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Full Length Research Paper

# Microstructure formation and rheological properties of bread containing medium-chain triacylglycerols (MCT) and its comparison with long-chain triacylglycerols (LCT) and butter containing bread

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The microstructure formation and rheological properties of bread containing medium-chain triacylglycerols (MCT) were investigated in comparison with long-chain triacylglycerols (LCT) and butter. Baked breads absorbed moisture in storage, and consequently water activity ( $a_W$ ) decreased, reaching an equilibrium after eight days; the  $a_W$  of LCT bread was 0.61. In contrast, the MCT and butter breads showed  $a_W$  of 0.76 and 0.72, respectively. Assessment of the rheological properties of LCT bread showed greater hardness than breads containing MCT or butter; a significant difference (p<0.05) was observed between the MCT and LCT breads. In contrast, no significant differences in the adhesion of breads were observed. With the addition of various lipids, changes were observed in the bread microstructure. With respect to the fine surface structure, a smooth surface was formed in the case of the MCT bread, as compared to that with LCT and butter. This suggests that the properties of MCT make it applicable to bread making.

**Key words:** Medium-Chain, triacylglycerols (MCT), long-chain triacylglycerols (LCT), microstructure formation, rheological properties, water activity.

# INTRODUCTION

Medium-chain triacylglycerols (MCT) composed exclusively of medium chain fatty acids (C8 and C10) were first used in the 1950s for the dietary treatment of malabsorption syndromes caused by the inadequate absorption of nutrients. Since then, these diseases have been widely studied. Moreover, although a large number of reports have been published, the majority have focused on the issue from a clinical nutrition or biochemical standpoint (Leveilie et al., 1967; Chanez et al., 1991; Kris-Etherton and Yu, 1997; Ecelbarger et al., 1991; Papamandjaris et al., 1998). In contrast, very few studies have been conducted from a food science standpoint.

Wheat gluten proteins are mainly comprised of gliadin and glutenin (Schofield, 1986). During dough mixing gluten

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution License 4.0</u> International License proteins are hydrated and form a three-dimensional network, which is responsible for the unique visco-plasto-elastic property of bread. Gluten contents are involved large as a factor that determines the quality of the bread, and as a factor of the other, involvement of lipids is considered. However, detailed studies as at present, are few. We previously reported (Toyosaki et al., 2008, 2010, 2013; Toyosaki, 2014) that MCT is implicated as an important factor in this phenomenon. It is know that MCT plays a role in a number of dough properties. However, information regarding the interaction between MCT and bread dough requires to be studied.

The purpose of this study was to determine the functional food properties of MCT in baked bread in comparison with long-chain triacylglycerols (LCT) and butter by assessing the molecular structure and rheological properties of bread.

#### MATERIALS AND METHODS

#### Materials

Medium-chain triacylglycerols (MCT), and LCT were a kind gift from Nisshin OilliO Group Ltd. (Kanagawa, Japan). Spring wheat flour (Super King brand) was obtained from Nisshin Flour Milling Inc. (Chiyoda, Tokyo, Japan). The proximate analysis of the flour indicates the contents of protein, ash, lipid and water were 13.1% (Kjeldahl, N x 6.25), 0.4%, 1.8% and 15.0%, respectively. More than 95% of flour granules were sifted through a 132 mm mesh sieve. Dry yeast (*Saccharomyces cerevisiae*) was purchased from S. I. Lesaffre (Marcq-en-Baroeul, France). Other reagents used in this study were of analytical grade and were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

#### Preparation of baked bread and freezing conditions

Bread dough was prepared using commercially available ingredients and by employing the straight dough method. The dough ingredients were comprised of lipids (MCT, LCT, and butter, at 4.0%), hard flour (80%), yeast (2.5%), water (70%), sugar (8%), salt (2.0%) and skimmed milk powder (4.0%). Dough temperature at the completion of mixing was 26°C, and the dough was fermented for 90 min at 28 to 30°C. It was then molded and subjected to a final fermentation for 60 to 70 min at 36°C and 75% humidity, followed by baking for 35 to 40 min at 230°C (top of oven) to 210°C (bottom of oven). After baking, samples were frozen and freezer-stored under conditions typically found in the baking industry. Also, pieces of baked bread (200 g) were frozen in a blast freezer at -40°C for 4 h. The temperature in the center of the sample reached -20°C after 2 h and -40°C after 2 h of freezing. Thereafter, the samples were packaged in plastic bags and stored in a walk-in freezer at -40°C. During the 60-day storage period, samples were taken at regular intervals and analyzed by cryoscanning electron microscopy (cryo-SEM).

#### Cryo-SEM

Samples were removed after 2 h of freezing in a blast freezer and immersed in liquid nitrogen or after 1 day and 40 days of frozen storage. Small rectangular pieces (120 mm<sup>3</sup>) were cut from a frozen baked bread sample in the walk-in freezer to avoid thawing. All samples were taken from the center of the baked bread section,

except in one case, where samples were also taken from the outer zone. For further cooling and transport, samples were stored in liquid nitrogen. The fresh reference sample (100 g) was frozen in liquid nitrogen for 20 min after proofing of the baked bread.

Samples were fixed on a precooled holder with O.C.T compound and fractured under liquid nitrogen. The holder was transferred under nitrogen atmosphere to the cryo preparation unit (SCU 020; Bal-Tec AG, Balzers, Liechtenstein). The sample was sputter-coated with 20 nm of gold at -40°C and then transferred to the cold stage of the SEM (JSM-7800F; JEOL Ltd., Japan). The samples were observed at temperatures below -60°C and at an acceleration voltage of 10 kV.

#### Water activity (aw)

The water activity ( $a_w$ ) of baked bread was determined at 25°C with a Hygro Palm 2 (Rotronic AG, NY, USA). Determinations were made in at least duplicate.

#### Determination of rheological properties of baked bread

The hardness and adhesion of the baked bread were measured using a rheometer (TPU-2S; YAMADEN Co., Ltd., Tokyo, Japan). The baked bread was placed in a rheometer cell, 42 mm across and 16 mm high. A cylinder-type plunger (diameter of 15 mm) compressed the dough in the cell at 5-mm intervals and at a compression rate of 1 mm per second. Quadruplicate replicates were carried out on each sample within 5 min of one another. Each value is expressed as the mean  $\pm$  standard deviation.

#### Statistical analysis

All data were expressed as the mean  $\pm$  standard deviation. Statistical analysis was performed using the unpaired student's *t*-test (KaleidaGraph, Ver. 4.0; Synergy software, PA, USA). Differences in mean values among groups were assessed using the Tukey-Kramer multiple comparison test (Instat Ver. 3.0; GraphPad software, Inc., CA, USA). The level of significance was set at p<0.05 for all statistical tests.

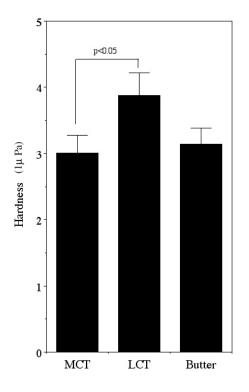
#### **RESULTS AND DISCUSSION**

#### Rheological properties of baked bread

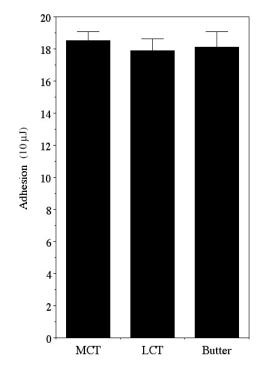
The hardness and adhesion of baked bread containing MCT, LCT or butter were rheometrically determined. The hardness of the LCT bread was increased compared to the MCT and butter breads (Figure 1); a significant difference (p<0.05) was observed between the MCT and LCT breads. In contrast, the adhesion of the breads did not significantly differ (Figure 2). These results confirm that type of lipids used for the preparation of bread is a major factor in its physical properties.

#### Water activity $(a_W)$ of baked bread

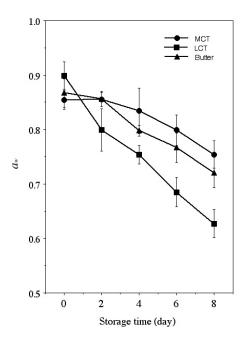
Water absorption is the main limiting factor in the shelf life of baked confectionery products and bread. Baked breads absorb humidity in storage, consequently *a*<sub>W</sub> decreased,



**Figure 1.** Effects of MCT, LCT or butter on the hardness of baked breads. Each value represents the mean  $\pm$  standard deviation of triplicate measurements.



**Figure 2.** Effects of MCT, LCT or butter on the adhesion of baked breads. Each value represents the mean  $\pm$  standard deviation of triplicate measurements.



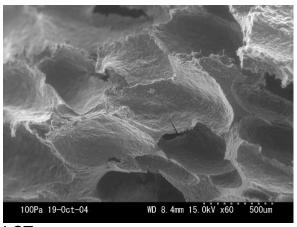
**Figure 3.** Effects of MCT, LCT or butter on the water activity ( $a_W$ ) of baked breads stored for 8 days. Each value represents the mean  $\pm$ standard deviation of triplicate measurements.

reaching an equilibrium after eight days; the  $a_W$  of LCT bread was 0.61 (Figure 3). In contrast, the MCT and butter breads showed  $a_W$  of 0.76 and 0.72, respectively. The decrease in  $a_W$  means a decrease of the mobility of the water molecule. The  $a_W$  is an important parameter in the microbiological stability of products (Fennema, 1993; Battaiotto et al., 2012). The  $a_W$  maximum of 0.6 as described in the present work, is sufficiently low to prevent microbial growth. However, in case of bread containing MCT or butter, the observed  $a_W$  maximums were insufficient to prevent microbial growth. Hence, the results of this study indicate that the differences in the properties of the lipid affect the level of water activity in the breads incorporated with them.

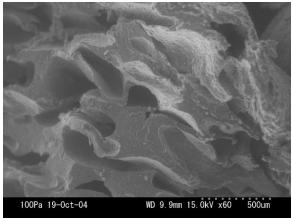
### Microstructure of baked bread

As shown in Figures 4 to 6, cryo-SEM was employed to observe the fine structure of the baked breads and indicates the differences in the properties of lipids which affect the microstructure of breads. It is likely that differences in the fine structure are closely involved in the textural quality of the bread. In the cryo-SEM images of Figure 4, a number of pores were observed in the MCT bread. On the other hand, in bread containing butter, smaller numbers of pores were observed. Considering the fact that porosity had a major impact on the textural properties of the bread, the texture of the MCT bread was

# MCT



LCT



Butter

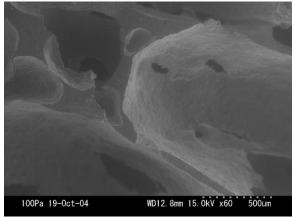
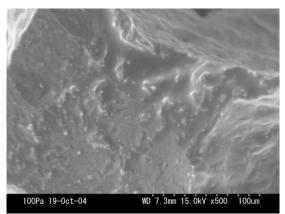


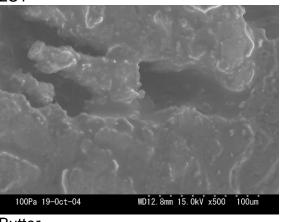
Figure 4. The microstructure of breads prepared with MCT (top), LCT (middle) or butter (bottom). Scale bars represent \* 500  $\mu m$ 

presumed to be the best. Figures 5 and 6 show enhanced details of the fine structure of breads shown in Figure 4. Changes were observed in the bread microstructure depending on the lipid added. With respect to the fine structure surface, in the case of MCT bread, a smooth

# MCT



LCT



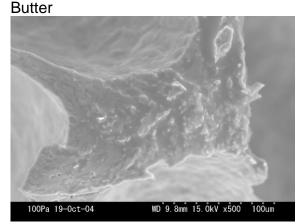


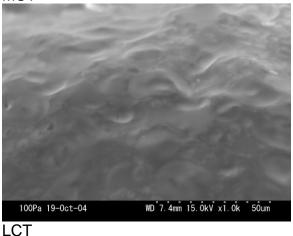
Figure 5. The microstructure of breads prepared with MCT (top), LCT (middle) or butter (bottom). Scale bars represent \*100  $\mu$ m.

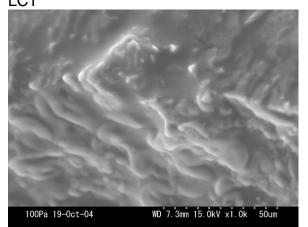
surface was formed compared to bread with butter or LCT. This may be that bread with MCT has good texture. Furthermore, the microstructure results are consistent with the results of bread properties (Figures 1 and 2).

# Conclusions

The main aim of this research was to ascertain the







**Butter** 

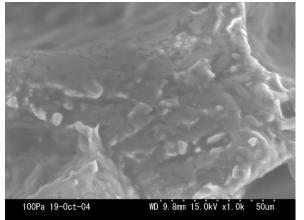


Figure 6. The microstructure of breads prepared with MCT (top), LCT (middle) or butter (bottom). Scale bars represent  $*50 \ \mu$ m.

functional characteristics of MCT in bread production. The microstructure formation and rheological properties of MCT bread were compared with those of bread containing LCT or butter. While the role of lipids in bread making has previously been poorly understood, the present study clarified that lipids affect the physical properties and microstructure of breads. This study suggests that the properties of MCT can be functionally applied to bread making.

# **Conflict of interests**

The authors did not declare any conflict of interest.

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