the added glycerophospholipids from soya cells (Table II). The aim of this analysis was to find out whether or not the acyl moieties of phosphatidylcholines and phosphatidylethanolamines isolated from curd and whey were isomerized between the *sn*-1 and *sn*-2 positions of the glycerol backbone. Lipases of microbial origin catalyzing such deacylation–reacylation sequences are well-known (Pieringer, 1983). The results given in Table II show that the distribution of radioactive acyl moieties in phosphatidylcholines and phosphatidylethanolamines of curd broadly reflects that of the added glycerophospholipids indicating that these compounds remained almost unchanged during cheese making. The glycerophospholipids of whey, however, showed some minor changes in the distribution of labeled acyl moieties.

The acyl moieties of mixtures of phosphatidylcholines and phosphatidylethanolamines from curd and whey were transmethylated, and the resulting methyl esters of fatty acids were analyzed by radio gas chromatography. Methyl linoleate was the only labeled methyl ester found in the glycerophospholipids added and in those isolated from curd and whey (Table II). These findings again support the view that positional distribution as well as composition of labeled acyl moieties of phosphatidylcholines and phosphatidylethanolamines in both curd and whey remained almost unchanged.

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Thermal and Compositional Changes of Dry Wheat Gluten–Carbohydrate Mixtures during Simulated Crust Baking

I. Irving Ziderman[1] and Mendel Friedman*

A model system was designed to simulate crust baking at 215 °C for 72 min. The effect of admixtures of food carbohydrates on compositional changes and thermal stability of wheat gluten was investigated. Exothermic browning was accompanied by volatilization and nonuniform depletion of amino acids. Three transformations could be discerned: above 161 °C (internal temperature measured with a thermocouple), volatilization of nonnitrogenous compounds; above 189 °C, volatilization of nitrogenous products; above 235 °C, conversion of amino acid residues to nonvolatile nitrogenous products. The latter was the main overall fate of the protein. Above 300 °C, however, volatilization was the predominant change. Thermal reactivity of gluten–carbohydrate mixtures increased in the following order: L-ascorbic acid < potato amylose ≃ wheat starch ≃ cellulose ethers ≃ glucose ≃ fructose ≃ lactose ≃ maltose < sucrose < cellulose. The possible implications of these findings for the mechanism of browning and for baked foods are also discussed.

INTRODUCTION

In addition to its unique functional role in the formation of dough and the crumb and crust of bread and other foods, gluten is the major source of dietary protein in cereal products (Hansen et al., 1975; Betschart, 1978). The nutritional functionality of gluten may be impaired by its chemical reaction with other flour components and with baking additives, most of which are carbohydrates, including starch, the main component of flour (Block et al., 1964; Mauron et al., 1960; Gotthold and Kennedy, 1964; Jansen et al., 1964; Audidier, 1968; Palamadis and Markakis, 1980; Tsen et al., 1982, 1983).

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710.

[1]Foreign Research Associate, 1983–1984. Permanent address: Israel Fiber Institute, 91080 Jerusalem, Israel.

During baking, the mixture of water, protein, and carbohydrates in dough is exposed to two distinct transformations. Desiccation of the surface on its exposure to temperatures reaching 215 °C results in formation of a crust. The crust in turn encloses the bulk of dough in a steam phase at approximately 100 °C, forming the crumb. The nutritional impairment of gluten occurs particularly at the crust, which comprises nearly 50% of dry weight of whole bread (unpublished results). In hard biscuits, the bulk behaves thermally in a similar fashion to bread crust (Audidier, 1968).

In this study, a variety of food carbohydrates are compared in respect to their effects on the chemical stability of gluten in a model system simulating the dry conditions of crust formation. The carbohydrates investigated include widely used nutritive sugars (D-glucose, D-fructose, lactose, maltose, sucrose), vitamin C (L-ascorbic acid, sodium L-ascorbate), starches (potato amylose, wheat starch), and nonnutritive bulking agents and additives (cellulose, hy-

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drocellulose ("microcrystalline cellulose"), sodium (carboxymethyl)cellulose, [(hydroxypropyl)methyl]cellulose).

Sucrose itself is not found in bread dough since it is hydrolyzed to D-glucose and D-fructose by yeast invertase. D-Fructose is present in many breads as high-fructose corn syrup and is expected to behave similarly to D-glucose in thermal reactions with gluten. Since wheat flour contains only 10% gluten, it was necessary to use a 4:1 ratio of gluten to carbohydrate in this study, in order to provide a sufficient amount of treated protein for the animal-feeding studies that are to be reported separately.

The involvement of classical Maillard-type reactions in nonenzymatic browning during crust formation may be curtailed (Labuza and Saltmarch, 1981) by the essentially anhydrous conditions of the crust during baking. Furthermore, heating in a forced convection oven may enhance reactions with atmospheric oxygen and thereby change the course of browning reactions from the pathway generally described hitherto (Friedman, 1982) for Maillard-type reactions.

In addition to monitoring changes in chemical composition of the gluten–carbohydrate mixtures due to degradation of carbohydrate and amino acid residues, we have paid special attention to thermal changes within the substrate, by emplanting thermocouples within the test material during heating.

MATERIALS AND METHODS

Wheat gluten was supplied by U.S. Biochemical Corp. (Cleveland, OH). Maltose monohydrate was from Fisher Scientific (Chicago, IL). ICN Nutritional Biochemical (Cleveland, OH) supplied anhydrous β-D-glucose (dextrose), β-D-fructose, D-ascorbic acid, sodium L-ascorbate, α-lactose monohydrate, sucrose, cellulose (Alphacel, nonnutritive bulk composed of finely ground cellulose for addition to animal diets), hydrocellulose (a microcrystalline hydrolyzed Alphacel), and wheat starch. The commercial sucrose was milled in a Wiley mill using a 1-mm screen. Potato amylose, type II practical grade, was from Sigma Chemical Co. (St. Louis, MO). [(Hydroxypropyl)-methyl]cellulose (HPMC) was Dow Methocell K4M premium grade (number average degree of polymerization 460; number average molecular weight 89 000; methyl degree of substitution 1.1–1.6; hydroxypropyl molar substitution 0.1–0.3; maximum water 8%; maximum sodium chloride content 0.75%). Purified sodium (carboxymethyl)cellulose (CMC) was Hercules high-viscosity food grade cellulose gum type 7HF (purity ca. 99.6%; degree of substitution ca. 0.7; maximum water 8%). All graphs were plotted by computer.

Purification of Commercial Wheat Gluten. Gluten (20 g) was dissolved in acetic acid (0.5 M, 500 mL) by vigorous stirring in a blender. The centrifuged supernatant was neutralized with sodium hydroxide (1 M) to coagulate the gluten that was then washed with water, lyophilyzed, and ground in a mortar (yield 44%). The properties of the product as listed in Table I show that a protein essentially free of carbohydrate and lipid was obtained.

This purification was performed in order to corroborate the initial analytical data obtained for commercial gluten with regard to lipid and carbohydrate composition. Although high molecular weight protein would not be solubilized in this technique, the purified gluten was found to be representative of the commercial material in regard to amino acid composition. Commercial gluten was used in most experiments to model practical conditions in crust baking.

Compositional Analyses. Nitrogen, crude fiber, free lipids, and ash content of untreated and treated materials were assayed by standard techniques (AOAC, 1980). Water content was determined by heating for 70 min in a 130 °C air oven.

Table I. Purification of Wheat Gluten

	% compn[a]	
	commerc gluten	purified gluten[b]
fiber	0	0.17
water	9.16	6.92
free lipids	1.68	0.4
total lipids	7.3, 8.1	1.4, 1.0
water-satd. butanol extractables	13.6, 12.1	2.2, 7.4
ash	0.91	0.64
total carbohydrates	10.50, 10.82	0.27, 0.26
starch	5.6	0.0
nitrogen	12.53, 12.54	14.4
amino acid res[c]	74, 77	91
nitrogen recovd as amino acids[c]	95, 96	100
emp factor for converting % N to protein cont	6.25	6.25

[a] Two values are from duplicate determinations. [b] Yield 44%. [c] Calculated from amino acid analysis.

Starch and glucose were determined as described by Thivend et al. (1972), total lipids by the procedure of Bekes et al. (1983), and amino acids by ion-exchange chromatography (Smith and Friedman, 1984). The sulfur amino acids (half cystine and methionine) were analyzed separately as cysteic acid and methionine sulfone, respectively, after performic acid oxidation of the proteins (Moore, 1963; Friedman et al., 1979). Total sugars were extracted with ethanol (80%) and then assayed with anthrone–sulfuric acid (Loewus, 1952). Total carbohydrate was dissolved in sulfuric acid (72%) then hydrolyzed by aqueous dilution, and assayed with anthrone–sulfuric acid.

Procedure for Simulated Crust Baking. Commercial wheat gluten was mixed for 1 h with each carbohydrate as an air-dry powder (4:1, w/w, air dry basis) in a Hobart Model N-50 mixer at slow speed setting. The initial moisture content of the mixtures was 7–9% depending on the nature of the carbohydrate. Samples weighing 200 g each were evenly spread in layers approximately 7 mm deep in enamel pans 29 × 19 × 5 cm. An Omega glass-braid-insulated, 0.02-in. diameter, exposed-junction iron–constantan (J) thermocouple was embedded in each sample, and its wire was anchored to the pan's rim with adhesive tape in order to immobilze the junction. The uncovered pans were placed on the shelves in a Fisher Isotemp thermostat forced-draft convection oven, Model 177, that had been preheated to 215 °C as measured by another thermocouple in the oven cavity. The oven door seal was closed directly onto the thermocouple wires. The wires were connected to an Omega Model 199 10-channel digital thermometer, whereby temperatures were monitored for 72 min. The pans were then removed from the oven, left to cool in the hood to ambient temperature, and then weighed. Products were ground with use of a Wiley mill with a 1-mm screen and stored in Ziploc plastic bags below 0 °C.

All heat treatments were conducted in the air convection oven, unless it is specified that a vacuum oven was used, in which case the oven was evacuated under oil pump suction.

In each experiment, five pans containing the same gluten–carbohydrate mixture were heated in the oven together with one pan of pure carbohydrate. The temperature of each pan was monitored with a separate thermocouple. Temperature profiles of the five replicates were very similar, differing only due to fortuitous variations in the exact depth of the embedded thermal junction: the highest

Table II. Weight Changes and Probe Temperature Peaks during Baking of Pure Carbohydrates and of Wheat Gluten in Admixture with Various Carbohydrates

	carbohydrates only			gluten plus carbohydrates	
substrate	peak temp, °C	wt loss,[a] %	unchanged substrate in res,[a] %	peak temp, °C	wt loss,[a] %
starch	219	9.4	62[d]	273	30.1 ± 3.3[i]
amylose	nd[j]	95	18[d]	282	29.1 ± 3.4[i]
HPMC	230[i]	3.4	78[e]	284	31.4 ± 0.9[i]
CMC	224	6.0	20[e]	296	32.6 ± 2.1[i]
glucose	233	12	1.0[f]	280[h]	35.5 ± 1.3[i]
fructose[b]	nd	nd	nd	284[h]	28.4 ± 1.9[h]
maltose	220	23	43[g]	305	34.0 ± 4.7[i]
lactose	220	10	35[g]	306	33.5 ± 0.4[i]
sucrose	216	14	79[g]	309	38.3 ± 2.1[i]
cellulose	254[i]	6.8	13[e]	377	51.3 ± 1.3[i]
hydrocellulose	239	4.2	7.8[e]	342	49.5 ± 1.6[i]
sodium ascorbate	460	55	nd	250	20.2 ± 0.8[i]
ascorbic acid	223	<43	nd	283	30.4 ± 0.4[i]
gluten only[c]				218	8.3
gluten only				269	24.3 ± 0.57[i]

[a] Air-dry basis. [b] Fructose alone caramelized into a syrupy mass during heating. [c] Based in vacuum oven. [d] Determined as starch. [e] Determined as carbohydrate. [f] Determined as glucose. [g] Determined as total sugars. [h] Average from three determinations. [i] Average from five determinations. [j] nd = not determined.

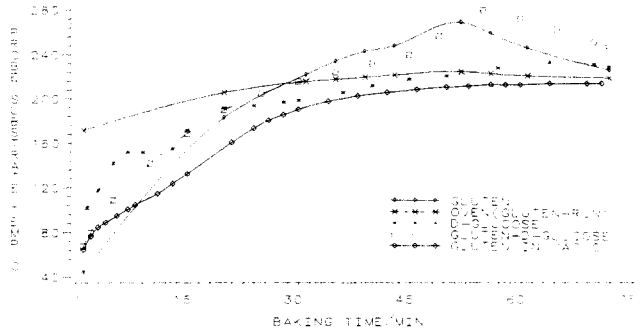

Figure 1. Thermogram of gluten baked with D-glucose.

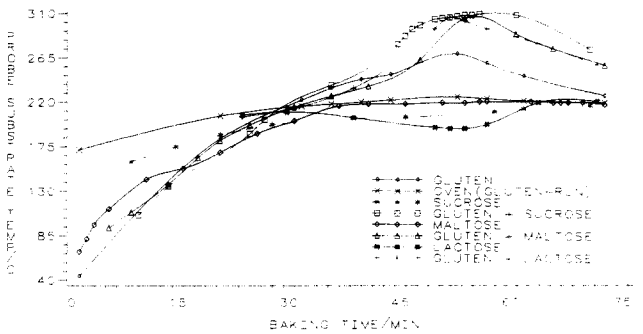

Figure 2. Thermograms of gluten baked with disaccharides (lactose, maltose, sucrose).

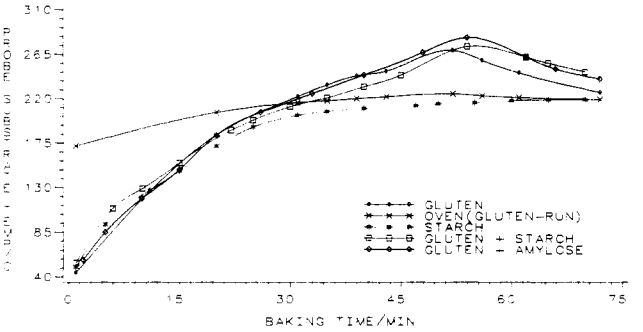

Figure 3. Thermograms of gluten baked with starches.

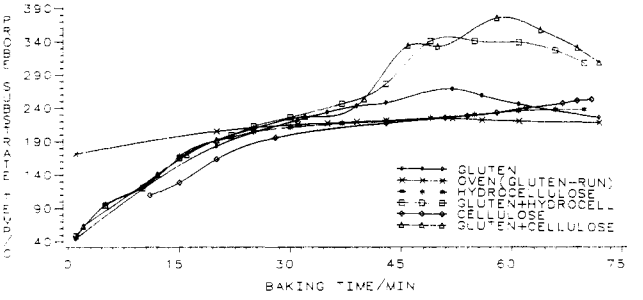

Figure 4. Thermogram of gluten baked with celluloses.

temperature achieved is reported in the Results and Discussion. Weight loss data in Table II are the averages of five separate weighings.

Experimental attempts to increase water activity were unsuccessful, since the gluten dough, on heating, swelled uncontrollably and filled the oven cavity.

RESULTS AND DISCUSSION

Thermochemical Studies. In contrast to a passive warming of the pure carbohydrates in response to exposure to oven temperatures (Table II; Figures 1–4), their mixtures with gluten rose dramatically in temperatures above oven temperature and also above the temperature profile of commercial gluten heated alone (Table II; Figures 1–4). Pure sodium ascorbate was exceptional in that it underwent an extremely vigorous combustion in which a temperature of over 460 °C was generated within the powder layer within 22 min, and half of the material was volatilized. The pure sugars merely caramelized under the same conditions. The temperature of the mixtures, however, began to soar above oven temperature after approximately 40 min, and their thermograms exhibited two-phase kinetics. A 72-min reaction time was chosen in order to permit measurement of the exothermic peak. The sodium ascorbate–gluten mixture behaved differently in that its thermogram exhibited a single peak at a temperature considerably lower than all the other materials tested in air including commercial gluten.

The vigorously exothermic behavior is attributable to chemical reaction of atmospheric oxygen with gluten and carbohydrate mixtures, since anaerobic organic reactions do not generate heat. The small exotherm exhibited by whole gluten may be due to interactions involving its own 11% carbohydrate content, 6% of which is starch, or even to interactions with the considerable amount of bound lipid present (Table I). When heated in an evacuated oven, commercial gluten did not generate heat or decompose (no weight loss beyond its water content) nor did it undergo

Table III. Percentage Composition of Commercial Gluten Baked in Admixture with Various Carbohydrates[a]

carbohydrate ingred (20%)	free lipids	total carbohydrates	starch	nitrogen	protein[m]	water	crude fiber	ash
none (unbaked)	1.7	11	5.6	12.5	76	9.2	0.0	0.91
HPMC (unbaked)	nd[n]	24	nd	10.1	59	6.6	nd	nd
none[b]	2.3	11	0.05	12.9	68	4.8	0.53	0.81
none (control)	4.9	3.1[l]	0.2	13.9	42	2.4	1.9	1.1
starch	1.3	7.5	0.03[j]	11.3	29	2.6	1.9	0.97
amylose	1.1	7.7	0.04[k]	11.2	27	2.9	11	1.2
HPMC	0.96	5.9	0.1	11.5	21	3.0	15	1.2
CMC	3.8	2.7[c]	0.1	11.3	24	2.4	5.4	6.4
glucose	1.3	0.90[d]	nd	12.4	19	1.6	28	0.94
maltose	1.5	(0.45)[e]	nd	12.5	17	0.93	3.8	3.6
lactose	1.3	0.92[f]	0.1	11.8	18	2.4	2.6	1.2
sucrose	0.91	0.48[g]	0.1	12.7	11	4.8	55	1.1
cellulose	2.0	1.4[h]	0.0	12.6	5	3.3	73	1.4
hydrocellulose	1.1	3.0[i]	0.0	12.7	5	3.6	75	1.4
sodium ascorbate	2.5	5.0	2.4	10.9	44	2.0	0.74	11
ascorbic acid	5.5	1.6	0.0	12.5	25	2.0	4.4	1.1

[a] Air-dry basis. Samples of Table III. [b] Vacuum oven. [c] Unbaked mixture 9.2%. [d] Glucose content 0.65%; unbaked mixture 18%. [e] Determined as total sugars; unbaked mixture 19%. [f] Total sugars 0.4%; unbaked mixture 16%. [g] Total sugars 0.1%; unbaked mixture 21%. [h] Unbaked mixture 9.5%. [i] Unbaked mixture 9.4%. [j] Unbaked mixture 25%. [k] Unbaked mixture 18%. [l] Total sugars 1.5%. [m] Bone-dry basis, from amino acid analysis (excluding tryptophan). [n] nd = not determined.

much compositional change such as amino acid degradation (Tables II–V). This stability, in contrast to exothermic decomposition in air, is consistent with the latter being essentially an *oxidative* process.

A comparison of thermograms (Figures 1–4) of the gluten–carbohydrate mixtures, their peak probe temperatures and weight losses (Table II), indicates that the chemical reactivity of the gluten mixtures decreases, depending on the carbohydrate used, in the following order: cellulose ≃ hydrocellulose > sucrose > lactose ≃ maltose ≃ glucose ≃ fructose ≃ CMC ≥ HPMC ≃ amylose ≃ starch ≃ ascorbic acid > (whole gluten) > sodium ascorbate mixtures. The protective functionality of sodium ascorbate in *stabilizing* gluten to thermal stress is noteworthy.

Compositional Studies. The heated products were analyzed for amino acids, starch, ash, crude fiber, free lipid, carbohydrate, nitrogen, and water content (Tables II–V). Some of the data for pure carbohydrates after baking in the absence of gluten appear in Table II. The value for "crude fiber" in excess of cellulose content evidently represents cross-linked products insoluble in aqueous sulfuric acid and sodium hydroxide solutions.

The possibility that the presence of carbohydrates may interfere with analytical studies of the heated gluten was tested by analyzing an unheated mixture of gluten with [(hydroxypropyl)methyl]cellulose. The results for Kjeldahl nitrogen (Table III, lines 1 and 2) and amino acid analysis are as to be expected for a 20% dilution of gluten with inert material. This indicates that no interference in assays is expected due to the presence of unreacted carbohydrates.

Commercial gluten alone exhibited a 24% loss in weight. Its admixtures with all carbohydrates (except sodium ascorbate) had augmented weight losses in the range 28–51% (Table II). For comparison, the weight losses for pure carbohydrates were all less than 24% (except for ascorbic acid and sodium ascorbate) (Table II). Evidently, the presence of carbohydrate synergistically enhances the formation of volatile compounds during heating of protein–carbohydrate mixtures.

Amino acid analysis of the products (representative examples in Table IV) revealed that aerobic heating of whole commercial gluten destroyed half (48%) of the amino acid initially present. In the presence of carbohydrate, this damage was augmented to values in the range 60–88%. Again, sodium ascorbate was exceptional in *decreasing* the extent of gluten destruction to 36%. While the relative proportions of all amino acids in untreated gluten were greatly altered after baking, all heated products nevertheless exhibited a uniformly similar relative compositional profile. The most decomposed mixture was gluten + cellulose (4:1). The least damaged mixture was gluten + sodium ascorbate (4:1). The other mixtures had intermediate values proportionate to their total amino acid content. Those amino acid residues most sensitive to decomposition during crust baking were completely absent in many cases. They are threonine, methionine, arginine, cysteine, histidine, lysine, and serine—in approximately decreasing order of sensitivity to decomposition.

These observations suggest that amino acid residues in gluten that contain nitrogen, oxygen, and sulfur functionalities are much more susceptibile to heat-induced degradation in the dry state than are neutral amino acids such as alanine and phenylalanine. This may be a general phenomenon since we have previously reported similar findings with heated casein–carbohydrate mixtures (Smith and Friedman, 1984).

Table IV also shows that destruction of the amino acid residues in wheat gluten was much less severe after heating under a vacuum rather than in a forced-air draft oven. This result implies, as stated earlier, that oxygen exerts an enhancing effect on the heat-induced degradation of gluten. Since oxygen-dependent degradation appears to affect all amino acid residues, the mechanism of this effect may involve formation of unstable, peroxide-type free-radical intermediates, a well-recognized phenomenon in protein chemistry (Delincee, 1983). Alternatively, combustion reactions may be involved.

The destruction of amino acids in heated gluten–carbohydrate mixtures was accompanied by the formation of new ninhydrin-positive compounds, whose amounts and elution positions are listed in Table V. The chemical nature of these compounds and their mechanism of formation remain to be established (cf. Scharf and Weder, 1984). We have previously reported similar findings with heated casein–carbohydrate mixtures (Smith and Friedman, 1984).

Correlation between Thermal and Compositional Changes. Regression analysis was carried out for the dependence of three chemical parameters on peak substrate temperature (internal temperature), namely (a) total nitrogen loss, (b) non amino acid nitrogen formed, and (c) weight loss. The data are listed in Table VI. Figure 5 shows the computer-plotted graphs: the formation of non

Table IV. Amino Acid Composition (g/16 g of N) of Commercial Gluten, Heat-Treated Glutens, and Heat-Treated Mixtures of Gluten with Various Carbohydrates[a]

amino acid	commerc gluten (untreated)[b]	air-heated gluten[b]	vaccum-heated gluten	gluten + glucose	gluten + lactose	gluten + maltose	gluten + sucrose	gluten + ascorbic acid	gluten + sodium ascorbate	gluten + starch	gluten + cellulose[c]	CMC[c]
Asp	3.68	1.78	3.20	1.08	0.98	0.93	0.68	1.40	1.94	1.57	0.169	0.98
Thr	2.55	0.00	1.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ser	4.74	0.11	1.61	0.09	0.09	0.08	0.09	0.12	0.13	0.13	0.07	0.14
Glu	35.22	21.50	36.42	11.90	12.24	10.60	6.67	14.85	30.68	20.06	2.70	16.79
Pro	12.11	7.42	12.53	3.79	3.79	3.28	2.09	4.58	10.94	6.46	1.31	5.77
Gly	3.46	1.98	3.50	1.32	1.37	1.13	0.89	1.58	2.86	1.77	0.36	1.72
Ala	2.72	1.90	3.15	1.04	1.01	0.86	0.57	1.33	2.63	1.55	0.28	1.26
Cys	2.13	0.46	0.28	0.21	0.00	0.22	0.00	0.13	0.57	0.13	0.18	0.00
Val	4.25	2.61	4.27	1.42	1.41	1.18	0.75	1.85	3.50	2.33	0.27	1.79
Met	1.87	0.33	1.57	0.18	0.12	0.12	0.00	0.23	0.73	0.17	0.00	0.00
Ile	3.72	1.99	3.64	0.98	0.93	0.84	0.43	1.30	3.06	1.71	0.12	1.28
Leu	7.20	4.30	7.31	2.14	2.08	1.83	1.05	2.85	6.10	3.75	0.38	2.77
Tyr	3.45	1.79	3.37	0.78	0.77	0.66	0.19	1.14	2.68	1.44	0.15	1.12
Phe	5.18	3.27	5.26	1.73	1.73	1.47	0.91	2.13	4.46	2.89	0.53	2.32
His	2.15	0.61	1.73	0.14	0.21	0.21	0.00	0.18	0.92	0.30	0.00	0.22
Lys	1.81	0.40	0.84	0.16	0.21	0.14	0.00	0.24	0.55	0.33	0.00	0.29
NH_3	3.71	3.18	4.08	2.96	2.90	2.35	2.75	2.77	3.58	2.80	1.54	2.86
Arg	3.69	0.22	2.25	0.00	0.00	0.00	0.00	0.15	0.22	0.22	0.10	0.17

[a] 215 °C/72 min. [b] Duplicate determinations. [c] Data with amylose were similar to those of cellulose, except for Arg (0.00). Data with HPMC were similar to those of CMC, except for Ser (0.00), Cys (0.48), His (0.79), and Arg (0.00).

Table V. Elution Positions and Concentrations (in g/16 g of N Based on Leucine Equivalents) of New Peaks on Amino Acid Chromatograms of Hydrolysates of Baked (215 °C/72 min) Commercial Wheat Gluten and Gluten-Carbohydrate Mixtures

	X^a	X^b	X^c	X^d	X^e	X^f
air-heated gluten	0.69	0.58	0.72			0.34
vacuum-heated gluten		0.43	0.36		0.15	
gluten +						
glucose	0.42	0.50	0.37			0.52
lactose	0.46	0.183	0.42			
maltose	0.35	0.45	0.33			
sucrose	0.19	0.55	0.22			0.28
ascorbic acid	0.53	0.61	0.57			0.40
sodium ascorbate	0.60	0.12	0.94			0.29
		0.15[g]				
amylose	0.61	0.60		0.12		0.41
starch	0.61	0.63	0.59			
cellulose	0.10	0.19	0.12			
hydrocellulose	0.09	0.18	0.11	0.09		0.26
carboxymethylcellulose	0.59	0.47	0.60			0.38
hydroxypropylmethyl-cellulose	0.38	0.57	0.46			
		0.29[g]				

[a] Elutes before Ile. [b] Elutes before His. [c] Elutes before Lys. [d] Elutes before NH_3. [e] Elutes before Asp. [f] Elutes after NH_3. [g] Two peaks.

Table VI. Data for Regression Analysis for Heated Wheat Gluten-Carbohydrate Mixtures[a]

carbohydrate ingred (20%)	peak temp, °C	wt loss, %	N loss,[b] %	non amino acid N formed,[c] %	amino acids lost,[d] %
none (unbaked)				(4)	
none	269	24	15	37	48
starch	273	30	22	42	60
amylose	282	29	21	43	60
HPMC	284	31	21	49	66
CMC	296	33	25	44	65
glucose	280	36	21	51	68
maltose	305	34	18	58	72
lactose	306	34	23	51	70
sucrose	309	34	17	62	75
cellulose	377	51	39	53	88
hydrocellulose	342	50	37	55	88
sodium ascorbate	250	20	14	26	36
ascorbic acid	283	30	13	53	62

[a] See Figures 5 and 6 and Table VII. [b] Nitrogen loss = 100 − [(% N in product)(100 − % weight loss)(% N in unbaked sample)$^{-1}$]. [c] Non amino acid nitrogen in product as % N in unbaked sample = (100 − % N recovered as amino acids)(1 − fractional N loss). [d] (N loss) + (non amino acid N formed) − 4. Data not used in regression analysis.

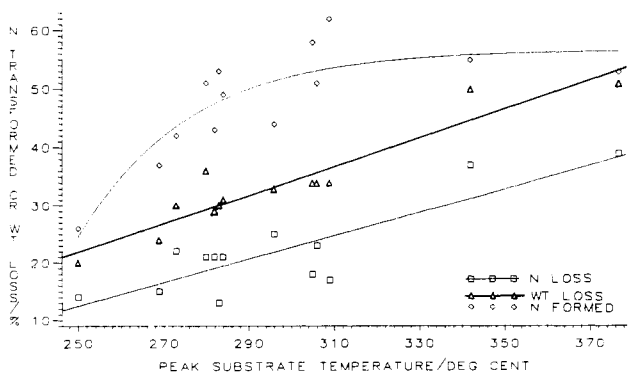

Figure 5. Regression of (A) nitrogen loss, (B) non amino acid nitrogen formed, and (C) weight loss on peak substrate temperature.

amino acid nitrogen increases asymptotically with peak internal temperature, while loss of both nitrogen and weight increase rectilinearly with peak internal tempera-

Table VII. Regression Equations for Figures 5 and 6[a]

param[b]			values		
y	x	equation	R^2	P	x (y = 0)
1	2	$y = -38.8 + 0.205x$	0.72	0.0002	189 °C
3	2	$y = -39.8 + 0.247x$	0.88	<0.0001	161 °C
4	2	$y = 56.8 - 539000e^{-0.0389x}$	0.77	0.0006	235 °C
1	3	$y = 5.66 + 0.825x$	0.81	<0.0001	6.9%
4	3	$y = 56.2 - 457e^{-0.134x}$	0.75	0.0010	15.6%

[a] Calculated from data of Table VI. [b] Key: 1 = nitrogen loss; 2 = peak substrate temperature; 3 = weight loss; 4 = nonamino acid nitrogen formed.

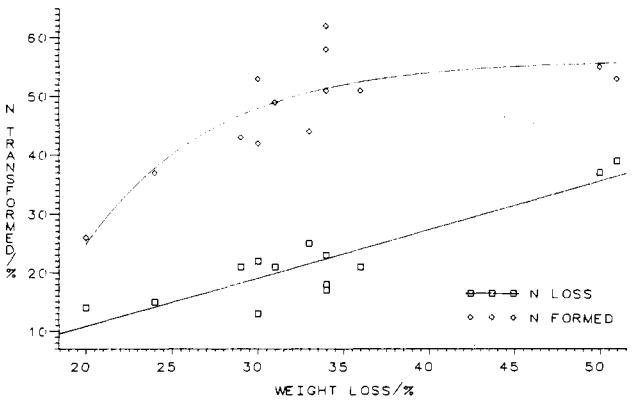

Figure 6. Regression of (A) nitrogen loss and (B) non amino acid nitrogen formed on weight loss.

ture. Table VII gives the regression equations calculated by the computer. All regressions are more than 99.9% significant.

The values of independent variable (x) were calculated for a zero value of each dependent variable (y = 0). Where x was based on the peak substrate temperature, its y = 0 values reflect the temperature at which a measurable chemical change does not occur. The y = 0 values in Table VII (last column) indicate therefore that weight loss would not occur below 161 °C, nitrogen loss would not occur below 189 °C, and formation of non amino acid nitrogen would not occur below 235 °C. These threshold temperatures may accordingly indicate sequential initiation of these chemical changes as the substrate internal temperature rises. In other words, an initial weight loss is followed at higher temperature by nitrogen loss (volatilization), which itself precedes initiation of the conversion of amino acid residues in protein to bound non amino acid nitrogenous material. Being asymptotically dependent on internal temperature, however, the transformation of gluten to bound non amino acid nitrogenous material is limited to a lower internal temperature range (<300 °C). Above this temperature, volatilization of the substrate (as measured by both weight loss and nitrogen loss) continues to increase monotonously. Oxidative processes would be largely involved in these transformations.

Regressions for (A) nitrogen loss and (B) non amino acid nitrogen formation on weight loss are plotted in Figure 6, and their equations are given in Table VII. These correlations are supportive of the scheme outlined above, of an interdependent empirical relationship for the three chemical transformations studied during heating of dry gluten–carbohydrate mixtures.

The yields of insoluble polymers ("crude fiber" in Table III) are evidently greatest in those mixtures that have undergone a more severely exothermic reaction. This parameter has not yet been treated for regression analysis.

If the extent of gluten degradation depends on the peak internal temperature, a predominant factor in determining the extent of reaction will be the heat of reaction. This conclusion has prompted us to undertake a study of the differential scanning calorimetry of the combustion of gluten and gluten–carbohydrate mixtures.

Mechanism of Browning. The possible involvement of classical Maillard-type reactions (i.e., between primary amino groups and reducing sugar functions) in the production of browning in this study is not consistent with our finding that a "nonreducing" polysaccharide such as cellulose is more effective than reducing sugars in enhancing protein decomposition. Nor is it consistent with the similarity in reactivity found for reducing and nonreducing sugars such as glucose and sucrose, respectively, and for the reactivity of nearly all other amino acids in addition to lysine, the presumed partner in the Maillard-type reactions (Finot, 1982; Friedman, 1982; Saltmarch and Labuza, 1982). In fact, reactions of this nature are usually curtailed as the water activity decreases below a value of 0.6 (Labuza and Saltmarch, 1981). The essentially desiccated condition of the materials heated in this study would accordingly not be conducive to their browning by a Maillard mechanism.

In the course of studying the nature of the brown products obtained, it was found that some are mutagenic in the Ames test and that the gluten–sodium ascorbate product inhibits growth when fed to mice. These observations will be communicated separately.

Implications for Baking. In bread doughs, the moisture content is close to half of the total weight. Mixing and fermentation develop a network of intimate contact at the molecular level between gluten, sugars, polysaccharides, and other flour and dough ingredients. As bread is baked, water moves from the interior to the surface and evaporates, tending to keep the dough relatively cool during the early stage of baking. On becoming sufficiently dry, crust temperatures rise and browning occurs.

The internal thermal kinetics of baking have been studied by Audidier (1968). Hard biscuit behaves like the crust of cake and bread in rapidly exceeding 100 °C after approximately 2 min. Hard biscuit reaches 120 °C internally at the end of an 8-min baking period, with more rapid response in its crust region. On baking a pan loaf, a temperature of 120–140 °C is reached 1 cm below the surface after a 16-min baking time. These thermal kinetic changes correspond essentially to the heating profiles measured in our model system during the initial stage of the process, indicating a significant simulation of the conditions prevailing during baking of hard biscuits and crust layers.

The model system uses gluten and a variety of carbohydrates that are typical of those found in bread and other dough formulas. The surprising lack of a demonstrable Maillard reaction between protein and reducing sugar, hitherto considered the accepted mechanism for browning during baking of bread, cake, and pastry products, is particularly significant. Baking of bread is often carried out for as long as 60 min; exothermic interaction between gluten and carbohydrates in our model system generally occurred after 40 min of heating. Compared to the model system, a baking crust normally experiences a higher water activity and consequently is unlikely to attain such a high temperature. On the other hand, the higher ratio of carbohydrates to gluten in flour and their more intimate molecular contact as dough will both presumably act to enhance destruction of protein in normal crust baking. While it is, therefore, difficult at present to assess quantitatively the contribution of model damage to changes taking place when bread and other flour products are

baked, the destructive phenomena now observed are considered to be highly relevant in a qualitative sense to artificial depletion of the nutritive value of food. The described studies should facilitate formulating baking conditions to minimize loss of nutrients and the formation of antinutrients.

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Registry No. HPMC, 9004-65-3; CMC, 9004-32-4; L-ascorbic acid, 50-81-7; amylose, 9005-82-7; starch, 9005-25-8; D-glucose, 50-99-7; D-fructose, 57-48-7; D-lactose, 63-42-3; D-maltose, 69-79-4; D-sucrose, 57-50-1; cellulose, 9004-34-6; hydrocellulose, 9034-34-8; sodium L-ascorbate, 134-03-2.

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Phosphine Residue and Its Desorption from Cereals

Jois R. Rangaswamy

The extent and the pattern of desorption of PH_3 residue in wheat, raw polished rice, raw unpolished rice, and parboiled rice were followed at laboratory level for 60 days. The PH_3 residue on each day of storage was computed by knowing the total amount of PH_3 desorbed over 60 days. The PH_3 desorption patterns from four types of cereals are discussed under three stages: up to 19 days, 20–49 days, and 50–60 days of storage. The relationship between the logarithm of such residues vs. storage in days is linear over the first 19 days, except in the case of residue from raw polished rice, which is only up to the first 9 days. During the period of linearity the desorption follows first-order kinetics. Between days 20 and 49 there is a waxing and waning in the amount of PH_3 desorbed. Between days 50 and 60 there is a regular fall in PH_3 residues due to uniform decreased desorption, except in raw unpolished rice in which desorption is a bit sluggish up to 56 days.

Although phosphine liberated from aluminium phosphide preparation has been in use as fumigant for cereals and their products since 1936, nothing is known about the manner and the rate of its residue elimination during subsequent storage after airing, except for the claim that PH_3 disappears completely on aeration (Degesch, 1962). As it has now been conclusively established that PH_3 is not a "nonresidue" fumigant, the manner of its elimination from fumigated cereals is important to assess the possibility of any physically bound toxic PH_3 residues persisting in cereals.

Cereals or their products absorb PH_3 gas depending on their gas-holding capacity; for example, wheat (Rangaswamy, 1984), raw polished rice, and paraboiled rice (Rangaswamy, 1985) absorb about 62%, 27%, and 84%,

Infestation Control and Pesticides Discipline, Central Food Technological Research Institute, Mysore 570 013, India.

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