空気分級した小麦粉中のグリアジン及びグルテニンの含有比が粘弾性に与える影響(農業工学科)

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Effect of Ratio of Gliadin and Glutenin Contents of the Air Classified Wheat Flour on the Visco-elasticity

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I INTRODUCTION

More than ninety-five percent of wheat consumed in Japan is imported from various countries according to the suitability of varieties for various purpose. This situation often invites a food shortage problem whenever there is a change in the policy of wheat supplying countries due to their own conditions or a change in the international situation. In order to solve this problem, it is necessary to develope some technique by which any variety of wheat can be used for several purpose. The most possible technique for this purpose is air classification.

Though air classification was studied by Jones[5], Gracza[1] and others, since physical property of the air classified flour which is one of the most important fundamental properties to utilize the air classified flour was not clear, the authors[8] studied on rheological behavior of the air classified flours, and found that dynamic visco-elasticities of those flours were not always proportional to their protein contents as shown in Fig.1. Since more wedge type protein bodies distribute in fine fraction and more adhesive

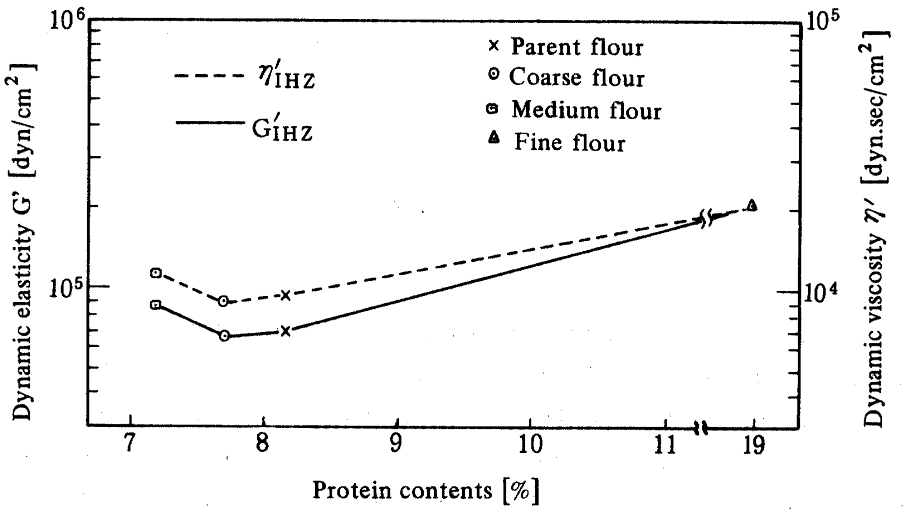

Fig. 1. Relationship between protein contents and dynamic visco-elasticity of the air classified flour

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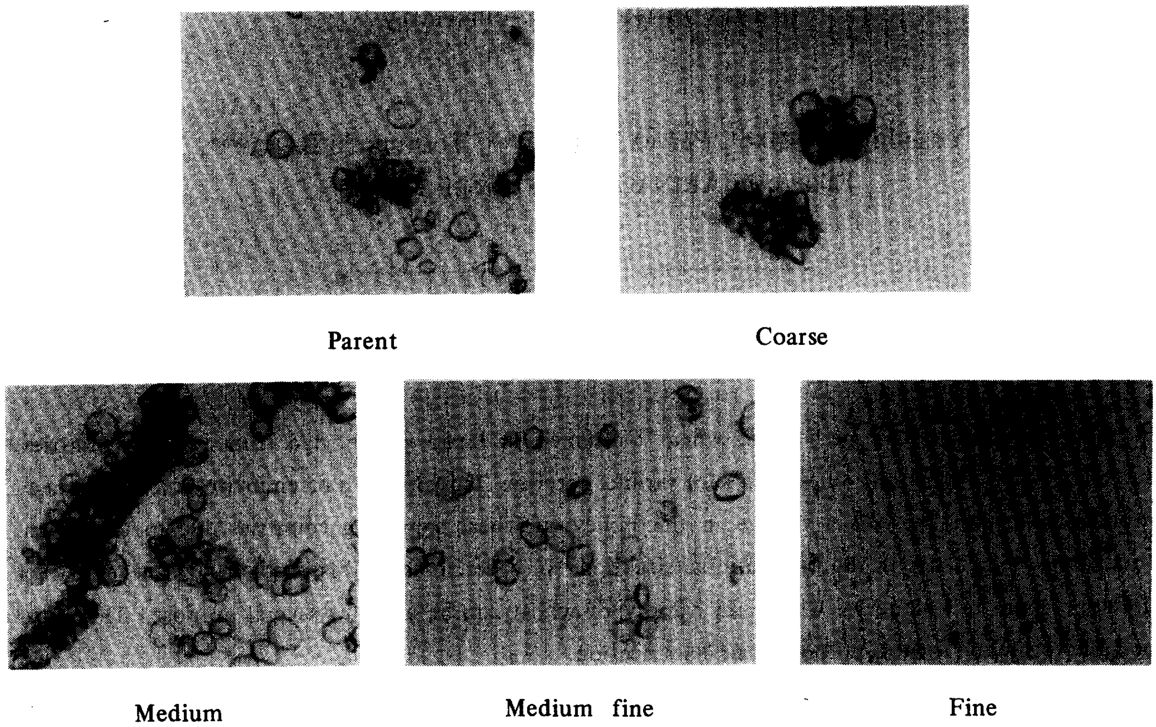

Fig. 2. Microscopic view of the air classified wheat flour.

protein in middle fraction in the air classified flour as shown in Fig.2 by Kohda (1979), it seems to be possible that ratio of gliadin and glutenin contents in the air classified fractions might be different and this might be a cause of different visco-elasticity in fractions of the same protein contents. In order to certify this hypothesis, an attempt was made to explore if there is any change in gliadin and glutenin contents of the air classified wheat flour fractions.

II EXPERIMENTAL

1 Material

Variety selected for study was Western White imported from the U.S.A. Sample material was milled in an industrial system, and was air classified with cyclones in Kumamoto Seifun Co. into three fractions, namely, coarse, middle and fine. The parent flour and these three fractions were named A-1, A-2, A-3 and A-4 respectively. Another sample of flour which consists of 70% of Western White and 30% of domestic wheat was air classified with Mikroplex separator in Tofuku Milling Co. into four fractions which were named B-1 (parent), B-2, B-3, B-4 and B-5. The later samples were used only for amino acid analysis.

2 Fractionation of Gluten

Gluten was obtained from these flours by hand washing method according to AACC standard and was fractionated into gliadin and glutenin by following two methods.

a) Isopropanol method: Gluten obtained as above mentioned was lyophilized and grinded into fine powder, and was used for fractionation into gliadin and glutenin, according to the method described by Murphy and Dahle[10]; by using 0.25 g of powdered gluten. This gluten was stirred in 40 ml of 35% v/v isopropanol for 35 min. Mixture was centrifuged at 800 rpm (80xG) for 3 min and supernatant was collected. Residue was extracted twice in the same way with isoparopanol, and then supernatants were pooled together and concentrated under vacuum untill about 10 – 15 ml of the material was left. Concentrated material was analyzed for nitrogen contents, converted into protein (N x 5.7) and reported as gliadin. Residue left over after gliadin was washed several times in distilled water, and analyzed for nitrogen contents, converted into protein (N x 5.7) and reported as glutenin.

b) 70% ethanol method: Hand washed gluten obtained from all the fractions of flour were solubilized by cutting into small pieces and stirring in 40 ml of 0.005 N lactic acid untill all the gluten was disolved (5 hrs), according to the method described by Hosney et al[3], with the exception of high protein flour. In that case 60 ml of lactic acid was used. After all the gluten was disolved, suspension was centrifuged at 1000 rpm (123 x G) for 20 min to remove entrapped starch. This residue was collected, dried and protein contents was estimated, and reported as non-extractable protein.

Acid soluble gluten was further used for gliadin and glutenin fractionation by making solution in 70% ethanol and neutralizing with 0.1 N Na_2CO_3. Neutralized solution was allowed to stand over night at 4 °C. The glutenin precipitated was collected by centrifugation and then freeze dried. Protein contents of dried material was estimated and reported as glutenin. Solution collected by decantation was vacuum distilled at 25 °C to remove ethanol, and then freeze dried, and protein contents was estimated and reported as gliadin.

So, above mentioned gliadin and glutenin means isopropanol soluble and insoluble fractions or 70% ethanol soluble and insoluble fractions, namely, crude gliadin and crude glutenin.

3 Water Soluble Protein

In all the sample of group A, water soluble proteins were calculated by difference between percentage of flour protein and that of gluten protein. Ratio of water soluble protein and gluten protein was calculated and reported.

4 Amino Acid Analysis

Since there are some difference of particular amino acid contents between gliadin and glutenin, amino acid composition of the air classified sample group B were determined, and were refered to compare gliadin-glutenin ratio in each fraction. Amino acid analysis was carried out by Hatano's method[2], with JLC-6AH (Nippon Denshi Co. Japan) after 5 mg of dried gluten was hydrolyzed in sealed vacuum tube for 48 hr at 105 °C with 3 ml of 6 N HCl containing 0.02% of mercaptoethanol.

5 Particle Size Distribution

Particle size distribution of the air classified flours were measured by sedimentation method using 70% ethanol at the concentration of 0.5%. Sedimentation rate was measured optically by photospectrometer and size distribution was calculated.

III RESULTS AND DISCUSSION

1 Effect of The Air Classification

Particle size distribution and protein contents of the air classified flours are shown in Fig.3, Fig.4 and Table 1 respectively. In these figures and tables, particle size distributions of each fraction are

Table 1. Composition of samples

Fraction	Moisure %	Ash* %	Protein* (N x 5.7) %	Lipid* %
A-1 (Parent)	14.0	0.4	8.5	0.7
A-2 (Fine)	11.9	0.4	18.2	0.8
A-3 (Medium)	13.2	0.4	8.2	0.7
A-4 (Coarse)	13.0	0.4	7.5	0.8
B-1 (Parent)	15.4	0.4	8.5	1.2
B-2 (Coarse)	14.4	0.3	8.4	0.8
B-3 (Medium)	15.3	0.4	8.1	0.9
B-4 (Medium fine)	14.5	0.5	25.1	2.3
B-5 (Fine)	13.6	0.5	25.3	1.4

* As-is basis

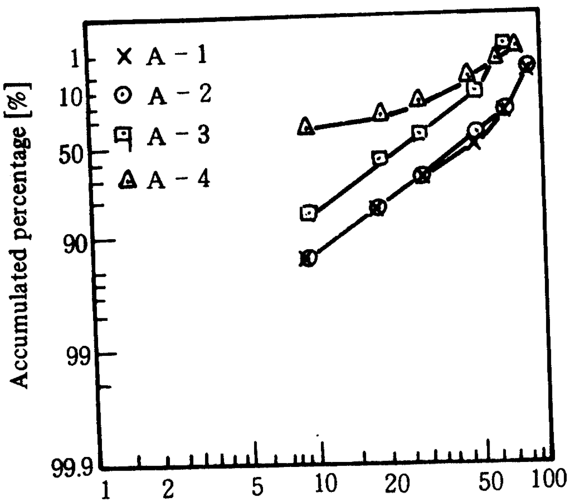

Fig. 3. Particle size distribution of the air classified flour (Sample A)

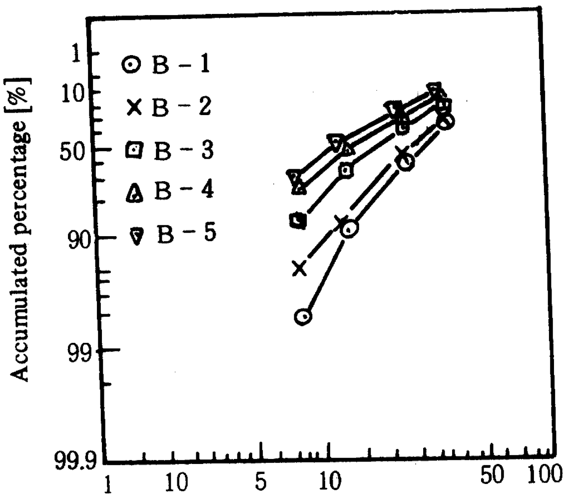

Fig. 4. Particle size distribution of the air classified flour (Sample B)

obviously different, and protein contents of the sample A changed from 8.56% of parent flour to 7.75 – 19.80%, and that of sample B changed from 8.5% of parent flour to 8.1 – 25% respectively, which shows that air classification could effectively made a protein shift. Gluten protein of flour, water soluble protein and their ratio of sample A are reported in Table 2, which shows gluten protein contributed 75 – 79.8% of total flour protein in case of parent flour and fine flour fraction, which is

Table 2. Composition of gluten and water soluble fraction in the air classified flour. (as-is basis)

Fraction	Flour protein %	Dry gluten %	Protein content of gluten % (G)	Gluten protein % (G)	Water soluble protein % (W)	G/W
A-1 (Parent)	8.56	8.65	6.42	74.25	2.14	3.00
A-2 (Fine)	19.80	20.29	15.79	77.82	4.01	3.94
A-3 (Medium)	8.23	8.23	6.33	77.01	1.90	3.33
A-4 (Coarse)	7.75	8.11	6.11	75.32	1.64	3.72

identical to the results reported by Mussarat and Kauser in previous paper[11]. Total soluble fractions ranged from 20.1 to 25% in case of fine fraction and parent flour respectively as compared to 19.8 – 27.0% and 13 – 22% reported by Kauser and Chandhry[7]. Ratio of water soluble and gluten protein was the highest in the case of fine flour (1:3.94) followed by coarse flour (1:3.72) and the lowest parent flour (1:1.30), which shows water soluble protein is less responsive to air classification than gluten protein. However, water soluble protein will not effect to visco-elasticity of wheat flour dough.

2 Ratio of Gliadin and Glutenin Contents

Contents of gliadin and glutenin extracted from sample A are reported in Table 3, which shows rartios of the both components obtained by isopropanol method and are65.3:35.4,64.6:33.8,65.8:35.7and 62.1: 41.1 respectively. These values are almost constant. On the other hand, ratios obtained by 70%

Table 3. Ratio of gliadin and glutenin in the air classified flour fraction.

Fraction	35% isopropanol fractionation		75% ethanol fractionation	
	gliadin %	glutenin %	gliadin %	glutenin %
A-1 (Parent)	65.27	35.37	41.77	58.23
A-2 (Fine)	64.55	33.79	42.14	57.86
A-3 (Medium)	65.78	35.69	41.64	58.32
A-4 (Coarse)	62.11	41.09	41.94	58.06

ethanol method were 41.8:58.2, 42.1:57.9, 41.6:58.3 and 41.9:58.1. These values are almost constant and coinside with the results reported by Hoseney et al[3] which is 43:57. Although these ratios varied with the method of fractionation, ratios in the same method were constant. It means that air classification did not affect the ratio of gliadin and glutenin in the flour, which agrees with the finding of Jones and Dimler[6], who reported the results when high and low protein glutens were compared by starch gel moving boundary electrophoresis had identical fractions and the same relative concentration.

3 Amino Acid Analysis

In order to confirm above results, amino acid composition of the air classified flour was investigated with the sample B. As Wu and Dimler[12] and others reported, it is known that contents of proline, glutamic acid, threonine and methionine are different in gliadin and glutenin. So, amino acid composition of each fraction of the air classified flour should be different if each fraction had different ratio of gliadin and glutenin contents. However, air classified flours fractionated into gliadin and glutenin fractions, when analyzed for amino acid composition, did not show statistically significant difference in the above mentioned amino acid composition as shown in Table 4.

Table 4. Amino acid composition of the sample B groupe.

Flour Fraction	B-1		B-2		B-3		B-4		B-5	
Amino Acid	μg*	%	μg*	%	μg*	%	μg*	%	μg*	%
Phenyl-alanine	28.92	3.57	26.54	3.29	26.57	3.66	24.71	3.37	17.47	3.87
Tyrosine	20.61	2.54	21.83	2.70	21.47	2.96	19.09	2.60	10.61	2.37
Leucine	50.86	6.27	48.05	5 .95	44.89	6.19	47.19	6.43	29.58	6.58
Isoleucine	33.47	4.12	28.58	3.54	23.51	3.24	27.89	3.80	16.91	3.76
Methionine	6.06	0.75	11.24	1.39	7.57	1.04	7.02	0.97	4.49	1.00
Valine	29.09	3.58	31.54	3.90	25.79	3.56	28.95	3.95	17.74	3.95
Alanine	19.51	2.40	20.33	2.52	15.76	2.17	16.53	2.25	10.76	2.39
Glycine	27.26	3.36	26.83	3.32	22.87	3.15	23.30	3.18	14.10	3.14
Proline	179.30	22.09	186.95	23.14	141.93	19.58	170.90	23.29	106.12	23.61
Glutamic acid	217.41	26.79	222.98	27.60	214.58	29.60	220.98	30.12	131.78	29.32
Serine	12.91	1.59	25.80	3.19	17.82	2.46	16.84	2.30	7.20	1.60
Threonine	16.78	2.07	16.82	2.08	16.17	2.23	14.16	1.93	7.47	1.66
Aspartic acid	32.86	4.05	34.11	4.22	32.97	4.55	25.39	3.46	15.28	3.40
Arginine	25.13	3.10	24.44	3.03	19.56	2.70	23.09	3.15	14.81	3.29
Ammonia	58.63	7.22	44.59	5.52	49.49	6.83	33.54	4.57	22.94	5.10
Histidine	26.55	3.27	17.56	2.17	15.13	2.09	17.49	2.38	11.37	2.53
Lysine	26.14	3.22	19.66	2.43	25.94	3.58	16.58	2.26	10.84	2.41
Total	811.51	—	807.85	—	725.02	—	733.65	—	449.52	—

Tryptophan and Cysteine contents were not determined.
*μg amino acid/mg protein

According to these findings, it may be concluded that ratio of gliadin and glutenin contents in the air classified wheat flour is constant even though amount of wedge type protein and adhesive protein varies. It means that the phenomena that visco-elasticity of the air classified flour is not always proportional to the protein contents might depend on other factors. For example, effects of glyicolipid to interreaction between gliadin and glutenin in dough formation reported by Hoseney et al[4] might suggest a key to solve this problem. So, other factors including this should be investigated moreover to make clear the cause of this phenomena.

IV Summary

Dynamic visco-elasticities of air classified wheat flours are not proportional to their protein contents. Since it was supposed, as one of possible cause of this phenomena, that ratio of gliadin and glutenin contents which is the main factor of the visco-elasticity might change while air classification process owing to the difference of distribution of wedge type protein and adhesive protein, gliadin and glutenin contents in the air classified flours were investigated by 70% ethanol extraction, isopropanol extrction and amino acid analysis. However, significant change in the ratio of gliadin and glutenin contents was not found in the air classified flours. This fact suggests that above mentioned visco-elastic behavior owes to other cause.

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空気分級した小麦粉中のグリアジン及びグルテニンの含有比が粘弾性に与える影響

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要 約

空気分級した小麦粉の粘弾性は必ずしもそのタンパク質含量に比例しない。その原因のひとつとして，空気分級によって生じるクサビ状タンパク質と付着タンパク質の分布の差に伴ない，粘弾性の主要因となっているグリアジンとグルテニンの含有比が異なって来る可能性が考えられるので，エタノールとイソプロパノールによる抽出及びアミノ酸組成を求めることによって空気分級した小麦粉中のグリアジンとグルテニンの含有比を調べた。しかしながら，空気分級した小麦粉中でもグリジアジンとグルテニンの含有比に有意の差を見出せなかった。このことは空気分級した小麦粉が有する上述の粘弾性的挙動が他の要因によっていることを示唆している。

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