THE PROTEIN COMPOSITION OF DIFFERENT FLOURS AND ITS RELATIONSHIP TO NITROGEN CONTENT AND BAKING PERFORMANCE[1]

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ABSTRACT

The proteins in a total of 26 flour samples ranging in nitrogen content from 1.49 to 2.92% dry weight were fractionated by differential extraction with 0.01*M* sodium pyrophosphate (pH 7.0) and 0.05*M* formic acid (pH ~ 3.5). The distribution of nitrogen in these extracts was correlated with total nitrogen and with baking performance. Except for four flours in this group there was a general tendency for baking quality to rise with increasing total nitrogen content. The exceptions included a durum wheat. In all cases, including the anomalous flours, there was a marked negative correlation between the total nitrogen and the percentage of that total extracted into sodium pyrophosphate. When the results were expressed as g. of nitrogen extracted into each solvent from 100 g. of flour, a very highly significant correlation was observed between total nitrogen and both pyrophosphate- ($r = 0.592^{+++}$) and formic acid- ($r = 0.978^{+++}$) soluble nitrogen. Except for the four anomalous flours a positive but less highly significant correlation ($r = 0.632^{++}$) was also observed between the formic acid-soluble nitrogen (g./100 g. flour) and baking score.

Attempts have been made by several workers to relate the baking quality of wheat flours to their protein content (1–6,14,16,17,19–23, 26–28). In most cases the correlations observed have been between total flour nitrogen and baking quality as judged by loaf volume. However, Finney (15), Mattern and Sandstedt (19), Pence, Elder, and Mecham (21), and Sollars (26–28) have fractionated the flour proteins in an attempt to assign specific quality effects to specific protein components. Of the methods available for fractional extraction of the flour proteins, the dispersion of gluten into dilute acetic (9,10,18,26–28) or formic (12,13) acid appeared to be the mildest available. A modification of the methods developed by Coates and Simmonds (11) based on the use of formic acid and sodium pyrophosphate was therefore adopted in conducting a survey on the relationships existing between extractable nitrogen, total nitrogen, and baking quality in 26 Australian flours.

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TABLE I

SUMMARY OF ORIGIN AND CHARACTERISTICS OF FLOUR SAMPLES USED FOR EXTRACTION AND TEST BAKING STUDIES

Flour Sample No.	Variety and Origin	Protein (14% m.b.)	Baking Studies[a]		Dough Characteristics (Alveograph)	Bushel Wt.
			—	+		
		%				lb.
C 4251	Gabo; Curlewis, NSW	14.8	72	79	Strong; extensible	60
C 4266	Variety unknown; Tichborne, NSW	14.0	63	79	Medium; extensible	61.5
C 4740	Charter; Boggabri, NSW	12.7	78	71	Strong; stable	65.5
C 4747	Broughton; Quirindi, NSW	8.3	64	65	Strong; stable	65.75
C 4753	Sabre; Quirindi, NSW	7.8	52	49	Moderate; stable	64
C 6330	Charter; Allsopps, NSW	13.2	..	80	Strong; well balanced	65.75
D 5081	Variety unknown; Curlewis, NSW	13.6	78	89	Medium; very extensible	63.5
D 5176	Javelin; S.A., 1959	12.9	83	81	Medium; very extensible	61.75
D 5177	Dural (durum wheat); S.A., 1959	15.3	46	51	Strong; very extensible	64
D 5178	Gabo; S.A., 1959	12.8	70	76	Strong; rather extensible	62.75
D 5179	Insignia; S.A., 1959	12.1	73	79	Moderate; very extensible	65.25
D 6335	Gabo; Queensland, 1959	9.4	67	70	Medium; well balanced	58.25
D 6381	Variety unknown; Winton, Queensland	14.4	66	82	Strong; very extensible	64.5
D 6391	Gabo-Mentana-Kenya Crossbred; Winton, Queensland	15.0	52	55	Medium; very extensible	63.75
D 6912	Variety unknown; Glen Lossie, W.A.	8.9	58	63	Moderate; well balanced	62.5
D 6999	Variety unknown; Glen Lossie, W.A.	10.3	74	81	Medium; very stable	63.5
D 7026	Variety unknown; Glen Lossie, W.A.	8.4	68	69	Moderate; very stable	62.5
D 7171	Cappelle; France	7.5	62	56	Low; very extensible	..
D 7172	Lichtis; Germany	10.6	68	74	Moderate; extensible	..
D 7272	Claymore; Gunnedah, NSW	11.0	58	63	Strong; very stable	60.75
D 7279	Gabo; Gunnedah, NSW	11.5	66	75	Strong; very stable	61.5
D 8322	Variety unknown; Trangie, NSW	9.9	60	73	Low; very extensible	64.5
D 9585	Glenwari; NSW	12.2	49	53	Very low; very extensible	61
D 7173	Commercial blend; Manitoba No. 3, Canada	11.4	81	87	Strong; very extensible	..
D 7438	Commercial blend; Crago Mill, NSW	10.8	54	51	Moderate; very stable	..

[a] —, No bromate added during mixing; +, 10 p.p.m. potassium bromate added during mixing.

Materials and Methods

Flour Samples. The flours used were selected both from wheat and from commercially milled samples sent to the Bread Research Institute of Australia for baking and other physical tests. The wheat samples were milled to approximately 70% extraction on a Buhler laboratory mill. The identification numbers, origin, and characteristics of the samples are summarized in Table I.

Extraction Procedure. Preliminary experiments showed that 1 to 2% of the total nitrogen was extracted into the water-saturated butanol, 2 to 5% of the nitrogen was dialyzable against 10 volumes of distilled water for 24 hours at 4°C., and 1 to 2% remained in the final residue. The final procedure adopted is given below:

Samples of flour weighing 5 g. and containing 12 to 14% moisture were mixed with 15 ml. 0.01*M* sodium pyrophosphate using the "Vibromixer" (A.G. für Chemie Apparatebau, Zurich) for ½ to ¾ minute. The mixer was then washed with 5 ml. of pyrophosphate and the solution shaken gently on an end-to-end shaker for a further 10 minutes. At the end of this time the tubes were centrifuged at 2,000 G for 10 to 15 minutes and the supernatant decanted. Three further treatments with pyrophosphate were followed by six similar treatments with 0.05*M* formic acid. All pyrophosphate and all formic acid extracts were combined, the residues were freeze-dried, and samples of each were subjected to nitrogen analysis.

Test Baking. The standard baking test to which these samples were subjected has been described (11).

Results and Discussion

The fractionation procedure described by Coates and Simmonds (11) partitioned the flour nitrogen into butanol-soluble (lipid material), pyrophosphate-soluble (albumins, globulins, pentosan-protein complexes, nucleoprotein), acetic acid-soluble (gluten proteins), and sodium hydroxide-soluble (glutenin fraction). In a preliminary series of experiments between 15 and 20% of the total nitrogen was extracted with 0.2*N* sodium hydroxide after preliminary extractions with sodium pyrophosphate and formic acid, while 1 to 2% was left in the insoluble residue. Although there was a significant relationship at the 1% level between the amount of sodium hydroxide-soluble nitrogen (g. per 100 g. flour) and total nitrogen ($r = 0.960{+}{+}{+}$), no correlation with baking score could be observed and it was decided to simplify the procedure by extracting only with sodium pyrophosphate and formic acid. Under these conditions the small amount of nitrogen present in the lipid fraction (1 to 2%) would be extracted into the pyrophosphate solution, while the sodium hydroxide-soluble fraction would be left in the residue. Some overlapping of components in each extract undoubtedly occurs. Additional butanol and sodium pyrophosphate extracts contained small but significant amounts of nitrogen, derived probably from the gluten fraction. For these reasons reproducibility of the extraction procedure was limited to ± 5% at the pyrophosphate step and ± 10% at the formic acid step. Statistical analysis of the results of three separate surveys conducted on the flours listed in Table I has shown that, in spite of slight modifications in the extraction procedure employed, the trends shown in the figures below are significant at the 1% level or better. Figure 1 shows the relationship between total nitrogen and the percentage of that total extracted into pyrophosphate and into formic acid respectively.

The inverse relationship observed between pyrophosphate-soluble nitrogen (Y) and total nitrogen (x) ($Y = 35.73 - 6.51\,x$; $r = 0.808{+}{+}{+}$) was highly significant. However, the slight upward trend observed between formic acid-extractable nitrogen and total nitrogen was not significant in this case. In a previous survey (data not shown here) this relationship proved to be significant at the 1% level ($r = 0.584{+}{+}$). In this case, however, 0.05*N* acetic acid was used

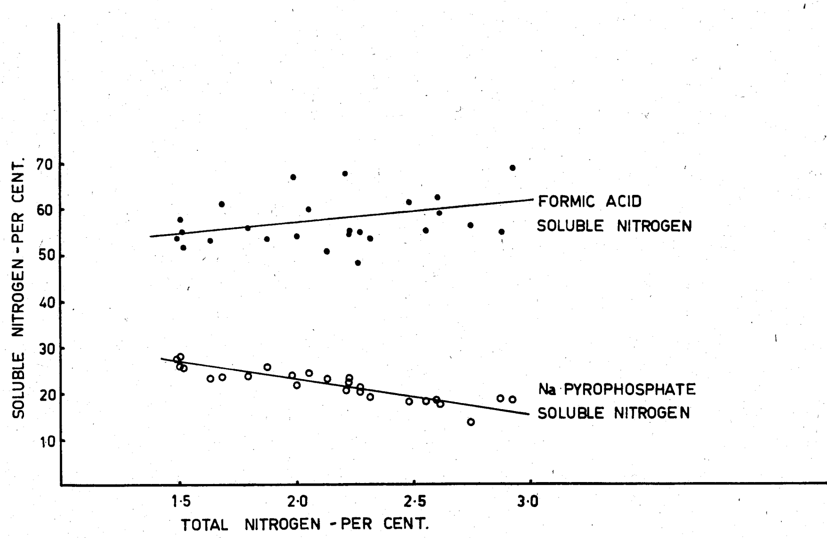

Fig. 1. Relationship between total nitrogen (percent) and nitrogen extracted (a) into 0.01*M* sodium pyrophosphate (pH 7.0) and (b) into 0.05*M* formic acid (pH ≈ 3.5). Extracted nitrogen is expressed as a percentage of total nitrogen taken in each case.

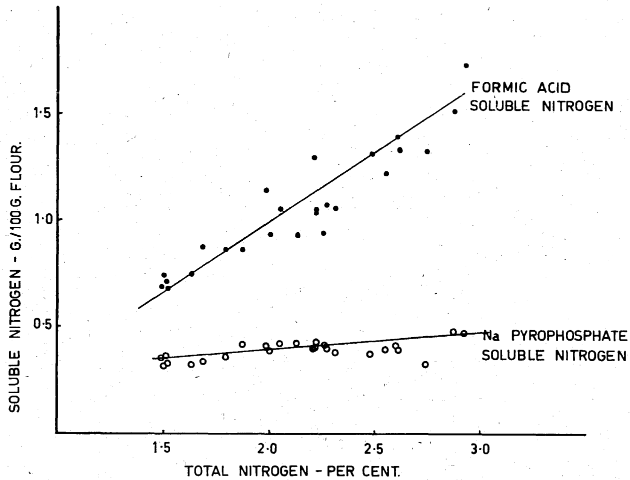

Fig. 2. Relationship between total nitrogen (percent dry weight) and nitrogen extracted (a) into 0.01*M* sodium pyrophosphate (pH 7.0) and (b) into 0.05*M* formic acid (pH $\simeq$ 3.5). The actual amount of nitrogen extracted is expressed as g. per 100 g. flour (14% moisture basis).

as extractant, and somewhat lower amounts of nitrogen were dissolved at this step.

Figure 2 shows the relationship between total nitrogen and the amount of nitrogen extracted into pyrophosphate and into formic acid from 100 g. of flour (adjusted to 14% moisture).

The amounts of both pyrophosphate- and formic acid-soluble nitrogen increase with increasing total nitrogen content, the latter very much more rapidly than the former. In each case the relationship is highly significant. For pyrophosphate-soluble nitrogen (Y) vs. total nitrogen (x) the regression equation was ($Y = 0.054\,x + 0.269$; $r = 0.592^{+++}$). For formic acid-soluble nitrogen, the equation was ($Y = 0.500\,x - 0.200$; $r = 0.978^{+++}$).

Figure 3 shows the relationship between total nitrogen and baking score for this group of flours.

When the nitrogen extracted by pyrophosphate and by formic acid (expressed as g. per 100 g. flour) is plotted against baking score, the relationships shown in Fig. 4 are obtained.

There appeared to be no significant correlation between the amount of pyrophosphate-soluble nitrogen and baking score. This is not surprising in view of the very different roles played by the pyrophosphate-soluble proteins as compared with the formic acid-soluble group. The former will contain those proteins and enzymes concerned directly or

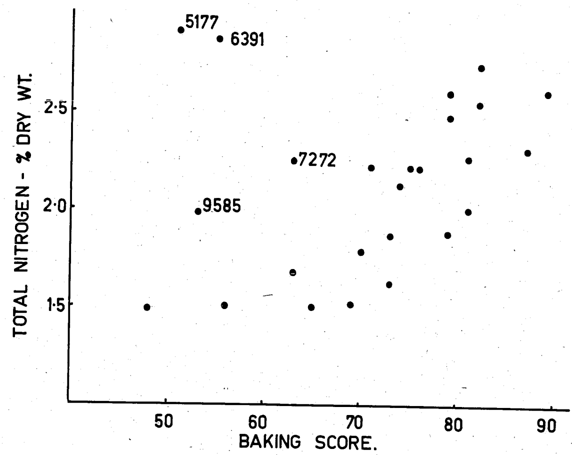

Fig. 3. Relationship between total nitrogen (percent dry weight) and baking score for the flour samples listed in Table I.

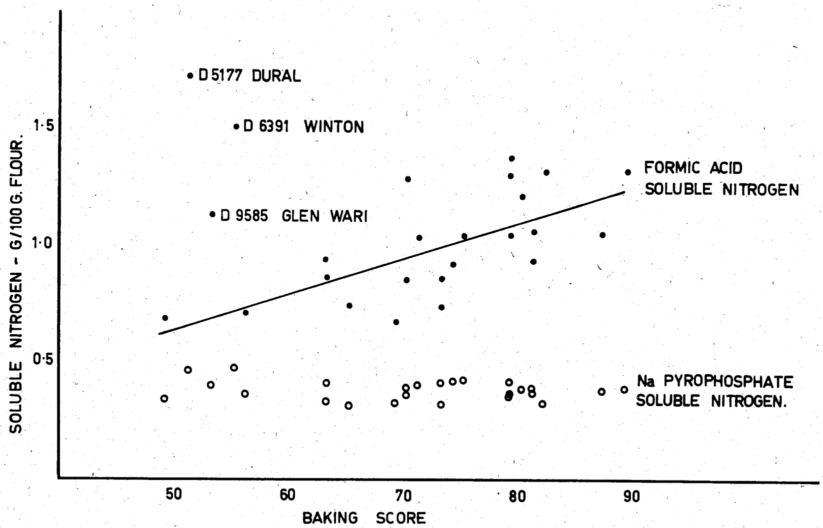

Fig. 4. Relationship between baking score and nitrogen extracted (a) into 0.01*M* sodium pyrophosphate (pH 7.0) and (b) into 0.05*M* formic acid. The actual amount of nitrogen extracted is expressed as g. per 100 g. flour (14% moisture basis).

indirectly with cellular metabolism, either with gluten and starch synthesis during grain maturation, or with their subsequent breakdown during germination. There is no evidence that any proteins of the pyrophosphate-soluble group are involved in the formation of the cohesive network characteristic of the gluten or formic acid-soluble group when flour-water doughs are prepared. Experiments with developing grain have shown that as maturity approaches the rates of gluten synthesis and deposition increase rapidly, these processes only being halted by the final desiccation of the endosperm cells. For this reason the proportion of enzymatic and "nonstorage" proteins would be expected to decrease with increasing total grain nitrogen. The correlation between formic acid-soluble nitrogen and baking score had about the same level of significance as that between total nitrogen (Y) and baking score (x) ($Y = 0.015\,x - 0.100$; $r = 0.632{+}{+}$). Flours D5177 and D6391 were omitted from the calculations in each case. The points shown in Figs. 3 and 4 show considerably more scatter than those relating formic acid-soluble nitrogen to total nitrogen, thus demonstrating the superimposed effect of a protein quality factor. In particular, flours D5177 and D6391, although fitting into the nitrogen classification, are clearly anomalous in baking behavior. Further experiments have shown these flours to give a normal pattern when their "acid-soluble" proteins are subjected to chromatography on carboxymethylcellulose (25), but to differ characteristically from normal flours when their pyrophosphate-soluble proteins are subjected to chromatography on DEAE-cellulose (24). In the case of the durum wheat, this may be the direct result of its different genetic constitution, but the explanation in the case of flour D6391 must await further work.

The results presented in this work have confirmed the validity of taking total flour nitrogen as the traditional index of baking quality and have shown the formic acid-soluble, rather than the pyrophosphate-soluble, nitrogen to be responsible for this relationship. The anomalous behavior of some flours in the survey, however, may be attributed to abnormalities in the pyrophosphate-soluble nitrogen, and further study of this fraction by chromatographic or electrophoretic techniques may reveal some possible explanations. Similar differences in the distribution of nitrogen among different classes of proteins have been observed by Bishop (7,8) in barley samples of widely differing nitrogen content.

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