国産麺用小麦粉を用いたパンの品質に及ぼす酸可溶性小麦タンパク質と中種法の改良の影響

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Effect of Acid-Soluble Wheat Protein and Improvement of the Sponge and Dough Method on Bread Quality Using Domestic Noodle Flour

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In this study, we used acid-soluble wheat protein (ASP), which is mainly composed of gliadin, to improve the sponge and dough method. We were able to use domestic noodle flour, hitherto considered unsuitable for baking, and obtain a bread quality comparable to that made with imported strong wheat flour. The loaf bread volume and specific volume were investigated, and the microstructure of the gas cells in the bread crumb was observed. Further analysis was conducted to determine the degree of starch gelatinization, and bread crumb rheological properties such as firmness and cohesiveness changed over time. The results showed that the volume of noodle wheat flour bread increased with ASP addition. Furthermore, to enhance the dough gluten strength of the noodle wheat flour, the following steps were conducted: salt, which is usually added at the dough mixing stage, was added to the sponge dough, and the amounts of oxidizing agent and compressed yeast were increased. This led to a substantial increase in the bread volume and specific volume. Microstructure observation showed that the gas cell shape in the noodle wheat flour bread was similar to that of the control, which used wheat flour for bread. It was also confirmed that changes over time in rheological properties were suppressed, and that overall bread quality approached that of the control.

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Key words: *domestic noodle flour, wheat protein, gliadin, bread, dough*
国産麺用小麦粉，小麦タンパク質，グリアジン，パン，生地

In Japan, domestic wheat varieties such as those used in the production of noodles are generally considered unsuitable for baking[1),2)], which typically demands better quality wheat than what is produced domestically. Bread quality can be improved by blending super strong flour or foreign wheat flour, or by using protein disulfide isomerase on improving bread quality[3)-6)]. In recent years, domestic wheat cultivars have begun to genetically modify wheat to render it suitable for bread production[7)]. Nonetheless, the production of wheat for use in bread remains low in Japan. Therefore, with the above in mind, the authors of this study attempted to develop a baking technique utilizing the sponge and dough method, which may promote the use of domestic noodle wheat flour in bread production. Noodle wheat flour has a lower protein content than bread wheat flour. Accordingly, when gluten, obtained from hard wheat grain, was added to the flour, its dough structure lost uniformity and the carbon dioxide gas generated through fermentation could not be sufficiently retained. Therefore, acid-soluble wheat protein (ASP), which is mainly composed of gliadin, was added to the flour to improve baking quality by enhancing dough extensibility[8)]. In this study, on the basis of previously developed techniques, baking with noodle wheat flour was tested under differing conditions

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(three test groups and a bread wheat flour control), and the quality of the bread was examined. The following points were examined: the volume and specific volume of the bread, the bread crumb microstructure observation, and changes over time during storage in both starch retrogradation and rheological properties.

Materials and methods

1. Samples

The flour used in the experimental groups in this study was domestic noodle wheat flour (9.8% protein, 0.39% ash; Akaboshi [Hokushin], Nippn Co., Ltd., Japan). The flour used for the control was imported wheat blended flour (12.2% protein, 0.38% ash; Eagle, Nippn Co., Ltd., Japan). For the ASP, which is mainly composed of gliadin, an industrial product (Glia A; Asama Chemical Co., Ltd., Japan) was used. The ASP was extracted from imported gluten that was prepared from hard wheat grains.

2. Baking method

Baking tests were performed using the sponge and dough method. The formulation and processes are shown in Table 1 and Fig. 1, respectively, following previous studies[8]. All ingredients were given in Baker's percentages based on total flour weight. Other materials were used for the preparation of bread; compressed yeast (Yongo Co., Ltd., Japan), ascorbic acid (Showa Chemical Co., Ltd., Japan), salt, granulated sugar, skim milk powder (Megmilk Snow Brand Co., Ltd., Japan), and shortening (Nichiyu Co., Ltd., Japan). For this study, the previously reported improved baking technique, as described below, was employed. First, the improved product prepared using domestic noodle wheat flour contained 3% ASP. Next, the amount of ascorbic acid, an oxidizing agent, added was increased to 100 ppm and mixed with, 2% salt (of total wheat flour), which is normally used in the final dough mixing, was added to the sponge dough phase. Finally, the water content was increased by 2% in the sponge dough. In addition, the amount of compressed yeast added was increased to 2.5%, and the final temperature of the mixture was raised to 27℃. This modified process was used to prepare the dough to retain the carbon dioxide gas produced during fermentation. The bread production for the tests was based on 4 kg of wheat flour. Two types of bread, loaf and pullman with tin were baked. The above baking test is shown in Test 3. As a comparison of the three test groups, CT was the control bread and was prepared with bread-grade wheat flour. Test 1 used noodle wheat flour with the standard sponge and dough method, and

Table 1 Material formula of baking

Baker's %

Material	CT	Test 1	Test 2	Test 3
Sponge				
Noodle wheat flour*1	–	70	70	70
Bread wheat flour*2	70	–	–	–
ASP*3	–	–	2.1	2.1
Salt	–	–	–	2
Compressed yeast	2	2	2	2.5
Ascorbic acid	0.001	0.001	0.001	0.01
Water	40	37.9	40	42
Dough				
Noodle wheat flour*1	–	30	30	30
Bread wheat flour*2	30	–	–	–
ASP*3	–	–	0.9	0.9
Sugar	6	6	6	6
Salt	2	2	2	–
Skim milk	2	2	2	2
Shortening	6	6	6	6
Water	24	16.1	17	15

*1 Domestic wheat cultivar (Hokushin)
*2 Imported wheat
*3 Acid-soluble wheat protein

Process	Condition
Sponge mixing*1	CT; L2M2, Test 1; L2M2, Test 2; L2M2, Test 3; L2M4H1 Final temperature of mixing, CT, Test 1, and Test 2 was 24℃, and only Test 3 was mixed at 27℃.
First fermentation	27℃, 75% RH, 4 h*2
Dough mixing*1	CT; L2M3H1, Test 1; L2M2H1, Test 2; L1M2H1, Test 3; L1M2H1 Shortening addition CT; L2M3H0.5, Test 1; M2, Test 2; M2, Test 3; M2H1 Final temperature of mixing 27±0.5℃, at four procedure
Floor time	27℃, 75% RH, 20 min
Dividing	Loaf bread; 450 g, pullman bread; 220 g×6
Rounding	
Intermediate proofing	15 min
Molding	
Final proofing	38℃, 85% RH Loaf bread; 10 mm height above the top of baking pan Pullman bread; 35 mm below the top of baking tin case
Baking	Loaf bread; 225℃, 25 min, Pullman bread; 225℃, 40 min
Cooling & packing	Packing after 90 min of cooling down at room temperature (20–25℃)

Fig. 1 Baking process via the sponge and dough method*3

*1 Mixing Condition; L, M, H indicated the rotation speed of the mixer, i.e. L is Low (135 rpm), M is Middle (203 rpm), and H is High (235 rpm).
The number after acronym indicated time (min).
*2 Final temperature of fermentation, CT; 28.5℃, Test 1; 27.8℃, Test 2; 27.9℃, Test 3; 28.0℃.
*3 Two types of bread were prepared, i. e., loaf bread and pullman bread.
The instruments used in the baking test are listed below.
Mixing; Vertical Mixer VM-3, Fermentation; Model OBS-D5,
Molding; Model WFS, Baking; Deck Oven, Model DOV-T 43,
(All equipments and machinery listed above are manufactured by Oshikiri Machinery Ltd.)

Test 2 went through the same production process as Test 1, but had an addition of 3% ASP.

3. Evaluation of bread quality

(1) Measurement of volume and specific volume of loaf bread Loaf bread volume (cm^3) was measured using a laser volume measurement unit (Selnac-Win VM; K-AXIS Co., Ltd., Japan) after the bread was baked and cooled for 30 min at room temperature (20–25℃). The specific volume (cm^3/g) was calculated by dividing the volume by weight.

(2) Microstructure Observation Using Scanning Electron Microscopy The bread crumb micro structure was observed by scanning electron microscopy (SEM, S-2340N; Hitachi, Ltd., Japan) after using the method described below. In each test group, the loaves of bread were sliced vertically to produce 15 mm thick slices, and the portion close to the crust was cut into a 10×10 mm square shape. These samples were then prepared for SEM observation, as follows: The samples were fixed with 2.5% (v/v) glutaraldehyde, rinsed with 100 mM phosphate buffer, fixed with 1% (v/v) osmium tetroxide, and rinsed again with 100 mM phosphate buffer. Samples were then dehydrated sequentially using 50%, 60%, 70%, 80%, 90%, and 95% ethanol (v/v), and ethanol was substituted with 2-methyl-2-propanol. Samples were then freeze-dried (ID-2; Eiko Co., Ltd., Japan), and subsequently gold-coated in an ion-coater (IB-3; Eiko Co., Ltd., Japan). Micrographs were taken at two magnifications (50× and 1,000×), both at an acceleration voltage of 15kV.

(3) Gelatinization degree measurement by enzyme degradability The degree of gelatinization in bread was measured using the β-amylase/pullulanase (BAP) method, following the method established by Kainuma[9]. This method is useful for determining the retrogradation of starchy foodstuffs during storage. Following this method, ethanol was added to bread crumb samples, that had been stored at 20℃ for 1 to 3 days, and dehydration was repeated three times. Finally, the samples were treated with acetone for dehydration, dealcoholization, and powderization. The powder samples were dispersed in water using a glass homogenizer, and then enzymatic reactions were carried out. The enzymes used for the reactions were soybean β-amylase (crude enzyme standard 5 IU / mg; Nagase ChemteX Corp., Japan) and pullulanase (crude enzyme 2 IU / mg; Hayashibara Co., Ltd., Japan). The reducing sugars produced by this reaction were measured using the Somogyi-Nelson method, and the total sugar was measured using the phenol-sulfuric acid method. The degradation rate was defined as the ratio of reducing sugar to total sugar. The degree of gelatinization was calculated using the following equation:

"Degradation rate of dispersed sample/degradation rate of alkaline gelatinized sample ×100"

(4) Measurement of rheological properties of bread After baking, the breads were cooled for 90 min and packaged in a polypropylene bag (0.03 mm). Thereafter, for each day of storage, four samples were placed in polyethylene bags (0.08 mm). The storage period was 1 to 3 days. Pullman bread was sliced to a thickness of 25 mm, and 25 × 25 mm squares of the bread crumb were cut from the center of the slice using an ultrasonic cutter (Model USC-3305; Yamaden Co., Ltd., Japan). Bread crumb firmness and cohesiveness were measured using a creep meter (RE2-33005S; Yamaden Co., Ltd., Japan). Firmness was measured using a circular plunger (ϕ 40 mm), with a measurement strain rate of 70% and a compression speed of 1 mm/s. All samples were compressed in the same direction, and the value at the 50% strain rate was determined as firmness. Cohesiveness was determined as the area ratio between the first and second waveforms expressed by the stress values when 70% compression was performed twice at a speed of 1 mm/s with a plunger (ϕ 16 mm).

4. Statistical analysis

Baking tests were carried out twice, and it was confirmed that the results of each test group showed the same trends. After one-way analysis of variance, Tukey's honest significant difference multiple comparison test was performed, and statistical significance was set at $p < 0.05$.

Results and Discussion

1. External appearance, volume and specific volume of loaf bread

The appearance of the different loaves under each baking condition was compared (Fig. 2). Test 1, which used noodle flour without ASP, had less volume than the control (CT), which was prepared using bread flour. The bread made in Test 2 with noodle flour plus ASP, expanded to the same level as CT; however, it had a less cracked upper crust than that of CT. It was assumed that this was due to the difference in the dough expansion ability during baking. The bread made in Test 3 expanded

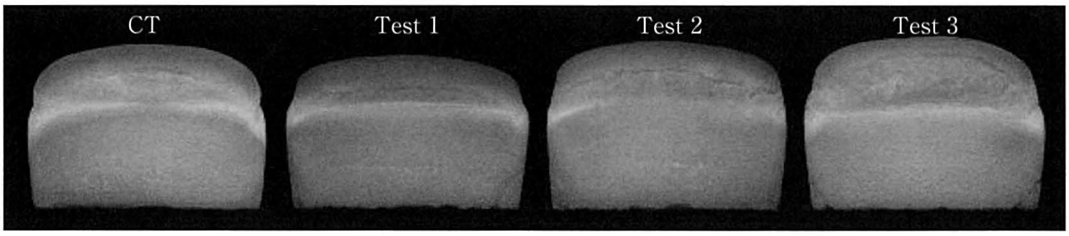

Fig. 2 External appearance of bread loaves

CT, Bread flour; Test 1, Domestic noodle wheat flour; Test 2, Domestic noodle wheat flour +3% ASP; Test 3, Domestic noodle wheat flour +3% ASP. CT, Test 1, Test 2, Standard sponge and dough method; Test 3, Improved sponge and dough method; ASP, acid-soluble wheat protein.

more than that the bread in CT, and the side cracks in the bread crust became larger. Usually in the case of loaf-shaped bread, when the dough expands rapidly during the baking process, cracks appear on the upper sides, and it is said that the larger the size of the cracks, the better the baking property[10]. Additionally, the specific volume of bread increases by reducing the gas-leakage rate of the dough[11]. Therefore, the difference in appearance of these breads was assumed to be related to differences in dough expansion in each of the test groups during the 4 h dough fermentation process. In a previous report, the Test 3 bread dough retained as much or more carbon dioxide gas that was generated during the fermentation process than CT. However, in both Tests 1 and 2, the rate of expansion of dough peaked 2–3 h after the start of fermentation, and became stable or gradually decreased[8]. It was found that Test 1 had weak dough due to lack of protein. The protein content of Test 2 was increased by the addition of ASP, but the dough could not sufficiently retain the carbon dioxide gas generated during fermentation solely through this increase of protein. However, dough expansion progressed due to the ability of ASP to increase dough extensibility. In general, the sponge and dough method requires a sponge dough with appropriate extensibility and elasticity. Normally, salt is added in the dough mixing process in sponge and dough method baking. However, it has also been reported that the addition of salt at the sponge dough stage, at a level of 0.5% or 1% of total flour, produced better bread compared to that produced with no salt addition or a salt addition of only 0.15% of total flour[12]. Salt has a water-soluble action that changes the state of existence of gliadin, and a further action that aggregates gliadin and strengthens the intermolecular action, and is considered to play a role in gluten formation[13]. Therefore, in the sponge and dough method using domestic wheat flour, Test 3 had ASP added to it, and in the sponge dough stage, 2% salt was added. Furthermore, the amount of ascorbic acid was also increased, resulting in a dough was sufficiently mixed to have high gas retention. As a result, the dough from Test 3 gained a viscoelasticity comparable to that of CT. Additionally, the volume of the bread increased, and loaf shape improved, with large cracks on the upper side surface. To objectively evaluate the bread, measurements were conducted on the volume and specific volume of loaf bread (Table 2). Test 3, which was improved by adding ASP and modifying its sponge formula, had substantially higher volumes and specific volumes than those the bread from any other test, and CT. Compared with Test 1, Test 2 showed similar values to CT, which used bread wheat flour. Similar tendencies were observed for

Table 2 Effect of addition of acid-soluble wheat protein (ASP) and improvement of the sponge and dough method on the volume and specific volume of bread prepared from domestic noodle wheat flour

	Type of wheat flour	Method	Addition of ASP (%)	Volume (cm^3)	Specific Volume (cm^3/g)
CT	Imported bread wheat flour	Standard sponge and dough	–	2,074 ± 10[b]	5.21 ± 0.05[b]
Test 1	Domestic noodle wheat flour	Standard sponge and dough	–	1,983 ± 39[c]	4.99 ± 0.09[c]
Test 2	Domestic noodle wheat flour	Standard sponge and dough	3	2,071 ± 42[b]	5.16 ± 0.11[cb]
Test 3	Domestic noodle wheat flour	Improved sponge and dough	3	2,187 ± 15[a]	5.51 ± 0.06[a]

The results showed the average value ± standard deviation (n = 3). The values followed by the same letter with the column are not significantly different (p < 0.05). The analysis of variance between the data was evaluated using IBM SPSS Statistics Ver. 25.

bread appearance (Fig. 2).

2. Bread crumb microstructure

Fig. 3 shows the SEM observations of the loaf bread crumb microstructure for each test group and the control. The observed parts are indicated by squares. Compared with CT, large, non-uniform gas cells were observed in the bread crumb microstructure from Tests 1 and 2. However, in Test 3, vertically extending gas cells, similar to those in CT, were observed. Enlarged surface segments of each gas cell wall are shown in the right column. In Tests 1 and 2, the surface was partially peeled off, and flat starch particles were exposed. In CT and Test 3, the surface peeling decreased, and the exposed starch particles were slightly elongated. The gluten protein, which forms the structure of the gas cell walls in bread dough, begins to undergo heat denaturation at 60–70℃, at

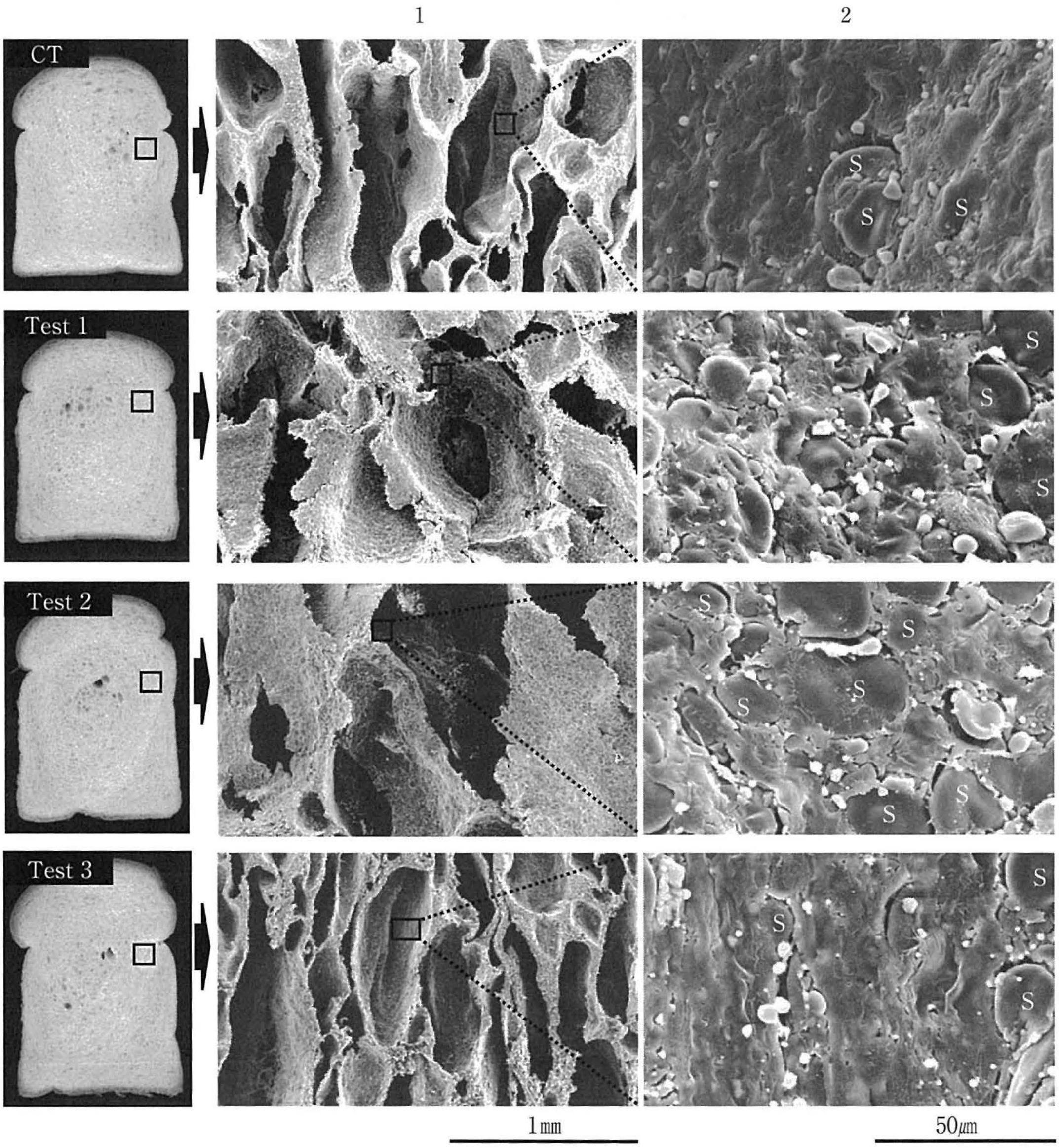

Fig. 3 Scanning electron micrographs of crumb microstructure of bread prepared from domestic noodle wheat flour using the sponge and dough method

Enlargement of the part shown in the bread pictures on the left column are shown in 1 (center column) and 2 (right column). 1: Structure of gas cell wall in crumbs (low-magnification, ×50). 2: Magnification of the surface of gas cell wall of the part in 1 (high-magnification, ×1,000). S indicates starch granules.
CT, Control (bread flour); Test 1, Domestic noodle wheat flour; Test 2, Domestic noodle wheat flour + 3% ASP; Test 3, Domestic noodle wheat flour + 3% ASP. CT, Test 1, Test 2, Standard sponge and dough method; Test 3, Improved sponge and dough method; ASP, acid-soluble wheat protein.

which point its hydration capacity is reduced, and released water is used for gelatinization of starch granules. As the gas cells grow larger, the starch granules elongate and deform, thus the gluten sheet becomes thinly stretched. The gelatinization of the starch granules that are uniformly embedded in the gluten matrix plays an important role in the regular expansion of dough during baking, thereby determining the crumb[14),15)]. A less uniform gluten matrix may not be able to withstand the regular expansion of gas cells during baking. During the early stages of baking, gas cells expand rapidly, coalesce, and then enlarge[16)], which affects the bread crumb grain structure. For Test 2 with added ASP, it could be inferred from microstructure observations that extensibility was higher than in Test 1. However, when enlarged, the starch particles exposed from the peeled part of the surface of the gas cells were flat, similar to those of Test 1, unlike those of CT. It has been reported that the higher the elasticity of gluten, the more the gas cells maintain a vertically elongated shape and volume during the gas cell structure becoming fixed through baking[17)]. It was inferred that the rheological properties of bread dough in Test 3 resembled those of CT, as opposed to the rheological properties of bread dough in Test 1 and Test 2. Observation of the bread crumb microstructure showed that the volume of Test 2 increased due to the addition of ASP, although flat starch granules exposed on the surface of the gas cells were also observed. Conversely, for Test 3, in which dough was prepared by adding ASP and improving the sponge dough, it was assumed that because the finely dispersed carbon dioxide gas could be retained, the resulting bread shape was similar to that of CT.

3. Changes in starch gelatinization degree in the bread crumb over time

Observation of the microstructure of the bread crumb gas cells indicated a difference in the existential state of starch granules in each of the test groups. Early bread retrogradation is assumed to be primarily affected by starch retrogradation[18)]. To examine the changes in the degree of gelatinization during storage of breads, starch was sampled from the crumb of baked breads stored at 20℃ after 1 or 3 days. The degree of gelatinization changes was measured using the BAP method[9),19)], which is particularly useful for determining the retrogradation of starchy food during storage (Table 3). The degree of gelatinization of Test 1, prepared using the sponge and dough method with noodle wheat flour, was 41% after 1 d. For Test 2, which had ASP added, it was 51%, for Test 3, it was 55%, and for the control, CT, it was 57%. After three

Table 3 Changes in the degree of gelatinization of bread prepared from domestic noodle wheat flour using the sponge and dough method during storage at 20 ℃

	Type of wheat flour	Method	Addition of acid-soluble wheat protein (%)	Degree of gelatinization (%) Storage period (day)	
				1	3
CT	Imported bread wheat flour	Standard sponge and dough	–	57.4 ± 5.4^a	28.6 ± 3.7
Test 1	Domestic noodle wheat flour	Standard sponge and dough	–	40.9 ± 2.9^b	20.3 ± 2.2
Test 2	Domestic noodle wheat flour	Standard sponge and dough	3	51.7 ± 2.6^a	25.7 ± 4.8
Test 3	Domestic noodle wheat flour	Improved sponge and dough	3	54.7 ± 0.9^a	26.8 ± 1.1

The results showed the average value ± standard deviation (n = 3). The values followed by the same letter with the column are not significantly different (p < 0.05). The analysis of variance between the data was evaluated using IBM SPSS Statistics Ver. 25.

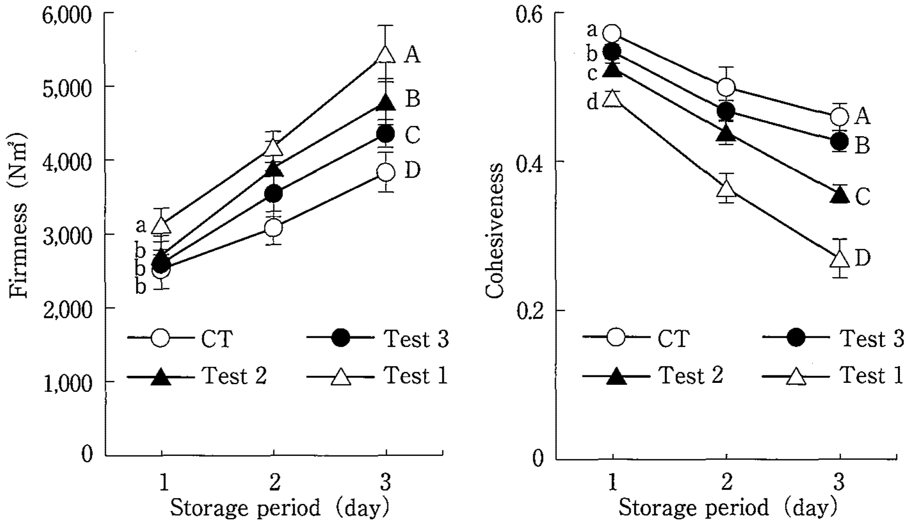

Fig. 4 Changes in crumb firmness and cohesiveness of bread during storage at 20℃, for bread prepared from domestic noodle wheat flour using the sponge and dough method

CT, Control (Bread flour); Test 1, Domestic noodle wheat flour; Test 2, Domestic noodle wheat flour +3% ASP; Test 3, Domestic noodle wheat flour +3% ASP. CT, Test 1, Test 2, Standard sponge and dough method; Test 3, Improved sponge and dough method. Vertical bars show the SD of each value (n=8). Bars labelled with different letters are significantly different ($p<0.05$) in between the tests groups. The analysis of variance between the data was evaluated using IBM SPSS Statistics Ver. 25. ASP, acid-soluble wheat protein; SD, standard deviation.

days, all values decreased to below 30%. However, compared to breads from Test 1, ASP-added Test 2 as well as Test 3 showed a reduced decrease in the degree of gelatinization. Thus, the progress of retrogradation in these breads was slightly suppressed. Adding ASP and improving the sponge dough resulted in higher elasticity of gluten in the dough, similar to that of CT. This resulted in better fermentation, improved burning during baking, and suppressed retrogradation of gelatinized starch.

4. Bread crumb firmness and cohesiveness and changes during storage

The changes in firmness and cohesiveness for each of the bread test groups stored at 20℃ for 3 days were measured. The firmness on the first day of storage was substantially higher for the bread from Test 1, while the firmness of the loaves from Test 2 and Test 3 was similar to that of CT loaves. On the 3rd day, there was a significant difference in firmness across all of the test groups, but Test 3 approached the firmness of CT. The cohesiveness was different from the first day for each of the test groups and it increased in the order of Test 1, Test 2, Test 3, and CT. The results for the changes over time in Test 1 showed the largest decline, followed by that of Test 2. Although there was a significant difference between Test 3 and CT, the decrease over time in both was suppressed in comparison to Test 1 and Test 2 (Fig. 4). Cohesiveness was defined as the area or energy ratio of the two compressions. The higher the value, the stronger the internal binding force of the bread. It has been reported that changes over time are related to a decrease in gluten-binding force[20], and a decrease in starch gelatinization. From the results of the SEM observation mentioned above, it was inferred that the structural difference in bread crumb gas cells also plays a role in this decrease.

Conclusion

In this study, baking tests were conducted to enable the production of high-quality bread using domestic noodle wheat flour, typically deemed unsuitable for baking. To investigate this, the sponge and dough method was improved, and the effect of addition of ASP to the dough was examined. The results of these tests showed that the volume, rheological properties such as firmness and cohesiveness, and changes in bread prepared using noodle wheat flour were comparable to those of bread prepared from bread wheat flour. Crumb microstructure SEM observation of the test bread showed that the state of gas cells and the shape of starch particles were similar to those of the control bread. To form such gas cells, the necessity of both ASP addition and modification of the sponge and dough method was confirmed. The gas cell wall of the bread crumb is composed of gluten and starch. Changes in the rheological properties of bread are also related to gluten and starch, which are key parameters in bread quality evaluation. Bread quality is determined by complex factors, such as retrogradation of starch and the interaction between gluten and starch. Investigation of the behavior of gluten and starch in the bread crumb gas cell wall, and its interrelationship with bread quality, is an area that requires further research.

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国産麺用小麦粉を用いたパンの品質に及ぼす酸可溶性小麦タンパク質と中種法の改良の影響

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製パンには不適とされる国産麺用小麦粉を用いて，品質の良いパンの製造を可能にするため，酸可溶性小麦タンパク質（ASP）を使用し，中種法を改良して製パン試験を行った。得られたパンは体積，比容積を測定し，走査型電子顕微鏡でパン内相の微細構造を観察し，デンプン糊化度並びに物性の経時変化を分析してパンの品質評価を行った。その結果，麺用小麦粉にASPを加えるとパンの体積は増加した。さらに生地のグルテン強度を高めるため，通常本捏で添加する食塩を中種に加え，酸化剤と酵母の添加量を増やし，十分な混捏を行い，中種生地を改良して製パンをした結果，パンの体積，比容積が有意に増加し，パン内相の硬さや凝集性などの物性の経時変化が抑制され，対照としたパン用小麦粉で作ったパンの品質に近づいていることを認めた。ASPを添加し，中種生地を改良して得られたパン内相の微細構造を観察すると，気泡の形状，気泡膜表面の状態，デンプン粒子の形状などがパン用小麦粉で作ったパン内相と近似していた。国産麺用小麦粉を用いた製パンにおいて，ASPの添加と中種生地の改良はパン内相の気泡形成に良い影響を及ぼし，パンの品質を高める一要因であると推察した。

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