HEAT-MOISTURE EFFECTS ON WHEAT FLOUR. I. PHYSICAL-CHEMICAL CHANGES OF FLOUR PROTEINS RESULTING FROM THERMAL PROCESSING[1]

L. P. HANSEN, P. H. JOHNSTON, and R. E. FERREL, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, CA 94710

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). Protein changes were studied by solubility in urea, gel filtration, disc electrophoresis, and amino acid analysis. The following effects were found: protein aggregation of lower-molecular-weight proteins; disappearance of albumins and globulins; the release of peptides; destruction of lysine, arginine, and cystine-cysteine. Temperature was the main parameter that determined the occurrence of these changes. An explanation for the chemical-physical changes of the proteins is presented involving the peptide and disulfide linkages.

Wheat plays a major role in feeding the world community but cannot be used without some kind of processing. The conversion of wheat to useful products is an on-going concern of the wheat industries and has brought about significant advances in wheat technology. New thermal processing methods such as extrusion, microwave heating, puffing, and spray drying that use high-temperature/short-time (HT/ST) processing conditions have been widely adopted. Protein concentrates from mill feeds and flours (1,2), usable in high-protein low-cost foods, can be processed using HT/ST methods. However, protein changes in flour due to thermal processing and their consequential nutritional effects have not been explored adequately.

Cereal chemists of the early twentieth century, Kent-Jones (3,4), Geddes (5,6), and others (7,8), investigated the favorable and unfavorable effects of heat (54°–100°C) on flour regarding baking quality and strength. Heat-induced protein changes were studied by viscosity and solubility methods. Their findings were not always in agreement (4,7). Mecham and Olcott (9) studied the effect of heat (110°–203°C for an 18-hr period) on wheat gluten to obtain information on its potential use in coatings, adhesives, and fibers. They reported a progressive decrease in amino groups of the heated gluten. Morgan (10) pioneered studies in the nutritional aspects of the heating effects on cereal protein using rat feeding tests. Rats fed heated gluten (150°C–30 min) had lower growth rates compared to those fed raw gluten.

The aims of this study are to demonstrate methods to assess the effects of thermal processing on flour proteins under controlled conditions of moisture, temperature, and time; to provide alternative conditions that may result in desired product characteristics with minimal nutritional damage; and to develop an understanding of the complex physical-chemical changes of flour proteins

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[2]Mention of firm names or trade product does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

that result from thermal treatments. The protein changes were examined by the following methods: urea solubility; disc electrophoresis; gel filtration; and amino acid analysis.

MATERIALS AND METHODS

Heat-Processed Flour Samples

Flour (HRW 1st clears, ash content 0.93%, N content dry basis 2.74%) was processed in a reversed-heat exchanger designed to control processing parameters of temperature, moisture, and time. The flour sample (186 g total) was heated at the specified temperature and time in a stainless-steel container with 21 individual tubes (0.95 cm i.d. × 16.5 cm) and then water cooled. The sample was frozen, freeze-dried, equilibrated to moisture conditions at ambient temperature, ground in a Wiley Mill[2] (40-mesh screen) and stored at 2° ± 1°C. A total of 45 flour samples were processed under the specified conditions (Table I). Times referred to in the text are holding times. A description and analysis of the hydrothermal processing will be published later by Johnston *et al*[3].

[1]Presented at the 56th Annual Meeting, Dallas, Oct. 1971.

TABLE I
Processing Conditions for HRW Flour

Flour Moisture %	108°C (5 psig) Time			150°C (55 psig) Time			174°C (111 psig) Time		
	Heat[a] min	Hold[b] min	Come-Down[c] min	Heat[a] min	Hold[b] min	Come-Down[c] min	Heat[a] min	Hold[b] min	Come-Down[c] min
12.7 ± 0.2	6.85	2	0.81	6.84	2	1.30	9.14	2	1.44
		5			5			5	
		10			10			10	
17.9 ± 0.2	5.53	2	0.86	5.88	2	1.34	6.26	2	1.45
		5			5			5	
		10			10			10	
23.8 ± 0.5	5.62	2	1.01	4.87	2	1.26	5.25	2	1.37
		5			5			5	
		10			10			10	
28.2 ± 0.2	3.96	2	1.01	4.91	2	1.34	5.15	2	1.28
		5			5			5	
		10			10			10	
33.1 ± 0.4	3.78	2	1.00	5.54	2	1.15	4.96	2	0.95
		5			5			5	
		10			10			10	

[a]Interval from time steam enters retort until processing temperature was achieved. Av times taken from time-temperature curves.
[b]Time at processing temp.
[c]Interval from end of holding time until room temp was achieved. Av times.

Solubility of Flour Proteins in Urea

Urea solubilizes flour proteins by breaking intermolecular hydrogen bonds and minimizes differences in partial specific volume and shape of proteins encountered in gel filtration molecular weight (mol wt) determinations. Proteins were extracted from a 500-mg flour sample with 10 ml buffer solution (3*M* urea–0.01*M* phosphate–pH 7.0) (11), shaken (Burrell Shaker) for 2 hr at 5°C, and then centrifuged (3016 × *g* for 1 hr at 5°C). Protein (0.03 ml supernatant aliquot) was measured at 500 nm using the procedure of Lowry *et al.* (12). This protein determination method was more sensitive for processed flour proteins than 280 nm absorbance determinations. Urea did not interfere with the Lowry protein determination as it did with the nitrogen determination of the Kjeldahl method. For total protein content of flour, protein from unheated flour was

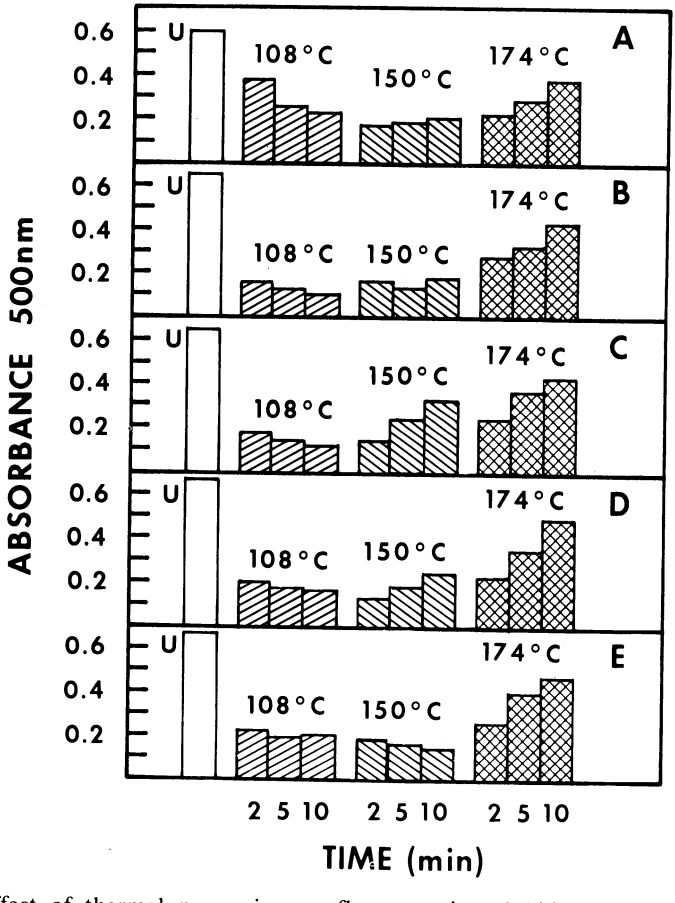


Fig. 1. Effect of thermal processing on flour-protein solubility in 3*M* urea-0.01*M* phosphate-pH 7.0 as measured by the Lowry procedure. Controls—protein solubilities of unprocessed flours adjusted to specified moistures prior to processing: A = 13% moisture content flour; B = 18%; C = 24%; D = 28%; E = 33%.

extracted with 1*N* NaOH instead of urea-phosphate solution by the same procedure.

Gel Filtration

An aliquot (4 ml) of the urea-phosphate-solubilized proteins was applied to a Sephadex G-100 column (Pharmacia, 40–120μ, 42×2.2 cm) and eluted with 3*M* urea–0.01*M* phosphate–pH 7.0 at ambient temperature. Effluent was collected in 2-ml fractions at 40 ml/hr for 6 hr. Peaks were detected by their 280 nm absorbance. Ribonuclease A (mol wt 13700), chymotrypsinogen A (mol wt 25000), and ovalbumin (mol wt 45000) were obtained from Pharmacia. Aliquots (2.5 mg of each protein) in 2.5 ml urea-phosphate buffer were used to calibrate the column for molecular-weight distributions (13). Sephadex G-150 and 200 were not used because of their slow flow rates.

Disc Electrophoresis

Electrophoresis of urea-phosphate-solubilized proteins (60 μl) was carried out by the Ornstein and Davis method (14) with the Canalco Model 12 apparatus. The proteins were run on 7% polyacrylamide gels (0.6 × 6.5 cm) with tris-glycine buffer pH 9.5 at 5 mA per gel for 40 min at ambient temperature. The gels were stained with 0.05% Coomassie® Blue–4.75% TCA–4.75% sulfosalicylic acid solution and destained with 5% TCA–5% sulfosalicylic acid solution of Chrambach *et al.* (15).

Acid Hydrolysate Preparation

The hydrolysate was prepared by adding 10 ml 6*N* HCl to 60 mg of 24-hr ball-milled flour in a constricted test tube (Corning No. 9860, 18 × 125 mm). Tube contents were frozen (Dry Ice-acetone mixture), twice evacuated to <15μ, flushed with nitrogen, and then thawed. They were evacuated again to <15μ and sealed at the constricted area. Sample was hydrolyzed at 110° ± 1°C for 24 hr, filtered (0.8-μ Millipore Filter), and rotoevaporated to dryness at 47°–50°C with four deionized water rinses.

Cystine, cysteine, and methionine were performic acid-oxidized by a modified Moore procedure (16) to cysteic acid and methionine sulfone. Hydrogen peroxide-30% (1 vol) was added to formic acid-88% (9 vol), held for 1 hr at ambient temperature, and then cooled to 0°C. Performic acid aliquot (2 ml) was added to a 30-mg flour sample in a constricted test tube and held overnight at 0°C. Hydrogen bromide-48% (0.30 ml) was slowly added to tube contents with swirling at 0°C. The HBr was removed by rotoevaporation at 40°C and the contents completely dried under vacuum. A 5-ml 6*N* HCl aliquot was added to the residue. Contents were frozen (Dry Ice-acetone), evacuated to <15μ, nitrogen flushed, reevacuated to <15μ, sealed, and hydrolyzed for 18 hr at 110° ± 1°C. Sample was filtered and rotoevaporated as above. Amino acid content of the acid hydrolysates was determined by a modification of the Spackman *et al.* procedure (17) on a Phoenix Amino Acid Analyzer.

RESULTS

Urea Solubility of Flour Proteins

Most of the protein (≅73%) from the unheated flour (control) was solubilized

TABLE II
Urea-Solubilized Flour Protein Measured by Absorbance at 500 nm and 280 nm

Processing Conditions			Absorbance	
Moist. %	Temp °C	Time min	500 nm/0.03 ml	280 nm/0.03 ml
13	0	0	0.595	0.145
13	174	2	0.224	0.186
13	174	5	0.293	0.236
13	174	10	0.404	0.324
24	0	0	0.644	0.152
24	174	2	0.241	0.175
24	174	5	0.351	0.247
24	174	10	0.457	0.396
33	0	0	0.659	0.156
33	174	2	0.253	0.222
33	174	5	0.337	0.247
33	174	10	0.457	0.480

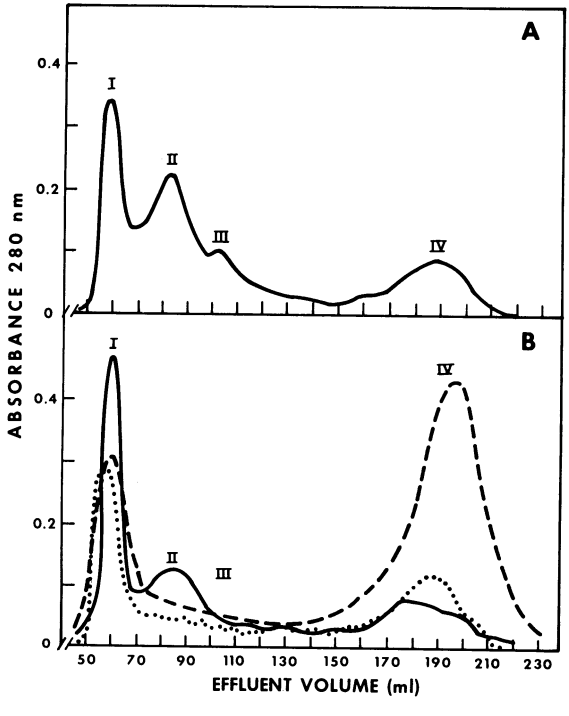


Fig. 2. Comparison of Sephadex G-100 chromatography of urea-phosphate-solubilized proteins of heated and unheated flours: A. Unheated flour proteins (13% moisture level flour); B. Heated flour proteins _____ 13-108-2, ... 13-150-2, - - - - - - - - 13-174-2 (% moisture-°C-min).

by the urea-phosphate solution. However, this solution did not solubilize the acetic acid-insoluble gel proteins. These proteins, constituting approximately 27% of the total flour proteins, have been characterized by Mecham *et al.* (18) and Cole *et al.* (19). The proteins from unheated flour, including the gel proteins, were solubilized with 1*N* NaOH. NaOH-solubilized protein gave an absorbance (500 nm) of 0.882 for a 0.03-ml aliquot.

Protein solubility in urea for all heat-treated flours at the specified moisture was affected by the heat treatment, as shown in Fig. 1. A comparison of flours at 13–33% levels processed at 108°C showed the lowest moisture level flour (13%) was the least affected by the heat treatment as measured by protein-urea-solubility. At 150°C–2-min flour processing, there was a marked decrease in protein solubility in urea at all moisture levels compared to the unheated samples. At 174°C, the protein solubility showed a linear increase as a function of time for all moisture levels. The 13%-moisture flour had the lowest rate of solubility change (absorbance 500 nm/min) at this temperature.

A comparison of urea-solubilized proteins determined by absorbance at 500 nm and 280 nm is shown in Table II. The increase in 280 nm absorbance of the processed samples was probably due to the formation of Maillard reaction products in addition to absorbance of tyrosine and tryptophan. Carbohydrates reacting with the amino groups of amino acids have an absorbance peak at 285 nm (20). Because of their available basic groups, lysine and arginine in the processed samples are particularly sensitive to the reaction.

Gel Filtration of Urea-Solubilized Proteins

Gel filtration on Sephadex G-100 of urea-solubilized proteins from unheated and heated flours was used to determine the relative molecular-weight changes of proteins as a result of processing. Four peaks were obtained for the unheated proteins as shown in the chromatogram (Fig. 2A). Peak I contained mostly glutenins, Peak II mostly gliadins, Peak III mostly globulins and albumins, and Peak IV mostly peptides by disc electrophoresis. Wright *et al.* (21) also obtained

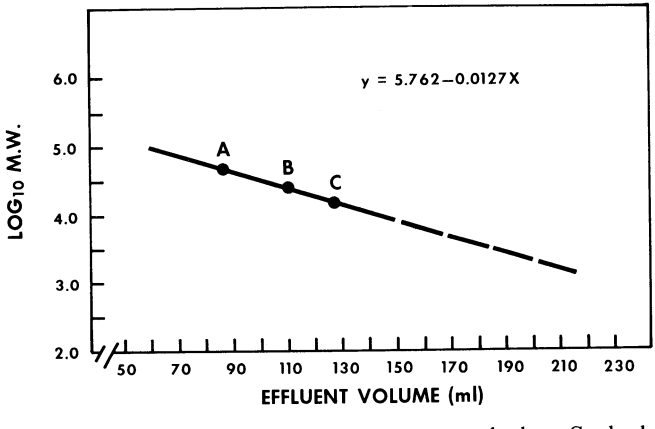


Fig. 3. Calibration curve for flour proteins chromatographed on Sephadex G-100: A. Ovalbumin; B. Chymotrypsinogen A; C. Ribonuclease A. y = $\log_{10}$mol wt; × = effluent vol (ml).

four peaks for aluminum lactate-solubilized flour proteins on Sephadex G-100.

From regression line equation in Fig. 3, Peak I appearing at the front has a mol wt 100000; Peak II was calculated to be 51200; Peak III 31000; and Peak IV 2000. Glutenins are reported to be 2–3 million in mol wt (22); α-gliadins in aluminum lactate 50000 by gel filtration, and 55000 by light scattering (23); albumins in 0.1*N* NaCl 28000 (24); and disulfide peptides 2000 (25).

The effects of thermal processing on flour proteins are shown in Fig. 2B. At 108° C–13% moisture, Peak I area had increased indicating that the lower mol wt proteins had aggregated. Peak II area, the gliadin fraction showed some decrease. Peak III area, the albumin and globulin fraction showed a substantial decrease. Peak IV area, the peptide fraction showed little change. The effect of processing became more pronounced at 150° C where Peak I area had decreased, the albumin and globulin Peak III was absent and a slight increase in the peptide

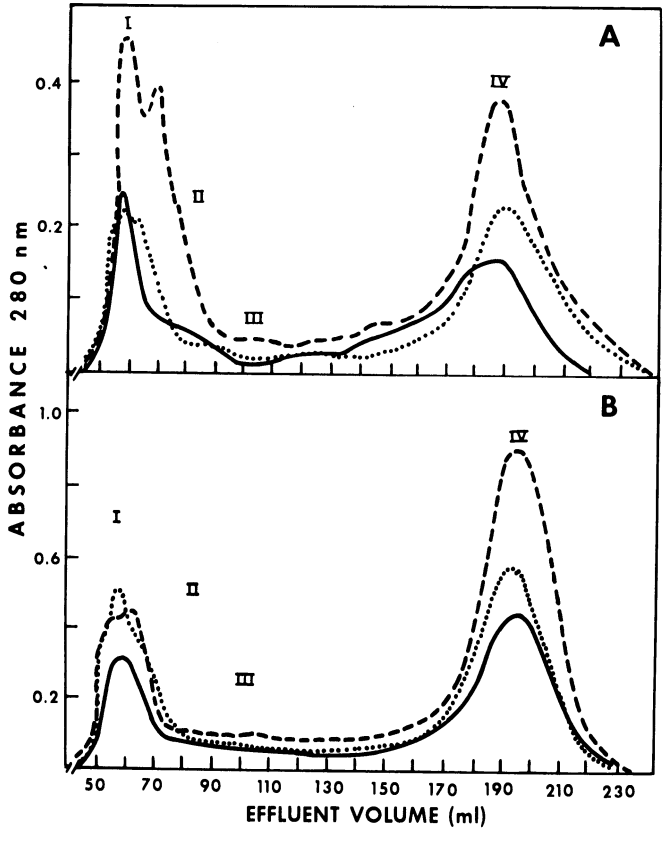


Fig. 4. A. Effect of flour moisture content during processing _____ 13-150-10, ... 24-150-10, - - - - - - - - - 33-150-10 (Peak I-opalescent). B. Effect of holding time of processing _____ 13-174-2, ... 13-174-5, - - - - - - - - - - - - 13-174-10 (% moisture-° C-min).

peak was noted. At 174° C, there was a substantial breakdown of the proteins to peptides (Peak IV).

Gel filtration can be used to measure processing effects on proteins with different flour moisture levels (Fig. 4A). The effect of time of processing on flour proteins can be studied by this method also (Fig. 4B). Peak IV, the peptide fraction, can be used to monitor protein breakdown from thermal processing by integrating the Peak IV area.

Fraction IV had a light yellow-brown color which was probably due to Maillard reaction products in the processed samples (150° C, 174° C). The urea-solubilized proteins from processed flour (13% moisture—174° C—10 min) were fractionated on Sephadex G-50 (fractionating range mol wt 500-10000). On

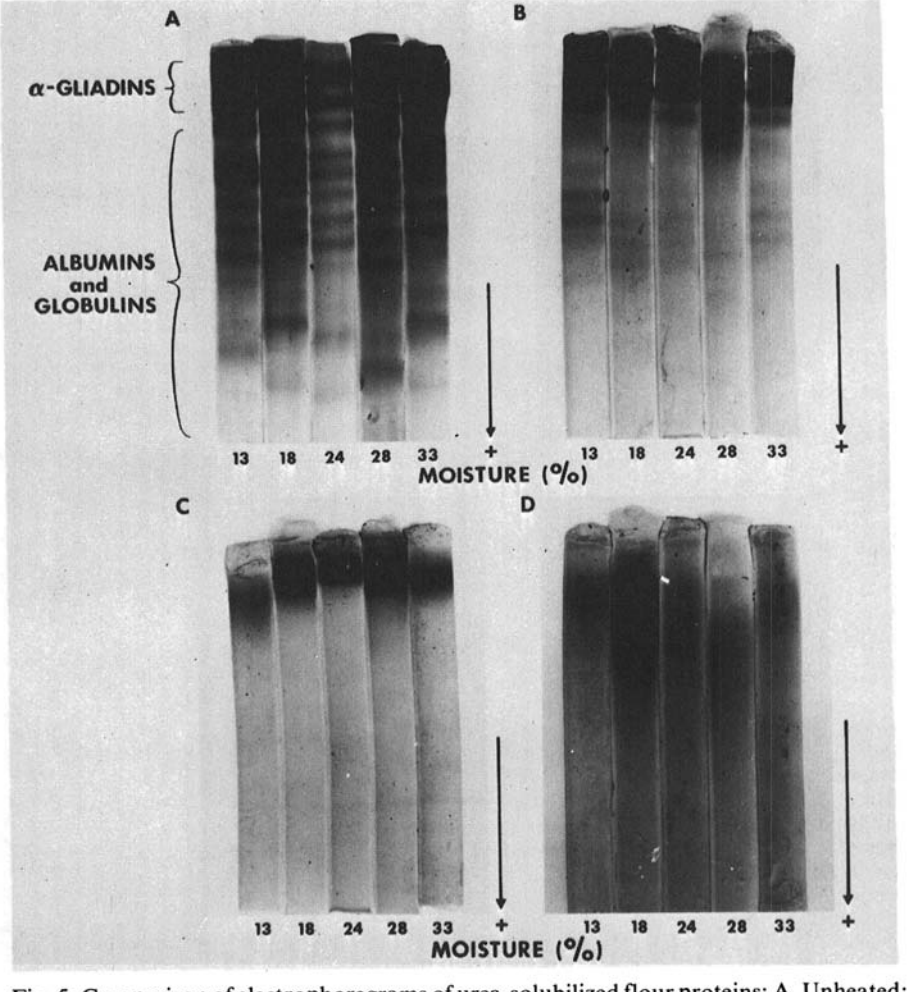


Fig. 5. Comparison of electrophoregrams of urea-solubilized flour proteins: A. Unheated; B. Processed 108° C-2 min; C. 150° C-2 min; D. 174° C-2 min.

Sephadex G-50 gel filtration, Peak IV was eluted as a single peak prior to amino acid indicator peak (tyrosine mol wt 181) indicating that Peak IV was composed of peptides.

Electrophoresis of Urea-Solubilized Proteins

The effect of heat on specific flour proteins was examined by disc electrophoresis as shown in Fig. 5. Eleven distinct protein bands that include the α-gliadins, albumins, and globulins were found for the urea extract of unprocessed flours. Glutenin did not migrate into the gel because of its large mol wt. At 108°C–2 min (Fig. 5B) the globulins and albumins showed a marked decrease in specific proteins and protein concentration. At 150°C–2 min (Fig. 5C), only the α-gliadins were present. At 174°C–2 min (Fig. 5D), there were no distinct protein bands. Electrophoregrams for the processed proteins at 5 and 10 min appeared similar to the 2-min runs except for a lighter staining of some protein bands.

Amino Acid Analyses

Amino acid destruction in the flour samples by the heat treatments is shown in Fig. 6 and Table III. Lysine, arginine, and cystine-cysteine had significant losses due to thermal treatments. Flour moisture content appeared to exert a greater influence on cystine-cysteine destruction than on arginine or lysine. Little destruction (25% or less) of the other amino acids was found for the flour processed at the maximum temperature and time, as shown in Table III.

TABLE III
Effect of Processing on Amino Acid Content of 24%-Moisture Level Flour

Amino Acid	Unprocessed	Processed	
	Control	174°C–10 min	
	g/16 g N	g/16 g N	% destruction
Lysine	1.94	1.04	46.4
Histidine	1.94	1.78	8.3
Ammonia	3.65	3.78	−3.6
Arginine	3.61	1.52	57.9
Aspartic acid	3.72	2.90	22.0
Threonine	2.46	2.21	10.2
Serine	4.24	3.65	13.9
Glutamic acid	32.44	29.88	7.9
Proline	10.73	10.08	6.1
Glycine	3.45	3.39	1.7
Alanine	3.02	3.09	−2.3
Cystine	2.74	1.30	52.6
Valine	4.01	4.07	−1.5
Methionine	2.18	2.10	3.7
Isoleucine	3.41	3.46	−1.5
Leucine	6.70	6.64	0.9
Tyrosine	2.82	2.96	−5.0
Phenylalanine	4.85	4.87	−0.4
Recovery (%N)	90	81	

Effect of Disulfide-Splitting Agent on Protein Solubility

Protein solubilized was much greater in 3*M* urea–0.01*M* phosphate–0.05*M* Na_2SO_3–pH 7.0 solution than in the absence of sodium sulfite as shown in Table IV. Processed flour samples were selected because of their low protein solubility in urea-phosphate solution (Fig. 1). Sodium sulfite split the SS bonds increasing the protein solubility of the processed flours. The protein solubilities of unheated flour in urea-phosphate-Na_2SO_3 and in 1*N* NaOH as measured by the Lowry procedure (12) were 6% and 27% higher, respectively, compared to the urea-phosphate solution.

DISCUSSION

In extrusion processing of flour-based foods, temperatures ranging from 100°–190° C are utilized in the production of snack foods, animal feeds, cereals, pet foods, protein supplements, and meat analogs. In multi-step processing of wheat flakes, temperatures from 27° to 154° C and moisture conditions 3–31% are utilized; wheat biscuits are processed at temperatures of 100°–121° C, and moistures of 11–60%; cereal granules at 27°–204° C; and puffed cereals at temperatures >260° C and 11–38% moistures (26). In commercial baking, oven temperatures of 191°–260° C for 20–30 min are used, dependent on the baked product. However, the crumb temperatures do not, in general, exceed 100° C in baked goods, while crust temperatures can reach 195° C (27).

In our study, the effects of thermal processing on flour proteins using controlled conditions of temperature, moisture, and time, in ranges that are commonly employed in commercial processing, are summarized as follows:

At 108° C, the albumins, globulins, and some gliadins were aggregating, forming large urea-insoluble complexes as indicated by decrease in protein solubility in urea (Fig. 1), increased area of Peak I, decreased areas of Peaks II and III of the gel filtration chromatograms (Fig. 2B), and the disappearance and decrease of many of the albumin and globulin bands in the electrophoregrams

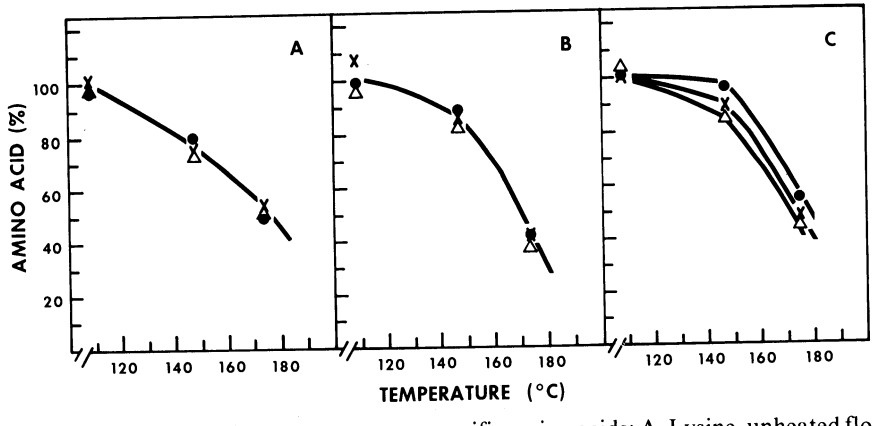


Fig. 6. Effect of processing temperature on specific amino acids: A. Lysine, unheated flour 2.10 g/16 g N = 100%; B. Arginine 3.68 g/16 g N; C. Cysteic acid 2.84 g/16 g N. • 13% moisture, × 24% moisture, Δ 33% moisture.

(Fig. 5B). At this temperature, for 10-min holding time and a moisture range of 13–33%, there was no apparent destruction of amino acids (Fig. 6).

At 150° C, the proteins appeared highly aggregated as evidenced by very low urea solubility, gel filtration chromatograms, and electrophoregrams (Fig. 1, 2B, 5C). The start of the breakdown of the aggregated protein was indicated by the increase in Peak IV (Fig. 2B, 4A). Peptide formation increased with higher flour moisture content (Fig. 4A). At this temperature, some destruction of arginine, lysine and cystine-cysteine was evident (Fig. 6).

At 174° C, the increased protein solubility in urea (Fig. 1) was due to the rapid breakdown of the aggregated protein to small peptides (Fig. 4B). There was a complete absence of proteins in 1000–100000 mol wt range as indicated by the electrophoregrams (Fig. 5D). There was significant destruction of arginine, lysine (the first-limiting amino acid in flour), and cystine-cysteine (Fig. 6).

The heat-produced protein aggregate might be due to the formation of peptide bonds between free amino and carboxyl groups by dehydrating the proteins and/or the formation of disulfide bonds from thiol groups (SH). The latter seems more likely since the processing conditions used held moisture constant. Urea used for solubilization would tend to disrupt other types of bonds. Earlier researchers (3,6) noted an increased viscosity of heated flour in ammonium fluoride and lactic acid solutions (54° — 100° C). The increased viscosity was probably due to heat-induced protein aggregation. Flour contains 100 μmol disulfide groups/g protein and 10 μmol thiol groups/g protein (28). Albumins and globulins, although only 20% of the total protein, contain 50% of the thiol groups in flour proteins (29). The thiol groups of albumins, globulins, and some gliadins, by formation of disulfide bonds and disulfide interchange, are probably responsible for linking the proteins into large aggregates at 108° C.

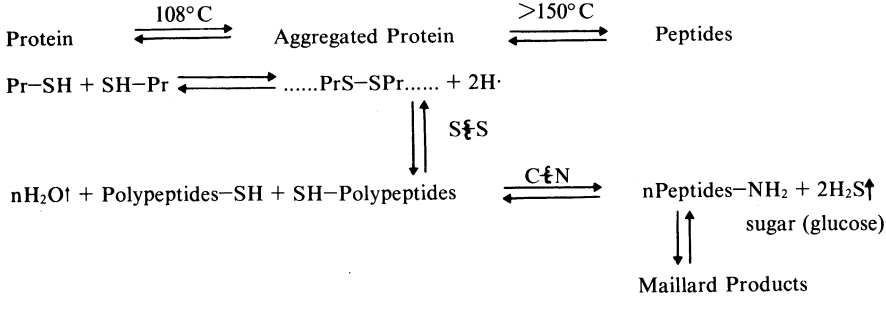
Heat has been used by Kent-Jones (3,4) as a flour improver. Chemicals such as potassium bromate, potassium borate, potassium persulfate, and chlorine dioxide are used by the baking industries, in countries that permit their addition, to increase the stiffness of dough. The generally accepted explanation of the effectiveness of the flour improvers is that they oxidize the SH groups of proteins to SS bonds. The effect of flour-heating conditions on dough improvement should probably be reevaluated for clues to an alternative to chemical oxidation.

TABLE IV
Effect of Sodium Sulfite on Protein Solubility

Processing Conditions			Solubility[a]	
Moist. %	Temp °C	Time min	no Na_2SO_3 %	Na_2SO_3 %
33	0	0	94.3	100[b]
33	150	2	14.9	50.5
33	150	5	22.4	54.1
33	150	10	23.0	53.8

[a]Lowry procedure—0.01 ml aliquots used for analyses.
[b]Taken as 100% for comparison purposes.

The breakdown of the aggregated protein to peptides by heat could be explained in part by the cleavage of SS bonds. This was assumed from the destruction of cystine and the hydrogen sulfide evolved. A mass spectrometry-thermal study by Kasarda and Black (30) of α-gliadin and other proteins under high vacuum from 100°–200° C showed the production of only H_2O, NH_3, and H_2S. Hydrogen sulfide was evolved from cystine or cysteine in 0.05*M* phosphate buffer-pH 7.0 on heating (31). If only SS cleavages were involved, protein products in the 20000–40000 mol wt would be expected from the gel filtration studies. Disulfide cleavage of glutenin resulted in 20000 mol wt products (32). Similar treatment of gliadin resulted in 22300 mol wt products (33). Since the heat-produced peptides were much smaller in mol wt, it appeared that peptide bond (C-N) cleavage was also involved. A suggested reaction scheme is:



There are critical temperatures that determine protein changes such as protein aggregation, peptide formation, and amino acid destruction. At any given temperature, the rate of protein change was a function of flour moisture content. The lower the moisture content of the flours, the less sensitive the proteins are to physical-chemical changes. The 13%-moisture flour had less protein changes even though the heating times were much longer (Table I). If very short times are used in processing products, the processing temperature will not be reached due to slow heat penetration rates in flour. For the production of high-protein foods, bread would appear to be a good vehicle because the internal crumb temperatures do not exceed 100° C where protein changes would be minimal. Processing at the higher temperatures (>150° C) would cause some amino acid destruction and, therefore, reduce the nutritional value of the flour product. The effects of these protein changes on enzymatic digestion of the heat-treated flours will be covered in a subsequent paper.

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