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Comparison of Rheological Properties of Dough Prepared with Different Wheat Flours

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ABSTRACT

Wheat flours milled from different wheat varieties and collected from different streams of milling system were used as raw materials in this study. The proteins of different flours were extracted and analyzed using electrophoresis for their composition. The dough rheological properties of different flours, including farinographic and extensographic properties, were also studied to correlate with their protein compositions. According to the results of electrophoresis, the flour protein compositions were grouped into six groups. The molecular weight ranges of the proteins in these six groups were 205.0~97.4, 97.4~66.2, 66.2~45.0, 45.0~36.0, 36.0~24.0, and 24.0~6.5 kDa, respectively. The protein contents of these six groups were found to be significantly correlated to some rheological values of wheat flour dough. These results revealed that the dough rheological properties of wheat flour were not only correlated significantly to the total protein content of the flour but also to the composition of the proteins.

Key words: wheat flour, flour protein, polyacrylamide gel electrophoresis, rheological dough properties, farinographic and extensographic properties.

INTRODUCTION

People in Taiwan have increasing demands for flour products in the recent years. With increased living standards, people require their flour products in better qualities. In response to these demands, large flour processing factories have urgent manufacturing needs to produce purpose-specific flours. The manufacturing of specific flours requires a full understanding of the protein compositions of flour and the rheological properties of dough in order to reduce problems arising from large quantities of non-specific usage flours. Proteins in flour are considered to influence the quality of flour products the most. Rheological properties of dough, such as farinographic and extensographic properties, reflect the processing adaptability of flours. Hence, to understand the relationship between protein composition of different flours and rheological properties of dough will encourage the manufacturing of specific flours and match to the needs for them.

Proteins in flour can be grouped into three main categories: glutenin, gliadin and albumin/globulin. Some researchers may further divide them into more detailed subgroups which are HMW glutenin subunit, ω-gliadin, LMW glutenin subunit, α-, β-, γ-gliadin and albumin/globulin[1-3]. Gliadin has a good extensibility, but lack elasticity. Glutenin has a better elasticity but a low extensibility[4]. Blending both of them in the dough brings a specific elasticity and extensibility, which can then be used in the processing of different flour products. When flour is mixed with water, glutenin swells and sucks in gliadin, mesonin and some water-soluble albumins and globulins. Along with mixing processes, a network structure of gluten is gradually developed[5-7].

Many studies indicated that gluten plays a key role in determining the quality of wheat flour[8-13]. Gluten proteins substantially control the quality of wheat flour products[14-16]. Wall[17] pointed out the relationship between gluten proteins and elasticity of dough, which also determines if this dough is suitable for bread making. Therefore, the quality of bread is greatly associated with the flour's gluten protein compositions. It is well known that rheological properties of dough are dictated by the amount of proteins contained. However, rheological properties differ even when the amount of protein remains unchanged. This fact implies that these properties relate not only to the proteins' quantity but also to their compositions[18, 19]. In the wheat flour research, many have focused on the influence of protein composition on the quality of flour products in some specific flour cases. However, it is seldom seen that the influence was analyzed after grouping different proteins from different wheat varieties and different streams of milling system. Hence, in this study, we extracted proteins from flours, which were from different wheat varieties and different milling streams, analyzed the composition by electrophoresis, discussed the farinographic and extensographic properties of different flours and summarized their relationships. We hope this study may serve as a reference for the manufacture of flour products.

MATERIALS AND METHODS

I. *Materials*

Materials in this study were kindly provided by

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Chiafha Co., Ltd. (Chingshui, Taichung, Taiwan). Major three sources of flours are:

1. Hard red spring wheat (A), which was milled on a commercialized large-scale mill (360 tons per day) from Buhler Co. (Switzerland). The flour was obtained mostly from DBR and B1~B5 milling streams with an extraction rate of 10~15%.
2. Hard red winter wheat (B), which was milled on a laboratory mill (Buhler MLU-202, Type 71-14-R4b). The flour was obtained mainly from B3 and C1 and with some from B2 and C2 milling streams with an extraction rate of 51.5%.
3. Soft winter wheat (C), which was milled on a commercialized large-scale mill (360 tons per day) from Buhler Co. (Switzerland). The obtained flour was straight flour with an extraction rate of 75%.

Furthermore, we prepared 7 flour samples, which were A, B, C, AB, BC, AC and ABC by mixing the three source flours in equal ratio.

II. *Analysis of Proximate Composition*

Water and ash content were determined respectively by AACC Method 44-15A[20] and AACC Method 08-01[20]. Crude protein was determined by AACC Method 46-12[20]. Damaged starch was analyzed by Medcaff and Gilles method[21]. Dry and wet gluten were determined by AACC Method 38-11[20].

III. *Analysis of Rheological Properties of Dough*

Rheological properties of flour, farinograph and extensograph, were determined by AACC Method 54-21[20] method and AACC Method 54-10[20] method respectively.

IV. *Extraction of Proteins*

According to the methods provided by Danno[22], Singh et al.[23] and Chang et al.[24], flour was suspended in 0.05 M phosphate buffer (pH 6.9) containing 2% SDS with a solid/liquid ratio of 1:20. The suspension was treated with a sonicator (Ultrasonic Processor XL, Misonix Inc., USA) for 5 min. at a 38 W output. After sonication, the suspension was stirred with a magnetic stirrer for two hr and then centrifuged at 12000 g for 20 min. The supernatant was collected as our protein extract liquid.

V. *Protein Analysis by SDS-PAGE Electrophoresis*

(I) *Electrophoresis*

Our approach followed the method provided by Gupta & MacRitchie[25] with a minor modification. The equipment used here were Electrophoresis apparatus Model AE-6450 (ATTO, Japan) and power supplier PS500XT with a 2.5 AMP input (Hoefer scientific instruments, USA). 10 μL protein extract liquid was loaded in a 15% acrylamide gel (gel concentration: stacking gel 4.5%, resolving gel 15%). Electrophoresis started at 70V and was tuned up to 140V after a tracer dye entered the resolving gel. This voltage was held until the tracer reached the bottom of the gel before the power was turned off. The gel was then immersed in Coomassie Blue for 2 hr and the color was stripped by a solution containing 7% methanol and 7% acetic acid.

(II) *Quantification of protein composition*

The color-stripped gel was scanned by a TLC scanner (Camag, Sweden) at 597nm. The scanned peaks were categorized into 6 groups according to their molecular weights, which were indicated by standard proteins and cut out from the recording paper for weight measurements. Relative protein content in each group was determined by the ratio of the peak's weight in each group over the peak's weight in the overall 6 groups.

VI. *Statistics*

Results in this study were analyzed by One-way ANOVA, Correlation and Stepwise regression functions in SAS[26].

RESULTS AND DISCUSSION

I. *Composition Analysis of Different Wheat Flours*

Table 1 shows the proximate compositions of 7 different wheat flours. These flours have significant differ-

Table 1. Proximate compositions of seven different wheat flours.

Sample No.*	Moisture (%)	Ash (%)	Crude Protein (% wet basis)	Damaged Starch (AACC %)	Wet Gluten (%)	Dry Gluten (%)
A	12.88^{a}**	1.02^{a}	17.24^{a}	7.03^{bc}	45.6^{a}	16.7^{a}
B	12.13^{b}	0.38^{g}	11.58^{d}	7.53^{a}	33.9^{e}	11.6^{e}
C	11.04^{d}	0.45^{e}	7.44^{f}	6.13^{f}	20.5^{g}	7.0^{g}
AB	12.75^{a}	0.69^{c}	14.22^{b}	7.23^{b}	39.6^{b}	14.2^{b}
BC	11.82^{c}	0.41^{f}	9.44^{e}	6.97^{cd}	27.8^{f}	9.3^{f}
AC	12.19^{b}	0.72^{b}	12.40^{c}	6.53^{e}	35.1^{c}	12.3^{c}
ABC	12.21^{b}	0.60^{d}	12.10^{c}	6.77^{d}	34.4^{d}	11.9^{d}

*: Samples A, B, C, AB, BC, AC, and ABC are the same as those shown in Figure 1.
**: Means with identical letter in the same column are not significantly different at $p > 0.05$.

ence in their compositions ($p < 0.05$) and therefore serve as our experimental samples. Table 1 shows that higher crude protein content brought higher ash content. This is because the ash of wheat is located in the bran and protein content is higher near the aleurone layer[27]. Sample A had higher ash content since it was taken from the aleurone layer. Sample C (straight flour, extraction rate 75%) has higher ash content than Sample B (from endosperm) because of different extracted portion of the wheat kernel. Damaged starch content varied in different samples. Hard red spring wheat (A) and Soft winter wheat (C) were milled on a commercialized large-scale mill. Hard red winter wheat (B) was milled on a laboratory mill. Damaged starch may originate from the damages caused by insects or microbial before milling, enzymatic degradation from wheat malting, or mechanical damage during the milling process. Soft winter wheat (C) has the lowest ash content due to its soft texture and therefore suffers less from mechanical damage. Also, Table 1 shows that wet/dry gluten content followed their corresponding crude protein content.

II. *Analysis of Farinographic and Extensographic Properties of Different Wheat Flours*

Table 2 shows the farinographic properties of 7 different wheat flours. The data were obtained by constant flour weight procedure and Japanese standard method. These 7 samples had significant differences in their farinographic properties ($p < 0.5$). Sample A absorbed the most water because of its high protein content and high damaged starch content. Some reports pointed out that damaged starch controlled the capability of water absorption[28-31]. Table 3 showed extensographic properties of 7 different wheat flours. Different aging times caused different resistance to extension (Rm). Basically, longer aging time led to greater resistance to extension. Extensibility (E) varied with aging time irregularly. In sample B, C, BC, extensibility decreased with increasing aging time in accordance with the results found by Chen and Chang[32]. Area (A-value) increased with increasing aging time.

III. *Protein Analysis by SDS-PAGE*

Proteins in 7 flour samples were extracted using a 0.05 M phosphate buffer (pH 6.9) solution containing 2% SDS. The proteins collected were subjected to SDS-PAGE (15% acrylamide gel) analysis. The results are shown in Fig. 1. We divided proteins into 6 zones according to different molecular weight ranges which were 205.0~97.4, 97.4~66.2, 66.2~45.0, 45.0~36.0, 36.0~24.0 and 24.0~6.5 kDa. Comparing the SDS-PAGE analysis studies done by Singh *et al.*[23], Batey *et al.*[33], Pasaribu *et al.*[34], Kasarda *et al.*[35] and Mimouni *et al.*[1] with acrylamide gel concentrations 10, 12, 12, 4~12 and 10~20% respectively, we assumed that zone 1 contained HMW glutenin subunit, zone 2 contained a little HMW glutenin subunit and ω-

Table 2. The farinographic dough properties of seven different wheat flours.

Sample	Farinographic Properties***						
No.*	WA	AT	PT	DT	VV	WE	ST
A	68.00^{a}**	5.75^{a}	7.50^{b}	13.00^{b}	69.95^{b}	44.00^{e}	7.25^{b}
B	63.61^{c}	5.63^{ab}	10.00^{a}	23.00^{a}	80.65^{a}	19.00^{f}	17.38^{a}
C	54.57^{g}	1.17^{e}	1.50^{f}	2.58^{f}	34.80^{f}	135.33^{a}	1.42^{f}
AB	65.20^{b}	5.19^{b}	7.50^{b}	11.50^{c}	69.75^{b}	48.00^{e}	6.31^{c}
BC	58.65^{f}	1.75^{d}	4.50^{e}	7.00^{e}	54.30^{e}	85.75^{b}	5.25^{d}
AC	60.97^{e}	3.50^{c}	5.00^{d}	6.61^{e}	57.80^{d}	77.33^{c}	3.11^{e}
ABC	61.88^{d}	3.50^{c}	5.50^{c}	8.17^{d}	60.83^{c}	64.33^{d}	4.67^{d}

*: Samples A, B, C, AB, BC, AC, and ABC are the same as those shown in Figure 1.

**: Means with identical letter in the same column are not significantly different at $p > 0.05$.

***: Farinographic dough properties:

WA = water absorption, AT = arrival time, PT = peak time, DT = departure time, VV = valorimeter value, WE = weakness, ST = stability.

Table 3. The extensographic dough properties of seven different wheat flours.

Sample	Extensographic Properties***								
No.*	R_m 45	R_m 90	R_m 135	E 45	E 90	E 135	A 45	A 90	A 135
A	301.17^{c}**	384.00^{c}	451.83^{c}	22.51^{a}	24.09^{a}	24.38^{a}	94.33^{b}	126.21^{b}	149.71^{a}
B	582.17^{a}	740.00^{a}	788.50^{a}	18.58^{d}	16.46^{e}	14.36^{d}	142.18^{a}	159.03^{a}	145.47^{a}
C	279.00d^{e}	373.83^{c}	430.00^{c}	15.38^{e}	14.28^{f}	13.86^{d}	61.11^{e}	74.81^{e}	82.57^{e}
AB	287.67^{cd}	356.33^{c}	387.00^{d}	22.44^{a}	22.83^{b}	22.13^{b}	91.77^{bc}	113.20^{c}	118.88^{b}
BC	367.67^{b}	475.00^{b}	536.33^{b}	17.75^{d}	16.84^{e}	16.04^{c}	85.99^{cd}	106.65^{c}	112.68^{bc}
AC	194.50^{f}	256.50^{e}	302.00^{f}	20.04^{c}	20.66^{d}	21.96^{b}	57.40^{e}	75.52^{e}	92.80^{d}
ABC	270.83^{e}	318.67^{d}	348.17^{e}	21.31^{b}	21.69^{c}	21.86^{b}	80.60^{d}	96.90^{d}	106.63^{c}

*: Samples A, B, C, AB, BC, AC, and ABC are the same as those shown in Figure 1.

**: Means with identical letter in the same column are not significantly different at $p > 0.05$.

***: Rm = Maximum resistance to extension after 45, 90, and 135 min aging. E = Extensibility after 45, 90, and 135 min aging. A = Area after 45, 90, and 135 min aging.

gliadin, zone 3 contained ω-gliadin, zone 4 contained LMW glutenin subunit, zone 5 contained α-, β-, γ-gliadin, and zone 6 contained albumin/globulin.

IV. *The Relationship between Flour Protein Compositions and Rheological Properties*

(I) *Protein composition analysis of 7 different wheat flour products*

Proteins from 7 different wheat flours were loaded and separated in electrophoresis gel, which was then analyzed by a TLC scanner. Fig 2 was the SDS-PAGE electropherograms of protein standards and the proteins from three wheat flours. Peaks in the electropherograms were categorized into 6 zones, which were I: 205.0~97.4, II: 97.4~66.2, III: 66.2~45.0, IV: 45.0~36.0, and V: 36.0~24.0, and VI: 24.0~6.5 kDa. Percentage compositions are also shown in Table 4. The protein contents in these samples were different in different molecular weight zones. Generally, when total protein amount in a wheat kernel changes, protein composition in different zones changes. When protein content was low, albumin and globulin occupied higher percentages. With protein content went up, albumin and globulin increased but not by as much as the storage proteins (gliadin and glutenin) did[36]. In zone 97.4~66.2 kDa, sample A had a higher percentage. It might be contributed to the fact that sample A was collected from the aleurone layer. Gliadin was stored in the aleurone layer and the external part of endosperm, and therefore brought sample A a higher gliadin percentage. Sample A also had a high percentage in zone 66.2~45.0 kDa. It might be because some gliadin was blended in.

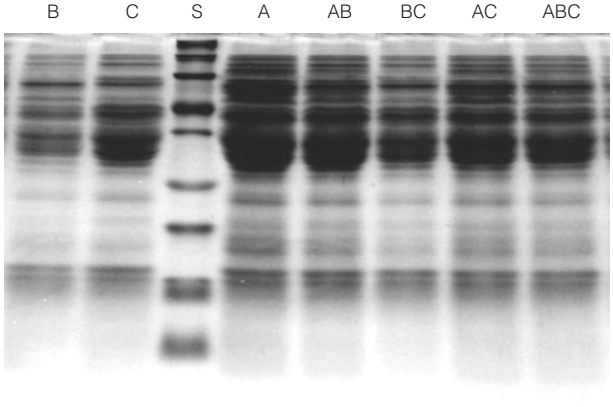

Figure 1. SDS-PAGE (15% acrylamide gel) patterns of proteins extracted with 0.05 M phosphate buffer (pH6.9) containing 2% SDS from seven different wheat flours.
Lane S: standard (205.0, 116.0, 97.4, 66.2, 45.0, 36.0, 24.0, 19.7/20.5, 14.4, and 6.5 kDa), Lane A: hard red spring flour (HRS), Lane B: hard red winter flour (HRW), Lane C: soft white flour (SW), Lane AB: HRS + HRW (1:1), Lane BC: HRW + SW (1:1), Lane AC: HRS + SW (1:1), Lane ABC: HRS + HRW + SW (1:1:1).

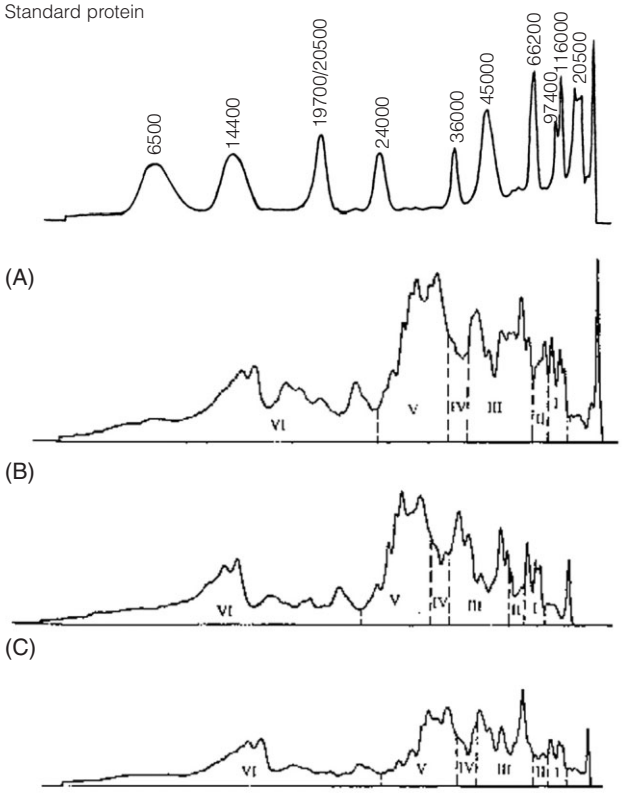

Figure 2. SDS-PAGE electropherograms of protein standards and the proteins from three wheat flours (samples A, B, and C) used in this study.

Table 4. Protein compositions of seven different wheat flours

Sample No.**	Protein compositions (%, based on flour weight)					
	Protein molecular weight*(kDa)					
	205.0~97.4	97.4~66.2	66.2~45.0	45.0~36.0	36.0~24.0	24.0~6.5
A	1.07^{a***}	0.79^{a}	3.97^{a}	0.96^{a}	5.26^{a}	5.19^{a}
B	0.80^{b}	0.32^{d}	2.75^{c}	0.81^{ab}	3.91^{bc}	3.00^{de}
C	0.44^{c}	0.24^{d}	1.87^{e}	0.45^{d}	2.05^{e}	2.39^{e}
AB	0.87^{ab}	0.60^{b}	3.30^{b}	0.82^{ab}	4.33^{b}	4.30^{b}
BC	0.68^{b}	0.32^{d}	2.22^{d}	0.63^{c}	2.82^{d}	2.77^{e}
AC	0.85^{b}	0.53^{bc}	3.06^{bc}	0.69^{bc}	3.72^{c}	3.56^{cd}
ABC	0.68^{b}	0.46^{c}	3.13^{b}	0.71^{bc}	3.50^{c}	4.02^{bc}

*: The molecular weight of proteins is grouped according to the results of electrophoresis (Figure 1).
**: Samples A, B, C, AB, BC, AC, and ABC are the same as those shown in Figure 1.
***: Means with identical letter in the same column are not significantly different at $p > 0.05$.

Table 5. The correlation coefficients between the farinographic dough properties and protein compositions of wheat flours

Protein compositions	Farinographic properties[a]						
	WA	AT	PT	DT	VV	WE	ST
205.0~97.4 kDa	0.9335**	0.8428*	0.7191*	0.5226	0.7616*	-0.7591*	0.3619
97.4~66.2 kDa	0.8374*	0.6728	0.4090	0.1568	0.4691	-0.4775	-0.0413
66.2~45.0 kDa	0.9323**	0.8223*	0.6138	0.3844	0.6704	-0.6855	0.1939
45.0~36.0 kDa	0.9933**	0.9368**	0.8551*	0.6932	0.8831**	-0.8884**	0.5446
36.0~24.0 kDa	0.9858**	0.9190**	0.7686*	0.5806	0.8025*	-0.8068*	0.4083
24.0~6.5 kDa	0.8769**	0.7181	0.4757	0.2302	0.5358	-0.5541	0.0357

a: Farinographic dough properties:
WA = water absorption, AT = arrival time, PT = peak time, DT = departure time, VV = valorimeter value, WE = weakness, ST = stability.
*: Correlation is significant at p= 0.05 level.
**: Correlation is significant at p= 0.01 level.

Table 6. The correlation coefficients between the extensographic dough properties and protein compositions of wheat flours

Protein compositions	Extensographic properties[a]		
	Rm135	E135	A135
205.0~97.4 kDa	-0.0649	0.8322*	0.7222
97.4~66.2 kDa	-0.4235	0.9654**	0.4761
66.2~45.0 kDa	-0.2011	0.8941*	0.6432
45.0~36.0 kDa	0.1698	0.6826	0.8739*
36.0~24.0 kDa	-0.0085	0.7972	0.7735*
24.0~6.5 kDa	-0.3089	0.9197**	0.5798

a: Rm135: Maximum resistance to extension after 135-min aging, E135: Extensibility after 135-min aging, A135: Area after 135-min aging.
*: Correlation is significant at p = 0.05 level.
**: Correlation is significant at p = 0.01 level.

(II) *The relationship between flour protein compositions and rheological properties*

The correlation coefficients between proteins in the 6 zones and their corresponding farinographic properties are shown in Table 5. It was clear that zone 1, zone 4 and zone 5 were positively correlated to arrival time (AT), peak time (PT) and valorimeter values (VV) but negatively correlated to weakness (WE). It implied proteins with higher HMW glutenin, LMW glutenin and α-, β-, γ-gliadin percentage are less susceptible to mechanical mixing and they also have lower weakness values.

The correlation coefficients between proteins in the 6 zones and their corresponding extensographic properties are shown in Table 6. Protein content in zone 2 (ω-gliadin) was negatively correlated to R-value and positively correlated to E-value. It suggested higher gliadin content brought proteins better extensibility and less resistance to extension. Zone 3 had the same trend as zone 2, negatively correlated R-value and positively correlated to E-value. It might result from some gliadin portion blended in the zone 3. Protein content in Zone 6 was also positively correlated to E-value. It implied that low molecular weight albumin and globulin contributed to higher extensibility. Protein contents in zone 4 and zone 5 were positively correlated to the A-value.

CONCLUSION

This study showed wheat flours from different wheat varieties and different streams of milling system had different protein compositions, which consequently affected the rheological properties of flours. It confirmed the point that rheological properties were not only determined by protein content but also by its composition. Hopefully, these results will serve as a reference for flour formulation, processing and their full utilizations.

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