooking was strained using a water strainer and the cooked rice sample is left at room temperature for cooking down (Anandharamakrishnan, Sethupathy, Sivakamasundari, & Moses, 2021) [2].

Texture of foods

Using a 10 kg load cell and the Texture Profile Analyzer TA. HD Plus (Stable Microsystems, UK), initial firmness of the food samples was assessed. A skin puncture strength test using P/5 cylindrical probe was conducted to measure the force required to puncture the surface of the food sample which is termed as the firmness of the food (represented in kg f or N). For the cooked rice samples, the sample was filled in a beaker and the puncture strength analysis was conducted. The test conditions for texture profile analysis are as follows, 2 mm/s pre-test speed, 3 mm/s test speed and 2 mm/s post-test speed with a data acquisition rate of 200 pps.

Selection of subjects for *in vivo* oral mastication studies

For *in vivo* experiments, 25 healthy volunteers (15 males and 10 females, all between the ages of 20 and 30) were chosen. The subjects had a pre-screening to make sure they were in good physical condition, had healthy natural teeth, had no significant dental issues, and had no trouble swallowing or chewing. Additionally, it was made sure that no drugs that could have affected salivary flow or muscle function had been taken prior to the trials. Before the study, each participant signed an informed consent form.

In vivo oral mastication attributes of food samples

For the mastication trials, subjects are given with a known portion of food sample. While rice was served freshly cooked for *in vivo* testing, commercial samples were served immediately without any further processing. The sample was given to the subjects to chew on until they start to swallow. When the bolus reached the proper consistency for swallowing, each subject spit it out while keeping track of the number of chewing cycles and chewing time. Different metrics were calculated from the obtained bolus. The hot air oven method (115 °C for roughly 5 hours) was used to measure the moisture content of the bolus; further information on other parameters and methodologies is provided in the following sections. Each participant counted their chews. The number of bites the subjects took (counting from the first bite) until the desired chewing stage (a bolus with swallow able consistency) was attained was inquired of them. All the participants were informed about the experimental procedure and the written consent were obtained. During oral processing trials, mastication characteristics like the no. of chewing cycles and total chewing time were tracked. Other mastication characteristics were identified, including chew cycle duration (Eq. 1) and eating rate (eq 2) (Aguayo-Mendoza *et al*., 2019; Oladiran, Emmambux, & de Kock, 2018) [1, 15]. The boluses were then used for the solid loss estimation (Eq. 3) (Anandharamakrishnan *et al*., 2021) [2].

$$\text{Chew cycle duration} = \frac{\text{food consumption time (s)}}{\text{number of chews}} \quad (1)$$

$$\text{Eating rate } \left(\frac{g}{s}\right) = \frac{\text{weight of food consumed (g)}}{\text{food consumption time (s)}} \quad (2)$$

$$\text{Solid loss during mastication (\%)} = \frac{\text{dry matter weight of food} - \text{dry matter weight of bolus}}{\text{dry matter weight of food}} \quad (3)$$

Properties of masticated boluses

Granulometry

Granulometry analysis based on the dry manual sieving method was performed to estimate the average particle size distribution of the unthawed model foods and *in vivo* collected boluses. The bolus was initially put onto a 0.3 mm muslin towel and rinsed under running water to spread the particles out and remove any residual saliva. The cleaned bolus was dried in a hot air oven for two hours at 45 degrees. The features of the produced bolus are the reason for the changes in the drying time needs compared to hard solid foods. The dried bolus was manually sieved by placing it on a stack of six sieves with openings of 2, 1, 0.710, 0.355, 0.125, and 0.09 mm from Jayant Scientific Industries in India. Then, the material that remained on each sieve was weighed. The weight was established in a cumulative curve as a proportion of cumulative particles to reflect the particles that pass through each sieve. The median particle size (D50) was calculated using the cumulative curves that were produced. D_{50} is the theoretical sieve opening that permits 50% of the bolus mass to pass through.

Texture

Using a Texture profile analyzer TA. HD Plus (Stable Microsystems, UK) with a 10 kg load cell, the texture characteristics of the food bolus samples were determined. Using a P/35 (35 mm diameter) cylindrical Aluminum probe with 1 g/mm of test force and 5 g/min of speed in a two-bite test with compression for 50% of starting strain, textural characteristics including hardness, cohesiveness, springiness, adhesiveness, gumminess, etc. were assessed. The acquired force-time curves were used to determine the textural characteristics (Rosenthal, 2010) [22]. For boli that resemble fluids, the boluses were put in a 50 mL standard beaker with a 5 mm sample height and examined under the similar conditions. Hardness was defined as the highest stress (determined by dividing the force by the plunger's bottom area) experienced during the initial compression. The area over the negative stress-strain curve following the initial compression, which reflects the work per unit volume, was used to calculate adhesiveness.

Statistical analysis

The experimental data were statistically analysed using one-way ANOVA (SPSS ver. 25; IBM Inc., Armonk, NY, USA). Duncan post-hoc test was used to determine significance, and a probability threshold of 0.05 was regarded as significant. Results from each experiment were triple checked, and the mean and standard deviation were used to express the data.

Results and discussion

Texture of foods

According to the analysis of skin puncture strength performed using a texture analyser, cooked rice, wheat bread, and cake have a firm texture, whereas cheese wafers and high-fibre biscuits have a hard texture (Table 1). For the simplicity of comparing the oral mastication qualities in the following sections, the model foods are categorised as hard, firm, and soft foods based on these observations. The minimum hardness of cooked rice is 1.12 kg. While higher amounts of hardness are seen in high-fibre biscuits.

Table 1: Texture of food samples

Sample	Hardness (kg f)
Cooked rice	1.12±0.35^{a}
Idli	1.02±0.04^{b}
Wheat bread	1.17±0.50^{a}
Cake	1.68±0.50^{c}
Cheese wafer	2.16±0.21^{d}
High fibre biscuits	5.68±0.68^{e}

Significant difference between the values is indicated by different letters in the column

In vivo mastication parameters

The influence of texture and granulometry on mastication parameters was studied (Table 2). Bite-size of the samples were found to be 8.35±1.25 g (cooked rice), 12.56±2.21 g (wheat bread), 6.5±1.25 g (high fibre biscuits) and 4.26±0.98 g (wafer biscuit). However, in order to ensure the comparison between the samples a bite size of 5 g was considered for all the samples. The higher chew cycles (number of chews) and consumption times were seen in the high-fibre bread, cheese wafer, cake, and biscuits. This can be attributed to the presence of the levels of hardness and the inner structure of foods. It was observed that the samples with higher hardness leads to the higher number of masticatory cycles. Bread and cake were observed with higher number of masticatory cycles though having the firm texture. This is attributes due to the presence of air cells at the inner porous structure of the sample which makes the need of more cycles for uniform incorporation of saliva and succeeding formation of the boluses. The similar results of high number of masticatory cycles were observed for bread as compared to brittle foods. For the cooked rice, the mastication time was little longer as compared to other soft textured foods which may be due to higher cohesiveness and adhesiveness of the cooked rice which results in longer chewing times (Park, Kim, Lee, & Park, 2017) [16]. The chewing rate was found to be higher for found to be higher for cooked rice followed by high fibre biscuit, wheat bread, cake and wafer biscuits. From all the observations, it is evident that the wafer biscuits require minimum mastication time and number of chews which is attributed due to their creamy internal structure and firm outer layer which makes it swallow able with minimum number of chews.

The rate of saliva incorporation is another important phenomenon that defines the oral processing behaviour of different foods. The saliva incorporation rate for different food samples is given in table 2. Though there is a close correlation observed between saliva incorporation of samples in relation to their texture, the levels of saliva incorporation are also being affected based on the internal structures of the food matrices as observed from the experiments.

Table 2: *In vivo* mastication parameters of different foods

Parameter	Cooked rice	Wheat bread	High fiber biscuit	Cheese wafer	Cake
Total chewing time (s)	12.56±1.25^{a}	30.28±2.45^{b}	38.02±1.98^{d}	33.45±2.25^{c}	28.76±1.85^{b}
Number of chews per portion	18.58±2.56^{a}	40.25±6.15^{b}	49.25±2.50^{c}	51.50±8.75^{c}	41.00±2.50^{b}
Rate of saliva incorporation	0.08±0.00^{a}	0.04±0.011^{c}	0.02±0.005^{b}	0.01±0.002^{b}	0.03±0.006^{c}
Chewing rate (chews/s)	1.48±0.70^{d}	1.33±0.07^{c}	0.82±0.12^{b}	0.79 ±0.15^{a}	0.83±0.18^{b}
Average particle size (D_{50})	1.23±0.05^{c}	0.28±0.02^{a}	0.36±0.03^{b}	0.26±0.01^{a}	0.42±0.01^{b}

Significant difference between the values is indicated by different letters in the rows

Bolus properties

Median particle size

The obtained bolus samples at the swallow able consistency are washed and each bolus sample is sieved through a set of sieves in the running water for the removal of saliva and disintegrate the agglomerated bolus fraction (Raja, Priyadarshini, Moses, & Anandharama Krishnan, 2022) [18]. During this process the individual particles that have got disintegrated retains under each sieve. The retained particles of food are then washed with ethanol (99.9%) for further separation of the particles. Then the separated particles are kept on a petri plate and the median particle size of the bolus samples were measured by plotting the cumulative particle size distribution curves. The average particle size of the samples was also measured by image processing technique using Image J software.

The results (table 3) reveal that the cooked rice samples were found with maximum median particle size after mastication. Sieve examination revealed the average particle size (D50; mm), while software-based particle size analysis revealed comparable tendencies. Additionally, it was discovered that all five types' expectorated boluses had significantly different average particle sizes from one another. D_{50} (mm) of cooked rice and high fibre biscuits were 1.23±0.05 mm and 0.36±0.01

mm, respectively.

Table 3: Median particle size of oral masticated boluses of food samples

Sample	Median particle size (mm)
Cooked rice	1.23±0.05^{e}
Wheat bread	0.28±0.02^{b}
Cake	0.42±0.03^{d}
Cheese wafer	0.26±0.02^{a}
High fibre biscuit	0.36±0.02^{c}

Significant difference between the values is indicated by different letters in the columns

Texture

The texture profile of masticated boluses determined using two bite tests are given in Table 4. The cohesiveness and adhesiveness of all the masticated boluses vary significantly based on the initial hardness of the samples, quantity of total saliva incorporated and average particle size of the final masticated boluses. Out of the studied model foods, cake and bread were identified to have higher levels of cohesiveness and adhesiveness, which would have been resulted due to the presence of high levels of starch in those matrices as compared to the other model foods. Starch rich foods when masticated undergoes the incorporation of saliva at higher rates and result in the formation of adhesive and slippery bolus.

Considering the textural attributes of high fibre biscuit masticated boluses, the cohesiveness, chewiness and gumminess were found to be less as compared to the other foods. This may be due to the presence of higher levels of fibre content which results in the formation of bolus that is not completely agglomerated. Similar results of increase in adhesiveness were reported by (Wada *et al*., 2017) [23]. Also, the cohesiveness is another important phenomenon representing the degree of bolus formation, as the bolus formation primarily involved the development of sticky cohesive agglomerated fraction of food. The cohesiveness of cooked rice (0.612±0.08), bread (0.586±0.06) and cake (0.546±0.05) were higher. Whereas, for low moisture foods such as wafer biscuit and high fibre biscuit, the cohesiveness levels were lesser. Cohesiveness can also be related to the hydration ratio of foods as reported by (van Eck *et al*., 2019).

Table 4: Texture profile of masticated boluses

Sample	Hardness (N)	Adhesiveness (N.s)	Cohesiveness
Cooked rice	1.36±0.02^{a}	-2.48±0.06^{a}	0.612±0.08^{a}
Wheat bread	1.28±0.02^{b}	-2.29±0.05^{b}	0.586±0.06^{a}
Cake	1.31±0.03^{a}	-2.46±0.06^{a}	0.546±0.05^{b}
Cheese wafer	1.42±0.03^{c}	-1.85±0.03^{c}	0.421±0.03^{c}
High fibre biscuit	1.56±0.05^{d}	-0.98±0.02^{d}	0.392±0.02^{d}

Significant difference between the values is indicated by different letters in the columns

Effect of texture on *in vivo* mastication parameters of model foods

Total chewing time

Total chewing time has been found to be influenced by the hardness (Fig 1). High fibre biscuit having higher hardness have the maximum chewing time whereas, the cooked rice having lower hardness have been determined to have the lower levels of hardness. However, for bread having lower hardness is observed with higher chewing time which would have been due to the porous internal structure which require more masticatory cycles for the processing of food.

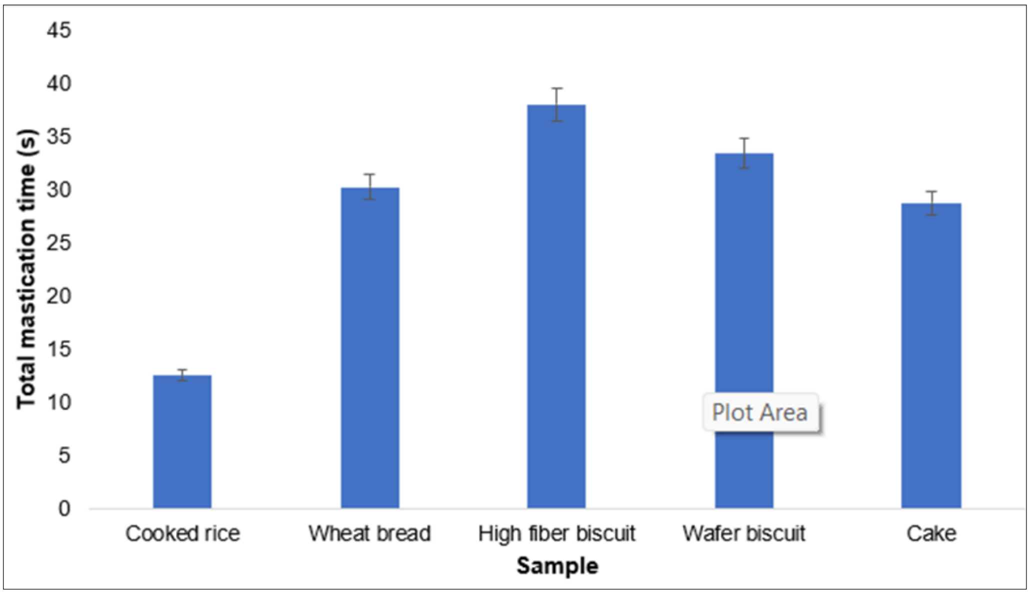

Fig 1: Total mastication time of different food samples

Rate of saliva incorporation

The quantity and rate of saliva incorporation was also found to be significantly influenced by the hardness of the samples. The samples with firm texture tended to have a higher quantity of saliva incorporated at a faster rate (Fig 2). However, cheese wafers having slightly higher hardness as compared to bread and Cake was found to produce lesser saliva at a very slower rate which may be attributed to their adhesive internal structure. It is also evident that the cooked rice tends to produce maximum levels of saliva during chewing. The high wafer biscuits required higher levels of saliva to be incorporated for mastication as they are dry and require more moisture to lubricate the food to reach its swallow able consistency. Similar results were reported by (Khalil *et al*., 2015) [11].

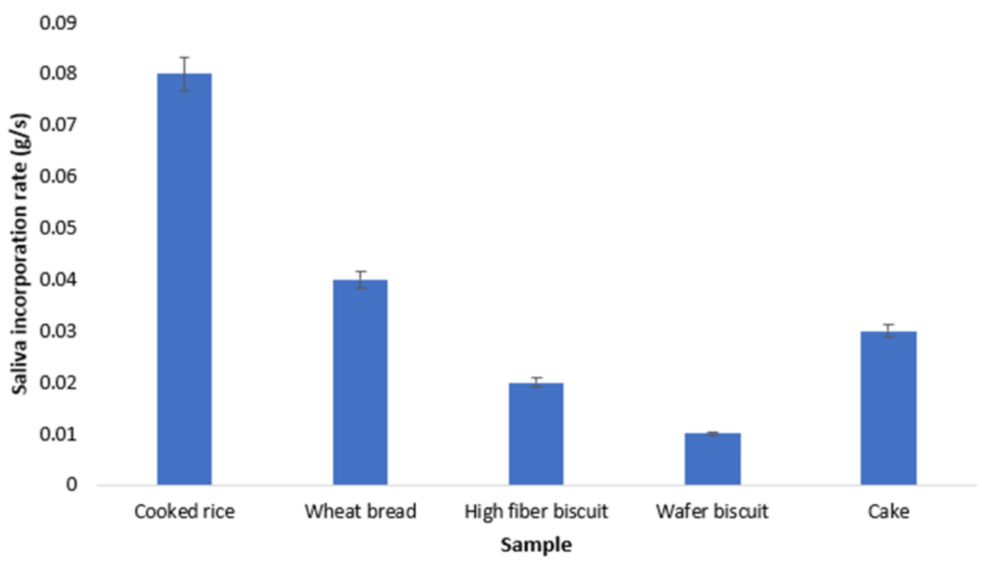

Fig 2: Rate of saliva incorporation vs chewing time

Conclusion

This study looked into how different food materials' oral mastication characteristics were impacted by their texture. Although it was found that the kinetics of mastication and bolus formation were directly controlled by food hardness, the microstructural characteristics of the food samples also had a substantial impact on the rate of saliva incorporation and other masticatory parameters. In order to comprehend oral mastication kinetics in humans and their effects on the pattern of food digestion in the human gastro-intestinal tract, it is crucial to have a grasp of how texture affects oro-sensory perception of various food items.

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