AMINO ACID COMPOSITION OF WHEAT FLOURS[1]

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ABSTRACT

Amino acid compositions are reported of six flours milled from four major types of Canadian wheats. All of the amino acid compositions were found to be quite similar. The results were obtained by automatic ion-exchange chromatographic analysis of $6N$ HCl and $6N$ Ba(OH)$_2$ hydrolysates. Recoveries of amino acid nitrogen were approximately 95%. Acid hydrolysis had to be carried out for various intervals, in order to correct for the decomposition of threonine and serine and to obtain an accurate estimate for ammonia content. Enzymatic hydrolysis was also carried out on one of the flours. Analyses of the enzymatic hydrolysates yielded the complete amino acid composition of this flour, including the amount of glutamine and asparagine present.

Although wheat flour is one of man's most common and important food sources, our knowledge of the amino acid composition of flour is limited. Besides some scattered studies on the occurrence of the essential amino acids, only a few relatively complete amino acid studies have been reported (1,2,3). A complete amino acid analysis of flour, including data for glutamine and asparagine, does not seem to be available.

The present work is divided into two parts. Part I describes a study of the amino acid composition, mainly by analysis of acid hydrolysates. Six flours were studied, covering a wide range of baking strength and behavior, milled from four types of wheat grown in Canada. Part II describes the further study, where enzymatic hydrolysates of one of the flours were also studied, in order to obtain the complete quantitative amino acid composition estimate.

PART I

Materials

Flour. The flours used in this study were all milled from sound samples of wheat. The flour extractions were all in the vicinity of 70%, except for the amber durum sample which was milled to a semolina of 57% extraction. The semolina was then reduced to flour. The flours are further described in Table I.

Reagents. All chemicals used in the preparation of buffer and analytical solutions were reagent grade.

TABLE I

DESCRIPTION OF FLOURS ANALYZED

NAME OF FLOUR	EXTRAC-TION	PRO-TEIN	MOIS-TURE	ASH	BAKING QUALITY
	% [a]	% [b]	% [c]	% [d]	
Hard red spring, commercial blend (HRS-G)	74	13.2	14.8	0.43	good
Hard red spring, Selkirk (HRS-S)	69	15.4	12.3	0.57	good
Hard red spring, experimental variety RL-2520 (HRS-R)[d]	68	14.9	13.2	0.45	poor
Alberta red winter, commercial blend (ARW)	74	11.0	14.9	0.42	fair
Ontario white winter, commercial blend (OWW)	unknown	9.25	12.9	0.43	poor
Amber durum, Western Canadian composite (AD)	57	12.4	14.3	0.60	poor

[a] 14% moisture basis.
[b] "As is," N × 5.7.
[c] "As is."
[d] This sample was grown on a small experimental plot and some of it had been labeled with sulfur-35.

Methods

Hydrolysis with HCl. Direct hydrolyses of flour with 6N HCl were carried out to obtain hydrolysates suitable for analysis for all of the amino acids present except for glutamine, asparagine, cystine, and tryptophan (4).

Hydrolyses were carried out by adding 4.00 ml. of twice-redistilled 6N HCl to 50–90 mg. of flour in 18-mm. Pyrex test tubes. After the mixture was frozen to −80°C., the test tubes were evacuated to less than 50μ and then sealed. Hydrolysis of different samples of flour was carried out at 100° ± 2°C. for 24, 48, and 72 hr. in an air oven.

The HCl was then rapidly removed from the hydrolysate mixture by placing the frozen mixture at −80°C. in a desiccator containing NaOH pellets and evacuating to approximately 0.1 mm. with a suitably protected oil pump. To avoid the appearance of any artifact peaks on the amino acid chromatograph, as is possible when the HCl is removed too slowly (5), the HCl was always removed rapidly (usually within 10 to 12 hr.) by using fresh NaOH pellets and a good vacuum. Citrate buffer, 0.20N Na+, pH 2.2, containing BRIJ-35 detergent and octanoic acid, was added to the residue, the insoluble humin being removed by filtering (vacuum) through Whatman No. 52 filter paper. The filtrate was accurately made up to volume with the pH 2.2 buffer and aliquots were analyzed for their amino acid content. The insoluble humin remaining in the filter paper was washed with water. After the humin was dry, its weight was estimated and the nitrogen content determined with a Coleman Nitrogen Analyzer. The recovery of humin obtained

was probably low because of its tenacious adherence to the filter paper.

Hydrolysis with HCl was also carried out on flour which had been previously oxidized with performic acid in order to analyze for total cystine-plus-cysteine content in the form of the more stable derivative, cysteic acid, as described by Schram et al. (6). The amount of cysteic acid was determined by analysis on the automatic amino acid analyzer, utilizing the 150-cm. ion-exchange column.

Hydrolysis with Ba(OH)₂. To analyze for tryptophan, hydrolysis with $Ba(OH)_2$ was carried out as described by Drèze (7), except that no starch was added, since it is already present in flour. After hydrolysis was complete, Ba^{++} was removed by adding cold, dilute H_2SO_4 to pH 2 and filtering or centrifuging away the $BaSO_4$. The filtrate or supernatant was accurately made up to volume with pH 2.2 buffer, and aliquots were analyzed for tryptophan content by analysis on the 15-cm. ion-exchange column on the amino acid analyzer.

Amino Acid Analysis. Aliquots of the hydrolysates were analyzed for their amino acid content by the method of Spackman et al. (8), with a Beckman-Spinco Model 120 amino acid analyzer. The amounts of amino acids were determined by comparing the recoveries of a standard amino acid mixture as provided by Beckman-Spinco. The double 4-liter oil bottle ninhydrin system provided with the amino acid analyzer was replaced with quadruple 4-liter water bottle system interconnected with thick rubber vacuum tubing in order to reduce the oxidation of the ninhydrin reagent by diffusion of atmospheric oxygen; and the ninhydrin storage bottle was also cooled to approximately 8°–12°C. to further retard decomposition of the ninhydrin reagent. These modifications resulted in the ninhydrin reagent's yielding virtually identical recoveries on analysis of standard amino acid mixtures, even when analyzed over a period of 30 days with the same lot of ninhydrin solution.

The values for the amount of ammonia present in determinations carried out in the present work have been corrected, where applicable, for the amount of ammonia present in the HCl used during acid hydrolysis, in the pH 2.2 citrate buffer used to make the hydrolysate samples or residues up to volume, and in the amount of pH 2.2 citrate buffer used to wash the sample onto the ion-exchange column, together with that contributed by the enzymatic control experiment.

Cysteine content, where determined, was analyzed as S-succinyl (-1-C¹⁴) cysteine, the latter derivative obtained by dispersing flour with $8M$ urea containing N-ethylmaleimide-1-C¹⁴, followed by acid hydrolysis. The analysis for the S-succinyl (-1-C¹⁴) cysteine present in the acid hydrolysate was carried out by automatic ion-exchange separation in

conjunction with simultaneous and continuous scintillation radioactive recording techniques (9,10).

Results and Discussion:

Amino Acid Compositions of Canadian Flours

Hard Red Spring. The results from analysis of acid and alkali hydrolysates of three hard red spring (HRS) flours are listed in Table II.

The present work indicates that, within the confines of the present experimental error, the amino acid compositions of these flours are closely similar. This is so even when results of the experimental variety HRS-R are compared; this variety, although possessing a reasonable protein content, has very poor baking properties, its loaf volume being approximately 60% that of good varieties like Selkirk.

The very consistent amino acid composition of different HRS flour samples is further illustrated by the similarity of the present results with some published results, as shown in Table II.

Four Canadian Wheat Types. Table III summarizes the results of

TABLE II
AMINO ACID COMPOSITION OF CANADIAN HARD RED SPRING FLOURS
(g. N per 100 g. total Kjeldahl N)

	PRESENT WORK			PUBLISHED WORK		
AMINO ACID	HRS-S	HRS-G	HRS-R	Manitoba No. 2: Wunnikhoven and Bigwood (1)	"Manitoba": McDermott and Pace (2)	"U.S." [a]: Hepburn, Lewis, Jr., and Elvehjem (3)
Tryptophan	0.77	0.68	0.77	0.64 [b]	....	0.874
Lysine	2.17	2.35	2.24	2.51	2.46	2.49
Histidine	3.50	3.69	3.70	3.58	3.89	3.18
Ammonia	20.36	20.76	19.96	19.58	22.25	...
Arginine	6.42	7.44	6.94	6.95	6.99	7.64
Aspartic acid	2.55	2.63	2.60	2.78	2.65	2.72
Threonine	1.87	2.04	1.86	2.06	2.06	2.07
Serine	3.77	4.31	4.09	3.57	3.81	4.53
Glutamic acid	21.01	22.33	23.10	19.54	19.56	20.53
Proline	8.98	9.76	9.72	8.69	9.19	8.90
Glycine	3.69	3.99	3.73	3.92	3.84	3.76
Alanine	2.76	2.85	2.90	2.66	2.86	2.73
Cystine	1.59	1.40	1.84	1.44	1.74	1.54
Valine	3.12	3.21	3.23	2.64	3.12	3.39
Methionine	0.83	0.97	0.88	0.87	1.12	1.02
Isoleucine	2.60	2.66	2.62	2.36	2.52	2.84
Leucine	4.48	4.66	4.57	4.45	4.69	4.66
Tyrosine	1.25	1.45	1.24	1.54	1.45	1.57
Phenylalanine	2.64	2.85	2.82	2.79	2.72	2.61
Recovery N, %	94.36	100.03	98.81	92.84	96.92	...

[a] Mean values of two HRS and two HRW wheat flours.
[b] Value taken from: Block, R. J., and Bollings, D.: The amino acid composition of proteins and foods. C. C. Thomas, Springfield, Ill., 1947.

TABLE III
AMINO ACID COMPOSITION OF FOUR CANADIAN WHEAT FLOURS
(g. N per 100 g. total Kjeldahl N)

AMINO ACID	HARD RED SPRING [a]	DURUM	ALBERTA WINTER	ONTARIO WHITE	AMINO ACID	HARD RED SPRING [a]	DURUM	ALBERTA WINTER	ONTARIO WHITE
Tryptophan	0.74	0.83	1.01	0.88	Glycine	3.80	3.29	3.74	3.94
Lysine	2.25	2.16	2.48	2.89	Alanine	2.84	3.04	3.00	3.06
Histidine	3.29	3.33	3.14	3.71	Cystine	1.61	1.86	1.81	1.78
Ammonia	20.36	20.28	20.20	19.96	Valine	3.19	2.91	3.15	3.20
Arginine	6.93	6.52	5.72	7.03	Methionine	0.89	0.85	0.95	1.02
Aspartic					Isoleucine	2.63	2.56	2.50	2.41
acid	2.59	2.73	2.62	3.15	Leucine	4.57	4.24	4.42	4.47
Threonine	1.92	1.79	2.08	1.90	Tyrosine	1.31	1.14	1.25	1.02
Serine	4.06	3.26	3.81	3.55	Phenyl-				
Glutamic					alanine	2.77	2.71	2.65	2.44
acid	22.15	20.22	19.83	20.38					
Proline	9.49	8.77	8.53	8.92	Recovered N, %	97.37	92.49	92.89	95.72

[a] Average of HRS-S, HRS-G, and HRS-R from Table II.

analysis of the four common types of Canadian wheat flours, HRS, durum, Albert winter, and Ontario white. The values listed in the column for the HRS flour were obtained by averaging the results from Table II. These results again illustrate similarity in amino acid composition of different types of flours analyzed, but there do seem to be some definite differences for flour milled from Ontario white wheat. These differences are more evident in Fig. 1, a bar graph of the
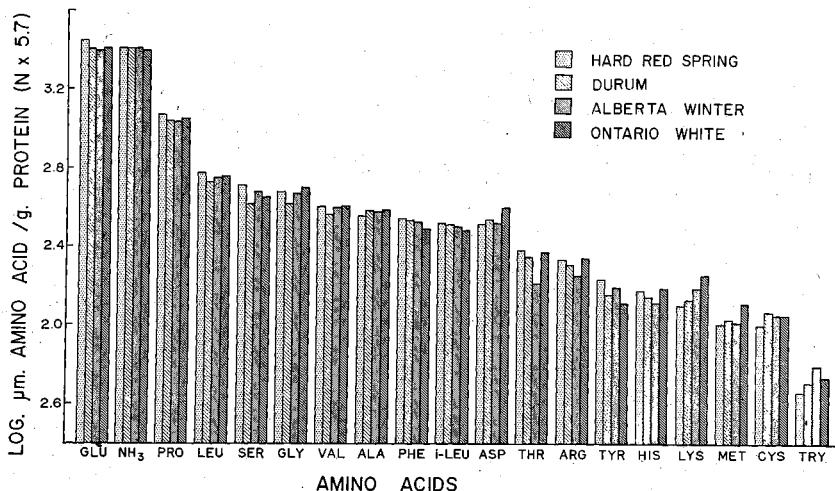

Fig. 1. Amino acid content of four types of flours. A mean value was used in plotting the bar graph for the HRS flour. The mean value was calculated from the values shown in Table III for the three HRS flours analyzed.

logarithm of the amounts of amino acids per g. protein in the various flours. This type of plot has been used previously to make a useful statistical examination of yolk lipoproteins which have similar amino acid compositions (11). Examination of Fig. 1 reveals that the Ontario white flour has more aspartic acid and lysine, and less tyrosine. The differences are small, however, and numerous accurate analyses of different samples would have to be available, for a determination as to whether these differences are typical. In general, the present results are in agreement with the composition of some United States HRS and winter wheats (3), and also with a related study on 17 glutens of widely differing quality (12).

Amino Acid Analysis of Flour

Effect of Acid Hydrolysis Time. To obtain accurate data with HCl hydrolysis, a minimum of three analyses of 24-, 48-, and 72-hr. hydrolysis duration is neccesary (13). Hydrolyses were carried out for various durations because the amounts of amino acids released by HCl are time-dependent, and some amino acids are destroyed during hydrolysis, particularly threonine and serine. The yield of ammonia, however, increases with the time of hydrolysis, most of the increase being due to the destruction of threonine, serine, and cystine and tryptophan. Accordingly, as illustrated in Fig. 2, the amounts of amide ammonia, threonine, and serine in the HCl hydrolysates were estimated by linear extrapolation to zero time of hydrolysis. In those instances where the recovery of amino acids was significantly higher at a certain time of hydrolysis, this maximum value was used as the amount of amino acid present. Where an increase or decrease in amino acid amount was not readily apparent, an average of values obtained by analysis of the 24-, 48-, and 72-hr. hydrolysates was used.

It was observed that the changes in the labile amino acids with hydrolysis time are different for different flour samples. Accordingly, it is not accurate to apply corrections calculated from results of one flour to those of another obtained at a single hydrolysis time. This variation can be expected, since different flours contain various amounts of inorganic salts (ash), which would tend to catalyze both the formation and decomposition of amino acids at different rates during hydrolysis. A varying rate of formation and decomposition of amino acids during hydrolysis with HCl has recently been observed even when crystalline protein enzymes have been hydrolyzed (14).

Probably all of the published amino acid compositions of flour are based an analyses of samples which had been hydrolyzed for a single fixed period. Because of the variations of amino acid yield with hy-
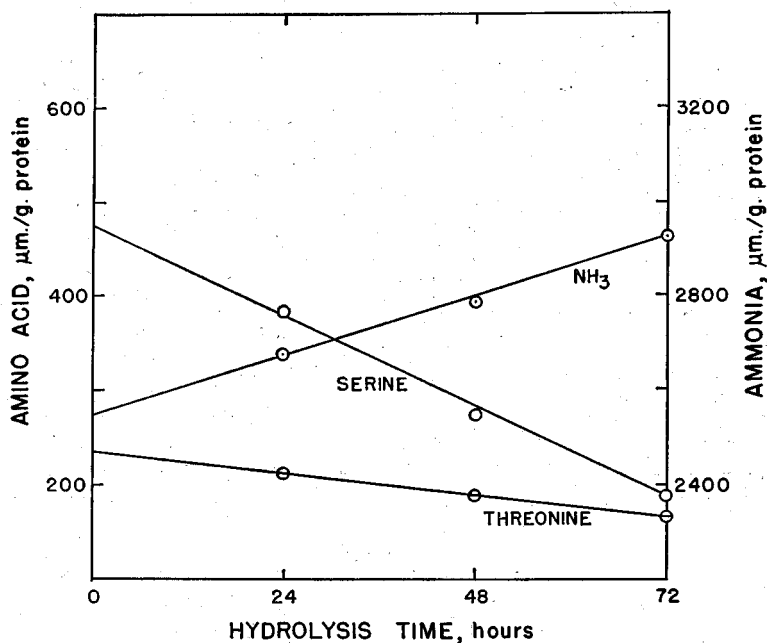
Fig. 2. Amino acid recoveries as a function of time of hydrolysis of Selkirk flour. The extrapolated recoveries at zero time for ammonia, threonine, and serine were found to be 2,550, 234, and 472 μm./g. protein.

drolysis time, these compositions must be reconsidered, particularly in the amounts of ammonia, threonine, and serine present.

Recovery of Amino Acid Nitrogen. The recovery of identifiable amino acid nitrogen (Tables II, III, and V) was approximately 95%. The nitrogen contained in the insoluble humin formed during 6N HCl hydrolysis contained, on the average, 2% of the total nitrogen. A small amount of nitrogen, estimated to be approximately 0.5–1% of the total, was present as unknown ninhydrin-positive constituents, as illustrated by the four small unknown peaks on the amino acid chromatograms. Thus approximately 98% of the total sample nitrogen was accounted for. The remaining 2% must be due to recovery losses, to ninhydrin-negative constituents, soluble humins (since the acid hydrolysates were faintly yellowish in color), and nonamino-acid-nitrogen constituents such as nucleotides, etc.

Precision of Amino Acid Analyses. The precision of averaged amino acid results from two analyses of one sample, when compared with a similar analytical result of a separately hydrolyzed sample, is approximately ± 3% for all amino acids except for ammonia, glutamic acid, cysteine, and perhaps tryptophan, where the error can be in the

vicinity of 5%. Precise results for ammonia and glumatic acid appear difficult to obtain in flour analysis because they are present in such relatively large amounts compared with the rest of the amino acids, and it is extremely easy for samples to be contaminated from traces of ammonia that are often present in a laboratory. A source of ammonia in this laboratory which could cause contamination was found to be a commercial liquid floor wax used for polishing floors.

PART II

This part of the paper describes an attempt at enzymatic hydrolysis of wheat flour. Enzymatic hydrolysis was carried out for a number of reasons. For example, a gentle hydrolysis method is desirable to isolate reactive and/or unstable reaction amino acid derivatives from flour doughs. It would be of interest to isolate glutamine and asparagine from flour, and this latter aspect is the main subject in this present paper. Finally it would serve as a useful check on the HCl hydrolytic method.

Materials

Flour. The flour used in this study was the Selkirk flour described in Table I.

Reagents. Reagent-grade chemicals were used in the preparation of all buffer and analytical solutions.

Proteolytic Enzymes. Leucine aminopeptidase was isolated from fresh swine kidney with the electrophoretically purified enzyme having an activity of $C_1 = 21.6$ by the methods as described by Hill *et al.* (15). Immediately prior to use, the enzyme was dialyzed overnight at 1°C. against several 200-ml. portions of $0.005M$ $MgCl_2$ and $0.005M$ Tris, pH 8.4, and centrifuged to get rid of any insoluble material.

Prolidase was isolated from the same lot of kidneys used for the isolation of leucine aminopeptidase with an activity of $C_1 = 28.9$, according to the method described by Davis and Smith (16). Immediately prior to use, the prolidase was dialyzed overnight at 1°C. against several 200-ml. changes of $0.05M$ $MnCl_2$, pH 7.3 Tris HOAc buffer, and centrifuged to get rid of any insoluble matter.

Crystalline papain was obtained from Worthington (Lot 5523, 2X, 19.3 mg. per ml.). Immediately prior to use, the enzyme was dialyzed at 1°C. against five changes of 100 ml., $0.04M$, pH 5.5 sodium acetate over a period of 24 hr.

Methods

Enzymatic Hydrolysis. Enzymatic hydrolyses were carried out in duplicate as described by Hill and Schmidt (17), with the necessary

modifications when flour was used as substrate. A freeze-dried dough of flour and water (347.2 mg., 58.53 mg. protein, moisture content 3.7%) was dispersed in 5 ml. 0.04M, pH 5.50 sodium acetate and 0.500 ml. 0.1M NaCN, pH 5.3. Papain, 0.25 ml., 4.8 mg. and a crystal of thymol were then added, and the mixture incubated for 26 hr. at 40°C. The extent of hydrolysis was followed by analyzing 25- λ aliquots of the reaction mixture at various time intervals with ninhydrin (Fig. 3). At the end of the digestion period, 0.60 ml. of the solution, taken after
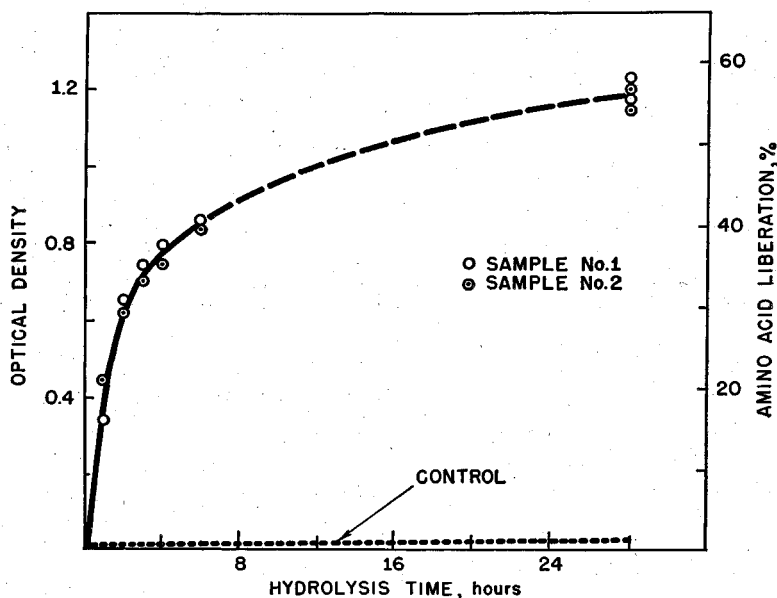

Fig. 3. Hydrolysis of flour by papain. Aliquots of the reaction mixture were treated with ninhydrin reagent, and the resulting absorbance measured at 570 mμ. The standard used to determine the extent of hydrolysis was an acid hydrolysate of gluten. An acid hydrolysate of flour cannot be used as a standard because of the presence of levulinic acid, which gives a yellow color with ninhydrin.

the starch had settled, was adjusted to pH 2 with dilute HCl, and the solution then freeze-dried. It was calculated that this 0.60-ml. aliquot contained 6.19 mg. of flour protein.

To the freeze-dried residue, 6.0 ml. of freshly dialyzed leucine aminopeptidase (9.90 mg., 20.9 units) solution, 0.005M Mg^{++}, 0.005M Tris, pH 8.3, and 0.25 ml. prolidase (1.44 mg., 6.25 units) solution, 0.01M Mn^{++}, 0.005M Tris, pH 8.5; a crystal of thymol added, and the mixture was incubated at 40°C. for 24 hr. The solution was adjusted to pH 2 with 1N HCl and the amino acids were removed by thin-layer dialysis with the apparatus described by Craig et al. (18) as modified

by Hill and Schmidt (17). The dialysate (200 ml.) was freeze-dried to give a residue which was then redissolved in approximately 10 ml. H_2O, adjusted to pH 2 with dilute HCl, and after addition of a crystal of thymol, made up to 25.0 ml., and stored at $-20°C$.

In the same manner and at the same time as the enzymatic hydrolyses were carried out, a control enzymatic digest was carried out with all constituents present except the substrate, to determine the amount of amino acids released by the action of the proteolytic enzymes on each other by cannibalistic digestion, and to account for any free amino acids that may still have been present with the enzymes even after dialysis. All analyses of enzymatic digests reported have been corrected for the amount of amino acids released during the control experiment. The amino acids released during the control run amounted to approximately 4% of the amino acids released from hydrolysis of the flour proteins.

Complete Amino Acid Composition of Flour from Selkirk Wheat

Analysis of Enzymatic Hydrolysates. The results from the analysis of enzymatic hydrolysates are given in Table IV. Since the enzymatic hydrolysate is expected to contain glutamine, asparagine, pyrrolidone carboxylic acid (17), and possibly some peptides, it was further hydrolyzed with $6N$ HCl. These results, along with a $6N$ HCl hydrolysis analysis, are also shown in Table IV.

From the data in Table IV, it can be observed that recovery of tryptophan, cystine, proline, and alanine from the enzymatic hydrolysates was poor. The very poor recovery of tryptophan was due at least in part to its instability in acidic aqueous solutions, since the enzymatic hydrolysate was stored at pH 2.2, a pH at which most amino acids are stable. This view was supported by the observation that successive analyses of the enzymatic hydrolysates yielded lower and lower recoveries of tryptophan. The low yield of cystine was partly due to its reaction with the small amounts of cyanide present (19), the cyanide being used to activate papain. The reason for the low recoveries of proline and alanine is not known. The higher amounts of proline and alanine present in the enzymatic + HCl, and HCl, hydrolysates would suggest that peptides of these two amino acids are present in the enzymatic hydrolysate.

As indicated above, the amino acid nitrogen recovery from analyses of enzymatic digests is not as good as that from analysis of acid hydrolysates, since the amino acid recovery was only 80%. The incomplete recovery of nitrogen (i.e., incomplete digestion) might be due to incomplete denaturation of the protein in the flour samples. In the present

TABLE IV

AMINO ACID RECOVERIES FROM ENZYMATIC AND ACID HYDROLYSATES OF SELKIRK FLOUR (HRS-S)

(μm. per 1 g. anhydrous protein [N $\times$ 5.7])

AMINO ACID	HYDROLYTIC METHOD								AMINO ACID COMPOSITION HRS-S
	Enzymatic		Enzymatic + HCl		6N HCl			Maximum, or Average, or Extrapolated Value	
					Hydrolysis Time, Hours				
					24	48	72		
Tryptophan	4.5	0	0	0	48			48	48
Lysine	145	140	182	174	137	133	137	136	136
Histidine	120	126	156	162	145	142	152	146	146
Ammonia	629	674	2,872	3,230	2,671	2,786	2,923	2,550	0
Arginine	212	206	226	232	207	194	201	201	201
Cysteine					11				11
Cysteic acid					198				0
Aspartic acid	185	184	352	339	316	307	328	319	185
Asparagine									134 a
Threonine	231	229	259	251	212	188	165	234	234
Serine	1,208	1,316	475	475	382	272	189	472	472
Glutamic acid	342	330	2,379	2,605	2,654	2,606	2,632	2,631	336
Glutamine									2,295 b
Proline	830	763	1,082	1,092	1,122	1,117	1,133	1,124	1,124
Glycine	409	412	503	493	460	458	469	462	462
Alanine	339	364	419	376	341	342	355	346	346
Cystine	37	34	19	18	17	12	16		94
Valine	337	378	441	399	382	393	398	391	391
Methionine	78	81	50	61	104	99	96	104	104
Isoleucine	304	307	363	338	310	330	334	325	325
Leucine	535	536	612	589	548	563	571	561	561
Tyrosine	172	164	183	151	157	103	108	157	157
Phenylalanine	300	300	345	332	326	332	334	331	331
Insoluble humin					3.75 mg.N	3.33 mg.N	(lost)		

a 319 − 185 = 134.
b 2,631 − 336 = 2,295.

work the samples were merely freeze-dried before enzymatic digestion, whereas Hill and Schmidt heated their samples to 95°C. for 3 min. before enzymatic digestion (17). Better amino acid recoveries might also be obtained by using the proteolytic enzymes with higher specific activity that are now commercially available.

The increase in the amino acid content on analysis of enzymatic + HCl hydrolysates indicates the presence of peptides. The appearance on the amino acid chromatograms of enzymatic hydrolysates indicates the absence of any significant amounts of peptides. A peptide (or peptides) could conceivably be eluted out together with an amino acid; however this did not seem likely, since changing chromatography

conditions did not reveal the presence of any additional peaks. This is evidence that any peptides present remain adsorbed on the ion-exchange resin.

Glutamine and Asparagine Contents of Flour. The enzymes papain, leucine aminopeptidase, and prolidase, if pure enough, are reported to have no glutamine or asparagine deamidase activities (17). Therefore, in using these enzymes to hydrolyze the proteins of flour, it should be possible to analyze for glutamine and asparagine present in flour. The presence of glutamine and asparagine is readily apparent in the present work when the elution curves from analysis of the enzymatic hydrolysates are examined and compared with elution curves obtained by analysis of an acid hydrolysate and enzymatic + HCl hydrolysates. Thus Fig. 4, A, an elution curve of an enzymatic hydrolysate, shows a relatively large "serine" peak; the peaks for glutamic and aspartic acids and for ammonia are small when compared to the "normal" elution curve obtained from an HCl hydrolysate, as illustrated in Fig. 4, C. The large serine peak is due to glutamine and asparagine being eluted out in the same position with serine in the elution scheme illustrated in Fig. 4, A. Quantitative amounts of glutamine and asparagine were obtained by hydrolyzing the enzymatic digests further with HCl, and observing the decrease in the "serine" peak size with the corresponding increase in the amounts of glutamic and aspartic acids and in ammonia without any decrease in the amount of any other amino acid present, as is illustrated in Fig. 4, B, and reported in Table IV. The amounts of glutamine and asparagine were quantitatively estimated by measuring the increase in the glutamic acid and aspartic acid peak sizes. The decrease in the "serine" peak size cannot be used for estimating glutamine and asparagine content, since at the pH of the buffers used for chromatography, glutamine is unstable and readily converts to the ninhydrin-negative pyrrolidone carboxylic acid derivative.

Glutamic and Aspartic Acid Contents of Flour. The evidence presented in this paper is the first quantitative estimate by the most direct means available of the amount of glutamine, glutaric acid, asparagine, and aspartic acid present in flour. Thus, in conjunction with the recent report that the amount of glutamine, glutarate, asparagine, and aspartate was 1,650, 25, 47.5, and 0 μm. per g. dry gliadin — the gliadin was approximately 56% pure (20) — there is now firm evidence for the long-held tacit assumption that the source of ammonia released during acid hydrolysis is due to glutaminyl and asparaginyl residues. This assumption was based on the demonstration some 32 years ago that glutamine was present in a partial enzymatic digest of gliadin (21),
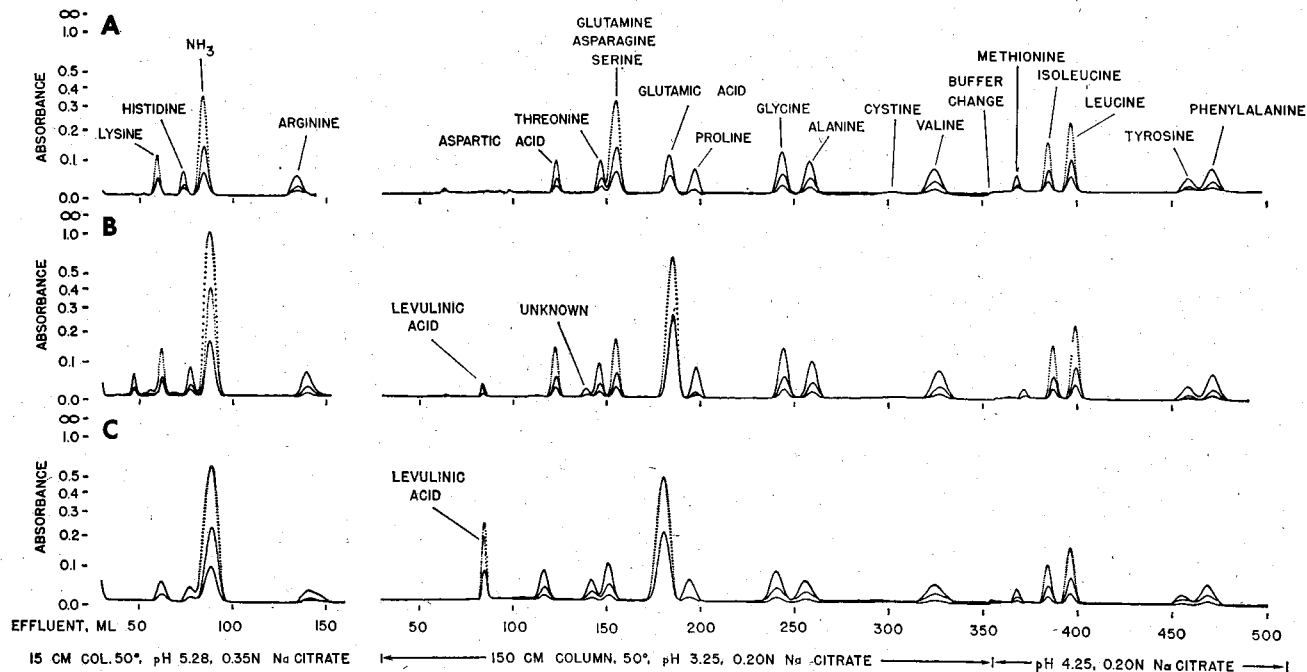
Fig. 4. Amino acid chromatograms of Selkirk flour hydrolysates. A. Analysis of an enzymatic hydrolysate equivalent to 0.493 mg. protein on each of the columns. B. Analysis of an enzymatic + HCl hydrolysate equivalent to 0.493 mg. protein on each of the columns. C. Analysis of 24-hr. 6N HCl hydrolysate equivalent to 0.463 mg. protein on each of the columns.

and on scattered reports that the amount of amide nitrogen in flour is approximately equivalent to the amount of glutamate and aspartate residues present.

It should be noted that not all of the glutamic and aspartic acid content of flour proteins is present in the amide form as is often assumed, but that a small portion of these two amino acids is in its free carboxylic acid residue form. In the present work, as is seen in Table V, 13% of the total glutamate and 40% of the total aspartate

TABLE V

AMIDE N, GLUTAMATE, AND ASPARATATE CONTENT IN SELKIRK FLOUR (HRS-S)
(μm./g. protein [N $\times$ 5.7])

RESIDUE	HYDROLYSATE	
	Acid	Enzymatic, Enzymatic + HCl
Aspartic acid	319	195
Glumatic acid	2,631	336
Asparagine	....	161 [a]
Glutamine	....	2,156 [b]
Amide N	....	2,317 [c]
Ammonia	2,530	....

[a] 346 — 185 = 161 (see Table IV).
[b] 2,492 — 336 = 2,156 (see Table IV).
[c] 2,156 + 161 = 2,317 (see Table IV).

residues in Selkirk flour are in their free carboxyl form. The number of glutamate, aspartate, and amide groups, as calculated from the analyses of acidic and enzymatic hydrolysates, is listed in Table V. It is seen that there is good agreement between the different analyses and between the values for ammonia and amide groups.

General Discussion:

Amino Acid Analysis of Flour

From evidence presented in this paper, it is apparent that there is still no simple or quick method for obtaining an accurate amino acid analysis of wheat flour. While the analytical method of automatic ion-exchange chromatography is capable of giving reasonably accurate and rapid results, the hydrolysis step presents considerable difficulty.

Proteins are generally hydrolyzed with 6N HCl because this treatment yields the best over-all recovery of amino acids. However, as described more fully elsewhere in this paper, at least a minimum of three separate hydrolyses of various durations is necessary to correct for the amounts of threonine, serine, and ammonia present. In addition, another acid hydrolysis of an oxidized protein sample and also a basic hydrolysis are necessary for the estimation of cysteine-plus-cystine and tryptophan.

Enzymatic hydrolysis of flour as carried out in the present work does not seem to offer much advantage over acid hydrolysis when one considers: the number of separate amino acid analyses that have to be carried out; the more demanding analytical procedures; and the facts that although two of the three enzymes used, papain and leucine aminopeptidase, are commercially available of sufficient purity, they are still relatively expensive, and that the remaining third enzyme, prolidase, has to be isolated from swine kidney. However, with the advent of commercially available enzymes of continually greater and greater purity, and further familiarity and knowledge of the technique, enzymatic hydrolysis will in the near future probably become a very useful tool. Enzymatic hydrolytic methods are also very useful in studies where it is essential to isolate those amino acids or amino acid derivatives which are unstable under the conditions of hot acid hydrolysis.

Relation of Amino Acid Composition to Flour Protein Quality

The generalization that the amino acid composition of many flours is similar should not be interpreted to mean that the same protein material occurs in these flours, since the occurrence of a large number of proteins in a mixture such as flour (22,23,24,25) indicates the possibility of the existence of a very large number of flours with identical amino acid composition but differing in the relative amounts of each protein component present, each with a characteristic amino acid composition. Furthermore, since the amino acid content in a heterogeneous mixture like flour can only be determined to within approximately 3 or 4%, one cannot expect to detect a significant variation in the amount of a protein component in flour by amino acid analysis, even if it possesses a characteristic amino acid composition. Furthermore, components with characteristic amino acid compositions such as some albumins or globulins (26) would not be expected to occur in large amounts in flour.

It is obvious that the differences in the amino acid composition of various flours are not large enough to relate them to flour quality. As it is generally thought that flour quality is significantly related to the properties of the proteins present, it would then appear that characterization of individual flour proteins would be more fruitful in relating flour quality to protein composition.

Acknowledgment

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