

Nitrogen Distributions of Some Wheat Flours

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Introduction

Among many factors influencing on breadmaking processes, the quality of wheat flour and the manipulation by baker are considerably important. The scientific control of breadmaking processes has been much improved recently but it is still unsatisfactory because of a lack of complete knowledge of flour. Farinograph and extensograph are very useful instruments to characterize the flour by recording graphically the rheological properties of a dough. It is, therefore, true that these instruments have made a great contribution to the industrial use of flour. However, it is also true that cereal chemists demand the theoretical elucidation of flour quality based on its chemical data.

Wheat flour is composed of carbohydrates, fat, proteins, fiber, mineral salts and small amount of other substances. Among them proteins play a leading role in mixing and leaving a dough and producing a bulky loaf of good texture.

The proteins in flour may be usually divided into four fractions such as albumin, globulin, gliadin and glutenin which may behave cooperatively in breadmaking processes because a slight variation in quantity of the individual protein is associated with important differences in the quality or physical nature of the flour.

Here, the analyses and the fractionations of proteins of some wheat flours different in variety, type and grade were carried out in our laboratory. From these experiments it is expected that a definite correlation between the quality and the protein fractions of flour is established.

Experimental

Materials

The wheats used in the experiment are listed in Tables 1-1 and 1-2 and were imported ones with exception of Norin No. 67. The flours shown in Table 1-1 were different in variety, type and grade, and bleached commercially milled patent. The flours shown in Table 1-2 were unbleached straight-run grade flours which were milled with test rollermill by courtesy of Nisshin Flour Milling Co..

Norin No. 67 (Garnet Ott. 652 × Kanto No. 20) is a variety of wheat most commonly cultivated in Kanto- and Chubu-District in Japan and is used mainly as a medium flour. This wheat was cropped in 1954 in Nagano Agricultural Experiment Station in Nagano Prefecture.

The flours thus obtained, were stored at laboratory temperature until the fractionation of flour proteins was set about. The period of storage was three or four weeks.

Table 1-1. Description and Nitrogen Contents of Commercial Wheat Flours.

Flour	Variety & Type	Grade	Yield %	Water %	Nitrogen %	Protein N×5.7 %	Nitrogen* in dry basis mg %	Protein* in dry basis mg %
A	Manitoba No. 3+No. 4	first patent	6	14.89	2.10	11.97	2470	14.08
B	U.S.A. Hard Red	second patent	54	14.69	1.96	11.17	2298	13.09
C	do.	do.	—	13.01	1.97	11.25	2263	12.90
D	U.S.A. Western White	first patent	—	13.27	1.23	7.01	1418	8.08
E	do.	second patent	—	12.48	1.37	7.81	1565	8.92
F	do.	do.	—	12.89	1.32	7.52	1515	8.63

* The calculation was made on the basis of dehydrated flour.

Table 1-2. Description and Nitrogen Contents of Laboratory-milled Flours.

Flour	Variety & Type	Grade	Yield %	Water %	Nitrogen %	Protein N×5.7 %	Nitrogen* in dry basis mg %	Protein* in dry basis mg %
G	Garnet No. 3	straight	87.0	13.64	2.01	11.46	2327	13.27
H	Manitoba No. 1	do.	66.9	14.82	2.03	11.57	2350	13.58
I	Western White	do.	62.8	14.97	1.18	6.88	1388	8.09
J	Norin No. 67	do.	50.0	14.13	1.59	9.06	1852	10.55

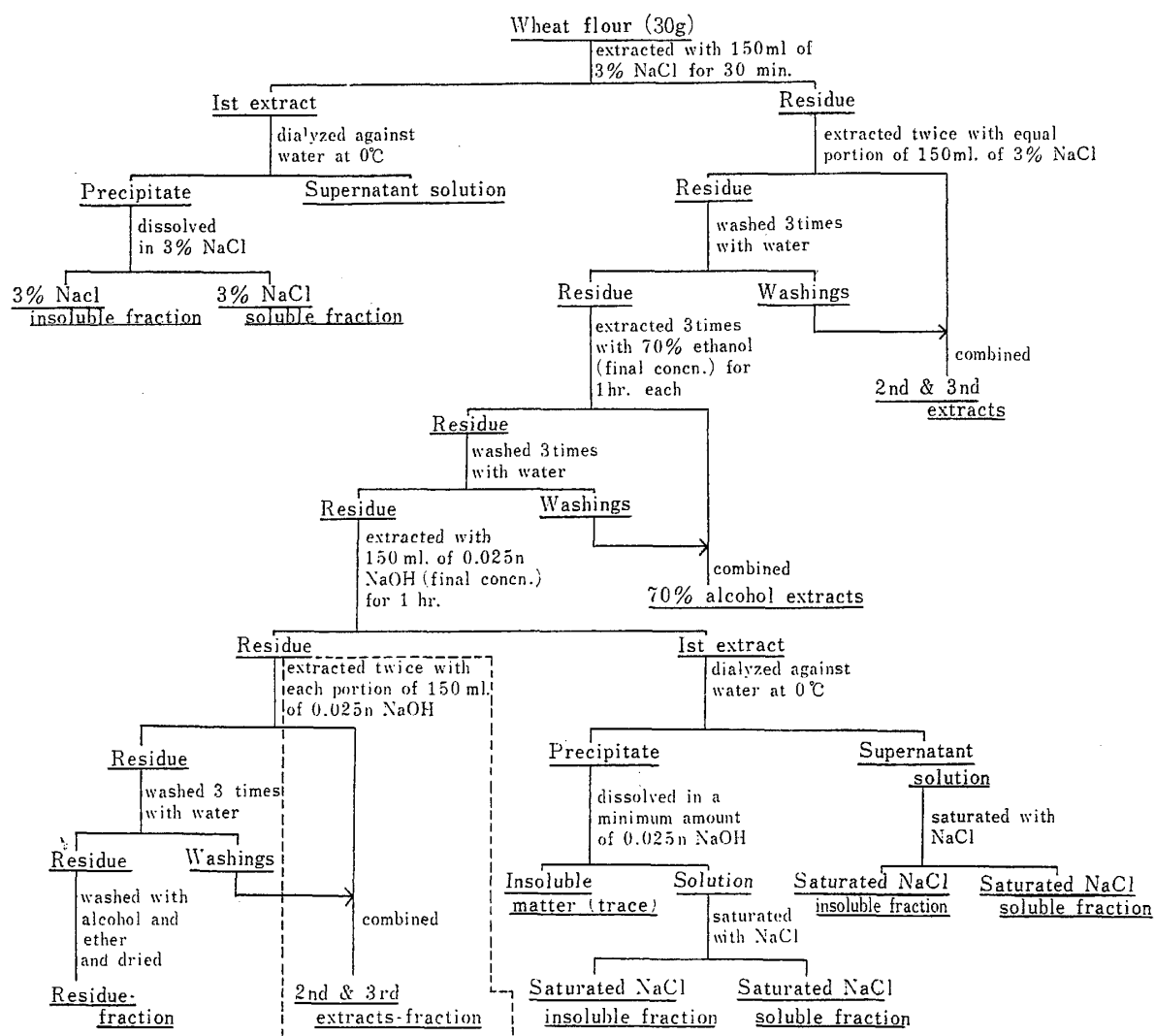
* The calculation was made on the basis of dehydrated flour.

Method of fractionation

The method of fractionation of flour proteins is shown schematically in Fig. 1: The flour was extracted with 3 percent sodium chloride, 70 percent alcohol and 0.025N sodium hydroxide solution successively¹⁾.

The extraction was repeated three times for each solvent. The first extracts of the sodium chloride and the sodium hydroxide extraction were used for further fractionation. The 1st NaCl extract was dialyzed with a collodion tubing against distilled water at 0°C. After the dialysis, a precipitate appeared. Although the supernatant solution was not fractionated further, the precipitate was divided into NaCl-soluble and -insoluble part by dissolving in 3% sodium chloride solution. The 1st NaOH extract was dialyzed in the same way as described above. The resultant supernatant solution was divided into NaCl-soluble and -insoluble part by saturating with sodium chloride. The precipitate obtained through the dialysis was dissolved with a minimum amount of 0.025N sodium chloride solution and divided also into two parts with saturation of sodium chloride. The residue after the 3rd NaCl extraction was rinsed with water exhaustively, washed with alcohol and ether and then dried at 100°C. An alternative method was adopted in some cases that the residue after the 1st NaOH extraction was washed and dried in the same way.

The nitrogen contents of all fractions including the washings and the residue were determined by the semimicro Kjeldahl method using cupper sulfate and mercuric sulfate as catalysts.



The processes enclosed with broken lines were omitted in the fractionations of commercial wheat flours.

Fig. 1. Flow Diagram of the Fractionation of Wheat Flour Proteins

Result

The nitrogen contents of all fractions were summarized in Tables 2-1, 2-2, 3-1 and 3-2. Tables 2-1 and 2-2 show the nitrogen distributions of commercial flours calculated on the basis of 100g of dehydrated flour and of 100g of total nitrogen respectively. Tables 3-1 and 3-2 show those of laboratory-milled flours calculated on the same bases as Tables 2.

Discussion

Flour A, B and C shown in Table 1-1 are all strong flours and among them Flour A is a first patent flour. Hence, Flour A must have the best or the near best quality for breadmaking and therefore, must have characteristically a well-balanced nitrogen distribution of flour proteins.

As seen in Table 2-2, the characteristics of the nitrogen distribution of Flour A were found in that a large majority of Precipitate-N of 1st NaOH extract transferred to Saturated NaCl-insoluble fraction and a small amount of the nitrogen to the soluble fraction. Any character-

Table 2-1. Nitrogen Distributions of Commercial Flours.
(milligrams per 100 gram of dehydrated flour)

Flour		A	B	C	D	E	F			
		mg	mg	mg	mg	mg	mg			
Total		N	2470	2298	2263	1418	1565	1515		
3 % NaCl extracts	1st NaCl extract	1st extract	N	368.0	361.9	340.3	230.4	305.6	238.1	
		Supernatant after dialysis	N	78.8	71.5	65.5	37.2	66.3	67.5	
		Preci- pitate after dialysis	3 % NaCl soluble	N	52.5	46.2	45.4	22.3	51.8	52.5
			3 % NaCl insoluble	N	32.0	21.5	29.9	21.7	23.8	29.2
		*Dialyzate	N	204.7	224.1	199.4	148.9	164.0	134.2	
	2nd & 3rd extracts	N	140.3	176.2	138.2	112.6	133.2	146.5		
	*1st, 2nd & 3rd extracts	N	508.3	538.1	478.5	343.0	438.8	429.6		
70 % alcohol extracts		N	674.7	617.6	758.9	521.3	461.2	420.3		
0.025 n NaOH extracts	1st NaOH extract	1st extract	N	830.3	775.7	808.1	432.9	503.7	496.7	
		Super- natant solution after dialysis	Supernatant	N	81.5	51.5	54.8	195.2	87.4	82.2
			Saturated NaCl soluble	N	65.7	33.5	26.8	21.4	23.1	63.3
	Saturated NaCl insoluble		N	11.2	17.7	24.5	171.2	64.2	13.0	
	Preci- pitate after dialysis	Precipitate	N	592.6	631.9	515.1	142.4	303.6	328.7	
		Saturated NaCl soluble	N	67.0	249.0	422.5	136.5	113.7	135.8	
		Saturated NaCl insoluble	N	508.3	339.7	86.8	5.3	187.2	191.1	
		*Dialyzate	N	156.2	92.7	238.2	52.7	112.5	80.7	
	**Residue		N	449.2	301.8	150.9	81.2	102.0	134.4	
	Total		N (Sum)	2462.5	2233.2	2196.3	1378.4	1505.7	1481.0	

*The nitrogen value of this fraction was calculated from the values concerned in the same column.

**This fraction contains 2nd & 3rd NaOH extracts-nitrogen.

istic differences were not found in 70 % alcohol extracts and all fractions of 3 % NaCl extracts. Essentially, the alcohol-soluble fraction consists of gliadin and the alkali-soluble contains a bulk of glutenin of flour proteins. Gliadin is a constituent of gluten along with glutenin and therefore, they are the important constituents of flour proteins. Gliadin confers mellow-ness and elasticity on the gluten, whilst glutenin provides the structure. Hence, the greater the amount of gliadin, softer the gluten. So gliadin to glutenin ratio determines principally the softness of gluten. However, it is too hasty a conclusion to estimate the gluten quality from the ratio because both gliadin and glutenin have been divided into several fractions²⁾ which should behave cooperatively in breadmaking processes. It will be discussed in detail after some more data would be collected.

Flour D, E and F are all soft flours. As Flour D is a first patent flour, this is presumed to have a superior quality for cakemaking among these flours. So, Flour D was compared with the other soft flours and the strong flours and the characteristic difference in the nitrogen

Table 2-2. Nitrogen Distributions of Commerical Flours*.
(Grams per 100 gram of Total Nitrogen)

Flour				A	B	C	D	E	F		
				%	%	%	%	%	%		
Total				N	100	100	100	100	100		
3 % NaCl extracts	1st	1st extract		N	14.9	15.8	15.0	16.2	19.5	18.7	
		Supernatant after dialysis		N	3.2	3.1	2.9	2.6	4.2	4.5	
	NaCl	extract	Precipitate after dialysis	3 % NaCl soluble	N	2.1	2.0	2.0	1.6	3.3	3.5
				3 % NaCl insoluble	N	1.3	0.9	1.3	1.5	1.5	1.9
			Dialyzate		N	8.0	9.7	8.8	10.5	10.5	8.9
	2nd & 3rd extracts				N	5.7	7.7	6.1	7.9	8.5	9.7
	1st, 2nd & 3rd extracts				N	20.6	23.5	21.1	24.1	28.0	28.4
	70 % alcohol extracts				N	27.3	26.9	33.5	36.8	29.5	27.7
0.025 n NaOH extracts	1st	1st extract		N	33.7	33.8	35.7	30.1	32.2	32.4	
		Super- natant solution after dialysis	Supernatant		N	3.4	2.2	2.4	13.8	5.9	5.4
	Saturated NaCl soluble		N	2.7	1.5	1.2	1.5	1.5	4.2		
	Saturated NaCl insoluble		N	0.5	0.8	1.1	12.1	4.1	0.9		
	NaOH	extract	Preaci- pitate after dialysis		N	24.0	27.5	22.8	10.0	19.4	21.7
			Saturated NaCl soluble	N	2.7	10.8	18.7	9.6	7.3	9.0	
			Saturated NaCl insoluble		N	20.6	14.8	3.8	0.4	12.0	12.7
			Dialyzate		N	6.3	4.1	10.5	6.3	6.9	5.3
Residue				N	18.1	13.1	6.7	5.7	6.5	8.9	
Recovery				%	99.7	97.2	97.1	97.2	96.2	97.8	

*The data shown in this table were recalculated from those given in Table 2-1.

distribution between them was found in the NaOH-soluble fractions. As shown in Table 2-2, Flour D showed higher nitrogen values in Supernatant fraction or its Saturated NaCl-insoluble fraction and less values in Precipitate-fraction or its Saturated NaCl-insoluble fraction of 1st NaOH extract than the other flours. In spite of the characteristic difference of Flour D, Flour E and F bear some resemblance in the nitrogen distribution to Flour B and C. Hence, if the total nitrogens of these soft flours were raised up to as same magnitude as those of the strong flours, Flour E and F might have the same flour quality as Flour B and C.

It should be mentioned here that soft flours are usually used for confectionery where bulky volume is not the first consideration and hence, flours for confectionery must have various types of character corresponding to various uses.

Among the laboratory-milled flours shown in Tables 3-1 and 3-2, Flour G is a Garnetian flour and Flour H is a Manitoban flour. These two flours differ distinctly in the nitrogen distribution. While the nitrogen distribution of Flour H resembles that of Flour A which was presumed to have the best quality for breadmaking, that of Flour G resembles that of Flour D which was presumed to have the best quality for cakemaking. The resemblance of

Table 3-1. Nitrogen Distributions of Laboratory-milled Flours.
(milligrams per 100 gram of dehydrated flour)

Flour			G	H	I	J		
			mg	mg	mg	mg		
Total			N	2327	2350	1388	1852	
3 % NaCl extracts	1st NaCl extract	1st extract	N	374.6	303.6	195.7	298.1	
		Supernatant after dialysis	N	68.2	97.8	55.4	76.0	
		Preci- pitate after dialysis	3 % NaCl soluble	N	49.2	37.7	9.1	45.6
			3 % NaCl insoluble	N	28.0	17.0	8.8	15.3
		*Dialyzate	N	228.9	151.1	122.4	161.2	
	2nd & 3rd extracts	N	169.6	145.1	102.6	140.6		
	*1st, 2nd & 3rd extracts	N	544.2	448.7	298.3	438.7		
70 % alcohol extracts			N	856.5	744.1	601.2	496.3	
0.025 n NaOH extracts	1st NaOH extract	1st extract	N	679.8	840.6	415.0	598.5	
		Super- natant solution after dialysis	Supernatant	N	325.8	62.0	55.2	72.1
			Saturated NaCl soluble	N	39.4	55.1	0	61.3
			Saturated NaCl insoluble	N	244.8	6.0	55.2	4.3
	Preci- pitate after dialysis	Precipitate	N	—	691.4	220.5	452.3	
		Saturated NaCl soluble	N	—	133.9	215.2	108.9	
		Saturated NaCl insoluble	N	—	547.4	5.2	328.1	
	*Dialyzate	N	—	87.2	139.2	74.1		
	2nd & 3rd extracts	N	—	253.6	35.2	255.8		
	*1st, 2nd & 3rd extracts	N	—	1094.2	450.2	854.3		
Residue			N	**235.3	99.6	16.8	71.5	
Total			N (sum)	2315.3	2386.6	1366.5	1860.8	

*The nitrogen value of this fraction was calculated from the values concerned in the same column.

**This fraction contains 2nd & 3rd NaOH extracts N.

Flour H to Flour A is natural because both the flours are Manitoban wheat flours. However, it can be found under careful observation of the nitrogen distributions of Flour H and A that Flour H is slightly poor in the 1st NaCl-soluble fraction and slightly rich in 70% alcohol-soluble and the 1st NaOH-soluble fraction.

The resemblance of Flour G to Flour D is very interesting. Flour G must be unsuitable for breadmaking.

As Flour D and E, Flour I is a American Western White flour. In comparison of Flour I with Flour D in the nitrogen distribution, similar relations as seen in comparison of Flour H with Flour A were observed in Table 2-2. Besides these facts, it was found that Flour I resembles rather Flour E than Flour D in the alkali-soluble fractions.

Table 3-2. Nitrogen Distributions of Laboratory-milled Flours*.
(Grams per 100 gram of Total Nitrogen.)

Flour				G	H	I	J		
				%	%	%	%		
Total				N	100	100	100	100	
3 % NaCl extracts	1st	1st extract		N	16.1	12.9	14.1	16.1	
		Supernatant after dialysis		N	2.9	4.2	4.0	4.1	
	NaCl	Precipitate after dialysis	3 % NaCl soluble	N	2.1	1.6	0.7	2.5	
			3 % NaCl insoluble	N	1.2	0.7	0.6	0.8	
	Dialyzate		N	9.8	6.7	8.8	8.7		
	2nd & 3rd extracts		N	7.3	6.2	7.4	7.6		
	1st, 2nd & 3rd extracts		N	23.4	19.1	21.5	23.7		
70 % alcohol extracts				N	36.8	31.7	43.3	26.8	
0.025 n NaOH extracts	1st	1st extract		N	29.2	35.8	29.9	32.3	
		NaOH	Super-natant solution after dialysis	Supernatant	N	14.0	2.6	4.0	3.9
				Saturated NaCl soluble	N	1.7	2.3	0	3.3
	Saturated NaCl insoluble			N	10.5	0.3	4.0	0.2	
	NaOH	Precipitate after dialysis	Precipitate		N	—	29.4	15.9	24.4
			Saturated NaCl soluble	N	—	5.7	15.5	5.9	
			Saturated NaCl insoluble	N	—	23.3	0.4	17.7	
	Dialyzate		N	—	3.5	10.0	4.0		
	2nd & 3rd extracts		N	—	10.6	2.6	13.8		
	1st, 2nd & 3rd extracts		N	—	46.6	32.4	46.1		
Residue				N	10.1	4.2	1.2	3.9	
Recovery				%	99.5	101.6	99.2	100.5	

*The data shown in this table were recalculated from those given in Table 3-1.

Flour J is a Japanese flour and belongs rather to a strong flour. As shown in Tables 2-2 and 3-2, Flour J bears a striking resemblance to Flour A and H in the nitrogen distribution. Flour J, if anything, is slightly more in 3% NaCl extracts and slightly less in Saturated NaCl-insoluble part of Precipitate-fraction than Flour A.

It has been supposed difficult to estimate the flour quality from the nitrogen distribution of the flour. However, as suggested in the discussion described above, this estimation is not impossible if a suitable method is applied to the fractionation of the flour proteins. In this meaning the present fractionation is still unsatisfactory. More detailed one must be established.

Summary

By successive extractions with 3% sodium chloride solution, 70% alcohol and 0.025N sodium hydroxide solution, followed by dialyses and salt precipitations, proteins of some strong and soft flours were fractionated and the characteristics of the nitrogen distributions of these flours were discussed. From the results some relation between the nitrogen distribution and the flour quality was established but it was emphasized that application of more detailed fractionation was necessary.

Acknowledgments

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References

- 1) T. B. Osborne: *The Vegetable Proteins* (1924). Longmans, Green, London.
- 2) J. H. Woychik, F. R. Huebner and R. J. Dimler; *Arch. Biochem. Biophys.*, **105**, 151 (1964).