DOCTORAL THESIS

Effect of trehalose on the mechanical properties of deep-fried foods

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List of abbreviations

T_g: glass transition temperature (ºC)

a_w: water activity (dimensionless)

a_{wc}: critical water activity (dimensionless)

w_c: critical water content (dimensionless)

GAB: Guggenheim, Anderson and de Boer

DM: dry matter

C: coefficient correcting monolayer sorption properties (dimensionless)

K: coefficient correcting multilayer sorption properties (dimensionless)

W_m: monolayer water content (g/100 g-DM)

ΔF: mechanical relaxation force-drop (N)

DSC: differential scanning calorimetry

TRA: thermal rheological analysis

CHAPTER 1 Introduction

1.1. Background

Deep-frying is one of popular cooking methods to produce dry foods having desirable and characteristic flavor and texture (Ziaiifar, 2008; Alnadif et al., 2014; Dana & Saguy, 2006; Van Koerten et al., 2015). Deep-frying is achieved by immersing food stuff into the heated oil (approximately 150~200 ºC) (Fakas, 1994; Ziaiifar, 2008). In the case of "*tempura*", various physical changes (such as starch gelatinization, water-evaporation, and oil-sorption) occur competitively and/or synergistically in the batter during the deep-frying (Fakas, 1994; Ziaiifar, 2008; Bordin et al., 2013; Oke et al., 2018). The fried batter (fry coating) covers the food stuff of *tempura* products.

After the deep-frying, *tempura* is cooled down at room temperature, and the fry coating becomes glassy state at least at the outer part. The glassy fry coating shows a favorable brittle texture. When storage time is prolonged, the fry coating changes from brittle texture to ductile texture because glass to rubber transition (briefly, glass transition) occurs through water sorption originating from the atmosphere moisture and/or from the food stuff. In food industry, it is important to maintain the brittle texture of the fry coating (glassy fry coating) as long as possible.

Temperature of which glass transition occurs is denoted as glass transition temperature (T_g). Typical glass transition behavior of food products is shown in Fig. 1-1. Food product is in glassy (solid-like) state at a lower temperature than the T_g (point A in Fig. 1-1-a). Glassy porous food shows brittle texture because of its high elasticity. The glassy food becomes rubbery (liquid-like) state at a higher temperature than the T_g (A to B in Fig. 1-1-a), and the texture changes to ductile one because of the reduced elasticity. Glass transition also occurs even at a constant temperature when water content changes, because the T_g of hydrophilic amorphous materials decreases with an increase in the water content because of water plasticizing effect. Thus, the glassy food becomes rubbery state, when the T_g becomes lower than the holding temperature as the result of water sorption (A to C in Fig. 1-1-a). Water content of which the T_g becomes 25 ºC (typical room temperature) has been described as critical water content (w_c). In addition, water activity (a_w) of which the T_g becomes 25 ºC has been described as critical water activity (a_{wc}). The higher w_c and a_{wc}, the greater resistance to the physical deteriorations induced by water sorption. To maintain brittle texture of fry coating as long as possible (A to C' in Fig. 1-1-b), it is important to elevate the w_c and/or a_{wc} of fry coating (w_c to w_c' in Fig. 1-1-b).

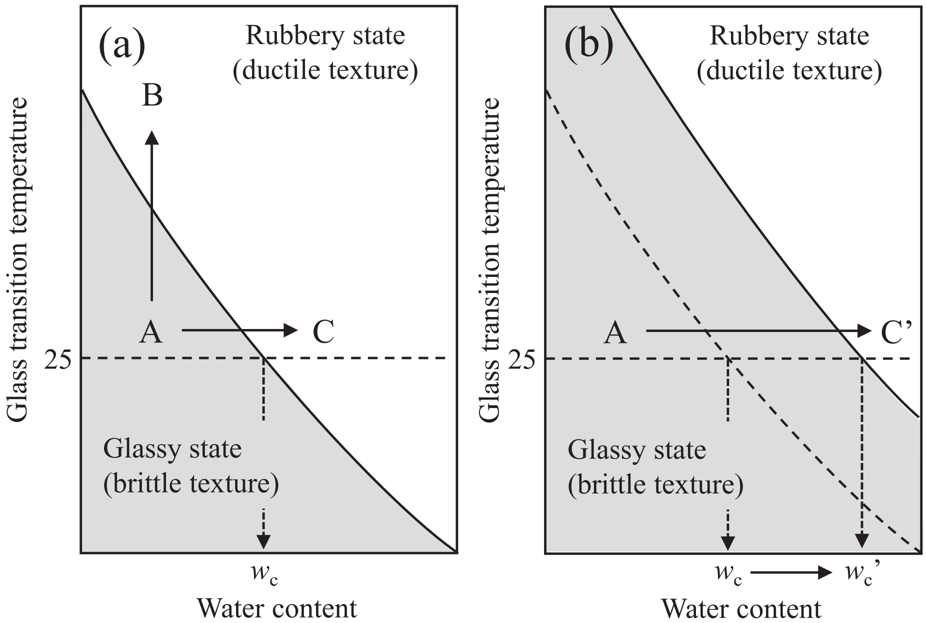


Fig. 1-1. Typical glass transition behavior of food products.

Differential scanning calorimetry (DSC) is the most common technique to determine the T_g of amorphous materials. Glass transition can be detected as an endothermic shift in DSC curve, and T_g is evaluated from the onset point of the shift. In the case of starch-based foods, it is difficult to determine the clear T_g because a minor endothermic shift is observed in a broad temperature range. In addition, multiple thermal responses due to heterogeneous components cover glass transition. In this case, thermomechanical approach is more effective (Kawai et al., 2014; Sogabe et al., 2018). In a previous study (Jothi et al., 2018), it was demonstrated that the glass transition of fried batter particles (i.e., "*agedama*" in Japanese) could be detected by thermal rheological analysis (TRA). In the TRA measurement, the food sample is compressed at a temperature below expected T_g and heated up to an enough high temperature. Then, T_g of the sample can be confirmed as a force drop due to the softening induced by glass transition. It is known that thermal T_g determined by DSC does not always agree with the T_g determined by thermomechanical approaches (Boonyai et al., 2007; Kasapis et al., 2007; Ross et al., 2002). Thus, the T_g determined by thermomechanical approaches is distinguished as "mechanical T_g".

It is known that the T_g of amorphous materials can be controlled by the compositions (Balasubramanian et al., 2016; Roos et al., 1996). For example, T_g of amorphous polymers decreases with an increase in small molecules because of their plasticizing effect (Figueroa et al., 2016, Carvalho & Mitchell, 2001; Fan et al., 1996). However, the plasticizing effect of small molecules on the mechanical T_g of the fried batter particles does not always obey to the plasticizing behavior. In our previous study (Jothi et al., 2020), it was demonstrated that the mechanical T_g of fried batter particles could be elevated by the addition of trehalose (disaccharide). As one of possibilities, it is suggested that trehalose filled the defects in the amorphous amylopectin (the major component of the fried batter particles) and the mechanical strength was enhanced (Fig. 1-2). This suggestion is based on "anti-plasticizing effect" explained in the previous studies (Slade & Levine, 1995; Figueroa et al., 2016).

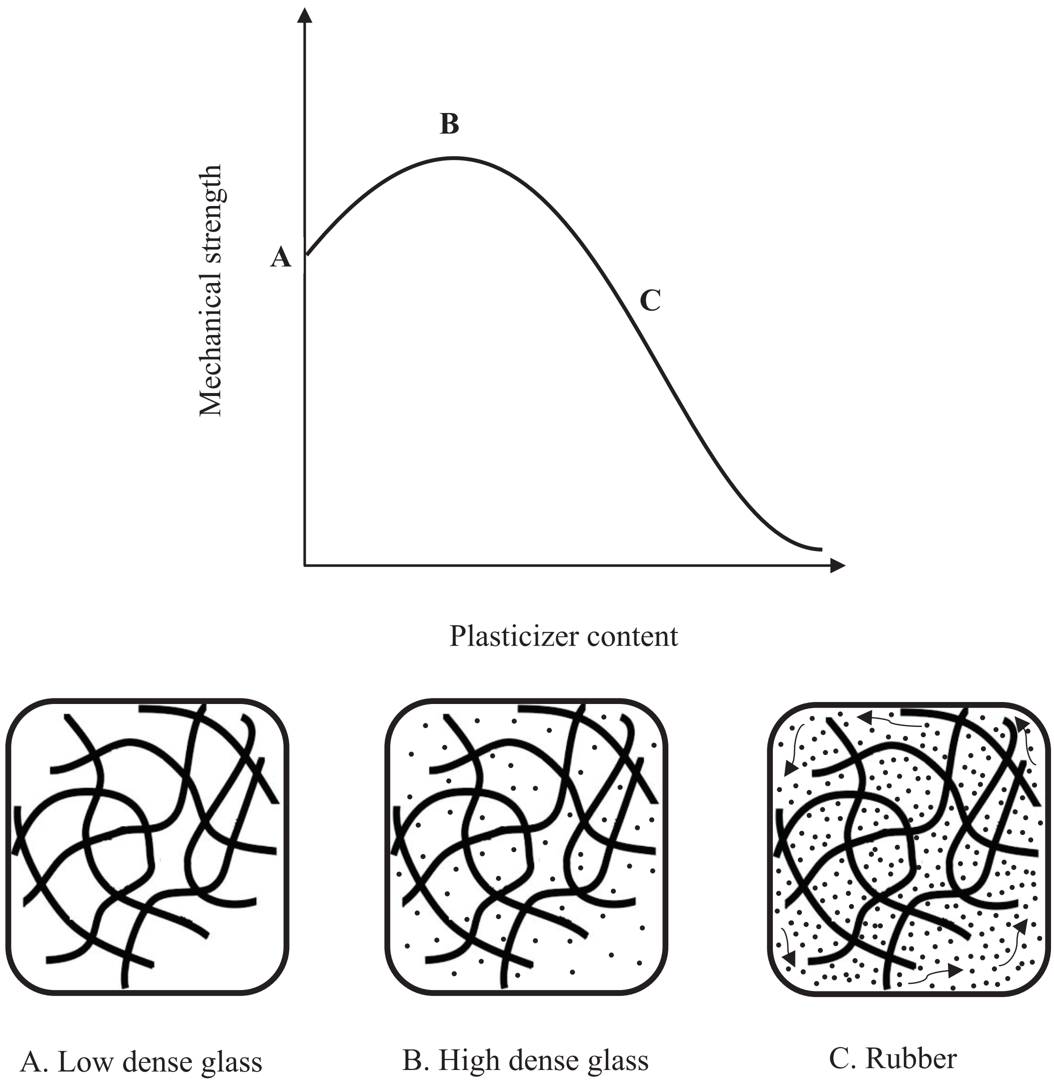


Fig. 1-2. Model drawing of anti-plasticizing effect of small molecules on the polymer.

1.2. Purpose

As explained above, it was demonstrated that the mechanical T_g of fried batter particles could be elevated by an addition of trehalose. In addition, the fried batter particles containing trehalose maintained a brittle texture at a higher water content compared to non-additive one.

As the mechanism of the physical modification induced by trehalose, anti-plasticizing effect of trehalose on starch is suggested.

To clarify physical modification effect of trehalose on the fried batter particles in more detail, this study aimed to understand effect of trehalose content on the mechanical properties of fried batter particles. In addition, freeze-dried waxy starch (amylopectin) was employed as a model of fried batter particles. The freeze-dried starch solid has numerous pores formed by ice sublimation and the initial water content can be used to alter the porosity of the solid. By dehydration, the porous solid becomes glassy state and has similar structure to fried batter particles. Amylopectin is the major component of fried batter particles, and chemical reactions and thermal degradation are highly diminished by freeze-drying. Thus, freeze-dried waxy starch was expected to be an effective model for the better understanding of the physical modification induced by trehalose addition.

In chapter 1 (this chapter), background and purpose of this study were stated. In chapter 2 (Fundamentals), materials and experimental techniques used in this study were explained. In Chapter 3, effect of trehalose content on the oil content, water content, a_w, mechanical glass transition, and texture properties of fry batter particles was reported. In Chapter 4, effect of trehalose content on the texture properties and true density of freeze-dried waxy starch was reported. In chapter 5, general discussion was provided according to the results. In Chapter 6, this study was concluded and outlook for the future was stated.

CHAPTER 2 Fundamentals

2.1. Batter

Batter for fry coating of "*tempura*" is prepared by the mixing of wheat flour and water. For the brittle texture of fry coating, the development of gluten network during processing should be suppressed as much as possible. Thus, this study employed wheat flour with a low protein content (Nisshin Flour Milling Inc., Nagoya, Japan). According to the product information, the wheat flour contains 8% protein and less than 1.7% lipid. In addition, batter was prepared by mixing wheat flour and chilled water in an iced water bath.

2.2. Trehalose

Trehalose has some advantages as a texture modifier of foods. Firstly, trehalose is chemically stable. Since trehalose is non-reducing disaccharide, non-enzymatic browning reaction (Maillard reaction) does not occur essentially in food system (Ledl & Schleicher, 1990). In addition, trehalose is resistant to hydrolysis. These are effective to diminish color change to deep brown during frying process. Secondly, trehalose has a high T_g. Anhydrous T_g of trehalose, sucrose, maltose, and lactose is 113 ºC, 68 ºC, 90 ºC, and 102 ºC, respectively (Kawai et al., 2005; Haque et al., 2006); trehalose is the highest anhydrous T_g among the disaccharides. This study employed trehalose dihydrate (Hayashibara Co., Ltd., Okayama, Japan).

2.3. Fried batter particles

This study employed fried batter particles (*agedama*) as a model sample for the fry coating of *tempura* according to our previous study (Jothi et al., 2020) with minor modifications. A micropipette with a 5 mm diameter hole and a digital oil bath with stirrer (EOS-200RD; AS ONE Co., Osaka, Japan) were used (Fig. 2.1). An enough amount of canola oil was placed in the oil bath, and the temperature was set to 180 °C with the agitation at 250 rpm by using a magnetic stir bar (diameter 8 mm and length 30 mm). The batter (approximately 4 ml) was dropped into the oil by the micropipette over a period of 30 to 40 s. The batter was deep-fried at 180 ºC for 4 min. The fried batter particles were scattered on a stainless-steel tray with mesh (70 mm × 70 mm), and they were cooled down at room temperature for approximately 4 min. The samples were kept in a bag until analysis.

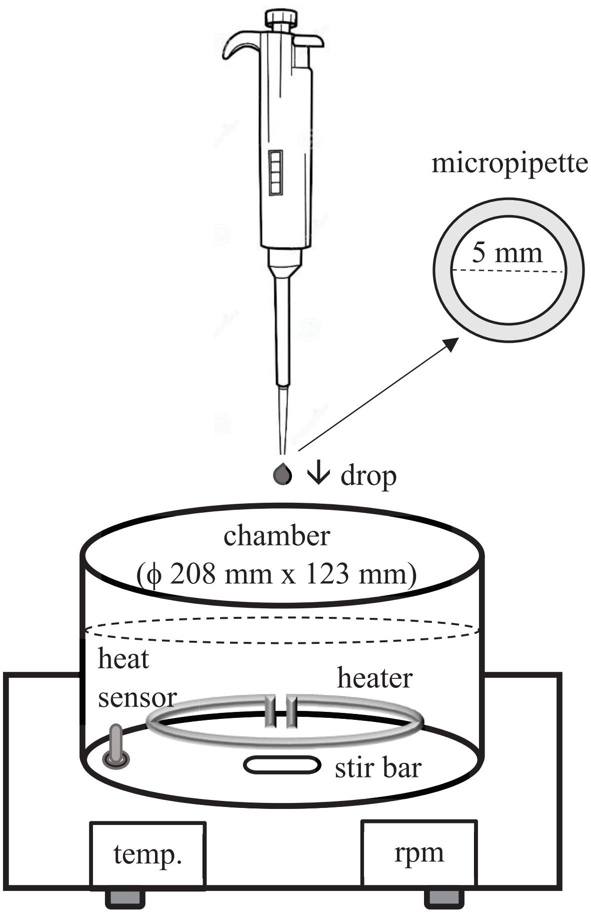


Fig. 2.1. Preparation of fried batter particles.

2.4. Freeze-dried waxy corn starch solid

Carbohydrate polymer becomes glassy state by dehydration. The freeze-dried starch solid has numerous pores formed by ice sublimation. Porosity of the solid can be adjusted by initial water content. Thus, freeze-dried starch solid can be employed as a model of fried batter particles. Freeze-dried waxy corn starch solid was prepared according to a previous study (Sogabe et al., 2018) with minor modifications.

Waxy corn starch was put into a vial, and distilled water was added to adjust water content. A magnetic stir bar was put into the vial, and the vial was covered by wrapping with film to prevent water evaporation. The vial was placed in a boiling water bath (98~100 ºC) and heated for 10 min with magnetic stirring to prevent the sedimentation of starch granules at the initial stage of the heating. The gelatinized waxy starch was cooled at room temperature.

The gelatinized waxy starch was put into cylindrical holes (diameter 20 mm and height 10 mm) in a mold and then frozen at -25 ºC for 16 h. The frozen starch was placed on a cooled stage (-35 ºC) in a freeze-dryer and freeze-drying was carried out at approximately 11 Pa with an increase in temperature from -35 ºC to 5 ºC over 34 h. The freeze-dried solids with numerous pores were carefully removed from the mold. In order to remove residual water, vacuum-drying was carried out at 110 ºC (stage temperature) for 8 h.

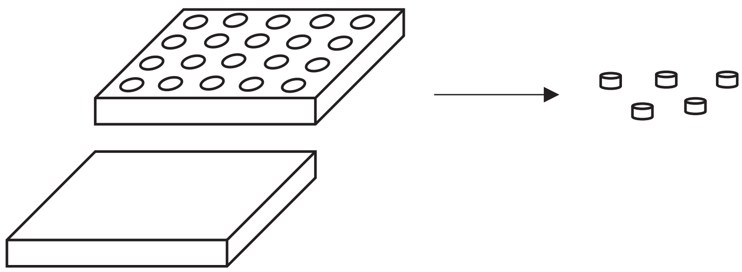


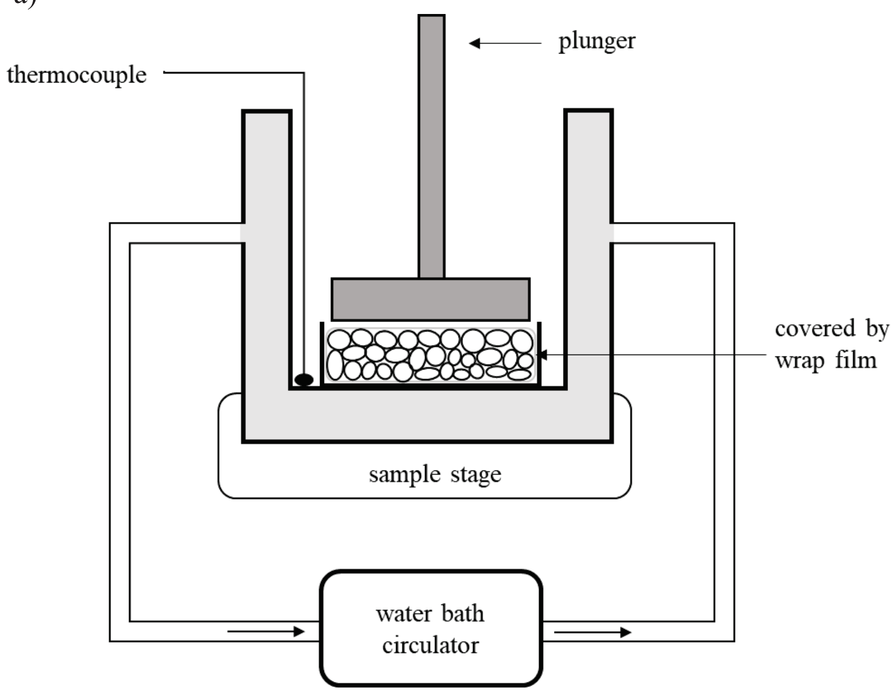
Fig. 2.2. Preparation of freeze-dried waxy starch solid.

2.5. Isothermal mechanical glass transition

As explained above, TRA is an effective tool to determine mechanical T_g of food system. From the effect of water content on the mechanical T_g, water content of which the T_g becomes 25 ºC (typical room temperature) can be evaluated as w_c. In addition, the w_c can be converted to a_{wc} though the effect of a_w on the equilibrium water content (i.e., water sorption isotherm). However, this approach to determine w_c and a_{wc} needs many experimental data. As more brief approach to determine w_c and a_{wc}, isothermal mechanical relaxation measurement was carried out using a rheometer (CR-150; Sun Scientific Corp., Ltd., Tokyo, Japan) according to our previous study (Jothi et al., 2020).

Schematic drawing of isothermal mechanical relaxation measurement is shown in Fig. 2-3. Fried batter particles (14-16 particles) were put into the sample cup (diameter 31.90 mm and height 9.95 mm) and then placed on the stage of the rheometer at 25 ºC controlled by water bath circulator (Fig. 2-3-a). The sample was compressed at a constant stress and held in the compressed condition. In the holding process, force to maintain the initial strain decreases because of mechanical relaxation. From the force-time curve, the force-drop (ΔF) between the initial force and the force after the holding was evaluated (Fig. 2-3-b). Glassy sample shows very little mechanical relaxation (low ΔF value) because of high elasticity. In contrast, rubbery sample shows great mechanical relaxation (high ΔF value) because of low elasticity. From effect of a_w on the ΔF of samples, a_w-range of which mechanical glass transition occurs can be understood.

a)



b)

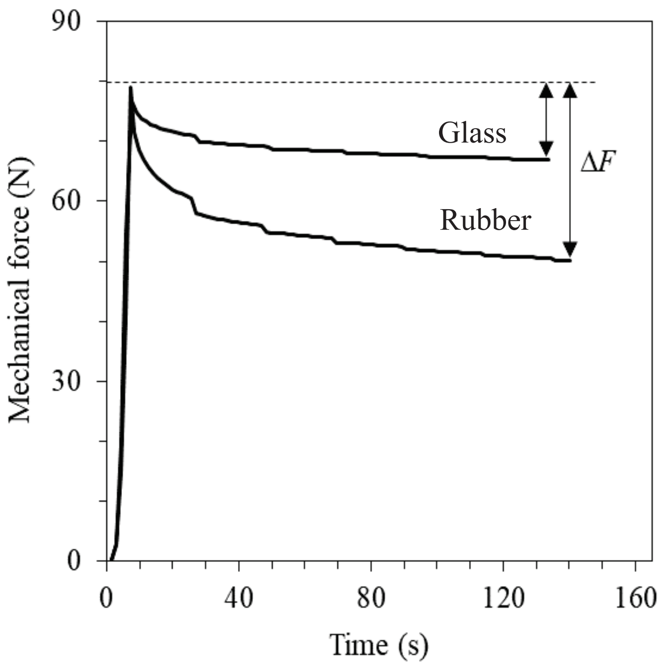


Fig. 2-3. Schematic drawing of isothermal mechanical relaxation measurement.

2.6. Texture

Texture of glassy porous food has commonly been investigated by rheometer (Jothi et al., 2018; Jothi et al., 2020; Sogabe et al., 2018). From the force-displacement curve, numerical fracture peaks are detected. One piece of the fried batter particles was compressed with a plate plunger, and fracture force was determined from the first fracture point. In addition, the total number of fracture peaks was counted in the displacement range of up to 2.5 mm (Jothi et al., 2018; Jothi et al., 2020). The fracture force and the number of peaks is expected to correspond to the hardness and brittleness, respectively.

2.7. Statistical analysis and fitting analysis

Analysis of variance (ANOVA) with Turkey's HSD test ($p < 0.05$) and fitting analysis were performed using KaleidaGraph software (version 3.6, Hulinks Inc., Tokyo, Japan).

CHAPTER 3 Effect of trehalose content on the mechanical properties of fried batter particles

3.1. Introduction

To maintain the brittle texture of fry coating as long as possible, it is important to elevate the T_g (w_c and/or a_{wc}) of fry coating. In our previous study (Jothi et al., 2020), it was demonstrated that the mechanical T_g of fried batter particles could be elevated by an addition of trehalose. In addition, the fried batter particles added trehalose maintained a brittle texture at a higher water content compared to non-additive one. To clarify physical modification effect of trehalose on the fried batter particles in more detail, effect of trehalose content on the oil content, water content, a_w, mechanical glass transition, and texture properties of fried batter particles was investigated.

3.2. Methods

3.2.1. Sample preparation

Fried batter particles were prepared as explained earlier (section 2.3). Wheat flour and chilled water were mixed at the weight ratio of 1.0 : 1.6, and trehalose (0, 5, 10, 20%, against dry wheat flour) was added into there in an iced water bath. The batter was dropped into heated oil and fried at 180 ºC for 4 min. The fried batter particles were scattered on a stainless-steel tray with mesh and cooled down at room temperature for approximately 4 min.

3.2.2. Water sorption

The samples were vacuum-dried at 110 ºC (stage temperature) for 8 h. The fully dried samples were equilibrated at 25 ºC for at least 2 weeks in a desiccator under various water activity (a_w) conditions adjusted by the following saturated salts (Greenspan, 1977): LiCl ($a_w = 0.113$), CH_3COOK ($a_w = 0.225$), $MgCl_2$ ($a_w = 0.328$), K_2CO_3 ($a_w = 0.432$), $Mg(NO_3)_2$ ($a_w = 0.529$), NaBr ($a_w = 0.576$), KI ($a_w = 0.689$), and NaCl ($a_w = 0.755$). Equilibrium water content (g/100 g-DM) of the samples was determined gravimetrically by vacuum-drying at 110 ºC (stage temperature) for 8 h. The measurements were performed in triplicate and the results were averaged.

3.2.3. Oil content

Oil content of the samples was investigated by the Soxhlet extraction method (AOAC, 2000). The samples were ground, and vacuum-dried at 110 ºC (stage temperature) for 8 h. The fully dried samples (1 g) were defatted with diethyl ether at 60 ºC for 12 h using Soxhlet extraction system (Yamato Scientific Co., LTD, Japan). The measurements were performed in triplicate, and the results were averaged.

3.2.4. Isothermal mechanical relaxation

The fried batter particles (14-16 particles) were set into the sample cup and then placed on the stage of the texture meter at 25 ºC (Fig. 2-3-a). The sample was compressed at 80 N with a plate plunger and held in the compressed condition for 3 min. From the force-time curve, ΔF (difference in the force between the initial force and the force after 3 min) was investigated. The measurements were performed in triplicate and the results were averaged.

3.2.5. Texture properties

One piece of the fried batter particles was put on the sample stage and then compressed with a plate plunger (diameter 30 mm) at 0.5 mm/s. From the force-displacement curve, the fracture force determined from the first fracture point and the total number of fracture peaks counted in the displacement range of up to 2.5 mm were evaluated (Jothi et al., 2018; Jothi et al., 2020). The measurements were performed five times, and the results were averaged.

3.3. Results

3.3.1. Oil content of fried batter particles

Effect of trehalose content on the oil content of fried batter particles is shown in Fig. 3-1. The oil content of 0% and 10% trehalose samples obtained in this study agreed well with the values (53% for 0% trehalose sample and 42% for 10% trehalose sample) reported in our previous study (Jothi et al., 2020). The oil content of fried batter particles significantly decreased and then increased slightly with increased trehalose content. Trehalose is a water-soluble carbohydrate, and thus will prevent the oil-sorption of deep-fried foods. Tavera-Quiroz et al. (2012) reported that water-soluble materials (methylcellulose-glycerol-sorbitol mixtures) reduce oil sorption during the frying process. Similar effects of corn gum mixture and wheat gum mixture (Sahin et al., 2005), wheat cellulose derivatives and wheat-sorbitol mixtures (Garcıa et al., 2002), and oat bran (Lee & Inglett, 2007) on the oil content of deep-fried samples are also reported.

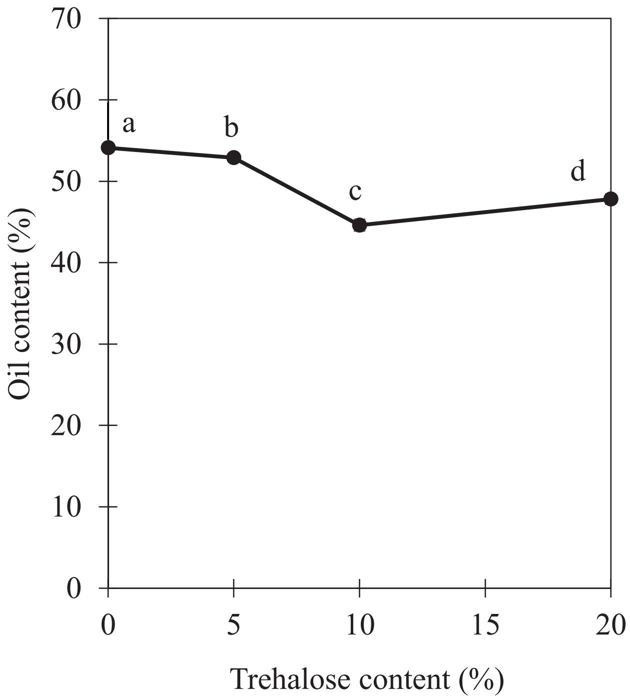


Fig. 3-1. Effect of trehalose on the oil content of fried batter particles. Values are expressed as mean ± SD. Different characters indicate significant difference ($p < 0.05$).

3.3.2. Water sorption, water content, and a_w of fried batter particles

Effect of a_w on the equilibrium water content of fried batter particles held at 25 ºC is shown in Fig. 3-2. The samples showed a sigmoidal water sorption behavior as similar to that for other amorphous starchy foods (Al-Muhtaseb et al., 2002; Kawas & Moreira, 2001). The solid curves (water sorption isotherm) were obtained by fitting data to the Guggenheim-Anderson-de Boer (GAB) model (Eq. 3-1),

$$W = \frac{W_m \cdot C \cdot K \cdot a_w}{(1 - K \cdot a_w) \cdot (1 + (C - 1) \cdot K \cdot a_w)} \quad \text{(Eq. 3-1)}$$

where W is the equilibrium water content (g/100 g-DM) and W_m, C, and K are constant. The W_m is the monolayer water content (g/100 g-DM). The C and K are correction factors for the sorption properties of the first layer with respect to the bulk liquid and for the properties of the multilayer with respect to the bulk liquid, respectively.

The GAB parameters (W_m, C, and K) are summarized in Table 3-1. The W_m values (2.48-2.71 g/100 g-DM) agreed well with the values (2.69 g/100 g-DM for 0% trehalose sample and 2.44 g/100 g-DM for 10% trehalose samples) reported in our previous study (Jothi et al., 2020). The C and K values were slightly higher and lower, respectively, than those of the previous study. As well-known, the GAB parameters are sensitive to differences in experimental values; even a small difference in experimental values results in large differences in GAB parameters (Fongin et al., 2007; Rahman & Al-Belushi, 2006). The GAB parameters were used to convert water content to a_w.

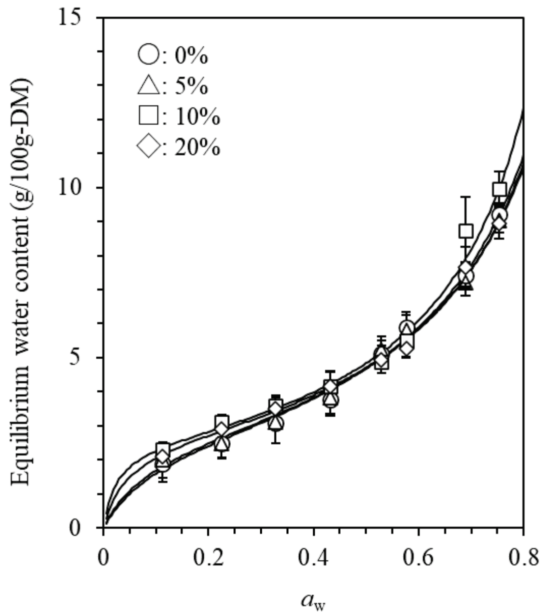


Fig. 3-2. Effect of a_w on the equilibrium water content of fried batter particles. Values are expressed as mean ± SD.

Table 3-1 GAB parameters for the fried batter particles

trehalose %	W_m g/100 g-DM	C	K	R^2
0	2.71	10.8	0.95	0.998
5	2.71	12.5	0.94	0.997
10	2.48	41.0	1.00	0.995
20	2.57	24.4	0.95	0.999

R^2: determination coefficient

Effect of the trehalose content on the water content and a_w of fried batter particles (unadjusted a_w) is shown in Fig. 3-3. The a_w of the samples was estimated from the water content through the water sorption isotherm with GAB parameters. The water content and a_w of 10% and 20% trehalose samples were significantly lower than that of 0% and 5% trehalose samples.

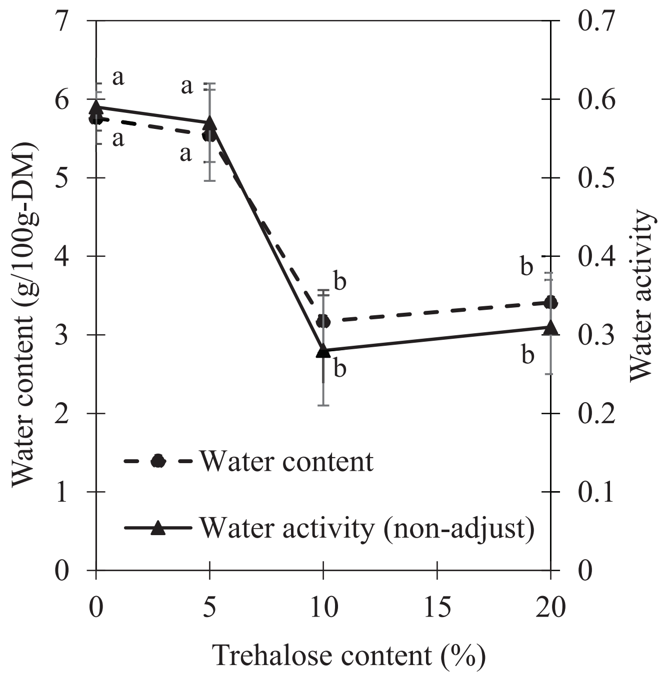


Fig. 3-3. Effect of trehalose content on the water content and a_w of deep-fried samples (unadjusted a_w). Values are expressed as mean ± SD. Different characters indicate significant difference ($p < 0.05$).

3.3.3. Isothermal mechanical glass transition of fried batter particles

Typical isothermal mechanical relaxation curves are shown in Fig. 3-4. The force of the samples decreased with increasing hold time due to mechanical relaxation. Mechanical relaxation nearly reached equilibrium within 3 min, and ΔF was evaluated from the isothermal mechanical relaxation curves. There was very little mechanical relaxation in the 0% trehalose sample having a_w 0.113; this suggests that the sample was in a glassy state (high elasticity). On the other hand, the 0% trehalose sample having a_w 0.529 showed a large mechanical relaxation; this suggests that the sample was in a rubbery state (low elasticity). The mechanical relaxation of 10% trehalose samples was much lower than that of 0% trehalose samples at each a_w.

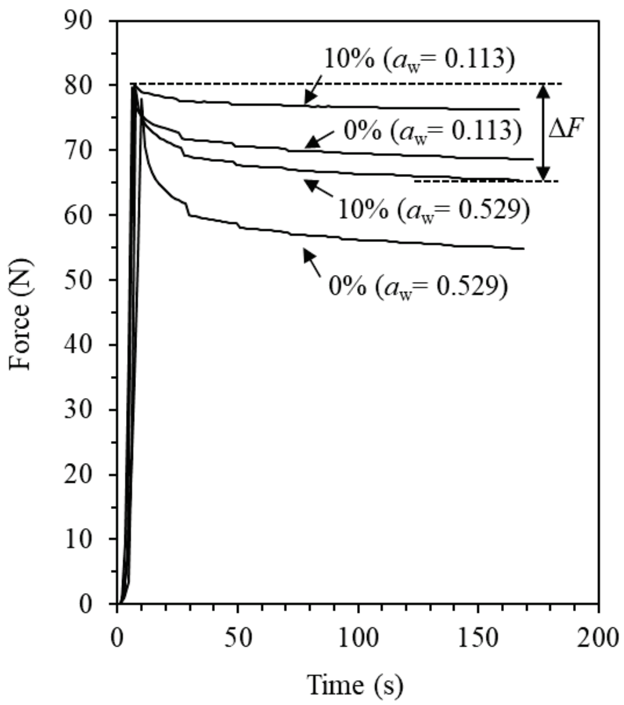


Fig. 3-4. Isothermal mechanical relaxation curves for fried batter particles.

Effect of a_w on ΔF of fried batter particles is shown in Fig. 3-5. The ΔF increased linearly with increasing a_w and became much greater above a certain a_w. This suggests that mechanical glass transition occurred at the a_w because of water plasticizing. As explained earlier, the a_w of which the mechanical glass transition occurs at 25 ºC is denoted as mechanical a_{wc}. The mechanical a_{wc} of 0% and 10% trehalose samples is reported to be 0.509 and 0.649, respectively (Jothi et al., 2020). The mechanical a_{wc} values are within the turning range of the a_w-dependency of ΔF. Taking this into account, 10% and 20% trehalose samples are higher mechanical a_{wc} than 0% and 5% trehalose samples.

In a comparison of the ΔF values among the glassy samples (open symbols in Fig. 3-5), 10% and 20% trehalose samples had much lower ΔF values than 0% and 5% trehalose samples. This suggests that 10% and 20% trehalose samples were more rigid glassy material than 0% and 5% trehalose samples.

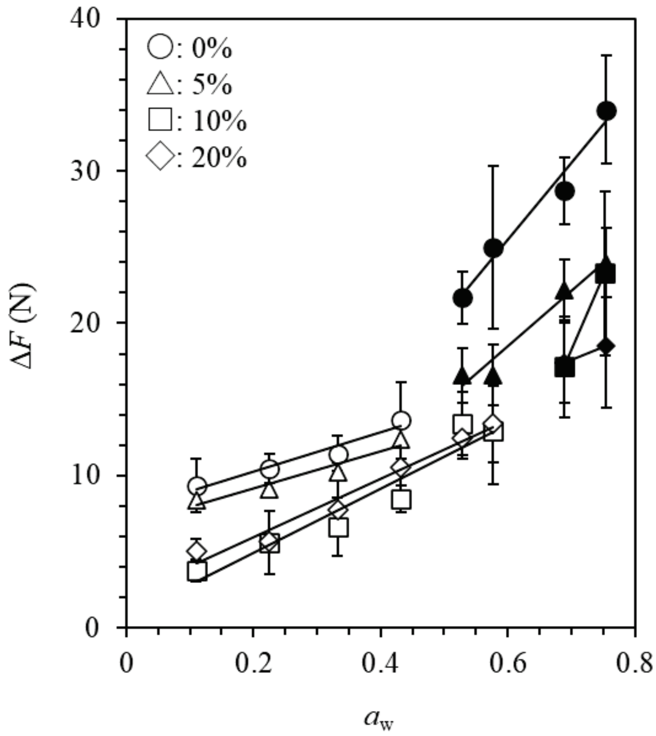


Fig. 3-5. Effect of a_w on ΔF of fried batter particles. Values are expressed as mean ± SD. Open and closed symbols mean glassy and rubbery samples, respectively.

3.3.4. Fracture properties of fried batter particles

Typical force-displacement curves for 0% and 10% trehalose samples having $a_w = 0.113$ and $a_w = 0.529$ are shown in Fig. 3-6. The 10% trehalose sample having a_w 0.113 (glassy state) showed numerous small fracture peaks (brittle texture). On the other hand, the 0% trehalose sample having a_w 0.113 (glassy state) showed larger and smaller number of peaks than the 10% trehalose sample. Although the 0% trehalose sample having a_w 0.529 (rubbery state) showed a large fracture peak (ductile texture), the 10% trehalose sample having a_w 0.529 (glassy state) still showed the fracture behavior due to brittle-like texture.

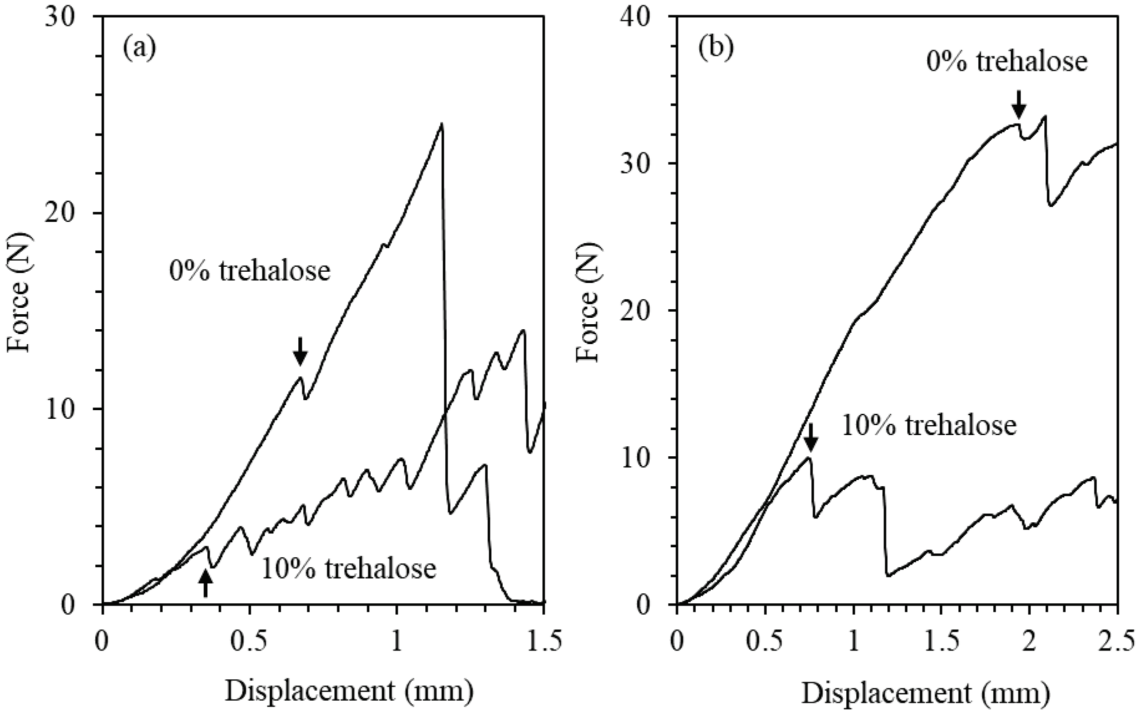


Fig. 3-6. Typical force-displacement curves for 0% and 10% trehalose samples having (a) a_w 0.113 and (b) a_w 0.529.

The effect of a_w on the first fracture force of fried batter particles is shown in Fig. 3-7-a. From Fig. 3-5, the samples indicated by open and closed symbols are expected to be in the glassy and rubbery states, respectively. The first fracture force of the glassy samples increased linearly with an increase in a_w. At each a_w in the glassy samples (0.113-0.432), there were no significant differences between 0% and 5% trehalose samples. The 10% trehalose sample showed a significantly lower value than the other samples (except for the 10% versus 20% trehalose samples having a_w 0.225 and a_w 0.432). At the rubbery state near the glassy state, a maximum first fracture force was observed because of the large displacement due to hard-ductile fracture. At higher a_w, the first fracture force largely decreased with increasing a_w because of the structural softening. There were no clear differences among the rubbery samples.

The effect of a_w on the number of fracture peaks for fried batter particles is shown in Fig. 3-7-b. From Fig. 3-5, the samples indicated by open and closed symbols are expected to be in the glassy and rubbery states, respectively. The number of fracture peaks of glassy samples decreased linearly with increasing a_w. At each a_w in the glassy state (0.113-0.432), the 10% and 20% trehalose samples were significantly higher values than the 0% and 5% samples (except for 5%, 10%, and 20% trehalose samples having a_w 0.225). There was no significant difference between 10% and 20% trehalose samples. In addition, there were negligible differences among the rubbery samples.

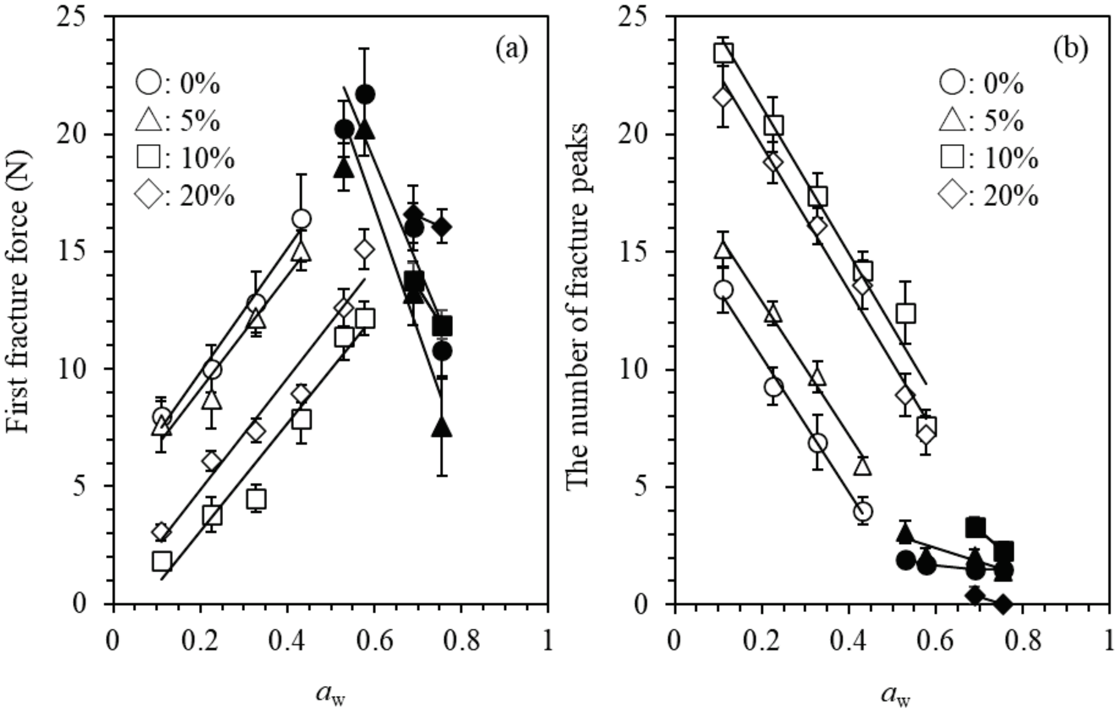


Fig. 3-7. The effect of a_w on the first fracture force of fried batter particles. Values are expressed as mean ± SD.

3.4. Conclusion of this chapter

The effect of trehalose content on the oil content, water content, a_w, mechanical glass transition, and texture properties of fried batter particles was investigated. From the results, it was found that 10% trehalose sample was the lowest oil content, water content, and a_w. In addition, 10% trehalose sample was higher mechanical a_{wc}, lower first fracture force, and higher number of fracture peaks than 0% and 5% trehalose samples. There were similar effects on the mechanical a_{wc} and fracture properties between 10% and 20% trehalose samples. This suggests that 10% trehalose is an optimum additive content for the brittle texture of deep-fried foods. The molecular mechanism of the results is discussed in Chapter 5 (General discussion).

CHAPTER 4 Effect of trehalose content on the fracture properties and true density of freeze-dried waxy starch-trehalose system

4.1. Introduction

In Chapter 3, the effect of trehalose content on the oil content, water content, a_w, mechanical glass transition, and texture properties of fried batter particles was investigated. To clarify physical modification effect of trehalose on the fried batter particles in more detail, freeze-dried waxy starch (amylopectin) was employed as a model of fried batter particles. Amylopectin is the major component of fried batter particles, and chemical reactions and thermal degradation are highly diminished in the freeze-dried sample. Thus, freeze-dried waxy starch was expected to be an effective model for the better understanding of the physical modification induced by trehalose addition. In this chapter, effect of trehalose content on the texture properties and true density of freeze-dried waxy starch was investigated.

4.2. Methods

4.2.1. Sample preparation for freeze-dried porous solid

The sample was prepared as described in the section of 2.4. In brief, waxy corn starch (5 g, dry basis) was put into a vial, and distilled water was added to produce 90% water content. For the preparation of the samples containing trehalose, 5%, 10%, and 20% trehalose (per waxy starch on dry weight basis) was added. Starch in the mixture was gelatinized, and the gelatinized mixture was put into cylindrical holes in a mold. The sample was freeze-dried, and porous solids were carefully removed from the mold. The solids were vacuum-dried, and the fully dried samples were equilibrated at 25 ºC for at least two weeks in a desiccator under a_w 0.113, 0.225, and 0.328. Since the purpose of this chapter was to understand the effect of trehalose content on the fracture properties of glassy trehalose-amylopectin system, the samples were adjusted to the low a_w values.

4.2.2. Fracture properties of freeze-dried porous solid

The fracture properties of freeze-dried porous solids were investigated as described in the section 2.6. In brief, freeze-dried solid was put on the sample stage and then compressed with a plunger (diameter 5 mm) at 0.5 mm/s. From the force-displacement curve, first fracture force and the total number of fracture peaks in the displacement range of 2.5 mm were evaluated. The measurements were performed five times, and the results were averaged.

4.2.3. Sample preparation for freeze-dried powder

The sample was prepared as described in the section 2.4. In brief, waxy corn starch (5 g, dry basis) was placed in a vial, and distilled water was added to produce 99% water content. For the trehalose-added samples, 5%, 10%, and 20% trehalose (per waxy starch on dry weight basis) was added. Starch in the mixture was gelatinized, and the gelatinized mixture was dispensed onto a stainless-steel tray. For the preparation of 100% trehalose sample, a 10% trehalose aqueous solution was dispensed onto stainless-steel tray. They were freeze-dried and ground to obtain powder by using a mill. The powder samples were vacuum dried at 60 ºC for 6 h.

4.2.4. True density of freeze-dried powder

The density of powder samples was determined using a gas pycnometer AccuPyc II 1345 (Shimazu Co., Japan). Helium was used as the displacement gas at an equilibration rate of 0.0345 kPa/min. The fully dried powder (0.05~0.25 mg) was put into the cell (diameter 11.4 mm and height 11.66 mm), and a filtered cap was set under nitrogen gas condition. The measurements were performed in triplicate and the results were average.

4.3. Results

4.3.1. Fracture properties of freeze-dried porous solids

The effect of a_w on the first fracture force of the freeze-dried porous solid samples is shown in Fig. 4-1-a. The first fracture force linearly increased with increasing a_w, depending on the trehalose content. At each a_w, the 0% trehalose sample was a significantly higher force than the other samples. In the contrast, the 10% trehalose sample showed a significantly lower force than the others (except for 10% versus 20% trehalose having a_w 0.113). There was no significant difference in the values between 5% and 20% trehalose samples.

The effect of a_w on the number of fracture peaks of the freeze-dried porous solid samples is shown in Fig. 4-1-b. The number of fracture peaks linearly decreased with an increase in a_w, depending on the trehalose content. At each a_w, the 10% trehalose sample was a significantly larger value than the others (except for 0% versus 10% trehalose having a_w 0.113). There was no significant difference in the values among the 0%, 5%, and 20% trehalose samples (except for 0% and 20% versus 5% having a_w 0.225).

As stated above, fried batter particles containing 10% trehalose were lower first fracture force and higher number of fracture peaks. This suggests that the 10% trehalose sample was more brittle texture than the others. A similar effect of 10% trehalose addition was also observed in the freeze-dried porous solid samples. This suggests that molecular interaction between trehalose and amylopectin is a major factor for the modified texture of fried batter particles.

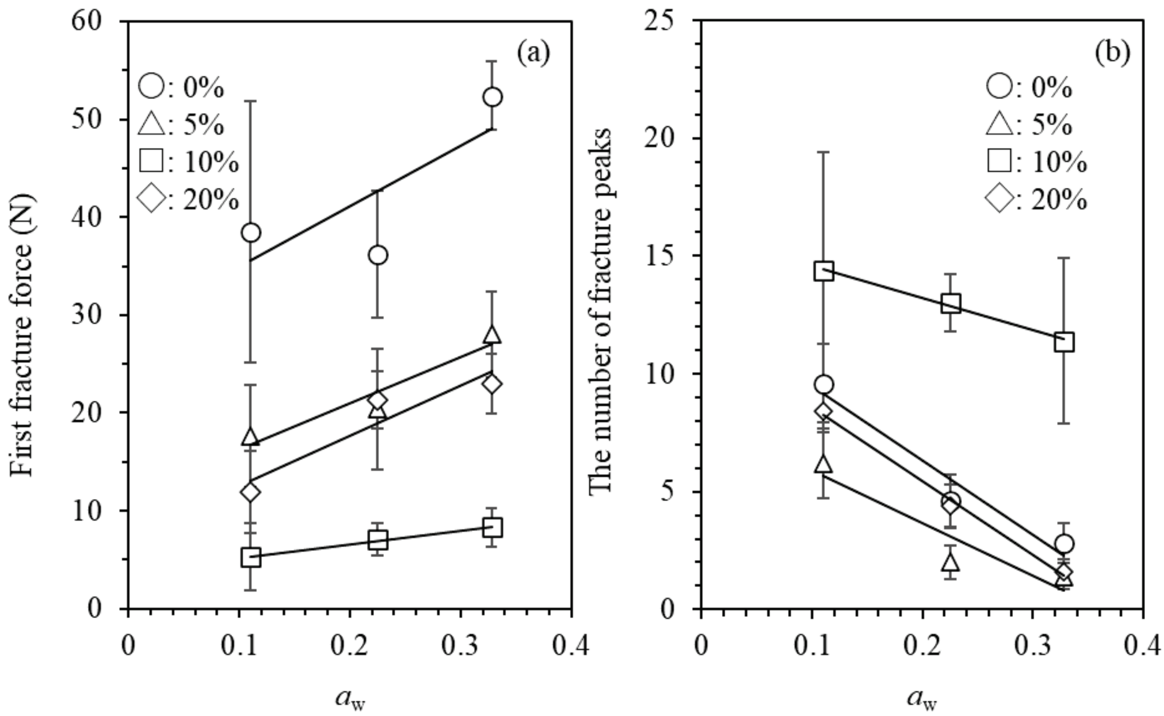


Fig. 4-1. The effect of a_w on the first fracture force and the number of fracture peaks of the freeze-dried waxy starch porous solids. Values are expressed as mean ± SD.

4.3.2. True density of freeze-dried powders

The effect of trehalose content on the true density of the freeze-dried powder samples is shown in Fig. 4-2. There is no significant difference in the true density between waxy starch and trehalose. Thus, it is expected that waxy starch-trehalose blend powder shows no or little change in the true density independent from trehalose content. However, 10% trehalose sample showed a significantly higher value than the others. This result indicates that 10% trehalose is embedded into the amylopectin. As stated above, it is suggested that trehalose fills the defects in the amorphous amylopectin and the mechanical strength is enhanced. This suggestion is based on “anti-plasticizing effect” explained in the previous studies (Slade & Levine, 1995; Figueroa et al., 2016). The fact that amylopectin containing 10% trehalose sample was significantly higher true density strongly supports the suggestion based on the anti-plasticizing effect.

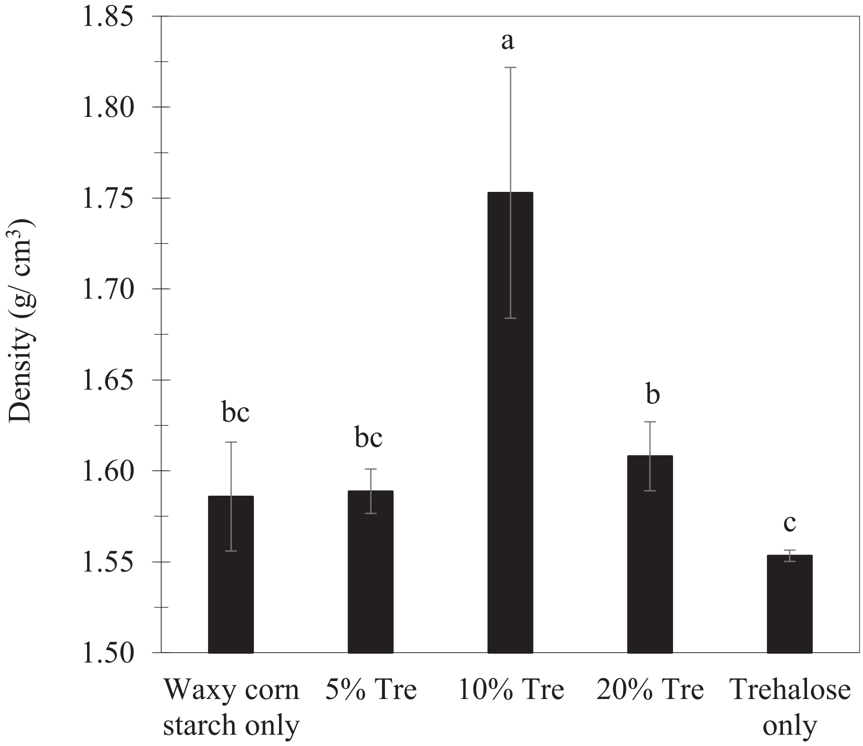


Fig. 4-2. Effect of trehalose content on the true density of the freeze-dried powders. Different characters indicate significant difference ($p < 0.05$).

4.4. Conclusion of this chapter

Effect of trehalose content on the fracture properties and true density of freeze-dried waxy starch-trehalose system was investigated. The first fracture force and the number of fracture peaks showed an almost equivalent behavior to those observed in fried batter particles. In addition, it was demonstrated that freeze-dried waxy starch containing 10% trehalose sample was significantly higher true density than the others. This result strongly supports the suggestion based on the anti-plasticizing effect; trehalose fills the defects in the amorphous amylopectin and the mechanical strength is enhanced. The molecular mechanism of the results is discussed in Chapter 5 (General discussion).

Chapter 5 General discussion

Effect of trehalose content on the oil content, water content, a_w, mechanical a_{wc}, and fracture properties of fried batter particles was investigated. From the results, 10% trehalose sample showed the lowest oil content, water content, a_w among the samples. In addition, 10% trehalose sample was higher mechanical a_{wc}, lower first fracture force, and higher number of fracture peaks than 0% and 5% trehalose samples. The similar effect of 10% trehalose addition was also observed in the fracture properties of freeze-dried porous solids. These results are discussed using model drawings as follows.

During deep-frying of batter, gelatinization of wheat starch occurs from approximately 60 ºC (Contardo et al., 2016; Yusop et al., 2011) and water evaporation occurs from approximately 100 ºC. A major component of wheat flour is amylopectin. Since amylopectin is a large polymer having many branched segments, oil is trapped in the many gap spaces of the branched segments under the dehydrated state.

When gelatinization of wheat starch occurs in the presence of trehalose, the trehalose is partially incorporated into the gelatinized wheat starch because of the hydrophobic interaction induced by oil. Thus, the trehalose molecules will act as "internal trehalose" (Fig. 5-1-a). The trehalose except for internal trehalose will locate at the interfacial parts of amylopectin.

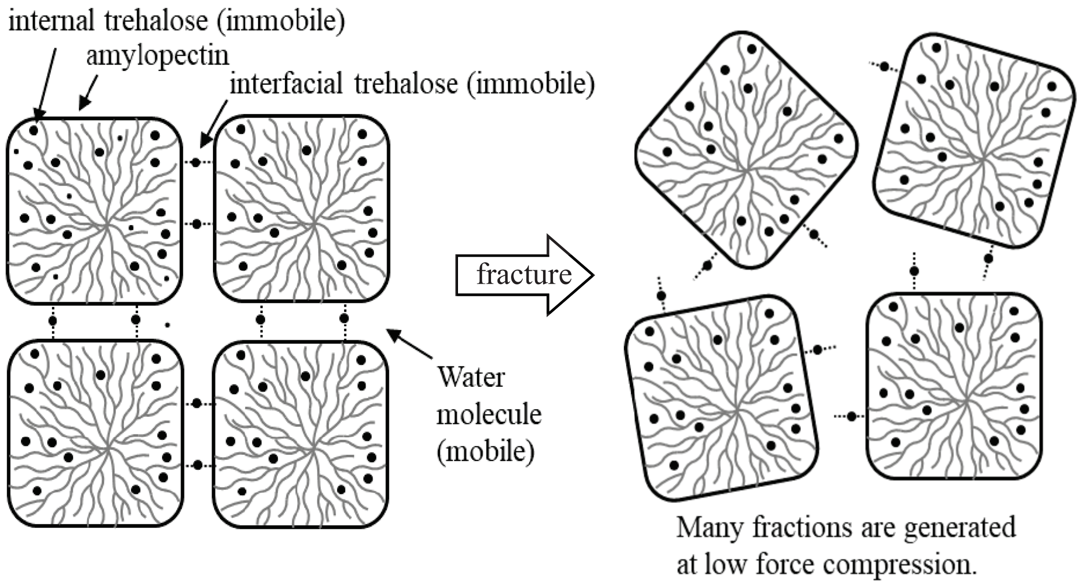
Since internal trehalose can fill the gap spaces of amylopectin, oil uptake is reduced. In addition, evaporation of water is promoted because water molecules are excluded from gelatinized wheat starch by internal trehalose. The fact that freeze-dried amylopectin containing 10% trehalose was the highest true density suggests that the gap space of amylopectin is almost saturated by 10% trehalose addition. This corresponds to the lowest oil content, water content, and a_w of fried batter particles containing 10% trehalose. In the case of 20% trehalose sample, it is thought that the amount of "interfacial trehalose" increased essentially and generated further oil-spaces and hydration sites in the system. Thus, 20% trehalose sample will have been slightly higher oil content than 10% trehalose sample.

According to the suggestion that internal trehalose fills the gap spaces of amylopectin, molecular structure of the amylopectin should be physically strengthened. This explains the reason why 10% and 20% trehalose samples were higher mechanical a_{wc} than 0% and 5% trehalose samples.

Since fractures intrinsically occur at the physically weak points in the structure, interfacial trehalose plays a role of fracture points. Thus, the first fracture force decreased, and the number of fracture peaks increased by the addition of trehalose. When excess amount of trehalose is added in amylopectin, the amount of interfacial trehalose increases, and the interaction between interfacial trehalose and amylopectin becomes physically strong. These suggestions explain the reason why 10% trehalose is an optimum amount to improve the brittle texture of fry coating.

Water intrinsically acts as similar to trehalose in amylopectin. However, the first fracture force increased linearly, and the number of fracture peaks decreased linearly with increasing a_w, even in the glassy state. In addition, ΔF increased linearly with increasing a_w. The different effect between trehalose and water can be explained by the differences in the molecular dynamics. Water is a much lower molecular size and much higher molecular mobility than trehalose. Water molecules within the glassy polymer are mobile at ambient temperature (Roudaut et al., 2004; Watanabe et al., 2019) and small enough to pass through the polymeric network. Thus, there will be an unclear distinction between internal and interfacial water molecules in glassy amylopectin. Water molecules will fill gap spaces in the internal and interfacial part of amylopectin that trehalose cannot sufficiently fill (Fig. 5-1-b), and thus fracture points attributed to interactions between interfacial trehalose and amylopectin can be physically strengthened by water. Consequently, the first fracture force increased linearly, and the number of fracture peaks decreased linearly with increasing a_w, even in the glassy state. In addition, amylopectin has branched segments with varying chain lengths, and water will enhance the flexibility of the chains from short chain segments to long chain segments with increasing a_w as a local plasticizer. Thus, ΔF increases with increasing a_w under the glassy state. When all chains become flexible by increasing a_w, a glass to rubber transition occurs, and the dependence of a_w on ΔF becomes more apparent. Trehalose, on the other hand, is immobile in the glassy matrix because the large molecules cannot pass through the polymeric network unless the polymer matrix becomes flexible (i.e., rubbery state).

(a) trehalose-added sample having a low a_{w} (glassy state)



(b) trehalose-added sample having a high a_{w} condition (glassy state)

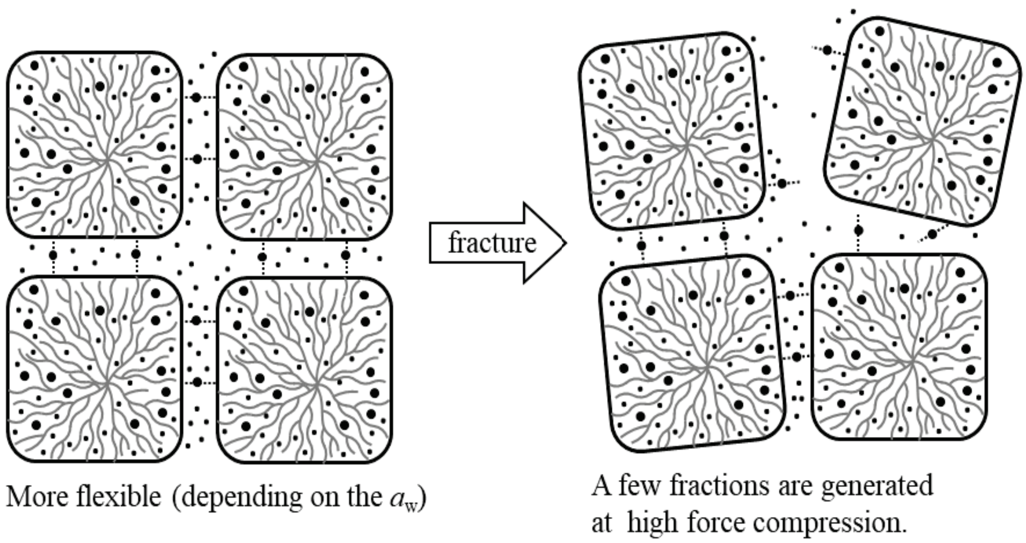


Fig. 5-1. Model drawings of molecular interactions between trehalose and amylopectin.

Conclusion

It is known that the mechanical T_g of fried batter particles could be elevated by an addition of trehalose. In addition, fried batter particles containing trehalose maintained a brittle texture at a higher water content compared to non-additive one. As the mechanism of the physical modification induced by trehalose, anti-plasticizing effect of trehalose on starch is suggested. This study aimed to understand effect of trehalose content on the mechanical properties of fried batter particles. For this purpose, freeze-dried waxy starch (amylopectin) was also employed as a model of fried batter particles.

Effect of trehalose content on the oil content, water content, a_w, mechanical a_{wc}, and fracture properties of fried batter particles was investigated. From the results, 10% trehalose sample showed the lowest oil content, water content, and a_w. In addition, 10% trehalose sample was higher mechanical a_{wc}, lower first fracture force, and higher number of fracture peaks than 0% and 5% trehalose samples. The similar effect of 10% trehalose was also confirmed in the fracture properties of freeze-dried porous solids. These results are discussed based on the concept of internal trehalose and interfacial trehalose.

This study employed fried batter particles as a fry coating model. Fry coating coats wet food stuff, and thus diffusion of water molecules from the food stuff to the fry coating occurs quickly. Since deep-fried foods are in non-steady state, there is a gradation of water content and temperature between inner and outer parts. It is practically important to understand the kinetic properties of fry coating. These are subjects in further studies.

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