

Fundamental Studies on Melt-in-mouth Textures of Baked Flour Products

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(小麦粉焼成品の口どけ感に関する基礎的研究)

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Abstract

Baked flour product such as bread, pancake is widely consumed food in the world. The expectations of consumers in terms of the quality, freshness, and nutritional and sensory properties of baked flour products have been increasing. Consequently, baked flour products quality and consumer acceptance are very important for companies maintaining and expanding their markets. Recently, "melt-in-mouth" has been used in industry of baked flour product as a sensory characteristic for advertising the quality of the products. "Melt-in-mouth" is also considered as physical properties which have changed in the mouth during chewing and has a high value for baked flour products quality analysis.

The objective of this dissertation was to obtain an insight into investigate "melt-in-mouth" textures of baked flour products and "melt-in-mouth" sensory attributes in bread. The final goal was to supply a scientific research results for applying "melt-in-mouth" on controlling quality of baked flour products.

I. Tongue and maxillary palatal models were used to investigate melt-in-mouth textures of baked flour products. Heat moisture treated flour and the compression area of food bolus between tongue and maxillary palate were varied. We found that the food bolus compression area was correlated, not with melt-in-mouth texture, but with food bolus cohesiveness during chewing. We conclude that the food bolus compression area is related to food bolus cohesiveness.

Keywords: baked flour product, heat moisture treatment, melt-in-mouth texture, food bolus compression area, cohesiveness

II. This study aimed to investigate "melt-in-mouth" sensory attributes in bread using four samples: freshly baked and after 1, 3 and 7 days of refrigerated storage (5 °C). The "melt-in-mouth" sensory attributes were evaluated as the intensities of "ease of dissolving in saliva" and "ease of swallowing", which both decreased during storage. The crumb firmness as measured by its elastic properties increased during storage but the water dissolution and water swelling rates of baked bread powder and starch powder decreased significantly. X-ray diffractograms of the baked bread powder showed that as refrigerated storage time increased starch retrogradation increased. During storage, starch retrogradation caused the crumb water absorption to decrease and the crumb firmness to increase, resulting in greater difficulty in dissolving in saliva and in swallowing, respectively.

Keywords: "melt-in-mouth", sensory evaluation, bread quality, starch retrogradation.

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Contents

List of Publications

Paper I: Nguyen Thi Huong Lan, Takahisa Nishizu, Yukihiro Iida, Akitoshi Katsumata, Noriko Kitaori, Lifen Zhang, Aiko Inukai, Kiyokazu Goto (2013). Melt-in-mouth Textures of Baked Flour Products and Their Compression Deformations between The Tongue and Palate. Journal of the JAPANESE SOCIETY OF TASTE TECHNOLOGY 12(1)10-15.

Paper II: **Nguyen Thi Huong Lan**, Manami Shibata, Takahisa Nishizu, Kiyokazu Goto (2013). The effects of starch retrogradation on the "melt-in-mouth" sensory evaluation of bread. Journal of the JAPANESE SOCIETY OF TASTE TECHNOLOGY. Accepted

List of Tables

List of Figures

Introduction

Baked flour product such as bread is widely consumed food in the world (Leray et al, 2010). The expectations of consumers in terms of the quality, freshness, and nutritional and sensory properties of baked flour products have been increasing (Sarah K, 2010). Consequently, baked flour products quality and consumer acceptance are very important for companies maintaining and expanding their markets. The quality of baked flour products are based on the flavor, scent and structure. The structure of the baked flour products includes: soft, moist and "melt-in-mouth" (kuchidoke), ect... There are many publications commented to the soft and moist but only a few publications mention about "melt-in-mouth" of the baked flour products. So that, I would like to present 2 aspects of "melt-in-mouth" in my thesis:

- (1) Evaluation the method to evaluate the "melt-in-mouth"
- (2) The factors affect to "melt-in-mouth"

As a definition, "melt-in-mouth" was divided into good and bad. Good "melt-in-mouth" includes: felling no food remains in the mouth, easy collapse, dissolve in saliva and easy swallowing. In addition, bad "melt-in-mouth" includes feeling the food remains in the mouth, difficult collapse, no dissolve in saliva and difficult swallowing.

The quality of baked flour product can be judged using sensory analysis by panels of trained assessors evaluating attributes such as texture, color, aroma and taste which play an important role in determining consumer preferences when purchasing baked flour product (Sarah K, 2010). In brief, knowledge of precise sensory characteristics is an important factor for bakery market development such as improving the quality of baked flour product and producing new kinds of baked flour product.

Chocolate confectionery manufacturers have often used the "melt-in-mouth" characteristic to advertise the quality of their products. Several studies have shown that it can be used as an essential parameter to evaluate chocolate product quality (Nebesny et al, 2005; Chetana et al, 2013). Recently, "melt-in-mouth" has been used in industry of baked flour product as a sensory characteristic for advertising the quality of the products (i). Recently, evaluating the "melt-in-mouth" sensory characteristic has become important to baked flour producers as they try to improve their product quality by making softer and moister (i). Therefore, "melt-in-mouth" might be important for evaluating not only chocolate but also baked flour product. Regarding the consumer's perception when chewing and swallowing bread, an acceptable "melt-in-mouth" characteristic is where the baked flour product dissolves easily in saliva and is easy to swallow, and an unacceptable "melt-in-mouth" characteristic is where the baked flour product is difficult to dissolve in saliva and difficult to swallow.

In addition, one of the problems of aging society is impairments of chewing and deglutition (Morais JA et al, 2013). Complications can be occurred such as dehydration, malnutrition, weight loss, and pneumonia (Sura L et al, 2012). One of severe complications is suffocation, which is an accident happened when swallowing food: act of killing by cutting of the oxygen supply; death caused by a lack of air or oxygen (Paul A O'Neill et al, 2000). Consequently, study "melt-in-mouth" is necessary to understand the physical properties of bolus and provide essential information for preventing the accident on eating in elderly people.

In general, "melt-in-mouth" is considered as an important of sensory evaluation so that it could be used for taste quantitation of baked flour products. However, definition of "melt-in-mouth" has been not fixed and measurement method of "melt-in-mouth" has been not established. In addition, the term "melt-in-mouth sensory" is mainly used for fatty food such as chocolate but in the baked flour products, predominant factor of "melt-in-mouth sensory" has not been elucidated. In this study, we have investigated the impact of starch properties on objective indicators of "melt-in-mouth sensory" of baked flour products.

I. "Melt-in-mouth" textures of baked flour products and their compression deformations between the tongue and palate

1. Introduction

'Taste' is reported as a major influence on customer's behavior (Tsukishima Foods Industry Co. Ltd, 2005). However, 'taste' is the sum of all sensory stimulation, includes not only taste but also smell, appearance and texture of food. Yanagimoto M. reported that taste and texture are the most important to contribute 'taste' (Yanagimoto M., 2002). For example, in bread: soft, moist, viscosity and "melt-in-mouth" are texture sensory but "melt-in-mouth" sensory evaluation is high value for bread quality analysis. Similarity to

bread, "melt-in-mouth" is considered as an important sensory evaluation for analysis flour baked product such as donuts and cake. In addition, study "melt-in-mouth" is necessary to understand the physical properties of bolus, especially in elderly people because they get difficulties in swallowing and food intake due to decrease in number of teeth and decrease in amount of saliva (Takumi H. et al 2002; Takumi H. et al, 2012). However, there is no "melt-in-mouth" definition as well as the method for evaluation of "melt-in-mouth".

From primitive study of bread, we found that there are a lot of people are interested in "dissolve in saliva quickly", "mixing saliva well". So that, "melt-in-mouth" is considered as physical properties which have changed in the mouth during chewing. Ishihara reported that the interaction between elastic of food and the mouth could effect to the food texture and food physical properties (Ishihara S., 2012).

There are five stages of eating and swallowing:

Stage-1 (Anticipatory stage): inserting food in to the mouth

Stage-2 (Oral preparatory stage): food is broken down by mastication action then dissolution with saliva

Stage-3 (Oral stage): to form the bolus

Stage-4 (Pharyngeal stage): bolus moves to pharyngeal area

Stage-5 (Esophageal stage): bolus goes through to the esophagus

In 5 stages, "melt-in-mouth" can be sensed in the stage-3. When the tongue approach to the maxillary palatal, the area of compressed bolus is larger, the

4

"melt-in-mouth" will be better. Consequently, we could use the area of compressed bolus to evaluate the "melt-in-mouth".

In this study we used tongue and maxillary palatal models to investigate and quantification of "melt-in-mouth" textures of baked flour products in the mouth. This is comparative study about deformation of food bolus in between tongue and maxillary palate. The purpose of our study is to investigate the relation between compressed bolus area and "melt-in-mouth" of baked flour products.

2. Materials

2.1. Preparation of pancake

In previous our study, we have found that heat moisture treated (HMT) flour is improved the "melt-in-mouth", so that in this study we used HMT flour in the study group and compared with normal flour in control group. And we prepared the samples flours with different percentage of HMT flour, and we have the different "melt-in-mouth" sample for studying.

Pancake was prepared according to a standard recipe as detailed in Table 1. Fresh whole eggs were purchased from Gifu University Field Science Center and fresh milk was purchased from Gifu university CO.OP supermarket. Premix for pancake was provided by Nisshin Food, Inc (Tokyo, Japan).The mean weight for one pancake was 13 g. During frying, temperature was kept at about 180oC. Pancakes were immersed into the deep-fryer for 1.5 min each side. The size of the average is 10mm thick and 40 mm in diameter.

Table 1 Recipe for pancake

2.2. Preparation of different "melt-in-mouth" pancake

From preliminary experiments, we have confirmed that when we add the heat moisture treated flour in to the "premix for pancake", the "melt-in-mouth" will be changed. Consequently, "melt-in-mouth" properties will be changed when adding heat moisture treated flour into normal flour with ratio 0%, 25%, 50%, 75%, 100%. In premix for pancake, the percentage of flour (including normal flour and heat moisture treated flour) was about 76%.

2.3. Bolus sample

We have measured the pancake weight before and after chewing by healthy men and found that 0.28g saliva mixed with 1 g pancake. Using this ratio as reference, 0.5g pancake was chopped by 3mm double-edged razor, then mixed with 0.14 g artificial saliva, actual ratio of pancake weight and saliva weight (37oC pre-warmed - Saliveht, Teijin Pharma Limited, Japan) in 1.5 ml Eppendorf tube by micro tube homogenizer $(23M - R25, ISO Corporation,$ Japan) for 40 seconds. For preparation of bolus sample, the mix was inserted into 11 mm \times 11 mm \times 4 mm (depth) mold made by silicon. The process of chewing sample preparation was shown in Figure 1. These samples were used for compression test and each sample was measured 5 times.

Figure 1. Preparation of bolus sample

2.4. Tongue pressure measurement

To measure the pressure of the tongue pancake during chewing, we used pressure measurement system (Octsen, Nitta Corporation, Japan). This system could be used for measured the pressure of incisive papilla with 9 mm diameter pressure receiving portion (Figure 2). Data was collected and transferred to computer (MY26X/L-H Mate, NEC Corporation).

Figure 2. Pressure measurement system

2.5. Tongue model

The tongue model was based on the shape of the adult healthy male tongue. Firstly, we have collected the data of adult male tongue by using X-ray. Secondly, we have sent the data to Tanac Co. Ltd, Japan for producing the tongue model made by polystyrene elastomer. The hardness of the tongue model during mastication is near JIS type E $10^{\circ} \pm 5$. The parameters of tongue model are shown in Figure 3.

Figure 3. Tongue model

2.6. Maxillary palate model

The impression of the adult male upper jaw was taken by dental practical impression agent (Phase Plus®, Zhermack). Then we used dental stone plaster (Youden Rock, Youdent Corporation, Japan) to prepare maxillary model template. Finally, dental acrylics composition (ERKODUR, ERKODENT® Erich Kopp Limited Liability Company) was used to cover the template (Figure 4).

Pressure sensor

Figure 4. Maxillary palate model

3. Methods

3.1. Comprehensive sensory evaluation of "melt-in-mouth"

A sensory analysis test was carried out to determine the difference of "melt-in-mouth" intensity in different percentage of heat moisture treated flour pancakes. Eight assessors in food processing company aged were provided with plate divided into 5 separated areas (0%, 25%, 50%, 75% and 100% heat moisture treated flour pancakes). Firstly, assessors chewed 0% heat moisture treated flour pancake (used as control sample); then chewed one of 4 other pancakes and evaluated the "melt-in-mouth": 3 - similar to control sample, 5 - very easy corruption; 4 - easy corruption; 1 - very difficult corruption; 2 - difficult corruption. During the panel, assessors rinsed their mouth with water before tasting next sample.

3.2. Sensory evaluation of ease of collapse

A sensory analysis test was carried out to determine the difference of "easy of collapse" intensity in different percentage of heat moisture treated flour pancakes. Eight assessors in Gifu university food processing laboratory aged from 20 to 30 were provided with plate divided into 5 separated areas (0%, 25%, 50%, 75% and 100% heat moisture treated flour pancakes). Firstly, assessors chewed 0% heat moisture treated flour pancake (used as control sample); then chewed one of 4 other pancakes and evaluated the "easy of collapse": 3 \cdot similar to control sample, 5 \cdot very easy corruption; 4 \cdot easy corruption; 1 - very difficult corruption; 2 - difficult corruption. During the panel, assessors rinsed their mouth with water before tasting next sample.

3.3. Compression test

Chewing sample was compressed like sandwich between maxillary palate model and tongue model. Maxillary palate model was connected to creep meter (RE-3305S, Yamaden Corporation Limited, Japan) by load cell and could movable (Figure 5b). The pressure sensor was placed in the front of maxillary palate model (Figure 4) to measure the direct pressure when tongue model compressing the chewing sample (Figure 5c). The compression rate was 0.5 mm/s and the compression will stop when pressure reaching 8 kPa. In pre-experiment, we have found that the pressure of human tough is around 8 kPa (Figure 5a), so that we have chosen 8kPa for this study. In addition, we have chosen 0.5 mm/s compression rate because if it is faster, we will not stop immediately compressing when pressure reaching 8 kPa. In our study (date not shown), the pressure of healthy adult male tongue is

(5a). Pressure of healthy human tongue in chewing process (pre-experiment)

(5b). Chewing sample was compressed like sandwich between maxillary palate model and tongue model.

(5c) Serial pictures of compression process Figure 5. Compression test

3.4. Measurement of compression sample area and measurement of particle size distribution of the granular solid

By using GIMP2 software (free download software in this site http://www.geocities.jp/gimproject2/download/gimp-download.html), we select and measure (in pixel) the area of compression sample (S1) and the area of (1 cm x 1 cm) square (S2) (Figure 6). Then the area of compression sample (in cm²) was calculated as follows:

The area of compression sample $(S0) = S1/S2$ (in cm²)

Compression sample

Area of compression sample $(S1)$ Area of $(1 \text{ cm } x \text{ 1 cm})$ square $(S2)$

Figure 6. Measurement of compression sample area by GIMP2

We considered that particle size distribution in the bolus during chewing may effect to the "melt-in-mouth" because it relates to the sensory feeling in the mouth. Eight ml 2-propanol was added into 0.5g pancake of chewing sample (above description),mixed for 2 min by vortexer, then placed in 8 stages of stainless steel sieve (75 mm in inner diameter, 20 mm depth, 1.00 mm, 0.71 mm, 0.50 mm, 0.30 mm, 0.212 mm, 0.15 mm, 0.10 mm, 0.053 mm

eye-opening, Iida-seisakusho Corporation, Japan) (Figure 7). After 2-propanol evaporation, we measured the mass of the sample remaining on the sieve. Opening size

Figure 7. The 8 stages of stainless steel sieve and measurement of particle size distribution.

4. Results and discussions

4.1. Relationship between "melt-in-mouth" evaluation and compression mass area

Figure 8 shows the relationship between "melt-in-mouth" evaluation and compression mass area. In the samples contained less than 75% of heat moisture treated flour, the more percentage of heat moisture treated flour was, the larger compression mass area was. On other hand, "melt-in-mouth" valuation of 25% heat moisture treated flour sample had the highest, and decreased gradually when increasing of the percentage of heat moisture treated flour. Percentage of heat moisture treated flour increased; the

"melt-in-mouth" became worse because of gluten denaturation during the process of flour treatment. So that, texture of pancake was weak and the "ease corruption" felling or "melt-in-mouth" has increased during chewing pancake. However, percentage of heat moisture treated flour increased over 25%, "melt-in-mouth" became worse dues to smaller particles absorbed with saliva resulted in increase the cohesive of the bolus and reduction of "melt-in-mouth". Therefore, we have found the negative relationship between "melt-in-mouth" evaluation and compression mass area.

Figure 8. Relationship between "melt-in-mouth" evaluation and compression mass area

4.2. The relationship between "melt-in-mouth" evaluation and particle size distribution in the sample granular solid

Figure 9a shows the relationship between "melt-in-mouth" evaluation and particle size distribution in the sample granular solid. The results reported that the amount of less than 100 μ m particles was similar in 5 mixing ratio sample. It reported that the human mouth sensory could detect the presence or absence of particles from 10μ m to 25μ m in diameter (Imai E., 2011).

In Figure 9b, the larger than 53 µm particle size residual rate was more than 77%, so that we considered that smaller than 53 µm particles could exclude when find the relation between "melt-in-mouth" evaluation and particle size distribution in the sample granular solid.

(9a) Frequency of particle curve (9b) Percentage of residual particle curve

Figure 9. The relationship between "melt-in-mouth" evaluation and particle size distribution in the sample granular solid

Figure 10 shows the relation between "melt-in-mouth" evaluation and

residual rate of larger than 53 µm particle. Strong relation $(r = 0.96; p <$ 0.05) was found and it means that the higher residual rate of larger than 53 µm particle is, the lower "melt-in-mouth" score is. If the residual large particle is high, it could be sensed by mouth sensory and results in reduction of "melt-in-mouth" score.

Figure 10. Relation between "melt-in-mouth" evaluation and residual rate of larger than 53 μ m particle (n=5)

4.3. The relationship between ease of collapse and compress mass area

Figure 11 shows the relationship between compress mass area and the mixing ratio of heat moisture flour sample. The compress mass area of bolus was increased during the higher mixing ratio of heat moisture flour sample and the largest in 75% of heat moisture flour sample.

Figure 11. The relationship between compress mass area and cohensive of different mixing ratio of heat moisture flour sample.

Figure 12 shows the relationship between ease of collapse and compress mass area of mixing ratio of heat moisture flour sample. The positive relation was found ($r = 0.67$) but not strong ($p < 0.21$).

Gluten in heat moisture treated flour was degenerated during processing, so that gluten formation was weak during pancake producing. Therefore, the "ease corruption" felling has increased during chewing pancake. In Figure 8, "melt-in-mouth" score increased in 25% heat moisture treated flour sample compared with 0% of heat moisture treated flour sample. However, when we increased the percentage of eat moisture treated flour; the smaller particles were produced during chewing. These smaller particles absorbed with saliva resulted in increase the cohesive of the bolus (Figure 11) and reduction of "melt-in-mouth" (Figure 8). Therefore, the food bolus compression area was correlated, not with "melt-in-mouth" texture, but with food bolus cohesiveness during chewing.

5. Conclusion

In this study, we have quantified the "melt-in-mouth" by using different "melt-in-mouth" samples with upper jaw and tongue palate model. The proportion of heat moisture treated flour was used to produce different "melt-in-mouth" samples. We have estimated the compression area of food bolus between tongue and maxillary palate in different "melt-in-mouth" samples. Compressed area of pancake bolus could not be used for comprehensive sensory evaluation of "melt-in-mouth", but may be used for sensory evaluation of collapse. Our results show that the food bolus compression area is related to food bolus cohesiveness.

II. The effects of starch retrogradation on the "melt-in-mouth" sensory characteristics of bread

1. Introduction

 Bread is one of the most widely consumed food products in the world (Leray et al, 2010). The expectations of consumers in terms of the quality, freshness, and nutritional and sensory properties of bread have been increasing (Sarah K, 2010). Consequently, bread quality and consumer acceptance are very important for bakery companies maintaining and expanding their markets. The quality of bread can be judged using sensory analysis by panels of trained assessors evaluating attributes such as texture, color, aroma and taste which play an important role in determining consumer preferences when purchasing bread (Sarah K, 2010). In addition, sensory evaluation can not only be an important part of bread quality assessment but also identify factors that can lead to the development of new products (Elía, 2011). Therefore, sensory evaluation is an important tool for the bakery industry, providing reliable information about product quality and consumer expectations. In brief, knowledge of precise sensory characteristics is an important factor for bakery market development such as improving the quality of bread and producing new kinds of bread.

 Chocolate confectionery manufacturers have often used the "melt-in-mouth" characteristic to advertise the quality of their products. Several studies have shown that it can be used as an essential parameter to evaluate chocolate product quality (Nebesny et al 2005; Chetana et al 2013). "Melt-in-mouth" of chocolate is resulted from fat and temperature. However, until now, although baked flour products have "melt-in-mouth" but the mechanism of "melt-in-mouth" of baked flour products is unknown.

Recently, evaluating the "melt-in-mouth" sensory characteristic has become important to bakery producers as they try to improve bread quality by making their products softer and moister (i). Therefore, "melt-in-mouth" might be important for evaluating not only chocolate but also bread. Regarding the consumer's perception when chewing and swallowing bread, an acceptable "melt-in-mouth" characteristic is where the bread dissolves easily in saliva and is easy to swallow, and an unacceptable "melt-in-mouth" characteristic is where the bread is difficult to dissolve in saliva and difficult to swallow. However, there have been few studies on using the "melt-in-mouth" characteristic to evaluate bread quality. The composition of bread include: water, starch and will affect to the "melt-in-mouth". When bread storage in fridge, starch will be retro-gradated and resulted in changes of "melt-in-mouth".

In this study, we aim to study the effects of starch properties during refrigerated storage on the "melt-in-mouth" characteristics of bread using sensory analysis, by measuring the water dissolution rate and water swelling rate as a reference point for starch properties and by using X-ray diffractometry to measure starch retrogradation.

2. Material

2.1. Bread making process and refrigerated storage

Bread was made according to a basic commercial recipe with 500 g wheat flour from Nisshin Flour Milling Inc. (Tokyo, Japan), 20 g shortening, 10.8 g salt, 4.5 g dry yeast (Saf-instant, Lesaffre Yeast Co., Milwaukee, WI, USA) and 360 ml water using a bread machine (PY-D432W, Twinbird Co., Tokyo, Japan). The bread was cooled at room temperature $(25^{\circ}C)$ for 2 h and then sliced to a thickness of approximately 1.5 cm using a slicing machine (FK-18N, Fujishima Koki Co., Ltd., Hyogo, Japan) (Figure13a). Slices were then stored in plastic bags to prevent moisture loss and refrigerated at 5°C. Samples were removed after 1, 3 and 7 days of storage and allowed to warm to room temperature before each experiment (Figure13b). In this study, we used only the crumb because the water absorption rate is different between crumb and crust. From each slice, a piece of crumb measuring $4 \text{ cm} \times 4 \text{ cm} \times$ 1.5 cm (Figure13c) was prepared for experiment (after 0, 1, 3 and 7 days of refrigerated storage). Figure 13 was used for showing the preparation process.

(13 a) Slicing to a thickness of approximately 1.5

(13 b) Samples were removed after 1, 3 and 7 days of storage

(13c) A piece of crumb measuring 4 cm × 4 cm × 1.5 cm

Figure 13. Preparation of $4 \text{ cm} \times 4 \text{ cm} \times 1.5 \text{ cm}$ piece of crumb

2.2. Preparation of baked bread powder

Bread slices (0, 1, 3 and 7 days of storage), warmed to room temperature after refrigerated storage, were freeze-dried (CS-110, Sakuma, Tokyo, Japan) and then milled using a grinder (3010-018, UDY Co., CO, USA) into baked bread powder.

2.3. Preparation of starch powder

One thousand grams of strong flour was mixed well with 300 ml water and washed by hand under running water. After washing, the precipitate was then dehydrated by washing in 100% ethanol and freeze-dried (CS-110, Sakuma, Tokyo, Japan). Twenty grams of the dried starch mixed in 80 ml water was autoclaved (LSX-500, Tomy Seiko Co., Tokyo, Japan) for 120 min at 120 °C. The gelatinized starch was cooled at room temperature for 2 h then stored in plastic bags to prevent moisture loss and refrigerated at 5 °C. Samples were taken after 1, 3 and 7 days of storage and freeze-dried, then milled by a grinder) into gelatinized starch powder.

3. Methods

3.1. Sensory evaluation

 A ranking test according to ISO 8587 (Tárrega and Costell, 2007) was used to determine any differences in the intensity of the "melt-in-mouth" characteristics, between bread samples $(4 \text{ cm} \times 4 \text{ cm} \times 1.5 \text{ cm} \text{ of} \text{ crumb})$ refrigerated for different periods. Nine assessors aged between 20 and 30 (3 males and 6 females) were provided with plates divided into 4 separate areas (samples from 0, 1, 3 and 7 days storage), marked by a random color code.

Assessors chewed each sample for 5 s and evaluated the two sensory attributes: the intensities of "ease of dissolving in saliva" (Rd) and "ease of swallowing" (Rs) (appendix 2 and appendix 3). The samples were ranked using numbers from 1: the easiest to 4: the most difficult. During the session, assessors rinsed their mouths with water before tasting each sample.

3.2. Elastic modulus

We also used $4 \text{ cm} \times 4 \text{ cm} \times 1.5 \text{ cm}$ pieces of crumb for measuring the elastic modulus. The uni-axial compression test was conducted using a rheometer (CR200D, Sun Scientific Co., Tokyo, Japan). The center of the upper side of the sample was compressed by a disc plunger (30 mm in diameter) at a constant speed of 4 mm/min. The compressive force data was recorded with a data logger (GL800, Graphtec Co., Kanagawa, Japan). The elastic modulus, E, was determined from the stress-strain diagram as the slope of the stress-strain curve before buckling.

3.3. Water absorption rate

A piece of crumb (4 cm \times 4 cm \times 1.5cm \cdot weight W0) was dipped into water at 25 °C for 5 second, and then surface water was wiped off with Kimtowel®. The weight of crumb after dipping, W1, was measured 5 times. The water absorption rate was defined as follows:

Water absorption rate $\left(\% \right) = W1/W0 \times 100$ (1)

3.4. Water dissolution rate and water swelling rate

One gram baked bread powder or starch powder $(W₀)$ was mixed in 1 ml methanol and then 50 ml water was added. After 20 min at room temperature, the sample was centrifuged for 30 min at 4500 rpm. The supernatant fluid was collected and dehydrated in an oven at 105 °C. The weights of the thickened sludge after drying the supernatant fluid (W_1) and the weight of the pellet after centrifugation (W_2) were measured 5 times. The protocol and the water dissolution rate, water swelling rate were defined as follows in Figure 14.

Water dissolution rate (Dr) $\left(\% \right) = W_1 / W_0 \times 100$

Water swelling rate $\left(\% \right) = W_2 / [W_0 (100 \cdot Dr)] \times 100$

Figure 14. Water dissolution rate and water swelling rate

3.5. Measurement the pasting properties using the RVA

Rapid Visco Analyzer (RVA Super 3, Newport Scientific pty. Ltd) was used to measure the pasting properties of baked bread powder. Slurries were prepared by dispersing 3.0g freeze-dried baked bread powder into 25 ml distilled water in RVA aluminum container. The samples were stirred manually using plastic paddle before the RVA run. Test profile was programmed according to the general pasting method. The slurries were first held at 30 °C for 15.5 min, heated to 93 °C within 6 min, held at 93 °C for 7 min, cooled to 50 °C within 4 min and held at 50 °C for 7.5 min, while keeping rotation speed of 160 rpm. The measurements were performed in fivefold and the averages were reported. RVA data in 30 °C stage was used to analyze pasting properties of starch.

3.6. X-ray diffraction (XRD)

 To study the crystalline structure of the starch in the bread samples and hence gain information on the degree of retrogradation, X-ray diffraction data were obtained using D8 Advance X-ray diffractometer (Bruker Corporation, Germany) fitted with a copper target X-ray tube set to 40kV and 40 mA. The baked bread powder samples (0, 1, 3 and 7 days of refrigerated storage) were scanned 5 times using a scattering angle range from 10 ° to 30 ° with a scanning speed of 0.45 °/s. After collecting, XRD data points were smoothed or deconvoluted (Ida T. and H. Toraya, 2002) by using Origin8J (Origin Lab Corporation, MA, USA). Then we estimated the integrated peak area at the diffraction angle (2θ) of 17 ° by using PeakFit v4.12 (SeaSolve Software Inc., CA, USA) (Figure. 15), because represents the crystalline portion on wheat flour starch (Zobel, H. F. et al, 1988).

Figure 15. Estimating the integrated peak area at the diffraction angle (2θ) of 17 ° by using PeakFit v4.12.

4. Results and discussion

4.1. Sensory evaluation

Sensory evaluation is often described using the definition of Institute of Food Technology. A scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Sarah K. et al, 2010).

In Table 2, the rank sum of both the "ease of dissolving in saliva" and "ease of swallowing" intensities increased during refrigerated storage. Using the Friedman test, the T statistic was 12.3 for Rd and 15.8 for Rs. Both values exceeded 11.34 (the critical value for Friedman test at the level $\alpha = 0.01$). therefore there was a significant difference between the samples at the level $\alpha = 0.01$.

To determine the differences between samples for Rd and Rs ordinal values, we used Fisher's least significant difference multiple comparison test for rank (LSRD) at the level α =0.05. In principle, samples whose rank sums were greater than 10.7 were deemed to be significantly different. According to Table 4, the Rd and Rs ordinal values increased insignificantly after a few days of storage, but increased significantly after 1 week of storage. This indicated that significant reductions in Rd and Rs ordinal values during refrigerated storage could be detected by assessors after 1 week of storage. After rescaling the data from the ordinal values to interval values (Nakamae, 2000) (Table 3), the mean values of Rd and Rs intensities were shown to

increase during refrigerated storage. A two-factor ANOVA analysis showed that there were significant differences between the 4 samples for Rd and Rs intensities $(p < 0.01$ and $p < 0.001$ respectively).

The identity of the different samples was then determined using the least significant difference multiple comparison test (LSD) at the level α =0.05. Samples, whose means differed by more than 0.66 for Rd and 0.58 for Rs, were considered to be significantly different. Similarly to the results from the ordinal data, the obvious feature in Table 3 showed that no significant differences were detected after 1 day of storage, but samples stored for 3 and 7 days were significantly different from the fresh (0 day) sample at the level $\alpha = 0.05$.

Therefore, there were significant differences between samples during refrigerated storage. The longer the bread was stored the more difficult it was for bread to dissolve in saliva and to be swallowed, both negative attributes for an acceptable "melt-in-mouth" sensory characteristic.

Table 2.Ordinal data for "ease of dissolving in saliva" intensity and "ease of swallowing" intensity from four samples stored for different times at 5 °C, ranked by nine assessors.

Storage time	"ease of dissolving in saliva" $R_{\rm d}$				"ease of swallowing" $R_{\rm s}$				Total
Assessors	0d	1 _d	3d	7d	0d	1 _d	3d	7d	
\rm{a}	$\mathbf{1}$	$\overline{2}$	3	$\overline{4}$	$\overline{2}$	$\mathbf{1}$	$\overline{4}$	3	10
$\mathbf b$	$\mathbf{1}$	$\overline{2}$	$\overline{4}$	3	$\overline{2}$	$\mathbf{1}$	3	$\overline{4}$	10
$\mathbf c$	$\mathbf{1}$	$\overline{2}$	$\overline{4}$	3	$\mathbf{1}$	$\boldsymbol{3}$	$\overline{2}$	$\overline{4}$	10
$\mathbf d$	$\mathbf{1}$	$\overline{2}$	3	$\overline{4}$	$\overline{2}$	3	$\mathbf{1}$	$\overline{4}$	10
${\bf e}$	$\mathbf{1}$	3	$\overline{2}$	$\overline{4}$	$\overline{2}$	$\mathbf{1}$	3	$\overline{4}$	10
$\mathbf f$	$\mathbf 1$	$\overline{2}$	$\boldsymbol{3}$	$\overline{4}$	$\mathbf{1}$	$\overline{2}$	3	$\overline{4}$	10
g	$\sqrt{3}$	$\overline{2}$	$\overline{4}$	$\mathbf{1}$	$\overline{2}$	$\mathbf{1}$	3	$\overline{4}$	10
\boldsymbol{h}	3	$\overline{2}$	1	$\overline{4}$	$\mathbf{1}$	3	$\overline{2}$	4	10
$\rm i$	$\overline{2}$	$\mathbf{1}$	3	$\overline{4}$	$\overline{2}$	$\mathbf{1}$	$\overline{4}$	3	10
R_i	14 ^a	18ab	27 _{bc}	31c	$15^{\rm d}$	$16d$	25 ^d	34 ^e	90
R_i^2	196	324	729	961	225	256	625	1156	
ΣR_i^2	2210				2262				
$\mathbf T$	12.3				15.8				
LSRD	$10.7\,$				10.7				

The samples were ranked using numbers from 1: the easiest to 4: the most difficult.

 R_i : Rank sum; Friedman T statistic $T = 12\Sigma R_i^2/[nt(t+1)] - 3n(t+1)$

 $t =$ number of samples, $n =$ number of assessors

Fisher LSRD =
$$
t_{\alpha/2}\sqrt{\frac{\text{nt}(t+1)}{6}} = 1.96\sqrt{\frac{9.4(4+1)}{6}} = 10.7
$$

The value for ta/2 at the level $\alpha=0.05$ is taken tables for Student's t distribution (1.96)

Samples with rank sums sharing the same letter within the same attribute are not significantly different at the level $\alpha=0.05$.

		"ease of dissolving in saliva"			"ease of swallowing"				
		$R_{\rm d}$			$R_{\rm s}$				
Storage time									
	0d	1 _d	3d	7d	0 _d	1 _d	3d	7d	
Assessors									
a	-1.03	-0.30	$0.30\,$	1.03	-0.30	-1.03	1.03	$0.30\,$	
$\mathbf b$	-0.30	-1.03	$0.30\,$	1.03	-1.03	-0.30	1.03	$0.30\,$	
$\mathbf c$	-0.30	-1.03	0.30	1.03	-1.03	0.30	-0.30	1.03	
$\mathbf d$	-1.03	-0.30	0.30	1.03	-1.03	1.03	-0.30	0.30	
\mathbf{e}	-1.03	$0.30\,$	-0.30	1.03	-1.03	-0.30	$1.03\,$	0.30	
f	-1.03	-0.30	0.30	1.03	-1.03	-0.30	0.30	1.03	
g	1.03	-1.03	$0.30\,$	-0.30	-1.03	-0.30	1.03	0.30	
$\mathbf h$	-1.03	1.03	0.30	-0.30	-1.03	0.30	-0.30	1.03	
\mathbf{i}	-1.03	-0.30	1.03	0.30	-0.30	-1.03	1.03	0.30	
Total	-5.74	-2.95	2.81	5.88	-7.80	-1.62	4.55	4.87	
Mean	$-0.64a$	$-0.33a^{b}$	0.31 bc	0.65c	$-0.87d$	$-0.18e$	0.51 f	0.54 f	
ANOVA	p < 0.01				p < 0.001				
(Two-factor)									
LSD		$0.66\,$			0.58				
$(a=0.05)$									

Table 3. Rescaling the ordinal data to interval data

Samples with means sharing the same letter are not significantly different at the level $\alpha=0.05$.

LSD: least significant difference.

In addition, sensory evaluation is very important for developing new products. It is estimated that 75% of new products fail within their first year on the supermarket shelf (Buisson P.D, 1995) and that, as a consequence, considerable resource invested in product development is squandered (Deschamps, J.P. & Nayak, P.R., 1996). Sensory attributes, whether the flavour of coffee, the smell of an air freshener, the texture of fabric or even the sound of a car door closing, are key determinants of product delivery including quality, functional and emotional benefits. Thus, a considerable proportion of product failure can be attributed to a mismatch between sensory properties and consumer needs or expectations. When integrated within the product development process, sensory and consumer testing allows cost effective delivery of acceptable products to consumers and thus reduces the risk of failure (Lawless, H.T. & Heymann, H., 1998).

So that, sensory and consumer testing is widely employed in the research arena. It is used at a more fundamental level to investigate new technologies to aid product development and to understand consumer behavior. Furthermore, multidisciplinary investigations linking sensory testing with, for example instrumental analyses, brain-imaging techniques, psychophysical tests and genomics provide a wider understanding of the mechanisms involved in sensory perception and the variations that exist within the population (Sarah K, 2010).

4.2 Elastic modulus

 To evaluate the quality of bread during refrigerated storage, crumb hardness has been considered as the critical quality index (Lainez et al., 2008). As shown in Figure 16, there were significant differences between the 4 samples which indicated that the quality of bread significantly decreased during refrigerated storage. During 1 week of storage, crumb hardness increased gradually to a significantly higher value ($p < 0.05$). Difficulty in swallowing might be caused by increased crumb hardness during storage, and contribute to a reduction in the "melt-in-mouth" sensory characteristic. Briefly, the present study suggests that "ease of swallowing" is a critical sensory factor for evaluating bread quality.

Figure 16. Effect of refrigerated storage on crumb firmness.

Values with the same letters are not significantly different at the level $\alpha = 0.05$.

4.3. Water absorption rate

 The effects of refrigerated storage on the water absorption of crumb are presented in Figure 17 which shows that water absorption decreased slightly during refrigerated storage with no significant difference between samples at the level $\alpha = 0.05$. This could cause a reduction in the ease with which bread dissolves in saliva. However, the structure of the crumb was similar to sponge, with many differently sized holes which may affect the water absorption rate significantly between the 4 samples. In addition, before swallowing, bread would be chewed into small pieces and mixed with saliva to form a bolus (Prinz and Lucas, 1995). Consequently, we experimented with bread powder samples made from the 4 crumb samples and flour starch.

Figure 17. Effect of refrigerated storage on water absorption rate of crumb.

Values with the same letters are not significantly different at the level $\alpha = 0.05$.

4.4. Water dissolution rate and water swelling rate

 The effects of refrigerated storage on the water dissolution rate and water swelling rate of baked bread powder are shown in Figures 18A and 18C. During storage, the water dissolution rate and water swelling rate decreased significantly ($p < 0.05$). Our results were similar to those of Martin et al. (1991) who showed that the water dissolution rate and water swelling rate of crumb was affected by the storage time. In the present study, the reduction in water dissolution rate and water swelling rate in baked bread powder during longer storage caused the water absorption in bread to decrease and bread hardness to increase, resulting in more difficulty in dissolving in saliva and swallowing, respectively. In addition, when bread is chewed to

become smaller in size and mixed with saliva to form a bolus in the mouth, it can only be swallowed after reaching a certain lubrication and particle size threshold (Prinz and Lucas, 1995). As a result, the changes in bread properties during refrigerated storage resulted in a reduction of the two "melt-in-mouth" attributes.

To determine to the reason for the reduction in the water dissolution rate and water swelling rate in baked bread powder, we repeated the experiment using starch powder. Figures 18B and 18D show that the water dissolution rate and water swelling rate of starch powder also significantly decreased in a similar way to the baked bread powder. Thus, the changes in starch properties during refrigerated storage might result in changes in the bread properties which in turn affected the "melt-in-mouth" sensory attributes of bread.

Many studies often refer to the critical role played by water in affecting bread firmness owing to its plasticizing effect on the crumb network (Ronda and Roos, 2011; He and Hoseney, 1990). During refrigerated storage, water is redistributed and affects the physical crumb structure by changing the amylose structure which acted as a cement to prevent water absorption in the crumb. Therefore, the reduction in the water dissolution rate and water swelling rate in starch during storage caused a reduction in the water absorption rate and increased the bread hardness, resulting in difficulty dissolving bread in saliva and swallowing.

35

Figure 18. Effect of refrigerated storage on water dissolution rate and water swelling rate of baked bread powder and starch powder.

Values with the same letters are not significantly different at the level $\alpha = 0.05$.

A and C: Water dissolution rate and water swelling rate of baked bread powder

B and D: Water dissolution rate and water swelling rate of starch powder

4.5. Measurement the pasting properties using the RVA

The pasting properties of baked bread flours were shown in Figure 19. The viscosity of baked flour was the highest in day 0 of storage and then decreased gradually during the time of storage. There was a significant different between 0 day of storage and 1 day, 3 day or 7 day of storage with p

 0.05 and $p < 0.01$. In our study, we used measurement of RVA pre-heating such as bolus formation with saliva activity in the mouth cavity. From the results, long storage crumb caused reduction of pasting of baked flour and more difficulty in bolus formation.

The results of pasting of baked flours agreed with the results of water absorption ratio, dissolution rate and swelling rate, which also decreased during storage. One more, water distribution was considered as an important role in the changes of physical structure of the crumb, which may affected to the sensory evaluation of the melt-in-mouth. Finally, melt-in-mouth sensory evaluation could be used to determine the changes of the structure of crumb including water distribution or the freshness of the bread during storage.

Figure 19. Effect of storage time on pasting property of baked bread flour

4.6. X-ray diffraction

 The X-ray diffractograms of the four samples stored for different times at 5°C are shown in Figure 20A. Every diffractogram showed a typical V-type X-ray diffraction pattern given by gelatinized starch. The longer the refrigerated storage time, the greater was the intensity of the peak at $2\theta=17^\circ$. The peaks both at $2\theta=17^\circ$ and $2\theta=20^\circ$ were extracted by deconvolution of the X-ray diffraction data. The ratios of peak area at $2\theta=17^\circ$ to peak area at $2\theta=20^\circ$ are shown in Figure 20B. As the refrigerated storage time increased, the peak area ratio increased, which indicated the progression of starch retrogradation (Zobel et al, 1988). In the present study, starch retrogradation during refrigerated storage resulted in a reduction of water absorption, which caused difficulty for bread to dissolve in saliva. Our results have suggested that with longer storage times, bread becomes harder and more difficult to swallow. As a result, the changes of starch properties during storage caused a reduction in the "melt-in-mouth" sensory characteristics.

Figure 20. The effect of refrigerated storage on the crystallinity of bread powder

- A: X-ray diffractograms of bread stored for different times at 5°C
- B: Relationship between peak area ratio (17°/20°) and storage time

5. Conclusions

 The results of this study showed that the intensity of two "melt-in-mouth" sensory attributes, "ease of dissolving in saliva" and "ease of swallowing", significantly decreased during refrigerated storage. During storage, a significant reduction in water absorption caused more difficulty for bread to dissolve in saliva and the slight increase in crumb hardness resulted in more difficulty in swallowing. The significant differences in water absorption and bread hardness between the 4 samples may be attributed to the reduction in water dissolution rate and the water swelling rate of starch during storage. This indicated that starch retrogradation during long storage times caused the water absorption of bread to decrease and bread hardness to increase, resulting in greater difficulty for bread dissolving in saliva and swallowing, respectively. The present study has found that the reduction of "melt-in-mouth" characteristics during refrigerated storage was caused by retrogradation of starch. Further studies would elucidate how changes in the crystalline structure of starch affect the intensity of "melt-in-mouth" sensory characteristics.

III. Summary

Baked flour product such as bread, pancake is widely consumed food in the world. The expectations of consumers in terms of the quality, freshness, and nutritional and sensory properties of baked flour products have been increasing. Consequently, baked flour products quality and consumer acceptance are very important for companies maintaining and expanding their markets. Recently, "melt-in-mouth" has been used in industry of baked flour product as a sensory characteristic for advertising the quality of the products. "Melt-in-mouth" is considered as physical properties which have changed in the mouth during chewing and has a high value for baked flour products quality analysis.

"Melt-in-mouth" is considered as an important of sensory evaluation so that it could be used for taste quantitation of baked flour products. However, definition of "melt-in-mouth" has been not fixed and measurement method of "melt-in-mouth" has been not established. In addition, the term "melt-in-mouth sensory" is mainly used for fatty food such as chocolate but in the baked flour products, predominant factor of "melt-in-mouth sensory" has not been elucidated. In this study, we have investigated the impact of starch properties on objective indicators of "melt-in-mouth sensory" of baked flour products.

I.

A compression tester using tongue and maxillary palatal models for adult male was developed to investigate the relations between the melt-in-mouth textures of baked flour products and the compression area of food bolus. The pancakes with different melt-in-mouth textures were prepared by changing additive amount of heat moisture treated flour (0%, 25%, 50%, 75% and 100%).The food bolus sample prepared using synthetic saliva was placed between tongue and maxillary palate and then compressed with a creep meter. We found that in the samples contained less than 75% of heat moisture treated flour, the more percentage of heat moisture treated flour was, the larger compression mass area was. On other hand, the "melt-in-mouth" of 25% heat moisture treated flour sample had the highest. Therefore, the melt-in-mouth texture is considered to be estimated not only by the compression area. Another sensory attributes were evaluated as the intensities of "ease of corruption in oral cavity". It had a positive correlation with the compression area of bolus. Then the granular solid size distributions in bolus prepared by mixing with synthetic saliva were evaluated by a sieve analysis. As a result, there was a strong positive correlation between the mass of granular solid whose diameter is over 3.5 um and the melt-in-mouth texture. Thus, even though the ease of corruption is considered to be one of factors of increasing in the melt-in-mouth sensory attribute, the coarse granules in bolus decreases the melt-in-mouth sensory attribute. Then "melt-in-mouth" decreased gradually when increasing of the percentage of heat moisture treated flour due to more smaller particles were produced and increased the cohesive of the bolus during chewing. Finally, we conclude that the food bolus compression area was correlated, not with melt-in-mouth texture, but with food bolus cohesiveness during chewing.

II.

Four types of bread samples: 0 day after baking and cooled in room temperature for 2 hours; 1 day, 3 day and 7 day after refrigerated storage (50C) were prepared for analyzing the relations between "melt-in-mouth" texture of bread and physical properties of bread starch. The "melt-in-mouth" sensory attributes were separately evaluated following 2 aspects (1) "dissolving easily in saliva" and (2) "being easy to swallow" by ranking method. The "dissolving easily in saliva" and "being easy to swallow" significantly decreased through refrigerated storage. The crumb firmness increased during refrigerated storage. The longer refrigerated storage, the water dissolution rate and the water swelling rate of baked bread powder as well as starch more significantly decreased. The X-ray diffract grams of baked bread powder showed that the longer refrigerated storage, the more enhance the area of peak at about $2\theta=17^\circ$, which indicated the generation of starch retrogradation. During storage, starch retrogradation caused decrease of crumb water absorption and increase of crumb firmness, then resulting in difficultly dissolving in saliva and more difficult to swallow, respectively. The present study found that the reduction of "melt-in-mouth" during refrigerated storage was caused by retrogradation of starch.

In this study, we found that the size of particle in the bolus is the physical property which effects to the "melt-in-mouth" and "ease to collapse" of baked flour products. Our results showed that "easy to swallowing" is related to the crystallinity starch dissolving in saliva. Finally, food bolus compression area in the tongue and maxillary palatal models suggested that "ease to collapse" may be an important factor of baked flour products "melt-in-mouth".

Appendix 1. Critical value table for Friedman test

The table lists the critical values for Friedmans test. Different numbers of assessors are shown in the left hand column and different numbers of samples are shown at the head of each column. The table also includes two different significance levels for the test. If the calculated value for 'T' is greater than or equal to the tabled value, reject the null hypothesis. (Sarah K. et al.,2010)

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