Bull. Univ. Osaka Pref., Ser. B, Vol. 16.
大阪府立大学紀要，農学・生物学，Jan., 1965.

Changes of Nitrogen Distributions of Wheat Flours During Storage and Effect of Milling Method on the Changes

By

Hiromu SHIMOMURA and Yukio AWO

Laboratory of Food Chemistry, College of Agriculture

(Received August 31, 1964)

Introduction

It is a well known fact that various food products gradually deteriorate during storage. This deterioration may have a profound effect[1] not only on nutritive values but also on physical and chemical properties of the products. In all cereal products these changes[2] are continually taking place regardless of how they are stored.

Accordingly the denaturation of proteins of wheat flour during storage should be also expected. If such denaturation takes place, the nitrogen distribution of flour proteins should be changed in proportion to the period of storage.

In the present work the change of nitrogen distribution during storgae was investigated with several kinds of wheat flours. In addition to this, the changes caused by milling method were also investigated.

Experimental

Materials

Four samples of wheat were used; Canadian Manitoba No. 1, Igachikugooregon cropped in 1953 and 1954, and Norin No. 67. Manitoba No. 1 was imported from Canada for commercial use and the crop year is uncertain. The same sample was used in previous paper[3]. Igachikugooregon (Igachikugo × Oregon) is a variety of wheat which is cultivated in Chubu District in Japan. Two samples of Igachikugooregon were cropped in Nagano Agricultural Experiment Station in Nagano Prefecture in Japan. Norin No. 67 (Garnet Ott. 652 × Kanto No. 20), a variety most commonly cultivated in Kanto District in Japan, was cropped in 1953 also in Nagano Agricultrual Experiment Station described above. These wheats were milled immediately after they arrived at our laboratory.

Milling

Manitoba No. 1 and Igachikugooregon 1954 were milled to straight-run grade with the test rollermill by courtesy of Nisshin Milling Flour Co.. Igachikugooregon 1953 and Norin No. 67 were milled with the Wiley-mill (Shimazu Manufacturing Co.) and passed through a 60 mesh sieve.

Storage periods

These flours were stored in rubber-stoppered glass bottles at laboratory temperature. The periods of storage were as follows.

	Crop year	Period	
Manitoba No. 1	?	from Nov. 4, 1954 to June 27, 1955	(205 days)

Igachikugooregon	1954	from Dec. 10, 1954 to June 13, 1955	(169 days)
Igachikugooregon	1953	from Nov. 16, 1953 to Feb. 15, 1954	(91 days)
Norin No. 67	1953	from Nov. 4, 1953 to Feb. 1, 1954	(89 days)

Method of fractionation

The fractionation of flour proteins was carried out at the beginning and the end of the storage-period. The fractionation at the beginning was actually done after 3 or 4 weeks of the milling. The lapse of this period is necessary to stabilize the flour quality. The method of fractionation was same as described in the previous paper[3]. The semimicro Kjeldahl method was used for the nitrogen determination.

Result

The analytical data of the four flours at the beginning teh of storge-periods are shown in Table 1 and the changes of the nitrogen distributions of these flours during storage are summarized in Tables 2-1 and 2-2.

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

Flour	Variety	Grade	Milling	Yield %	Water %	N *mg*%	Protein N×5.7 %	N in water-free basis *mg*%	Protein in water-free basis %
I	*Manitoba No. 1	straight	roller	66.9	14.82	2030	11.57	2383	13.58
II	Igachikugooregon 1954	do.	do.	50.0	14.09	1650	9.41	1921	10.95
III	Igachikugooregon 1953	do.	Wiley	56.5	13.77	1690	9.61	1960	11.17
IV	Norin No. 67	do.	do.	52.5	13.79	1500	8.55	1740	9.92

*This wheat is the same as shwon in Table 1-2 in the previous paper[3].

Every nitrogen change of a flour shown in the tables indicates a difference between two nitrogen values of a fraction obtained at the beginning and the end of the storage-period and the positive sign means nitrogen increase and the negative one nitrogen decrease of the fraction during storage.

Discussion

Comparison of the nitrogen distributions during storage between Flour I and II, both of which were milled with rollermill.

In the NaCl-soluble fractions shown in Tables 2-1 and 2-2, the NaCl-soluble nitrogens of these two flours decreased during storage with exception of Dialyzate-N. The increase of Dialyzate-N may be attributed to proteolysis during storage. The alcohol-soluble fraction of the two flours showed much increase during stroage. The amount of increase was 11.4% for Flour I and 12.2% for Flour II as shown in Table 2-2. Any further fractionation of Alcohol extracts was not tried.

In fractions of 1st NaOH extract, Precipitate-fractions of Flour I and II and Supernatant-fraction of Flour II showed fairly distinct nitrogen changes during storage. It can be seen in Table 2-2 that the change occurred in Saturated NaCl-insoluble fraction. The increase of Dialyzate-fraction may be attributed again to proteolysis during storage.

Table 2-1. Nitrogen Distributions of Flour Proteins During Storage. (*Milligrams per 100 gram of dehydrated flour*)

Flour		I			II			III			IV		
Method of milling		roller			roller			Wiley			Wiley		
Days of storage		0	205		0	169		0	91		0	89	
		mg	*mg*	difference *mg*	*mg*	*mg*	difference *mg*	*mg*	*mg*	difference *mg*	*mg*	*mg*	difference *mg*
Total	N	2383			1921			1960			1740		
3 % NaCl extracts, 1st extract: 1st extract	N	303.6	269.5	− **34.1**	294.3	288.2	− **6.1**	376.8	352.5	− **24.3**	463.3	437.3	− **26.0**
3 % NaCl extracts, 1st extract: Supernatant after dialysis	N	97.8	59.8	− **38.0**	75.8	59.9	− **15.9**						
3 % NaCl extracts, 1st extract: Precipitate after dialysis, 3 % NaCl soluble	N	37.7	23.4	− **14.3**	37.2	36.2	− **1.0**						
3 % NaCl extracts, 1st extract: Precipitate after dialysis, 3 % NaCl insoluble	N	17.0	10.8	− **6.2**	26.1	25.6	− **0.5**						
3 % NaCl extracts, 1st extract: *Dialyzate	N	151.1	175.5	+ **24.4**	155.2	166.5	+ **11.3**						
3 % NaCl extracts: 2nd & 3rd extracts	N	145.1	138.5	− **6.6**	142.6	123.5	− **19.1**	158.8	170.9	+ **12.1**	213.9	223.2	+ **9.3**
3 % NaCl extracts: *1st, 2nd & 3rd extracts	N	448.7	408.0	− **40.7**	436.9	411.7	− **25.2**	535.6	523.4	− **12.2**	677.2	660.5	− **16.7**
70 % alcohol extracts	N	744.1	1015.7	+**271.6**	503.1	723.3	+**220.2**	569.5	520.9	− **48.6**	320.7	248.0	− **72.7**
0.025 n NaOH extracts, 1st extract: 1st extract	N	840.6	751.0	− **89.6**	652.2	615.9	− **36.3**	669.7	723.8	+ **54.1**	465.1	520.5	+ **55.4**
0.025 n NaOH extracts, 1st extract: Supernatant solution after dialysis, Supernatant	N	62.0	59.9	− **2.1**	67.1	163.4	+ **96.3**						
0.025 n NaOH extracts, 1st extract: Supernatant solution after dialysis, Saturated NaCl soluble	N	55.1	52.9	− **2.2**	58.3	59.4	+ **1.1**						
0.025 n NaOH extracts, 1st extract: Supernatant solution after dialysis, Saturated NaCl insoluble	N	6.0	7.5	+ **1.5**	3.3	101.9	+ **98.6**						
0.025 n NaOH extracts, 1st extract: Precipitate after dialysis, Precipitate	N	691.4	578.8	−**112.6**	519.8	336.2	−**183.6**						
0.025 n NaOH extracts, 1st extract: Precipitate after dialysis, Saturated NaCl soluble	N	133.9	74.8	− **59.1**	88.2	90.1	+ **1.9**						
0.025 n NaOH extracts, 1st extract: Precipitate after dialysis, Saturated NaCl insoluble	N	547.4	496.9	− **50.5**	422.6	238.2	−**184.4**						
0.025 n NaOH extracts, 1st extract: *Dialyzate	N	87.2	112.3	+ **25.1**	65.3	116.3	+ **51.0**						
0.025 n NaOH extracts: 2nd & 3rd extracts	N	253.6	143.5	−**110.1**	262.9	93.1	−**169.8**						
0.025 n NaOH extracts: *1st, 2nd & 3rd extracts	N	1094.2	894.5	−**199.7**	915.1	709.0	−**206.1**						
Residue	N	99.6	62.0	− **37.6**	56.4	47.0	− **9.4**	197.3	193.3	− **4.0**	274.1	314.0	+ **39.9**
*Total	N (sum)	2386.6	2380.2		1911.5	1891.0		1972.1	1961.4		1717.1	1743.0	

Note: 1. A number shown in Gothic style indicates a difference between the two mnumbers, adjacent to each other in a line just on the left side of the number; positive sign indicates the increase and negaive sign the decrease during storage.

2. The nitrogen value of the fraction marked by * was calculated from the others of the same flour shown in this table.

3. In the experiments with Flour III and IV, 2nd and 3rd NaOH extraction were omitted. Therefore, Residue N includes nitrogens which should be removed by the 2nd and 3rd extraction.

Table 2-2. Nitrogen Distributions of Flour Proteins During Stroage.
(*Grams per 100 gram of Total Nitrogen*)

Flour	I			II			III			IV		
Method of milling	roller			roller			Wiley			Wiley		
Days of storage	0	205		0	169	difference	0	91		0	89	
	%	%	difference %	%	%	%	%	%	difference %	%	%	difference %
Total N	100			100			100			100		
3 % NaCl extracts, 1st extract: 1st extract N	12.7	11.3	− **1.4**	15.3	15.0	− **0.3**	19.2	18.0	− **1.2**	26.6	25.1	− **1.5**
Supernatant after dialyssi N	4.1	2.5	− **1.6**	3.9	3.2	− **0.7**						
Precipitate after dialysis: 3 % NaCl soluble N	1.6	1.0	− **0.6**	1.9	1.9	**0**						
Precipitate after dialysis: 3 % NaCl insoluble N	0.7	0.5	− **0.2**	1.4	1.4	**0**						
Dialyzate N	6.3	7.4	+ **1.1**	8.1	8.8	+ **0.7**						
2nd & 3rd extracts N	6.1	5.8	− **0.3**	7.4	6.5	− **0.9**	8.1	8.7	+ **0.6**	12.3	12.8	+ **0.5**
1st, 2nd & 3rd extracts N	18.8	17.1	− **1.7**	22.7	21.5	− **1.2**	27.3	26.7	− **0.6**	38.9	37.9	− **1.0**
70 % alcohol extrats N	31.2	42.6	+ **11.4**	26.2	38.4	+ **12.2**	29.1	26.6	− **2.5**	18.4	14.3	− **4.1**
0.025 n NaOH extracts, 1st extract: 1st extract N	35.3	31.5	− **3.8**	34.0	32.7	− **1.3**	34.2	36.9	+ **2.7**	26.7	30.0	+ **3.3**
Supernatant solution after dialysis: Supernatant N	2.6	2.5	− **1.0**	3.5	8.7	+ **5.2**						
Supernatant solution after dialysis: Saturated NaCl soluble N	2.3	2.2	− **0.1**	3.0	3.1	+ **0.1**						
Supernatant solution after dialysis: Saturated NaCl insoluble N	0.3	0.3	**0**	0.2	5.4	+ **5.2**						
Precipitate after dialysis: Precipitate N	29.2	24.3	− **4.9**	27.1	17.8	− **9.3**						
Precipitate after dialysis: Saturated NaCl soluble N	5.6	3.1	− **2.5**	4.6	4.8	+ **0.2**						
Precipitate after dialysis: Saturated NaCl insoluble N	23.0	20.9	− **2.1**	22.0	12.6	− **9.4**						
Dialyzate N	3.7	4.7	+ **1.0**	3.4	6.2	+ **2.8**						
2nd & 3rd extracts N	10.6	6.0	− **4.6**	13.7	4.9	− **8.8**						
1st, 2nd & 3rd extracts N	45.9	37.5	− **8.4**	47.6	36.9	− **10.7**						
Residue N	4.2	2.6	− **1.6**	2.9	2.5	− **0.4**	10.1	9.9	− **0.2**	15.8	18.1	+ **2.3**
Sum (Recovery)	100.3	99.8		99.5	98.3		100.7	100.1		98.7	100.2	

Note: The nitrogen values of all fractions besides those of the differences were recalculated from the corresponding values shown in Table 2-1.

Table 3. Balances of Nitrogen Differences of Fractions
(*Rollermilled Flours*)

		3 %NaCl extracts 1st, 2nd & 3rd extracts *N-difference*	0.025n NaOH extracts 1st, 2nd & 3rd extracts *N-difference*	Residue *N-difference*	Sum	70% alcohol extracts *N-difference*
Flour I	*mg*	− 40.7	−199.7	− 37.6	−278.0	+271.6
	%	− 1.7	− 8.2	− 1.6	− 11.5	+ 11.4
Flour II	*mg*	− 25.2	−206.1	− 9.4	−240.7	+220.2
	%	− 1.2	− 10.1	− 0.4	− 11.7	+ 12.2

As shown in Table 3, the increase of the alcohol-soluble fraction is approximately equal to the sum of the decreases of the other three fractions. In other words, NaCl-soluble, NaOH-soluble, and Residue-nitrogen were partly converted into the alcohol-soluble nitrogen during storage.

Comparison of the nitrogen distributions during storage between the Wiley-milled and the roller-milled flour.

The nitrogen distributions of the Wiley-milled flours were remarkably different from those of the rollermilled flours. As shown in Table 2-2, the nitrogen values of the Wiley-milled flours are larger in 3% NaCl extracts and smaller in 70% alcohol extracts than those of the rollermilled flours. These two flours were also different in the nitrogen changes. The alcohol-soluble fractions decreased and 1st NaOH extract-fractions increased in the Wiley-milled flours during storage, while the alcohol-soluble fractions increased and 1st NaOH extract-fractions decreased in the rollermilled flours. Especially the increases of the alcohol-soluble fractions of the roller-milled flours were much large.

As shown in Note 3 in Table 2-1, the nitrogen value of Residue-fraction of the Wiley-milled flour is the sum of the original Residue-nitrogen and the nitrogen of 2nd & 3rd NaOH extracts of the same flour. The sums for Flour I and II corresponding to those for Flour III and IV were calculated as shown below.

	Flour I			Flour II		
Days of storage	0 %	205 %	difference %	0 %	169 %	difference %
2nd & 3rd NaOH extracts N	10.6	6.0	−4.6	13.7	4.9	−8.8
Residue N	4.2	2.6	−1.6	2.9	2.5	−0.4
Sum	14.8	8.6	−6.2	16.6	7.4	−9.2

The results show that the change during storage of Residue-fraction of the Wiley-milled flour tends to the positive direction while that of the rollermilled flour distinctly to the negative one. Table 4 shows the nitrogen balances for the Wiley-milled flours calculated as in Table 3.

It is obvious from this table that the NaCl-soluble and the alcohol-soluble nitrogen were converted partly into the NaOH-soluble or Residue-nitrogen during storage. Therefore, the Wiley-milled flour is entirely different in the tendency of conversion from the rollermilled flour. This fact might be due to the difference between the milling methods.

Table 4. Balances of Nitrogen Differences of Fractions.
(*Wiley-milled Flours*)

		3% NaCl extracts 1st, 2nd & 3rd extracts *N-difference*	70% alcohol extracts *N-difference*	0.025n NaOH extracts 1st extract *N-difference*	* Residue *N-difference*	Sum
Flour III	*mg*	−12.2(−0.6)	−48.6(−2.5)		−4.0(−0.2)	−64.8(−3.3)
	mg			+54.1(+2.7)		+54.1(+2.7)
Flour IV	*mg*	−16.7(−1.0)	−72.7(−4.1)			−89.4(−5.1)
	mg			+55.4(+3.3)	+39.9(+2.3)	+95.3(+5.6)

The numbers put in parentheses are expressed in percentages.
* This fraction contains not only original Residue-N but also 2nd & 3rd NaOH-soluble N.

These effects of storage on the wheat flour proteins may be caused by enzymes. A enzyme which participates in the deterioration of the proteins during strorage may be a proteinase but its activity in wheat flour is fairly faint[4]. It is suggested from the small nitrogen changes of Dialyzate-fractions of 1st NaCl- and 1st NaOH-extract shown in Tables 2-1 and 2-2 that the flour proteinase is not a leading cause for the deterioration during storage.

Of particular intersest is the work of Jones and Gersdorff[2] on the changes occurring in proteins of wheat flour during storage. They reported the decreases in solubility and in digestibility of proteins during storage and the greatest decrease was found in solubility in 3% NaCl solution. They stated also that the alternation of the proteins is ascribed to the effect of enzymes and the oxidation. However, their results are strikingly contrasted with ours.

Cuendet et al.[5] demonstrated the development of oxidative rancidity of wheat flour during storage at various moisture levels. It is easily admitted that the intermediate products which were produced through the processes of the development of the rancidity would inevitably participate in the nonenzymatic browning reaction.

Summary

1. The changes of nitrogen distributions during storage were investigated with some rollermilled and Wiley-milled flours.
2. The change of rollermilled flour during stroage was largest in alcohol-soluble fraction but that of Wileymilled flour was generally small.
3. In the rollermilled flour NaCl-soluble and NaOH-soulble nitrogen were converted into alcohol-soluble nitrogen during storage, whilst in Wiley-milled flour NaCl-soluble and alcohol-soluble nitrogen were converted into NaOH-soluble or Residue-nitrogen.
4. The causes of the deterioration of flour proteins were discussed.

Acknowledgments

We gratefully acknowledge the supply of wheat samples from the Nagano Agricultural Experiment Station and the Nisshin Milling Flour Co.. We also owed the milling of the samples to the Nisshin Flour Milling Co..

References

1) R. S. Harris and H. bon Loesecke: "Nutritional Evaluation of Food Processing." Jchr.

Wiley and Sons, Inc., New York, 1960.
2) D. B. JONES and C. E. F. GERSDORFF: Cereal Chem., **18,** 417 (1941).
3) H. SHIMOMURA and Y. AWO: Bull. Uniu. Osaka Pref., Ser. B, **16,** 113 (1965).
4) B. D. HITES, R. M. SANDSTEDP and L. SCHAUMBERG: Cereal Chem., **30,** 404 (1953).
5) L. S. CUENDET, E. LARSON, C. G. NORRIS and W. F. GEDDES: Cereal Chem., **31,** 362 (1954).