VARIATIONS IN THE AMINO ACID COMPOSITION OF AUSTRALIAN WHEATS AND FLOURS[1]

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ABSTRACT

Variability in the amino acid composition of six samples of Australian wheat and of the flours milled from them has been determined. The following amino acids were found to vary between wheats: alanine, 2.76 – 3.43 (range) (2.5%: significance level); arginine, 5.34 – 8.98 (1%); aspartic acid, 3.02 – 3.49 (5%); glutamic acid, 12.95 – 16.08 (1%); glycine, 3.92 – 4.59 (1%); lysine, 2.68 – 3.82 (1%); phenylalanine, 1.79 – 2.24 (1%); and tyrosine, 1.10 – 1.23 (1%). The following amino acids were found to vary between the flour samples: alanine, 2.28 – 2.98 (5%); arginine, 6.63 – 8.44 (10%); aspartic acid, 2.51 – 3.04 (2.5%); glycine, 3.36 – 4.10 (2.5%); leucine, 3.93 – 4.34 (10%); lysine, 2.30 –2.90 (2.5%); and threonine, 1.60 – 1.92 (5%). All figures are expressed as amino acid nitrogen as a percentage of the total nitrogen present. Proteins high in lysine and arginine were extracted into sodium pyrophosphate (albumin-globulin group); proteins rich in glutamic acid were found in the gluten group.

Many amino acid analyses have been carried out on whole wheat, on flour or on gluten derived from it (2,3,5–16,18,21–23), and in several cases attempts have been made to assess the variations existing between flours, or wheats of different protein contents or qualities (3,12,15,16,18,21,22). The work described in this paper was undertaken in an attempt to study the extent and cause of variations in amino acid composition occurring in Australian wheat flours. For this purpose amino acid analyses were carried out on six Australian wheats and the flour samples milled from them. The proteins occurring in two of the six flour samples were fractionated by differential extraction in order to locate the groups of proteins responsible for the variation in individual amino acids. A similar study on some British and Canadian wheats has recently been reported by McDermott and Pace (15).

Materials and Methods

Wheat and Flour Samples. Wheat samples 1 to 6 and flour samples 1 to 6 milled from them on a Buhler laboratory mill were obtained from the Bread Research Institute of Australia, Sydney. Their protein content, baking store (4), and dough characteristics are summarized in Table I.

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TABLE I

ORIGIN AND CHARACTERISTICS OF WHEAT AND FLOUR SAMPLES SELECTED FOR AMINO ACID ANALYSIS

VARIETY AND ORIGIN	MOISTURE	N DRY WT.	BUSHEL WT.
	%	%	*lb*
	Wheat samples		
Crossbred (Tichborne, N.S.W.)	12.1	2.81	61½
Gabo (N.S.W.)	12.1	3.14	60
Charter (Allsopps, N.S.W.)	11.7	2.84	65¾
Charter (Boggabri, N.S.W.)	11.1	2.74	65½
Broughton (Quirindi, N.S.W.)	11.5	1.67	65¾
Sabre (Quirindi, N.S.W.)	11.6	1.57	64

ALVEOGRAPH PATTERN	Flour samples			
	N DRY WT.	EXTRACTION RATE	BAKING SCORE	
			— Bromate	+ Bromate
	%	%		
Medium, extensible	2.61	72	63	79
Strong, extensible	2.70	69	72	79
Strong, well balanced	2.64	73	..	80
Strong, stable	2.34	73	78	71
Strong, stable	1.60	70	64	65
Moderate, stable	1.61	73	52	49

Analytical Methods. Hydrolysis: Samples (50 mg.) of whole ground wheat or of flour were hydrolyzed, without defatting, for 20 hours by autoclaving in an evacuated sealed tube with 5 ml. of twice-distilled, constant-boiling hydrochloric acid at 110°C. At the end of this time, the tubes were opened, the contents were lyophilized, and the residue dissolved in 20 ml. of water. Nitrogen in the hydrolysate was determined by a micro-Kjeldahl procedure and samples corresponding to approximately 80 μg. of nitrogen were taken for loading onto the ion-exchange columns.

Amino acid analysis: Amino acids were determined by the modified ion-exchange chromatographic method of Moore, Spackman, and Stein (19), using the automatic equipment described by Simmonds and Rowlands (25,26).

Analyses for Cystine, Methionine, and Proline. Samples of flours 1 to 6 weighing 20–25 mg. were treated with 2 ml. performic acid which has been shown (24) to convert cystine to cysteic acid and methionine to methionine sulfone in 90–95% yield. After 20 hours at room temperature a small amount of water was added to each and the samples were freeze-dried. The oxidized residue was autoclaved in an evacuated, sealed tube with 5 ml. constant-boiling hydrochloric acid at 110°C. for 24 hours. The hydrolysates were freeze-dried and

quantitatively transferred to the top of a 150-cm. column of Dowex 50-X8 resin equilibrated in the sodium form with citrate buffer at pH 3.25. The analysis for cysteic acid and methionine sulfone was conducted in the automatic equipment by elution with pH 3.25 citrate buffer at 50°C. (19) until just after the emergence of threonine. At this point, the filter in the colorimeter was changed and the yellow color given by proline with the ninhydrin reagent (20) was estimated at 420 mμ. The analysis was then discontinued. Figure 1 shows the effluent graph obtained when the optical density values recorded by the print-out mechanism (25) were plotted against effluent volume. A synthetic mixture of cystine, methionine, and proline was

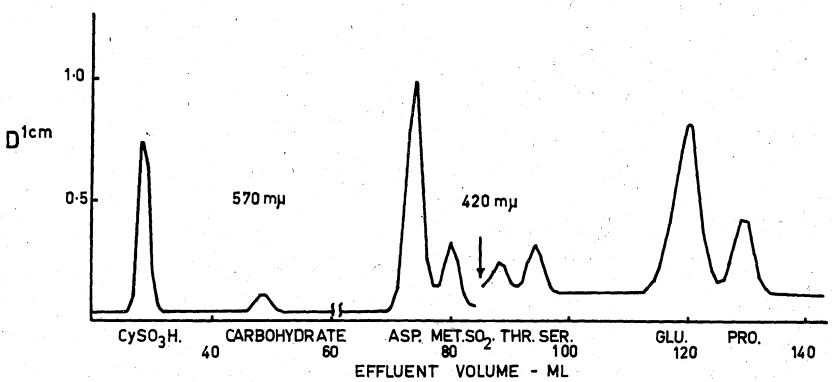


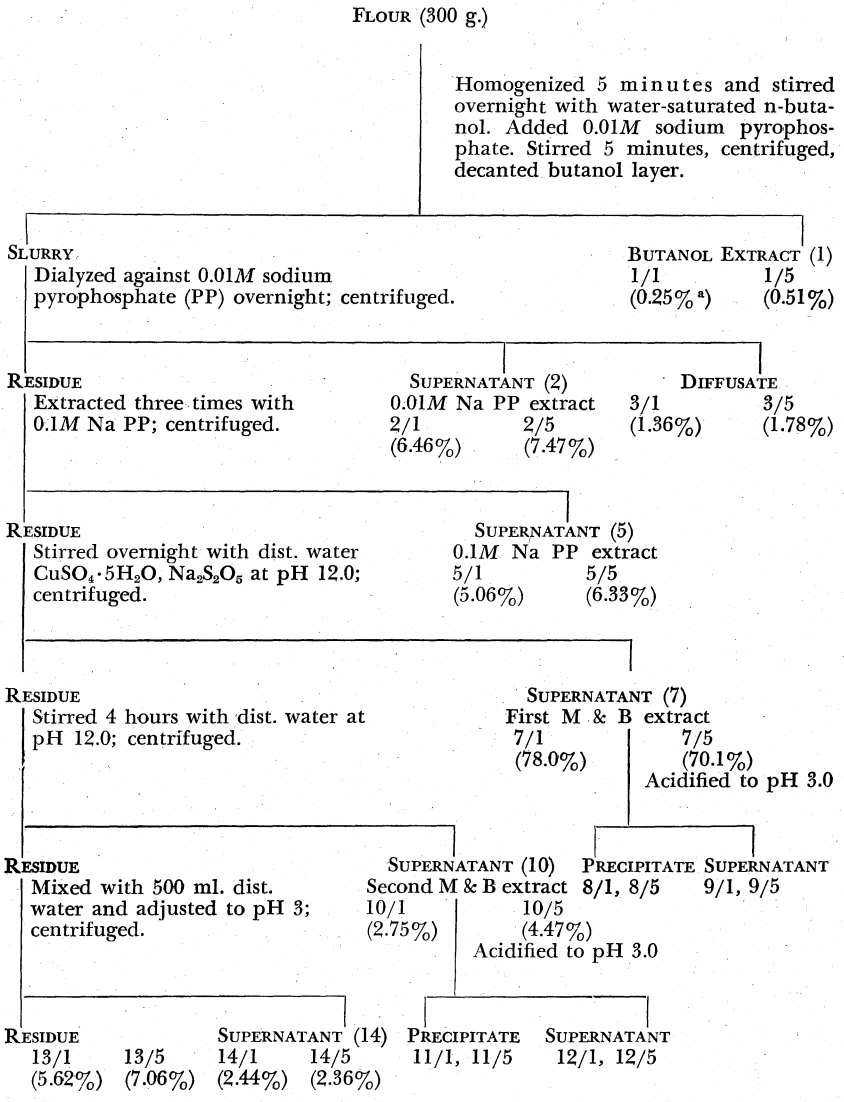
Fig. 1. Recording of effluent absorbance from a constant-boiling hydrochloric acid hydrolysate of an oxidized sample of flour. The arrow indicates where the filter of peak transmittance, 570 mμ, was replaced by one of 420 mμ.

carried through the above procedure, and the color values obtained were used to calculate the recoveries of the corresponding amino acids in the flour hydrolysates.

Fractionation Studies. Flours 1 and 5 were selected as differing most markedly in physical characteristics, nitrogen content, and general amino acid pattern. Samples of the gluten and nongluten proteins of these two flours were therefore prepared to allow inter-comparison of their amino acid compositions.

Lipids were first removed by overnight treatment with water-saturated n-butanol (600 ml. to 300 g. flour). The mixture was centrifuged, and the n-butanol decanted to give extracts 1/1 and 1/5 from flours 1 and 5 respectively. The residue was stirred for 5 minutes with 0.01*M* sodium pyrophosphate (Na PP). The mixture was dialyzed overnight against 0.01*M* sodium pyrophosphate, and centrifuged; the supernatant extract was decanted (extracts 2/1 and 2/5). A sample of

Diagram I. Fractionation I of Flour Samples 1 and 5



[a] Percentage of total nitrogen contained in extract.

the diffusate was also taken (3/1, 3/5). The residue was extracted three times with 0.1*M* sodium pyrophosphate (pH 9.5), with centrifuging between each extraction, and the supernatants were combined (0.1*M* pyrophosphate extract (5/1, 5/5)). The residue from these extractions was stirred overnight with a mixture of water (1,500 ml.), $CuSO_4 \cdot 5H_2O$

(8 g.), and sodium metabisulfite (1 g.), adjusted to pH 12.0 according to the method of Mertz and Bressani (17). The suspension was centrifuged and the supernatant (first M and B extract 7/1, 7/5) was adjusted to pH 3 with 4*N* HCl. The precipitated protein (first M and B precipitate, 8/1, 8/5) was recovered by centrifuging and a sample of the supernatant (first M and B supernatant, 9/1, 9/5) was taken for subsequent amino acid analysis. The residue from the alkaline extraction was re-extracted for 4 hours with 500 ml. distilled water at pH 12.0 and centrifuged. The supernatant was again adjusted to pH 3 to yield the second M and B precipitate (10/1, 10/5) and the second M and B supernatant (12/1, 12/5). After washing with water adjusted to pH 3, the residue was dried down to yield the final residue (13/1, 13/5) and supernatant washings (14/1, 14/5). The course of the fractionation, distribution of nitrogen in the various fractions, and numbering code for the fractions subsequently submitted to total and amino acid nitrogen determination is summarized in Diagram I.

Results

All results have been expressed as amino acid nitrogen as a percentage of the total nitrogen, to allow a comparison to be made between samples of differing nitrogen content. The amino acid composition of the six wheat samples is summarized in Table II.

The results of amino acid analyses carried out on the six flour samples derived from the wheats in Table II are summarized in Table III.

Figure 2 shows the variation in four amino acids among the various extracts obtained from fractionation I. These have been selected as representative of the types of variation observed.

Discussion

In agreement with other studies in this field, it is clear that wheat samples of widely differing protein content and type resemble one another quite closely in amino composition. The same observations apply to the flours milled from such wheats. Nevertheless, the differences reported in Tables II and III followed similar patterns in two separate sets of hydrolysates which were examined. The results presented here confirm those of Gunthardt and McGinnis (7), Lawrence *et al.* (12), Price (22), and McDermott and Pace (15), all of whom have noted an inverse relationship existing between the lysine and total nitrogen contents of whole wheat. The trend in the case of arginine is less marked, and no differences in histidine content between the various samples examined could be demonstrated. Glutamic acid

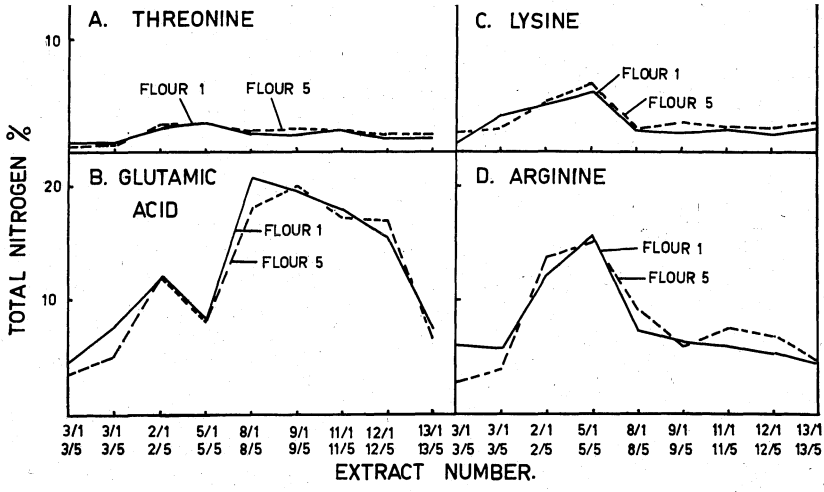


Fig. 2. Variation in amino acid composition of extracts obtained from fractionation I of flour samples 1 and 5. Amino acid nitrogen is expressed as a percentage of the total nitrogen in each case. A, variation in threonine content; B, variation in glutamic acid content; C, variation in lysine content; D, variation in arginine content. See text for details of extract numbers.

showed the reverse trend to lysine and arginine, in agreement with the results of McDermott and Pace. Although clear differences in glutamic acid content were observed between wheats, these differences could not be demonstrated between flour samples. This may be due to the slightly higher variability associated with the results in the latter case.

The fractionation procedure was devised to permit the maximum extraction of each group of proteins. Although it has been improved considerably in more recent work (4), it does allow some inferences to be made as to the nature of the proteins responsible for certain of the variations in amino acid composition observed. Alanine, glycine, histidine, isoleucine, leucine, phenylalanine, serine, threonine, tyrosine, and valine are fairly regularly distributed throughout the various fractions examined. The picture given by threonine (Fig. 2A) is typical of this group of amino acids. Furthermore, the two flour samples showed parallel amino acid composition in practically every extract examined. The only exception was in the amount of aspartic acid liberated after hydrolysis of the diffusible nitrogen from flour 1 which was somewhat higher than that from flour 5 (9.6% of the total nitrogen as against 6.0%).

This is in full agreement with the very complete analyses carried out on a series of gluten samples by Pence *et al.* (21), who were unable

TABLE II

AMINO ACID COMPOSITION OF SIX AUSTRALIAN WHEAT SAMPLES
(g. amino acid N per 100 g. total N)

AMINO ACID	WHEAT SAMPLE NO.												SIGNIFICANCE LEVEL OF DIFFERENCES
	1		2		3		4		5		6		
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	
	g	g	g	g	g	g	g	g	g	g	g	g	%
Alanine	2.76	0.11	3.08	0.08	3.21	0.09	3.00	0.03	3.24	0.02	3.43	0.23	2.5
Amide	15.22	0.88	14.53	0.49	13.85	0.35	13.79	0.48	13.40	0.62	13.42	0.16	n.s.
Arginine	5.34	0.52	7.87	0.33	8.60	0.14	8.98	0.07	8.21	0.12	8.30	0.76	1.0
Aspartic acid	3.22	0.15	3.21	0.06	3.12	0.07	3.02	0.05	3.31	0.08	3.49	0.06	5.0
Glutamic acid	14.16	0.21	16.08	0.69	14.97	0.71	12.95	0.11	13.14	0.11	13.09	0.19	1.0
Glycine	3.92	0.15	4.33	0.08	4.33	0.09	4.10	0.01	4.35	0.07	4.59	0.10	1.0
Histidine	3.18	0.13	3.36	0.20	3.62	0.41	3.66	0.41	3.59	0.20	3.59	0.71	n.s.
Isoleucine	2.11	0.08	2.07	0.04	2.05	0.06	2.14	0.06	2.09	0.09	2.12	0.09	n.s.
Leucine	3.90	0.05	3.83	0.20	3.87	0.04	3.79	0.09	3.89	0.05	3.89	0.12	n.s.
Lysine	2.92	0.10	2.69	0.11	2.68	0.13	3.19	0.23	3.51	0.09	3.82	0.15	1.0
Phenylalanine	2.00	0.02	2.24	0.09	1.95	0.11	1.79	0.15	1.79	0.15	1.87	0.11	1.0
Serine	2.96	0.16	3.17	0.08	3.16	0.11	3.23	0.02	3.23	0.04	3.20	0.09	n.s.
Threonine	1.80	0.06	1.82	0.05	1.83	0.07	1.74	0.04	1.87	0.11	1.92	0.07	n.s.
Tyrosine	1.16	0.06	1.23	0.13	1.10	0.05	1.18	0.06	1.14	0.02	1.15	0.06	1.0
Valine	2.62	0.06	2.73	0.02	2.99	0.16	2.56	0.07	2.69	0.12	2.67	0.21	n.s.

TABLE III

AMINO ACID COMPOSITION OF FLOUR SAMPLES DERIVED FROM AUSTRALIAN WHEATS IN TABLE II
(g. amino acid N per 100 g. total N)

AMINO ACID	FLOUR SAMPLE NO.												SIGNIFICANCE LEVEL OF DIFFERENCES
	1		2		3		4		5		6		
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	
	g	g	g	g	g	g	g	g	g	g	g	g	%
Alanine	2.28	0.12	2.34	0.07	2.62	0.22	2.86	0.06	2.98	0.16	2.93	0.20	5.0
Amide	14.41	0.31	17.10	0.74	15.31	0.74	15.61	0.88	16.30	1.20	15.67	1.45	n.s.
Arginine	7.26	0.48	7.02	0.33	7.54	0.04	6.63	0.37	7.64	0.29	8.44	0.02	10.0
Aspartic acid	2.83	0.11	2.75	0.18	2.83	0.11	3.04	0.16	2.98	0.10	2.51	0.20	2.5
Cystine	1.66	...	1.65	...	1.59	...	1.65	...	1.73	...	1.77	...	..
Glutamic acid	16.48	0.55	16.14	0.09	17.77	0.84	17.70	0.40	16.19	0.30	15.80	0.32	n.s.
Glycine	3.36	0.14	3.42	0.12	4.10	0.01	4.00	0.06	3.97	0.15	3.76	0.23	2.5
Histidine	2.88	0.06	3.63	0.37	2.84	0.04	3.07	0.26	3.54	0.52	3.48	0.38	n.s.
Isoleucine	2.40	0.15	2.13	0.15	2.39	0.05	2.23	0.11	2.23	0.09	2.45	0.04	n.s.
Leucine	3.98	0.08	3.93	0.10	4.15	0.09	4.17	0.02	4.04	0.03	4.34	0.14	10.0
Lysine	2.30	0.03	2.82	0.16	2.32	0.001	2.47	0.14	2.90	0.19	2.86	0.08	2.5
Methionine	1.06	...	1.01	...	0.87	...	0.95	...	0.98	...	1.17	...	..
Phenylalanine	2.04	0.08	1.87	0.13	2.22	0.11	2.00	0.19	2.16	0.09	2.13	0.07	n.s.
Proline	8.16	...	8.20	...	8.41	...	8.78	...	8.05	...	8.70	...	..
Serine	2.87	0.18	3.11	0.07	3.10	0.06	3.04	0.004	2.96	0.08	2.86	0.14	n.s.
Threonine	1.75	0.09	1.60	0.07	1.89	0.05	1.92	0.04	1.90	0.09	1.70	0.03	5.0
Tyrosine	1.30	0.05	1.12	0.13	1.50	0.11	1.24	0.21	1.29	0.04	1.16	0.12	n.s.
Valine	2.62	0.14	2.34	0.23	2.74	0.28	2.56	0.16	2.80	0.11	2.75	0.16	n.s.

to find any notable differences in amino acid composition in samples prepared from flours of widely varying type and protein content. Their gluten preparations would correspond to the first and second M and B mother liquor samples (8 – 12/1, 8 – 12/5) shown in Fig. 2.

Examination of Fig. 2, B, C, and D shows some interesting trends between protein fractions with the amino acids lysine, arginine, and glutamic acid. The pyrophosphate-soluble proteins (albumin-globulin group) are richer in both arginine and lysine, while the pH 12, copper sulfite-soluble proteins (gluten group) are richer in glutamic acid. This offers an explanation for the fluctuations in composition of these amino acids. The flours of low total nitrogen content have a higher proportion of pyrophosphate-soluble proteins (1); those of higher total nitrogen have a considerably greater amount of the gluten group proteins present. This is the case in flours 1 and 5 which contained 11.5 and 13.8% of pyrophosphate-soluble nitrogen respectively. Later and more efficient procedures (4) have shown an even greater difference between the two flours (16.8 and 24.6% pyrophosphate-soluble nitrogen respectively), and this is almost certainly responsible for the variations in lysine, arginine, and glutamic acid contents which have been demonstrated in the over-all analyses.

The 0.01*M* sodium pyrophosphate extract contained a fraction rich in glutamic acid (Fig. 2, B; extracts 2/1, 2/5, and 4/1, 4/5). This has been identified in subsequent work (4) as peak A, a pentosan-protein complex readily extracted by this solvent and separable on DEAE-cellulose. The protein moiety of this complex contains a proportion of glutamic acid almost as high as that of gliadin.

A comparison of the results reported in the present paper with those of McDermott and Pace (15) shows the agreement to be satisfactory in most cases. Significant differences occur between the two sets of results in the cases of the amide, glutamic acid, phenylalanine, proline, and serine contents, which are 10 – 15% lower than the results quoted by McDermott and Pace, and lysine, which is higher. Some of these differences may be due to the different methods of hydrolysis employed.

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