HEAT-MOISTURE EFFECTS ON WHEAT FLOUR. II. AN EVALUATION STUDY OF HEAT-PROCESSING EFFECTS ON FLOUR PROTEINS BY DIGESTIVE ENZYMES—PEPSIN, TRYPSIN, AND TRYPSIN-CARBOXYPEPTIDASE-B[1]

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ABSTRACT

Wheat flour (hard red winter, 1st clears) was processed in a reversed heat exchanger designed to control processing parameters of temperature (108°, 150°, and 174°C), moisture (13–33%), and time (2, 5, and 10 min). Enzymatic methods were used to measure *in vitro* digestibility of thermally processed flour proteins. Peptide bonds of processed flour proteins were less accessible to pepsin and trypsin attack than the bonds of unprocessed flour proteins. The highest pepsin and trypsin digestion rates for the processed flour proteins were for flour processed with a 13% moisture content at 108°C for 2 min; more severe processing resulted in a progressive reduction in digestion rates. Lysine availability was measured by trypsin-carboxypeptidase-B digestion of heat-processed flour proteins. Lysine availability of processed proteins (150°, 174°C) was decreased.

Food proteins cannot be used by man unless they are broken down to small peptides and amino acids capable of being absorbed into the small intestinal brush border membrane covering the mucosal cells (1,2). Protein digestion is initiated within the stomach by pepsin (3), an enzyme with a broad specificity but favoring peptide bond hydrolysis of aromatic amino acids. The breakdown of denatured protein (heat and acid) and polypeptides is continued in the intestinal lumen by chymotrypsin (specificity similar to pepsin's) (3), trypsin (4), and the carboxypeptidases (5). Amino acids and peptides are then absorbed within the intestinal mucosa, where the peptides are further digested by the tri- and dipeptidases (6,7) before entry into the circulatory system.

The proteins of heat-processed flours with a 13–33% moisture content range have been altered extensively with increasing temperature (108°–174°C). The following effects were found by Hansen *et al.* (8): protein aggregation, peptide formation, and amino acid destruction, particularly lysine. Protein changes due to processing may so alter the substrate that digestive enzymes, particularly those with narrow specificities, such as trypsin and carboxypeptidase-B, may be unable to hydrolyze them. Therefore, the action of digestive enzymes on the heat-treated flour has been studied to: a) obtain indications of conformational changes of flour proteins induced by the heat-treatments, using initial rate determinations with pepsin and trypsin; b) determine lysine availability of heat-treated proteins by digestion with trypsin-carboxypeptidase-B (TCB) (since lysine is nutritionally limiting in wheat flour, any further decrease in lysine content by heat-damage affecting availability would lower its nutritive value); and c) suggest possible relationships to *in vivo* digestion of heat-processed flour proteins.

Pure enzyme preparations were used rather than multiple enzyme

[1]Presented at the 57th Annual Meeting, Miami Beach, Oct.-Nov. 1972.

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preparations, so that results obtained on flour proteins could be ascribed to a particular enzyme.

MATERIALS AND METHODS

Heat-Processed Flour Samples

Flour (hard red winter-1st clears, 14% protein [5.7 nitrogen factor used], ash content 0.93%), at moisture contents of 12.7, 17.9, 23.8, 28.2, and 33.1%, was processed in a reversed heat exchanger at 108°, 150°, and 174° C for 2, 5, and 10 min as described in a previous paper (8).

Enzymes

Porcine stomach pepsin (3 × cryst), [3.4.23.1], was obtained from Mann Research Laboratories; beef pancreas trypsin (2 × cryst), [3.4.21.4], from Nutritional Biochemicals Corp.; and porcine pancreas carboxypeptidase-B (175 units/mg COBC), [3.4.12.3], from Worthington Biochemical Corp. (9,10).

Enzyme Assay for Initial Rate Determinations

Flour (50 mg unheated or heated containing 7 mg protein) in 1.45 ml 0.05*M* glycine-HCl-pH 2.0 buffer was shaken and equilibrated at 37° C for 5 min before pepsin (100 μg/50 μl) was added. The reactions were stopped by deproteinization with 2.5 ml of 5.0% trichloroacetic acid (TCA) after 0, 2, 4, and 6 min, and centrifuged for 20 min. For the zero-time assay, TCA was added prior to the enzyme addition. Zero-time assays were used for peptide formation studies. Absorbance of TCA-soluble digestion products using Folin-Ciocalteu Reagent was measured at 660 nm (11). Trypsin assay was similar to pepsin assay except 1.40 ml 0.05*M* tris-HCl-pH 8.0 buffer and trypsin (100 μg/100 μl) were used in the incubation mixture for buffer and enzyme, respectively.

Enzyme Assay for Lysine and Arginine Availability

Flour (50 mg unheated or heated containing 7 mg protein) per ml 0.01*M* tris-HCl-0.01*M* $CaCl_2$-1.24×10^{-4}*M* merthiolate-pH 8.0 buffer was incubated and shaken with trypsin (50 μg/25 μl) buffer for a 2-hr period at 37° C, followed by a second addition of trypsin (50 μg/25 μl buffer) for a 3-hr period. Carboxypeptidase-B (54 μg/3 μl) was then added to the reaction mixture and the incubation continued for 15 hr. Reactions were stopped by adjusting incubation mixture to pH 4.0 with 1 *N* HCl (5° C) and immersing the reaction vessel in ice, followed by centrifugation at 3105 × *g* for 1 hr at 5° C. A supernatant aliquot (0.2 ml) was used for the released amino acid analysis.

Amino acid content of the supernatant was determined by a modification of the Spackman *et al.* procedure (12) on a Phoenix amino acid analyzer, Model K-8000 B. The basic amino acids were run on a Durrum Resin DC-2 (0.9 × 6.0 cm), eluted with a 0.35*M* sodium citrate buffer pH 5.35 at 70 ml/hr, 52.5° C. Total analysis time was 55 min. The acidic and neutral amino acids were run on a Beckman Spherical Resin PA-35 (0.9 × 50 cm) eluted with 0.20*M* sodium citrate at pH 3.50 and 4.4 at 70 ml/hr, 52.5° C. Total analysis time was 125 min.

RESULTS AND DISCUSSION

Pepsin Digestion of Heat-Treated Flour Proteins

Digestion by pepsin of unheated and heated flour proteins was measured as a

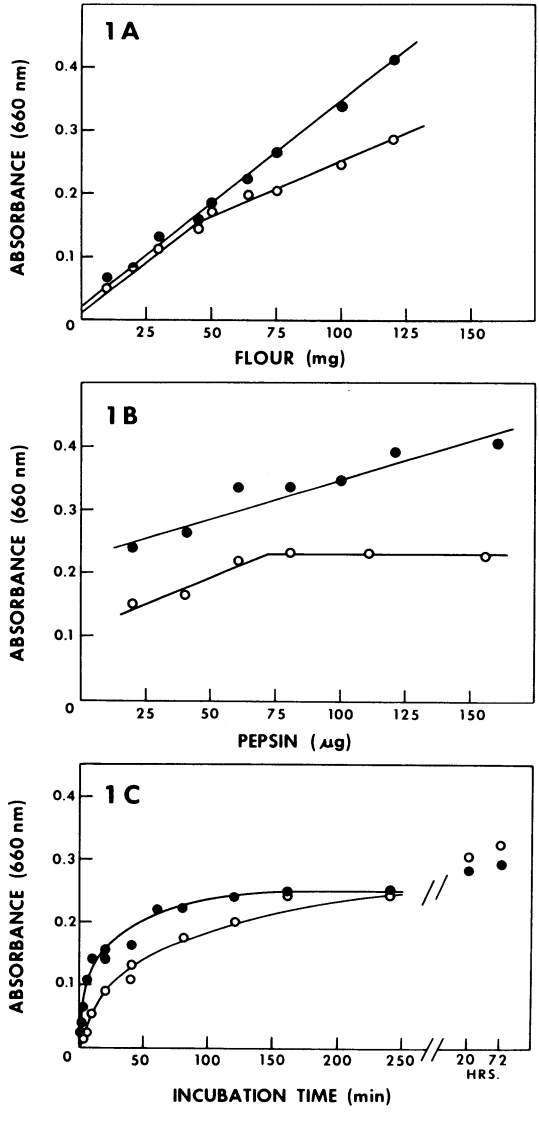

Fig. 1. Effect of thermal processing on pepsin digestion (pH 2.0) of flour proteins. • = Unheated flour; o = 18% moisture flour (150°C, 10 min). A = Function of flour concentration; enzyme (100 μg), incubation time (40 min, 37°C); B = function of enzyme concentration; enzyme incubation time (40 min, 37°C), flour (100 mg); and C = function of incubation time; flour concentration 50 mg (7000 μg protein), pepsin (100 μg).

function of flour concentration (Fig. 1A), enzyme concentration (Fig. 1B), and incubation time (Fig. 1C). The heated proteins (18% moisture flour, 150°C, 10 min) were highly aggregated, as shown by our solubility and gel filtration studies (8). When the flour concentration of the heated sample reached 50 mg in the incubation mixture (Fig. 1A), pepsin digestion rate of the protein slowed while the unheated sample continued to give a linear response. When the enzyme concentration (Fig. 1B) was increased and substrate concentration was held constant, the rate of pepsin digestion was faster for the unheated proteins than for the heat-aggregated proteins up to 75 μg pepsin. The addition of more than this amount of enzyme to an incubation mixture containing heat-aggregated protein resulted in no further increase in pepsin digestion rate, indicating that there were no available binding sites for the enzyme on the substrate, while the enzyme digestion of unheated protein continued to increase. Peptide bonds of heat-aggregated protein were, therefore, shown to be less accessible to pepsin attack than those of unheated protein. An explanation for the decreased pepsin digestion of heat-aggregated proteins could have been protein-starch interaction. A predigestion of unheated and heated flour with α-amylase was tried to release protein for subsequent pepsin digestion. The rate of protein digestion by pepsin was not changed for either heated or unheated proteins by the α-amylase treatment. With denatured proteins (urea), the pH optimum of pepsin is shifted to pH 3.0–3.5 (13). Pepsin activity was much lower at pH 3.0 for both unheated and heated flour proteins than at pH 2.0, with heat-treated flour proteins considerably lower than unheated. Pepsin digestion as a function of time (Fig.

TABLE I
Effect of Thermal Processing on the Digestion Rates of Flour Proteins by Pepsin

Flour Moisture %	Processing Temperature °C	Pepsin Rates[a] Processing time 2 min	5 min	10 min
13	108	67	68	31
18	108	41	51	40
24	108	36	34	44
28	108	44	41	44
33	108	53	53	44
13	150	59	51	56
18	150	61	53	35
24	150	34	35	25
28	150	30	28	34
33	150	46	46	18
13	174	41	41	41
18	174	59	31	52
24	174	55	63	43
28	174	43	58	59
33	174	43	46	48

[a]Based on rate constants (least squares analyses of increased absorbance (660 nm) as a function of time (min). The digestion rate (15.8×10^{-3} 660 nm/min) of unheated flour proteins by pepsin was taken as 100.

1C) was much slower for the heated proteins in the initial time periods. After 120 min, the pepsin digestion of the unheated proteins was 83% of maximum hydrolysis achieved, while it took approximately 240 min to achieve the same amount of digestion with the heat-aggregated protein. Based on Fig. 1C data, the heat-treated flour proteins compared to unheated flour proteins were scanned for pepsin digestibility using the initial time interval (0–10 min), where the absorbance (660 nm) as a function of time was essentially linear.

Initial digestion rates of heat-treated flour proteins by pepsin were lower than those of unheated proteins (Table I). There was less aggregation of the proteins of the 13% moisture flour for a 2-min heating period at 108°C, as evidenced by solubility, electrophoresis, and gel filtration studies (8), than of the other heated proteins and therefore a correspondingly faster rate was obtained. At the 150°C heat-treatment, the flour protein at all moisture conditions was highly aggregated and the pepsin digestion rates were lowered. At the 174°C heat-

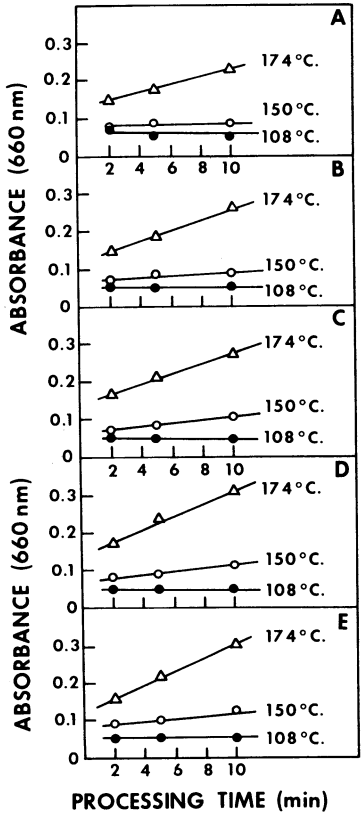

Fig. 2. Formation of heat-produced peptides from flour proteins as a result of thermal processing. Trichloroacetic acid supernatants zero-time absorbance (660 nm) as a function of thermal processing time. A = 13% Moisture flour, B = 18%, C = 24%, D = 28%, and E = 33%.

treatment, the flour proteins at all moisture conditions had partly broken down to peptides, as shown by gel filtration studies (8), and the slower pepsin digestion rates obtained were probably due to the decreased amount of proteins and the highly aggregated state of the proteins.

Peptide Production from Heat-Treated Flour Proteins

The increase in absorbance (660 nm) of the zero-time assays with increasing processing temperatures, times, and flour moisture contents gave further evidence of protein breakdown to peptides (Fig. 2) in addition to the gel filtration studies (8). There are peptides normally present in flour (14), which are not precipitable by 5% TCA, that give an absorbance of 0.05 at 660 nm with the unheated flour-protein. The kinetic data of peptide production resulting from heat-processing of flour proteins is summarized in Table II. Rates of peptide formation increased with processing temperature and flour moisture content. Activation energies showed a small increase with flour moisture content. The degree of heat-treatment given a flour product could be measured by the amount of peptides produced.

Trypsin Digestion of Heat-Treated Flour Proteins

Digestion by trypsin of flour proteins was also measured as a function of flour concentration (Fig. 3A), enzyme concentration (Fig. 3B), and incubation time (Fig. 3C). At any flour concentration (Fig. 3A), the trypsin digestion of the unheated protein was greater than that of the heated protein (33% moisture flour,

TABLE II
Kinetics of Heat Formation of Peptides from Flour Proteins

Flour Moisture %	T °C	k[a] $\Delta A_{660\ nm/min}$	log k/$\frac{1}{T}$[b]	Activation Energy[c] cal/mole
13	108	1.0×10^{-4}	-5.160×10^{3}	23,614
	150	16.8×10^{-4}	...	...
	174	108.0×10^{-4}	...	...
18	108	1.0×10^{-4}	-5.160×10^{3}	23,614
	150	23.6×10^{-4}	...	...
	174	154.6×10^{-4}	...	...
24	108	2.0×10^{-4}	-5.623×10^{3}	25,733
	150	45.1×10^{-4}	...	...
	174	136.4×10^{-4}	...	...
28	108	1.0×10^{-4}	-5.792×10^{3}	26,507
	150	64.4×10^{-4}	...	...
	174	164.7×10^{-4}	...	...
33	108	1.0×10^{-4}	-5.869×10^{3}	26,859
	150	46.1×10^{-4}	...	...
	174	180.4×10^{-4}	...	...

[a]Correlation coefficients 0.981–0.999 for ΔA (660 nm/min) the zero-order rate constant.
[b]Correlation coefficients 0.928–0.997.
[c]Activation energy = $-2.303\ R\ (\log k/\frac{1}{T})$, where R is the gas constant (1.987 cal degree^{-1}mole^{-1}) and T is the temperature (°K).

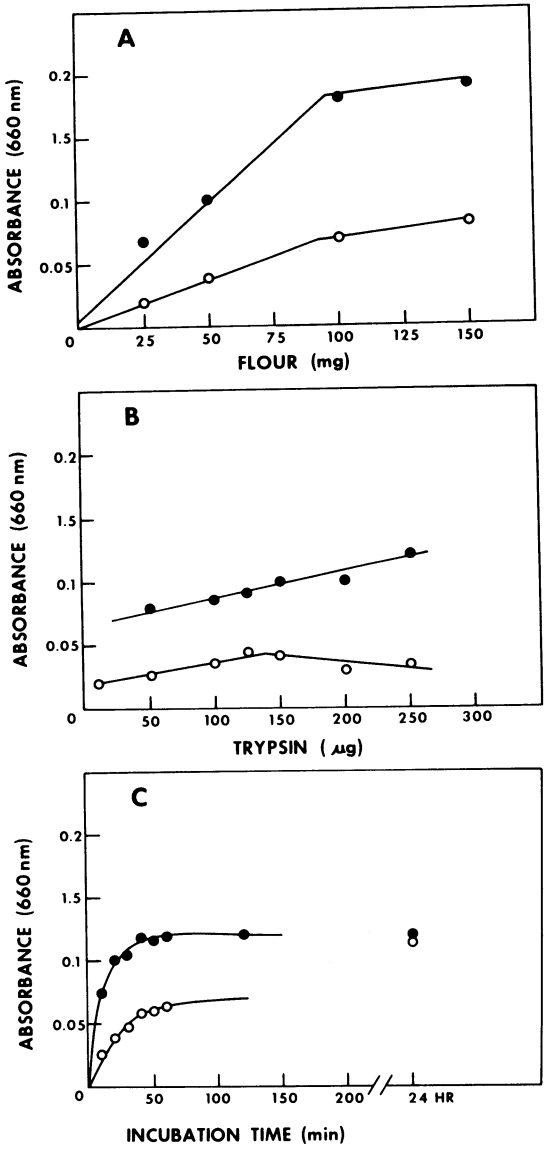

Fig. 3. Effect of thermal processing on trypsin digestion (pH 8.0) of flour proteins. • = Unheated flour; o = 33% moisture flour (150°C, 10 min). A = Function of flour concentration; enzyme (100 μg), incubation time (10 min, 37°C); B = function of enzyme concentration; enzyme incubation time (10 min, 37°C), flour 50 mg (7000 μg protein), trypsin (100 μg); and C = function of incubation time; flour concentration 50 mg (7000 μg protein), trypsin (100 μg).

150° C, 10 min). Like pepsin, trypsin showed a decreased amount of digestion of the heated protein compared to that of the unheated protein as a function of enzyme concentration. The addition of more than 150 μg trypsin to the incubation mixture resulted (Fig. 3B) in a decreased rate of protein digestion of the heated flour, indicating that the heat-aggregated protein was less accessible to trypsin attack than the unheated protein. Like pepsin, the heat-treated sample was digested at a slower rate by trypsin than the unheated flour proteins were (Fig. 3C). After a 24-hr digestion period, the amount of digestion product was the same for heated and unheated flour proteins.

There was less digestion of flour protein (heated or unheated) by trypsin than by pepsin, which was probably due to 1) the narrow specificity of the enzyme for lysyl or arginyl peptide bonds compared to the broader specificity of pepsin, and 2) the low lysine content of flour proteins, particularly the gliadins (15,16).

The initial trypsin digestion rates of heat-treated proteins, as with pepsin, were lower with increasing processing temperatures and time (Table III). Peptide bonds of the heat-aggregated protein (150° C) were even less accessible to trypsin attack than those of pepsin. With 174° C processing, there was less protein due to the heat-produced peptides for trypsin to act on, and more lysine destruction (8), so the protein digestion rates would be expected to be considerably reduced, as found.

Trypsin, because of its specificity, could be useful as a rapid but general indicator of heat-damage to proteins resulting from processing, using the unprocessed proteins as a control (initial rate determinations).

TABLE III
Effect of Thermal Processing on the Digestion Rates of Flour Proteins by Trypsin

Flour Moisture %	Processing Temperature °C	Trypsin Rates[a] Processing time 2 min	5 min	10 min
13	108	96	94	86
18	108	61	52	51
24	108	51	46	50
28	108	50	71	84
33	108	70	65	50
13	150	30	17	25
18	150	27	33	25
24	150	24	34	20
28	150	29	33	24
33	150	36	39	36
13	174	23	31	14
18	174	24	23	5
24	174	26	28	17
28	174	32	24	24
33	174	37	18	37

[a]Based on rate constants (least squares analyses of increased absorbance (660 nm) as a function of time (min). The digestion rate (14.2×10^{-3} 660 nm/min) of unheated flour proteins by trypsin was taken as 100.

Trypsin-Carboxypeptidase-B (TCB) Digestion of Heat-Treated Flour Proteins

Carboxypeptidase-B specifically releases carboxyl terminal lysine and arginine from trypsin-digested flour proteins, as shown by the digestion reactions in Fig. 4. To test lysine availability, unprocessed flour proteins were incubated with trypsin for 5 hr at pH 8.0, followed by carboxypeptidase-B digestion for 15 hr. The results of TCB digestion of unheated flour proteins are given in Table IV. Lysine was 70% released; arginine 79%; and histidine 21%; the other amino acids were less than 26% released by TCB digestion. The small amounts of other amino acids released (Table IV) were probably due to carboxypeptidase-A and chymotrypsin contamination of the carboxypeptidase-B preparation (10). The release of basic amino acids from unheated flour proteins by TCB as a function of time (Fig. 5) was nearly complete 30 min after the carboxypeptidase-B addition to the incubation mixture. Unheated flour proteins digested with trypsin alone for a 20-hr period showed that lysine and arginine release was slow in the absence of carboxypeptidase-B (Table IV). Both enzymes are highly specific with respect to their substrate requirements.

The TCB digestion chromatograms and data of the heat-treated flour proteins are given in Fig. 6 and Table IV, respectively. Mild heat-treatment (108° C) of flour facilitated the release of lysine and arginine from the proteins by TCB. At higher processing conditions, 150° and 174° C of flour, the release of lysine and arginine from the proteins was substantially reduced. This decrease in release of lysine and arginine from tryptic peptides was probably due to the change in the substrate. The free basic groups of lysine and arginine in proteins probably reacted with sugars (Maillard reaction) during thermal processing. Carboxypeptidase B has specific substrate requirements, and thus the digestion of the heat-treated proteins (150° and 174° C) has been impaired because of the chemical modification of the protein by heat.

Chemical modification of proteins may occur during the processing of protein foods, for example, in solubilizing or texturizing vegetable proteins (meat analogs). There is the possible formation of lysinoalanine, which may be toxic (17,18), when severe NaOH treatments are given to the soybean protein. Since

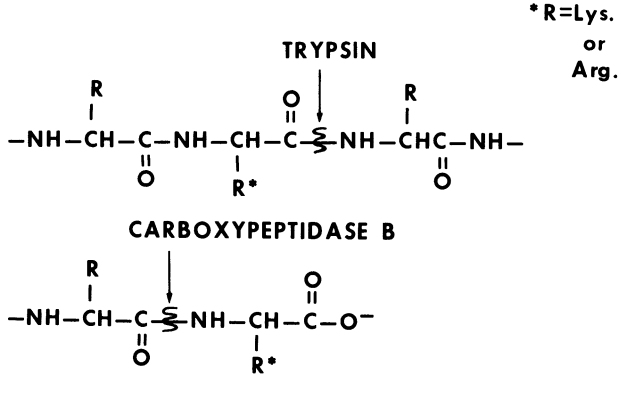

Fig. 4. Digestion reactions of peptide bonds of flour proteins by trypsin-carboxypeptidase-B (TCB).

TABLE IV
TCB Enzymatic Release of Amino Acids from Unprocessed and Processed Flour Protein

Amino Acid	6*N* HCl Unheated[a] μmoles/ml	Trypsin Unheated %	Trypsin-Carboxypeptidase-B Unheated %	13-108-2[b] %	13-150-2[b] %	13-174-2[b] %	33-108-10[b] %	33-150-10[b] %	33-174-10[b] %
Lys	1.16	7.8	69.8	81.0	59.5	12.9	74.3	34.6	7.8
His	1.05	0.0	21.0	20.0	15.2	10.5	19.1	17.2	15.3
NH_3	16.33	1.3	2.1	1.7	2.1	5.3	1.5	3.6	8.8
Arg	1.84	8.7	79.4	91.9	76.6	43.5	86.0	62.0	38.0
Asp	2.34	3.9	10.7	7.7	6.4	3.4	...	...	...
Thr	1.68	0.0	0.0	0.0	0.0	0.0	...	...	...
Ser	3.21	0.0	0.0	0.0	0.0	0.0	...	...	...
Glu	17.92	0.3	1.4	0.9	0.7	0.3	...	...	...
Pro	7.36	0.0	0.0	0.0	0.0	0.0	...	...	...
Gly	3.79	0.5	3.7	2.9	2.6	1.6	...	...	...
Ala	2.88	1.4	14.6	13.2	11.1	7.3	...	...	...
Cys	1.30	0.0	0.0	0.0	0.0	0.0	...	...	...
Val	2.69	0.0	14.9	15.2	10.4	6.7	...	...	...
Met	0.98	0.0	20.4	22.5	15.3	8.2	...	...	...
Ile	2.04	0.0	11.3	11.8	8.3	5.9	...	...	...
Leu	4.00	0.0	17.0	16.0	12.8	8.0	...	...	...
Tyr	1.27	0.0	26.0	28.4	30.7	20.5	...	...	...
Phe	2.24	0.0	17.9	19.2	16.5	11.6	...	...	...

[a]Amino acids released from unheated flour by 6*N* HCl = 100% for comparison purposes.
[b]% Moisture-°C-min.

carboxypeptidase-B appears to be highly specific in its substrate requirements, a modification of lysine (reactions with ϵ-amino group) by such chemical treatments of food proteins would be expected to result in decreased or altered enzyme activity.

Since, in *in vivo* digestion, pepsin digestion precedes trypsin digestion, the flour proteins were preincubated with pepsin at pH 2.0 for 2 hr before trypsin was added to the incubation mixture followed by carboxypeptidase-B addition. The digestion results (Table V) indicated that the release of arginine and lysine was essentially the same after a 24-hr incubation period, as with TCB digestion alone. However, there was an increased amount of alanine, valine, methionine, leucine,

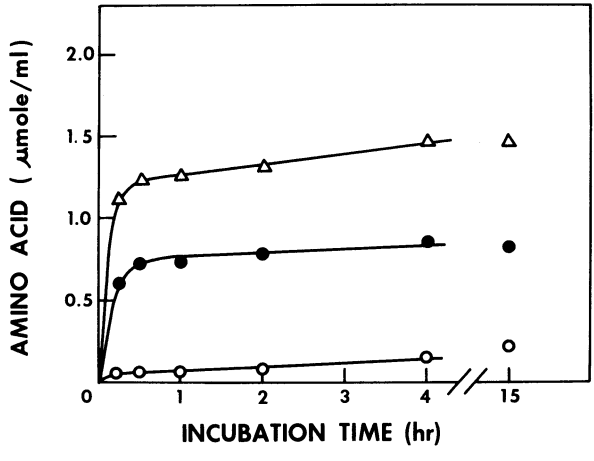

Fig. 5. The release of basic amino acids by TCB digestion of unheated flour proteins as a function of incubation time at 37°C, after the addition of carboxypeptidase-B. Δ = Arginine, • = lysine, o = histidine.

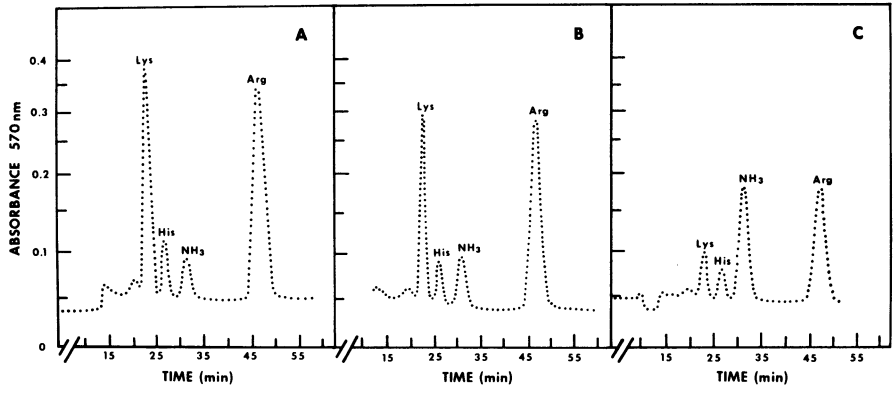

Fig. 6. Chromatograms (amino acid analyses) of TCB digestion of flour proteins processed at A = 108°C, B = 150°C, and C = 174°C, each for 2 min.

TABLE V
Effect of Predigestion with Pepsin on TCB Digestion of Flour Proteins

Amino Acid	Unheated TCB μmoles/ml	Unheated P + TCB μmoles/ml	13-108-2[a] TCB μmoles/ml	13-108-2[a] P + TCB μmoles/ml	13-150-2[a] TCB μmoles/ml	13-150-2[a] P + TCB μmoles/ml	13-174-2[a] TCB μmoles/ml	13-174-2[a] P + TCB μmoles/ml
Lys	0.81	0.83	0.94	1.02	0.69	0.75	0.15	0.16
His	0.22	0.24	0.21	0.33	0.16	0.24	0.11	0.18
Arg	1.46	1.58	1.69	1.77	1.41	1.62	0.80	0.89
Asp	0.25	0.20	0.18	0.17	0.15	0.14	0.08	0.11
Thr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ser	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glu	0.25	0.23	0.16	0.24	0.12	0.17	0.05	0.14
Pro	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00
Gly	0.14	0.15	0.11	0.13	0.10	0.09	0.06	0.09
Ala	0.42	0.68	0.38	0.65	0.32	0.52	0.21	0.48
Cys	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Val	0.40	0.61	0.41	0.57	0.28	0.46	0.18	0.39
Met	0.20	0.40	0.22	0.39	0.15	0.31	0.08	0.22
Ile	0.23	0.39	0.24	0.37	0.17	0.29	0.12	0.30
Leu	0.68	1.59	0.64	1.56	0.51	1.33	0.32	1.19
Tyr	0.33	0.54	0.36	0.54	0.39	0.43	0.26	0.36
Phe	0.40	0.65	0.43	0.64	0.37	0.52	0.26	0.47

[a]% Moisture - °C - min.

tyrosine, and phenylalanine when pepsin was present in the TCB incubation mixture.

Amounts of lysine and arginine released from flour proteins by acid hydrolysis (6*N* HCl) were compared with those released by trypsin-carboxypeptidase-B (Table VI). Amounts of lysine and arginine released from the heat-treated flour (150°, 174°C) by TCB were smaller than those released by acid hydrolysis. Below is shown a suggested mechanism to explain the increased lysine content from acid hydrolysis compared to enzymatic hydrolysis (Table VI).

$$\text{Protein-lysine} \overset{> 150^\circ\text{C}}{\rightleftharpoons} \text{Protein-lysine-sugar}$$

Enzymatic (TCB)	Acid Hydrolysis (6*N* HCl-110°C)
release-lysine	release-lysine
	lysine–sugar ⇄ lysine+sugar

Lysine, in the heat-treated sample, appeared to be more affected by the heat-treatment than arginine with respect to TCB release. It appears that amino acid content, as measured by acid hydrolysis of a flour product and other foods (19), is not indicative of the lysine available to the consumer.

From the *in vitro* data of this study, the following suggestive relationships to *in vivo* digestion of heat-treated flour proteins can be drawn.

1. The initial rates of pepsin digestion of heat-treated flour proteins would be decreased compared to the unheated proteins. Since only a small part of the total dietary protein consumed is normally digested in the stomach, this would not be important, if other intestinal digestive enzymes were not also affected.

2. However, the digestion rates of heat-treated flour proteins by trypsin, a major digestive enzyme in the alimentary tract, are also decreased (initial rate studies). Carboxypeptidase-B action is impaired by the heat-treatment given the flour protein (150°, 174°C). Tryptic peptides from heated proteins appear to be an unsuitable substrate for this enzyme, due to a probable lysine-sugar reaction.

TABLE VI
Comparison of TCB and Acid Hydrolysis of Flour Proteins

Flour	Lysine		Arginine	
	6*N* HCl %	TCB %	6*N* HCl %	TCB %
Unprocessed	100	70[a]	100	79
13-108-2[b]	100	81[a]	100	92
13-150-2[b]	92	57[a]	100	77
13-174-2[b]	58	9[a]	96	44
33-108-10[b]	97	74	95	86
33-150-10[b]	72	35	82	62
33-174-10[b]	50	8	37	38

[a]Different TCB runs, same conditions as in Table IV.
[b]% Moisture-°C-min.

The result of this impaired action of carboxypeptidase-B would be an increased amount of small peptides in the intestinal tract. Proteolytic enzymes of the intestinal mucosa are reported to be amino acid specific dipeptidases and tripeptidases (20–24). The dipeptidases require a free carboxyl and amino group adjacent to the peptide bond to be hydrolyzed, and they require specific amino acids. The tripeptidases will hydrolyze tripeptides, but not di- or tetrapeptides. Leucine aminopeptidases of the intestinal mucosa (23,24) are capable of hydrolyzing both di- and tripeptides. If the peptides are large, they will probably pass into the circulatory system.

A biological value study (25) of gluten using rats showed that the food nitrogen retained in the body was higher for the unheated gluten than for the heated gluten (150°C, 30 min). Food nitrogen absorbed was slightly reduced, and fecal nitrogen slightly increased, but urinary nitrogen showed a greater increase for rats fed the heated gluten compared to those fed the unheated gluten. Applied to our study, it would appear that the chemically modified, indigestible peptides could be absorbed into the intestinal mucosa, enter the circulatory system, and be excreted in the urine, thus decreasing the nutritional value of the high-temperature processed flours.

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