Effect of pore structure and starch retrogradation on physical properties of breadcrumb
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ABSTRACT
The firmness of bread crumb and the changes in firmness during preservation were measured. An emulsifier (glycerol monopalmitate; GMP) was added to the bread dough to change the degree of starch retrogradation. The additive amount of yeast was regulated to change the cellular structure in the bread crumb. A proteolytic enzyme (papain) was added to change the viscoelastic properties of gluten in bread dough. The addition of GMP delayed the starch retrogradation and the bread hardening within a few days. After that, the degree of starch retrogradation was kept not to be increased, whereas the bread hardening increased. Generally, the bread crumb staling is mainly caused by starch retrogradation. However, such experimental results showed it might be caused not only by the starch retrogradation. Addition of appropriately amount of papain improved extensibility of the dough and increased the bread crumb porosity. Increase in porosity resulted in decreasing in the firmness of bread crumb. The addition of papain decreased the firmness of cell wall in the bread crumb. Therefore, as well as gluten might directly affect the firmness of cell wall as the material of bread crumb, it might affect the elastic properties of bread crumb indirectly through cellular structure.

Keywords: bread; GMP; papain; cellular structure; retrogradation

INTRODUCTION
The bread staling begins soon after baking, and the bread firmness increases during preservation. The bread staling influences the bread quality. As the bread crumb is the porous material, the substances related to its physical properties are the pore walls as well as their three dimensional structure called as cellular structure. The pore walls mainly consist of gelatinized starch and gluten. The wheat starch has a large influence on the physical properties of bread crumb, because of wheat components from 60% to 75% are made up of starches [1] which absorb about 50% of total water in the bread crumb. The water transfer from gluten to gelatinized starch [2] might be the cause of bread hardening during preservation. On the other hand, about 85%(v/v) of bread volume is air. Therefore, the porosity and the pore size distribution in the bread crumb have a large influence on the physical properties of bread crumb. That is to say, the physical properties of bread crumb might be affected by the cellular structure and the physical properties of gelatinized starch and gluten, both of which play roles as matrices of air-dispersed system. This study focused on effects on bread firmness and hardening with time from the following viewpoints; starch retrogradation, cellular structure and elastic property of gluten. The elastic moduli and the elastic collapse stresses of standard breads were compared with each of the three bread types; a GMP-added bread to delay starch retrogradation, a yeast-rich or yeast-reduced to change bread to change cellular structure of breadcrumb, and a papain-added bread to inhibit gluten development.

MATERIALS & METHODS
Materials
GMP-added bread
Bread was made by a straight method with a bread machine (HBH-100, MK seiko Co.). Dough of bread (GMP b) was made with 300 mg hard wheat flour, 190 mL distilled water, 4 g salt, 2.4 g dry yeast and 3 g glycerol monopalmitate (GMP). The normal bread (STD) as a control sample was prepared without GMP. After baking, the breads were cooled at room temperature, and then they were sliced at 1.8 cm thickness. The breads were refrigerated in sealed containers at 278 K which accelerates the starch retrogradation, for one week.

Yeast-rich bread and yeast-reduced bread
Dough of bread was made with 300 mg hard wheat flour, 190 mL distilled water, 4 g salt and yeast; 1.2 g yeast (bread A); 4.8 g yeast (bread B); 7.2 g (bread C).
**Papain-added bread**
Bread was made by straight method with a bread machine (PY-D432W, TWINBIRD Co.). Dough of bread (PI b) was made with 500 mg hard wheat flour, 360 mL distilled water, 10.8 g salt, 20 g shortening, 4.5 g dry yeast and 10 or 13.7 mg papain. The normal bread (STD) as a control sample was prepared without papain.

**Pore wall model**
Dough of pore wall model was made with 504.5 mg strong flour, 360 mL distilled water, 10.8 g salt, 20 g shortening and 10 or 30 mg papain. The normal pore wall model (STD) as a control sample was prepared without papain. After mixing (one hour), the dough was rolled out to 0.5 mm-0.8 mm thickness, sealed in a plastic bag and then heated in water bath at 373 K for 10 min. After heating, it was cooled in at room temperature, and then refrigerated at 278 K which accelerates the starch retrogradation, for one week.

**Methods**

**Degree of starch gelatinization**
A degree of starch gelatinization was measured with beta-amylase and pullulanase method (BAP method) [3]. Figure 1 shows a procedural flow diagram of a BAP method. The gelatinization degree is defined as the following equation.

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\text{Degree of starch gelatinization} \% = \frac{\text{concentration of maltose in normal sample} - \text{concentration of maltose in degradated sample}}{\text{concentration of maltose in normal sample}}
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![Figure 1. Procedural flow diagram of a BAP method.](image-url)
Moisture contents
Moisture content of bread was measured by an oven method (378 K, 24 hours).

True density
Volume of sample was measured with a gas comparison pycnometer (AccuPyc II 1340 Pycnometer, Micromeritics). True density of sample was determined by dividing its mass by its volume.

Specific volume
Mass of bread sample (4 cm × 4 cm × 1.8 cm) was measured with an electric balance. Lateral side and upside images of sample were captured by a digital camera (PC1226, Canon). The volume of sample was determined by the product of the upside area and the thickness which were calculated from the binarized image of sample. Specific volume of sample was determined by dividing its volume by its mass.

Elastic modulus & Elastic collapse stress
The samples in sealed containers were warmed to room temperature before measurement. The uni-axial compression test was conducted with a rheometer (CR200D, Sun Scientific Co.). The center of upper side of sample was compressed by a disc plunger (30 mm dia.) at a constant speed of 4 mm/min. The compressive force data were recorded with a data logger (midi LOGGER GL800, Graphtec Co.). An elastic modulus $E$ and elastic collapse stresses $\sigma_{c1}^*$ was determined from a stress-strain diagram. The $\sigma_{c1}^*$ is a stress at buckling of inner pore walls. This buckling point was determined as an inflection point on the stress-strain curve. The $E$ was determined as the slope of the stress-strain curve before buckling.

The elastic properties of pore wall model sample were measured by a tensile test.

Porosity & thickness of pore wall
An X-ray micro CT scanner (1172, Skyscan) was used for understanding cellular structure inside of bread. The 1 cm cubic specimen was covered by plastic wrap for preventing water evaporation under observation. From the reconstructed images (pixel size = 8.93 µm) the porosity of specimen and the average thickness of pore wall were calculated.

RESULTS & DISCUSSION
The changes in $E$ and degree of starch gelatinization in seven days after baking are shown in Figure 2. The addition of GMP slowed down the progression of starch retrogradation and delayed bread staling. Although the degree of starch gelatinization of GMP bread in 7 days was kept higher than of STD bread, the $E$ of GMP bread continued to increase. The moisture contents of GMP and STD bread did not change in seven days. The changes in bread firmness could be caused not only by the starch retrogradation.

The changes in $E$ of breads made with different amount of yeast in seven days after baking were shown in Figure 3. It shows that the additive amount of yeast affected the elastic modulus. The X-ray cross-sectional images of the normal bread and 1/2 yeast of bread (bread A) are shown in Figure 4 and Figure 5, respectively. The amount of pore volume and the average size of pore in bread A are smaller than in STD bread because the less than normal amount of yeast suppressed the gas generation by fermentation. The thickened pore walls might have strengthened the breadcrumb, and therefore, the elastic modulus $E$ should have be increased.
The changes in $\sigma_{el}^*$ of STD and PPI b were shown in Figure 6. The $\sigma_{el}^*$ of PPI b were lower than of STD, however, if the amount of papain was too much, the $\sigma_{el}^*$ was not decreased. Figure 7 and Figure 8 show the changes in specific volumes and the porosities, respectively, in seven days after baking. In case of addition of small amounts of papain, the specific volume was slightly increased. It means that the pore volume was increased. The improvement of dough extensibility by inhibition of gluten development might decrease the pore wall thickness and strength, whereas, the addition of much amounts of papain decreased both specific volume and porosity of bread crumb. It means that the pore volume was decreased. The inhibition of gluten development by much addition of papain could change the cellular structure and increase $\sigma_{el}^*$. The changes in $E$ of STD and PPI b were shown in Figure 9. The gluten development inhibition by addition of papain might have decreased elasticity of heat-treated pore wall.
CONCLUSION
The elastic moduli and the changes in $E$ during preservation at 278 K were measured for GMP-added breads, yeast-rich or yeast-reduced breads and papain-added breads. The addition of GMP delayed the starch retrogradation and the bread hardening within a few days. After that, the degree of starch retrogradation was kept not to be increased, whereas the bread hardening increased. The addition of inappropriately amount of yeast decreased the pore wall thickness and increased the elastic modulus of bread crumb. The addition of papain increased the porosity of bread crumb and decreased the $\sigma_{el}$ of the bread crumb. Whereas, the addition of much amounts of papain decreased both specific volume and porosity of breadcrumb, but did not decrease $\sigma_{el}$. Therefore, as well as gluten might directly affect the firmness of the pore wall as the material of the bread crumb, it might affect the elastic properties of the bread crumb indirectly through the cellular structure such as pore wall thickness.

REFERENCES