Thermal Interaction of Ascorbic Acid and Sodium Ascorbate with Proteins in Relation to Nonenzymatic Browning and Maillard Reactions of Foods

I. Irving Ziderman,† Kay S. Gregorski, Stephen V. Lopez,‡ and Mendel Friedman*

Western Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 800 Buchanan Street, Albany, California 94710

Previous work showed that baking of proteins at 215 °C in admixture with sodium L-ascorbate severely aggravated the antinutritional effects in mice but that L-ascorbic acid ameliorated their toxic activity. In the present work, thermal analysis of these materials was performed by differential scanning calorimetry (DSC) in oxygen in order to clarify their different reactivities. DSC curves of gluten and casein were similar but differed from that of soybean protein isolate. While additions of ascorbic acid did not alter the DSC curves of the three proteins, sodium ascorbate (20%) produced new exothermic peaks, indicating the occurrence of chemical reaction with the proteins. Baked ascorbate contained mainly sodium oxalate and carbonates, which were largely absent in the baked protein–ascorbate blends. In contrast, ascorbic acid behaved similarly to carbohydrates in not reacting directly with proteins during dry heating. Observed exothermic self-heating is explained by a kinetic control mechanism that involves the rates of heat generation and loss. Nonenzymatic browning due to self-heating does not involve Maillard reactions. Since sodium ascorbate has a potentially antinutritional effect on protein baking, ascorbic acid is a preferable food additive.

Nonenzymatic browning that occurs when foods containing protein and carbohydrate are heated is generally considered to be initiated by a Maillard reaction between primary amino groups and reducing sugar, resulting in formation of brown polymeric melanoidins (Friedman, 1982). The amino–carbonyl mechanism is characterized as having an absolute requirement for moisture (Hodge, 1953; Mauron, 1981; Taylor et al., 1984). While, in general, the amino–carbonyl reaction between amino acids and reducing sugars is responsible for subsequent browning, the relevance of this reaction to the browning of actual foods, especially in the dry state, has been questioned (Hargraves and Pariza, 1984; Taylor et al., 1984, 1986). Moreover, there is evidence that a nonreducing sugar (sucrose) is just as active as D-glucose in browning proteins at 121 °C (Smith and Friedman, 1984).

When heated in air at 200 °C (simulated crust baking), proteins undergo self-heating (exothermic process), causing weight loss, amino acid destruction, and dark browning. These effects are aggravated in the presence of reducing sugars, nonreducing sugars, or polysaccharides (Ziderman and Friedman, 1985; Friedman et al., 1987). The reactivity of amino acid residues in this reaction decreases in the order threonine, methionine, arginine, cysteine, histidine, lysine, and serine, showing no selective participitation of primary amine in a putative amino–carbonyl reaction. Since our studies showed that browning in the dry state is dependent on the presence of air (oxygen) and occurs in the absence of moisture, high-temperature browning of proteinaceous foods is evidently not mediated by a classical Maillard reaction (Ziderman and Friedman, 1985). Previous differential scanning calorimetry studies of carbohydrates and their admixtures with wheat gluten in oxygen suggested that self-heating may be caused by the high enthalpy of the pure carbohydrates in their 200–400 °C exotherm (Ziderman et al., 1987).

Such thermal modification of the protein at 200 °C has toxicological consequences as well. It causes antinutritional effects when fed to rats and mice as food supplement (Gumbmann et al., 1983; Friedman et al., 1987, 1988; Ziderman et al., 1988). These antinutritional effects are alleviated when carbohydrates are present during the baking in an inverse relation to the protein degradation. However, heating proteins with sodium L-ascorbate, but not with free ascorbic acid or dehydro-L-ascorbic acid, severely aggravated the antinutritional effect, despite the fact that self-heating, volatilization, and amino acid decomposition during heating were curtailed (Friedman et al., 1987, 1988; Ziderman and Friedman, 1985; Ziderman et al., 1988). The present report describes DSC of protein–ascorbic acid and –sodium ascorbate (vitamin C) blends and discusses thermochemical aspects of crust baking and high-temperature food browning.

EXPERIMENTAL SECTION

Materials and methods are described in Friedman et al. (1987) and Ziderman et al. (1987). Protein–vitamin C mixtures (4:1) for DSC were prepared by grinding the dry powders together using pestle and mortar. A Perkin-Elmer DSC-2C was used with oxygen flow at 50 mL/min and heating rate of 20 °C/min. Baking experiments were performed for 72 min in a Fisher Isotemp 177 forced-draft convection oven or in a National vacuum oven, Model 5851. Internal temperatures were monitored by an Omega Model 199 digital thermometer with iron–constantan thermocouples.

RESULTS

Ascorbic Acid and Sodium Ascorbate. When sodium ascorbate was baked in air at 215 °C, a maximum internal temperature of 467 °C was recorded (Ziderman and Friedman, 1985). The residue (44% yield) contained oxalate (4.8%), carbonate (22%), and bicarbonate (13%),

† Visiting scientist. Permanent address: Israel Fiber Institute, Ministry of Industry and Trade, P.O. Box 8001, 91080 Jerusalem, Israel.

‡ Work done as part of a course, Nutritional Field Studies, Department of Nutritional Sciences, University of California, Berkeley, CA.

Table I. Baking Proteins with Vitamin C (4:1, w/w)[h]

sample baked[d]	temperature, °C		residue compn, %				
	oven	peak	sample yield	vit C	oxalate[b]	carbonate	bicarbonate
sodium ascorbate	180	180	100	99	0.01	0	0
sodium ascorbate	200	381	48	<0.1	1.7	18	0
sodium ascorbate	215	467	44	<0.03	4.84	22	13
sodium ascorbate[a]	215	246	59	0.5	3.9	6.0	2.1
ascorbic acid[g]	215	223	68	0.07	0.04	0	0
dehydroascorbic acid[f]	215	219	64	nd	0.02[c]	0	0
gluten	180	188	93				
gluten	200	212	88				
gluten	215	269	76				
gluten[a]	215	218	92				
gluten/ascorbic	215	283	70	0.04	0.01		
gluten/dehydroascorbic[e]	215	322	62	nd	0.08	0	0
gluten/ascorbate	180	184	94	20		0	0
gluten/ascorbate	200	244	79	0.65	1.6	0	0
gluten/ascorbate	215	247	79	0.15	0.3	0	0
gluten/ascorbate[a]	215	223	84	2.4	0.8	0	0
casein	200	200	89				
casein	215	218	86				
casein/ascorbate	180	180	91	19		0	0
casein/ascorbate	200	200	89	18	0.15	0	0
casein ascorbate	215	248	80	<0.03	1.14	0	0
soybean protein	180	185	95				
soybean protein	200	280	78				
soybean protein	215	230	79				
soy/ascorbate	180	180	93	21		0	0
soy/ascorbate	200	262	78	<0.05	0.5	2.5	0
soy/ascorbate	215	391	41	<0.03	0.4	3.6	0

[a] In vacuum oven. [b] Calculated as oxalic acid. [c] Unbaked DAA contained 0.01% oxalate. [d] 200-g samples unless otherwise indicated. [e] 50-g sample. [f] 12.5-g sample. [g] 11.6-g sample. [h] Data partly from Ziderman and Friedman (1985) and Friedman et al. (1987).

but no remaining ascorbate (Table I). Calculation showed that these constituent anions represented a sodium content of 25%, corresponding to 93% of the sodium originally present in the sodium ascorbate; the corresponding sodium salts would account for 65% of the residue mass. Sodium ascorbate decomposed similarly at 200 °C but less vigorously, without formation of bicarbonate. No decomposition at all occurred at 180 °C. The same products were also formed by heating sodium ascorbate in a vacuum oven at 215 °C, but the carbonate content was lower than in air and little heat was generated (Table I). Sodium ascorbate is presumably oxidized to the dehydro species before it is converted to oxalate by oxidative scission between C-2 and C-3, when L-threonic acid is also formed (Bauernfeind and Pinkert, 1970). The sodium ascorbate may be converted further to L-tartaric acid (Kirk-Othmer, 1984). The primary product of thermal decomposition of sodium oxalate is carbonate; an exothermic peak often occurs in air or oxygen (Dollimore, 1987).

The DSC curve of sodium ascorbate in oxygen was a well-resolved doublet (Figure 1; Table II). The peak at 242 °C possibly was due to formation of sodium oxalate, while the 608 °C peak may mark its conversion to sodium carbonate, with possible partial formation of bicarbonate when available sodium was exhausted. The DSC curve for ascorbic acid (Figure 1; Table II) had a sharp endotherm at 203 °C due to latent heat of fusion (56 ± 2 cal/g), followed by a poorly resolved triplet with peaks of increasing intensity at 253, 340, and 498 °C. These peaks may have been due to serial oxidative decompositions. Baking ascorbic or dehydroascorbic acids in air at 215 °C (Table I) led to a small rise in internal temperature. Any oxalic or carbonic acids formed would have vaporized.

Commercial Gluten. Commercial wheat gluten containing 70% protein, 11% carbohydrate, and 7.7% lipids (Ziderman and Friedman, 1985) exhibited exothermic transitions at 356 °C (ΔH = −169 cal/g), 532 °C (ΔH = −1687 cal/g), and 643 °C (ΔH = −18 cal/g) (Figure 2; Table III).

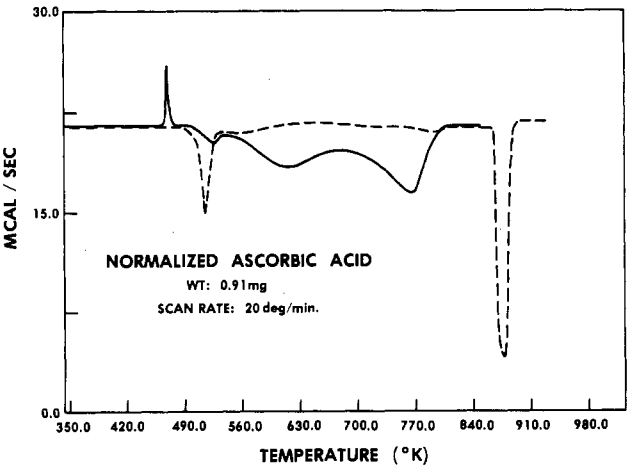

Figure 1. Differential scanning calorimetry curves (heat capacity in millicalories per second versus temperature) of ascorbic acid (unbroken) and sodium ascorbate (broken line).

Purified Gluten. After precipitation from aqueous acetic acid solution with alkali, the gluten was 85% protein, 0.27% carbohydrate, and 1.2% lipids (Ziderman and Friedman, 1985). The DSC curve of this purified gluten (Figure 3) was very similar to that of commercial material (Figure 2), but its heat of combustion was somewhat lower due to a diminution of the major transition (Table II). Purified gluten components gliadin and glutenin have quite different DSC curves. Each lacks, for example, the weak 643 °C transition (Ziderman et al., 1987). After trituration to a gum with water, followed by lyophilization, commercial gluten lost this 643 °C exotherm (Figure 3) and the lower temperature transition changed shape with the peak moving from 356 °C (Figure 2) to 305 °C (Figure 3), as previously reported (Ziderman et al., 1987).

Protein–Ascorbic Acid and –Sodium Ascorbate Blends. *Gluten.* The effect of vitamin C admixture on the DSC of commercial wheat gluten is shown in Table

Table II. DSC Data for Purified Gluten and Vitamin C

figure	sample	parameter	peak I	peak II	peak III	peak IV	total combustion
1	ascorbic acid (AA)	temp range, °C	184–212	220–274	272–417	391–543	213–549
		peak temp, °C	203	253	340	498	497
		$(\Delta H)_2$, cal/g	+55.9	-45.7	-340	-743	-2186
		SD	±1.8	±0.2	±31	±4	±15
1	sodium ascorbate (SA)	temp range, °C		201–370	370–633		198–631
		peak temp, °C		242	608		608
		$(\Delta H)_4$, cal/g		-362	-977		-1320
		SD		±9	±42		±49
3	purified gluten	temp range, °C		280–429	419–604	599–667	267–668
		peak temp, °C		360	511	643	509
		$(\Delta H)_2$, cal/g		-183	-1253	-81.3	-2161
		SD		±27	±22	±2.0	0

[a] $(\Delta H)_n$ = average value from n number of runs.

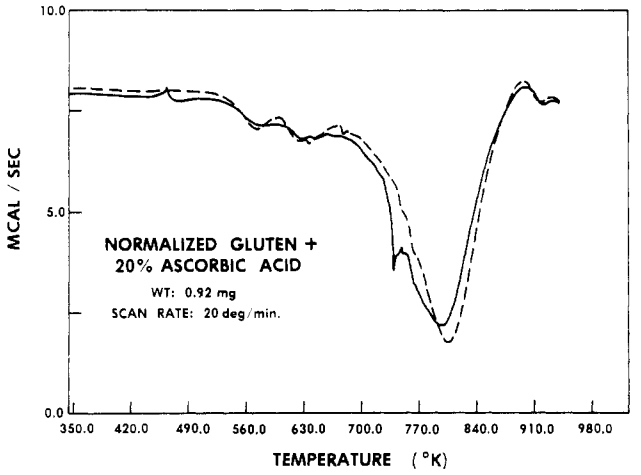

Figure 2. Differential scanning calorimetry curves of gluten (broken) and gluten–ascorbic acid mixture (unbroken line).

III. In commercial practice, the useful level of ascorbic acid is about 0.1% based on gluten. Addition of 1% ascorbic acid or sodium ascorbate did not alter the curve, except for moving the lower temperature peak from 356 to 299 °C. The DSC curve of the 4:1 gluten–ascorbic acid mixture was essentially the same as for gluten alone, with the addition of a very weak endothermic transition (ΔH = 8.3 cal/g) at 191 °C, due to ascorbic acid fusion (Figure 2). The corresponding blend with sodium ascorbate, however, altered the DSC curve of gluten considerably (Figure 4). A small peak at 241 °C was due to sodium ascorbate decomposition, ascribed to oxalate formation. Since the enthalpy of this exotherm corresponds to reaction of 7% of the sodium ascorbate in the blend, it appears that much of the 20% vitamin originally present reacted chemically with gluten. The weak gluten exotherm at 643 °C was absent, and the two major peaks were replaced by new exotherms at 413 and 554 °C.

Casein. Casein gave DSC results similar to those of gluten (Figure 5; Table IV), although the weak transition at 643 °C was absent. As with gluten, 1% ascorbic acid or sodium ascorbate addition had no effect, except that the lower temperature peak increased from 333 to 360 °C. Casein also behaved similarly to gluten with 20% ascorbic acid (Figure 5; Table IV). A very weak endotherm appeared at 194 °C for latent heat of fusion, and there were essentially unchanged exothermic peaks for the protein. The similarity to gluten was even more striking on addition of 20% sodium ascorbate to casein (Figure 6). Thus, in addition to the small peak at 242 °C for sodium ascorbate decomposition that correspond to transformation of 7% sodium ascorbate, the two major peaks were replaced by new exotherms at 412 and 556 °C.

Soybean Proteins. The DSC curves of soybean protein isolate (Figure 7) were completely different from those of gluten and casein. There were three main exothermic peaks, at 363 (major), 490, and 605 °C. These were unchanged by the addition of 1% (Table V) or 20% ascorbic acid (Figure 7). However, soybean protein reacted with 20% sodium ascorbate (Figure 8) just as did gluten and casein; there was a weak exotherm at 241 °C that corresponded to conversion of 6% sodium ascorbate, and the two higher temperature transitions of pure protein were replaced by new exotherms at 415 and 531 °C.

Loss of Amino Acids. Admixture with ascorbic acid or dehydroascorbic acids increased the internal temperature rise and weight loss of gluten during aerobic baking at 215 °C (Table I). While no reaction took place when proteins and sodium ascorbate were heated separately or blended at 180 °C, exothermic decomposition occurred at 200 and 215 °C (215 °C only for casein), when small amounts of oxalate were formed in the blends (Table I). Baking gluten (Ziderman and Friedman, 1985) or gluten–ascorbate blend (Table VI) in vacuum largely prevented protein degradation, presumably due to the absence of atmospheric oxygen. The results in Tables I and VI were obtained with 200-g samples. When smaller amounts of gluten or gluten–cellulose were baked in air at 215 °C, weight loss was greatly reduced (Table VII) to values near the samples' initial water content, exothermic decomposition to volatile products was not evident, and the products were brown but not as blackened as the 200-g samples.

DISCUSSION

Thermal analysis has rarely been used in relation to nonenzymatic browning of foodstuffs (Biliarderis, 1983). Raemy et al. (1983) ascribed the 100–160 °C exothermic peak in DSC curves of casein–lactose mixtures and milk powders to Maillard reactions; sealed cells containing a limited amount of air were used, and the highest enthalpy (-20 cal/g) was obtained with a casein to lactose ratio of 3:2. Emmerich et al. (1984) examined glutens in a static nitrogen atmosphere and found a 30–170 °C endothermic transition with its peak at 92 °C (ΔH = 53 cal/g) for dried powders and at 130 °C (ΔH = 253 cal/g) for gum glutens. We found no such transitions in our examinations of protein and protein–sugar blends, which were performed under dynamic oxygen flow favoring oxidation and volatilization and thereby resembling food cooking and processing.

In differential thermal analysis at high oxygen pressure (25 bar), casein and casein–lactose (3:2) ignite spontaneously at 185 °C without participation of Maillard reactions (Raemy et al., 1983). When oxygen supply is limited by conducting DSC in a sealed cell with air, cereals

Table III. DSC Data for Commercial Gluten and Mixtures with Vitamin C

figure	sample	parameter[a]	peak I	peak II	peak III	peak IV	total combustion
2	gluten	temp range, °C		253–407	403–626	626–659	220–625
		peak temp, °C		356	532	643	531
		$(\Delta H)_2$, cal/g		−169	−1687	−17.8	−2413
		SD		±15	±72	±2.7	±121
	gluten + 1% ascorbic acid	temp range, °C		250–413	396–629	634–662	216–632
		peak temp, °C		299	534	649	533
		$(\Delta H)_2$, cal/g		−188	−1464	−11.1	−2407
		SD		±2	±7	±0.2	±15
2	gluten + 20% ascorbic acid	temp range, °C	173–206	251–401	387–629	632–662	229–630
		peak temp, °C	191	352	525	646	525
		$(\Delta H)_2$, cal/g	+8.28	−89.1	−1596	−10.6	−2312
		SD	±0.38	±4.2	±197	±0.5	±33
	gluten + 1% sodium ascorbate	temp range, °C		254–417	396–625	631–661	221–628
		peak temp, °C		299	533	646	533
		$(\Delta H)_1$, cal/g		−218	−1373	−8.11	−2345
4	gluten + 20% sodium ascorbate	temp range, °C	212–260	263–442	434–616		212–632
		peak temp, °C	241	413	554		553
		$(\Delta H)_2$, cal/g	−26.3	−217	−1136		−2562
		SD	±1.2	±3	±18		±5
	gluten + 25% sodium ascorbate	temp range, °C	217–258	260–447	442–604		213–634
		peak temp, °C	241	329	547		547
		$(\Delta H)_3$, cal/g	−24.9	−196	−1050		−2620
		SD	±1.3	±27	±49		±41

[a] $(\Delta H)_n$ = average value from n number of runs.

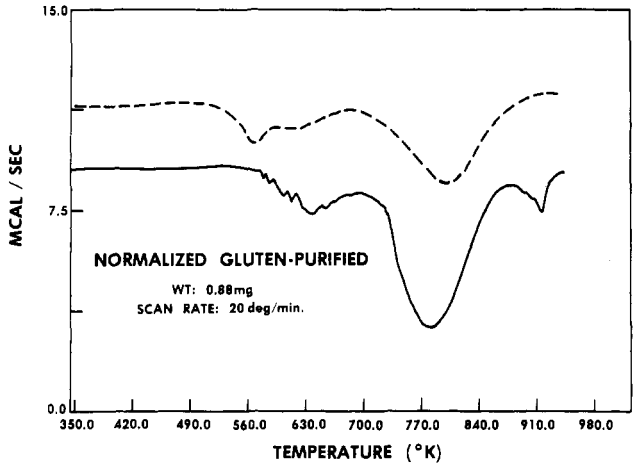

Figure 3. Differential scanning calorimetry curves of lyophilized gum gluten (broken) and purified gluten (unbroken line).

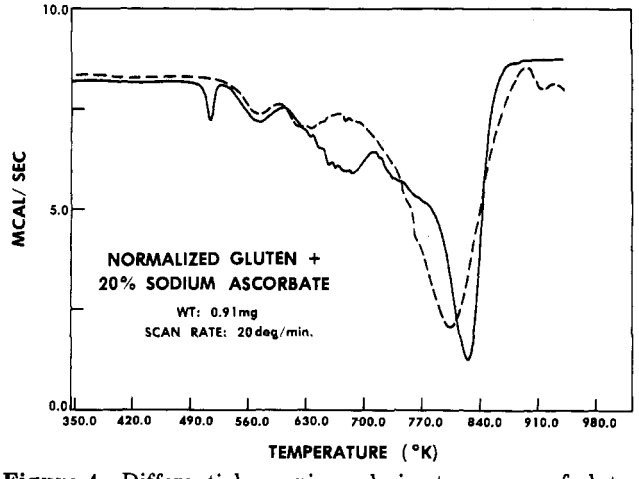

Figure 4. Differential scanning calorimetry curves of gluten (broken) and gluten–sodium ascorbate mixture (unbroken line).

undergo intense exothermic reactions at 200–250 °C (ΔH = −130 to +160 cal/g). These reactions have been attributed to oxidation of polysaccharides without involvement of protein (Raemy and Lambelet, 1982). Our results

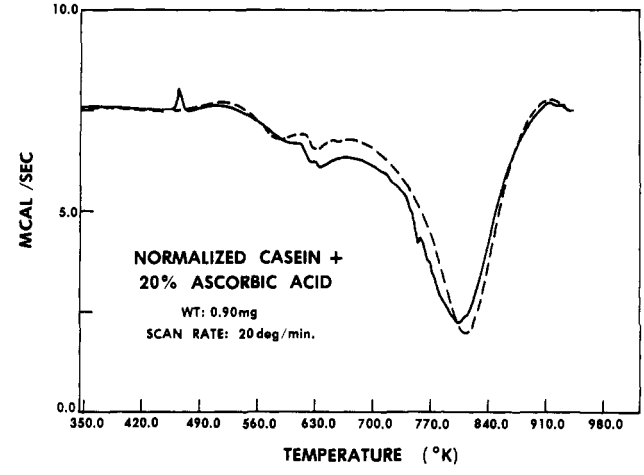

Figure 5. Differential scanning calorimetry curves of casein (broken) and casein–ascorbic acid mixture (unbroken line).

show that protein also is oxidized when more oxygen is available and volatile breakdown products are formed.

The two exothermic peaks in the DSC curves of gluten and its gluten–ascorbic acid mixtures also were found with the gluten–carbohydrate blends previously studied (Ziderman et al., 1987). The graphs resemble the biphasic kinetic curve of internal sample temperature during baking of these materials (Ziderman and Friedman, 1985). The higher temperature peak was stronger in these doublets. By contrast, the lower temperature peak was stronger for the baking kinetic curves for sodium ascorbate and gluten–sodium ascorbate mixtures (Figure 9).

Since blending with ascorbic acid probably did not alter the DSC curves of gluten, casein, or soybean protein, new chemical reactions did not occur between the proteins and ascorbic acid. Ascorbic acid thus behaved in the same way as sugar and polysaccharides in their thermal interaction with gluten (Ziderman et al., 1987). Since the thermochemical behavior of gluten mixtures with ascorbic acid or carbohydrates during baking did not correlate with the DSC curve data of the mixtures, their synergistic baking interactions may not involve chemical reaction of protein with ascorbic acid or carbohydrate (Ziderman et al., 1987). It was suggested that the high heat of combus-

Table IV. DSC Data for Casein and Mixtures with Vitamin C

figure	sample	parameter[a]	peak I	peak II	peak III	total combustion
5	casein	temp range, °C		254–418	399–642	242–647
		peak temp, °C		333	541	541
		$(\Delta H)_2$, cal/g		−139	−1478	−2275
		SD		±6	0	±40
	casein + 1% ascorbic acid	temp range, °C		262–418	399–642	247–645
		peak temp, °C		360	544	544
		$(\Delta H)_2$, cal/g		−130	−1418	−2185
		SD		±3	±6	±3
5	casein + 20% ascorbic acid	temp range, °C	181–207	258–420	395–642	234–645
		peak temp, °C	194	364	534	533
		$(\Delta H)_3$, cal/g	+9.12	−123	−1354	−2185
		SD	±0.57	±23	±135	±199
	casein + 1% sodium ascorbate	temp range, °C		255–421	399–641	236–647
		peak temp, °C		362	540	542
		$(\Delta H)_2$, cal/g		−155	−1368	−2178
		SD		±5	±5	±19
6	casein + 20% sodium ascorbate	temp range, °C	224–260	260–450	447–664	218–664
		peak temp, °C	242	412	556	555
		$(\Delta H)_2$, cal/g	−25.2	−307	−1344	−2425
		SD	±1.2	±20	±26	±9

[a] $(\Delta H)_n$ = average value from n number of runs.

Table V. DSC Data for Soybean Protein and Mixtures with Vitamin C

figure	sample	parameter[a]	peak I	peak II	peak III	peak IV	total combustion
7	soy protein	temp range, °C		218–428	408–536	541–639	200–642
		peak temp, °C		363	490	605	364
		$(\Delta H)_2$, cal/g		−823	−413	−381	−2629
		SD		±4	±9	±1	±8
	soy protein + 1% ascorbic acid	temp range, °C		217–428	408–537	541–644	203–645
		peak temp, °C		362	490	617	616
		$(\Delta H)_2$, cal/g		−811	−414	−375	−2601
		SD		±4	±8	±1	±23
7	soy protein + 20% ascorbic acid	temp range, °C	161–200	223–427	404–537	545–641	212–641
		peak temp, °C	186	359	490	617	360
		$(\Delta H)_2$, cal/g	+10.8	−688	−461	−334	−2533
		SD	±1.1	±12	±5	±10	±13
	soy protein + 1% sodium ascorbate	temp range, °C		221–430	413–536	542–643	190–651
		peak temp, °C		359	485	617	618
		$(\Delta H)_2$, cal/g		−828	−352	−376	−2606
		SD		±14	±20	±2	±41
8	soy protein + 20% sodium ascorbate	temp range, °C	227–259	254–396	393–438	438–578	197–591
		peak temp, °C	241	357	415	531	530
		$(\Delta H)_2$, cal/g	−22.4	−524	−58.0	−583	−2566
		SD	±3	±5	±1.5	±24	±54

[a] $(\Delta H)_n$ = average value from n number of runs.

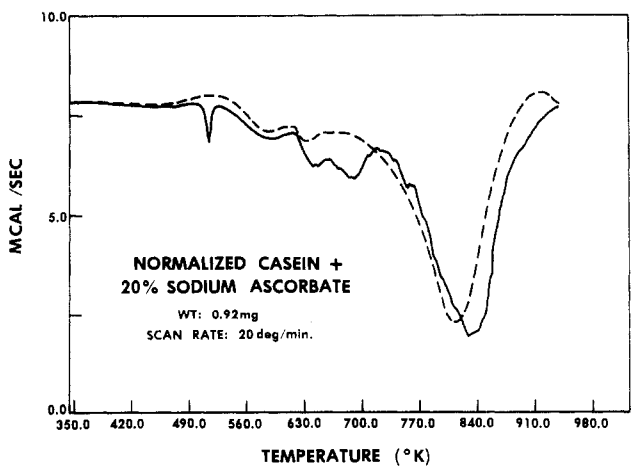

Figure 6. Differential scanning calorimetry curves of casein (broken) and casein–sodium ascorbate mixture (unbroken line).

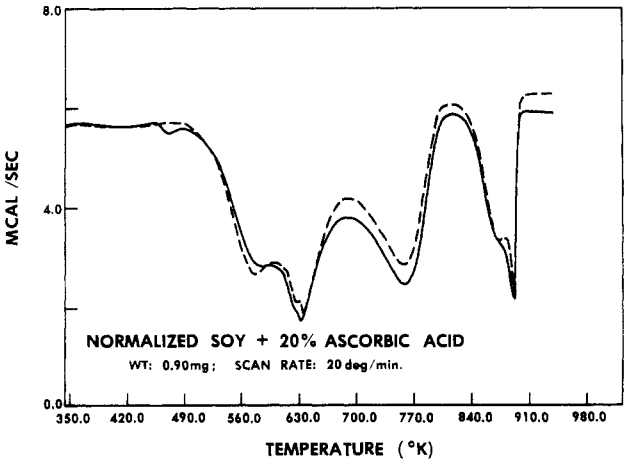

Figure 7. Differential scanning calorimetry curves of soybean protein (broken) and soybean protein–ascorbic acid mixture (unbroken line).

tion of pure carbohydrates in their 200–400 °C exotherm might tend to elevate the internal temperature of the mixture so as to aggravate protein decomposition. However, the lack of a quantitative correlation between enthalpy and self-heating indicates that kinetic rather than thermodynamic control is more likely to be involved in the mechanism of self-heating.

Spontaneous heating of dried foodstuffs will occur when the rate of heat generation in exothermic oxidation exceeds

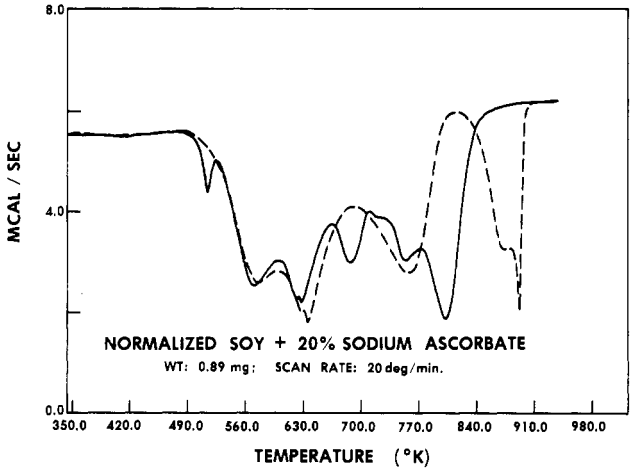

Figure 8. Differential scanning calorimetry curves of soybean protein (broken) and soybean protein–sodium ascorbate mixture (unbroken line).

Table VI. Amino Acid Composition (Grams/16 g of N) of Gluten–Sodium Ascorbate Mixture (4:1, 200 g) Baked at 215 °C for 72 min

		heated gluten–sodium ascorbate	
parameter	unheated gluten[b]	forced-draft oven[c]	vacuum oven
peak temp, °C		250	223
wt loss,[a] %		20	16
moisture content,[a] %	9.2	2.0	4.9
N content,[a] %	12.5	10.9	9.6
amino acid			
Asp	3.68	1.94	2.77
Thr	2.55	0.00	1.10
Ser	4.74	0.13	2.06
Glu	35.22	30.68	40.10
Pro	12.11	10.94	13.44
Gly	3.46	2.86	3.54
Ala	2.72	2.63	3.11
Val	4.25	3.50	4.25
Met	1.87	0.73	1.50
Ile	3.72	3.06	4.24
Leu	7.20	6.10	8.15
Tyr	3.45	2.68	3.41
Phe	5.18	4.46	5.42
His	2.15	0.92	1.51
Lys	1.81	0.55	0.81
Arg	3.69	0.22	1.32
total	97.80	71.40	95.89

[a] Air-dry basis. [b] From Ziderman and Friedman (1985). This and a related paper (Friedman et al., 1988) list extensive amino acid analysis data for wheat gluten heated at various temperatures.

the rate of heat loss to the environment in cooling (Raemy et al., 1982; Bilbao et al., 1987). The existence of a threshold ambient temperature (ca. 200 °C) below which self-heating does not occur (Table I) is explained by the differing temperature dependence of the two rates. The rate of heat generation increases exponentially (Arrhenius) and is initially greater than heat loss. At ambient temperatures below the threshold, as the internal temperature rises, the rate of heat loss will increase linearly at a faster rate until a stable thermal equilibrium is established (Semenov, 1959; Fardell and Lukas, 1987). Above the critical ambient temperature, however, the rate of heat generation is always higher than the rate of heat loss, and self-heating will occur. This hypothesis for explaining spontaneous heating at high oven temperatures (Table I) is supported by the lack of decomposition of small samples (Table VII) that can generate less heat than larger samples and therefore would require correspondingly higher temperatures for initiation of self-heating.

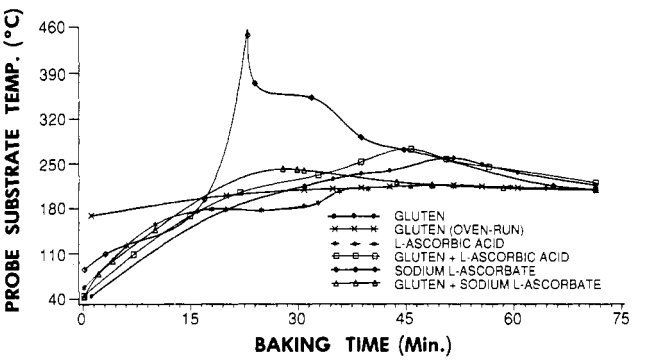

Figure 9. Temperature kinetics during baking (forced convection oven) of gluten, sodium ascorbate, ascorbic acid, and their blends (20%) with gluten.

Comparison of the changes in amino acid composition of baked gluten-sodium ascorbate mixtures (Table VI) with the same data for gluten baked in the absence of sodium ascorbate (Ziderman and Friedman, 1985) shows differences in protein breakdown, as do the analyses for gluten, soy protein, and casein heated at various temperatures with and without sodium ascorbate (Friedman et al., 1988). These differences are not uniform but are dependent on the protein used or the oven temperature, as is the apparent toxicity of the protein–sodium ascorbate heated products relative to the proteins heated in the absence of sodium ascorbate (Friedman et al., 1987). The experimental findings therefore indicate that the thermal reactivity of sodium ascorbate with proteins is specifically dependent on the unique structure and/or composition of each protein experiment.

The appearance of new exothermic peaks at ca. 413 and 550 °C in the DSC curves for protein–sodium ascorbate mixtures indicates that new chemical reactions occur between the ingredients. These reactions may form the toxicant(s) revealed in animal feeding studies (Friedman et al., 1987). Oxalate and carbonate, the major prod-

Table VII. Dependence of Weight Loss and Composition on Amount of Material Baked

material baked[a]	init wt, g	wt loss, %	N content,[c] %	protein content,[c,e] %
purified gluten protein[b]	4	13	15.6	69.5
gluten[d]	15	13	13.1	50.4
gluten[d]	80	10	nd	nd
gluten[d]	200	18	14.1	45.1
gluten[d] + cellulose (4:1)	4	11	10.6	45.2
gluten[d] + cellulose (4:1)	15	15	10.5	37.6
gluten[d] + cellulose (4:1)	60	8	10.2	42.9
gluten[d] + cellulose (4:1)	80	25	10.3; 12.9[f]	39.4; 8.8[f]
gluten[d] + cellulose (4:1)	200	48	13.0	2.4

[a] Baking at 215 °C for 72 min in air. [b] Lyophilized, initial hydrogen content 14.8%, initial protein content 79.2%. [c] Air-dry basis. [d] Commercial gluten. [e] From amino acid analysis, excluding cysteine and tryptophan. [f] After 72 min, this sample was only partly blackened by combustion; the two values refer, respectively, to uncombusted and combusted areas that were analyzed separately.

ucts of baking sodium ascorbate alone, are formed in very low yield in its protein mixtures. This difference, too, suggests that sodium ascorbate may react with proteins.

Rendleman (1986) has baked proteins and sugars (2-g samples) at 200 °C for 2.5 h and has found that the dark brown water-insoluble fraction of the products can complex polyvalent metal cations, suggesting that such complexation might play a role in reducing the uptake of metals through the intestinal wall and thus adversely affect food safety and nutrition (Pearce and Friedman, 1988).

CONCLUSIONS

During baking of bread, part of the dry weight becomes crust, which is heated above 100 °C and may undergo self-heating with concomitant nutritional damage to protein and possible formation of toxicants (Ziderman and Friedman, 1985; Friedman et al., 1987). In low-moisture products of baked flour mixtures, such as hard biscuits, the bulk behaves thermally in a similar fashion. Although levels of sodium ascorbate used in foods are generally lower than those used in our model crust-baking studies, it would, nevertheless, be prudent to use ascorbic acid rather than sodium ascorbate as a dough additive in bread baking to avoid potential antinutritional consequences of chemical reaction between gluten and sodium ascorbate.

In nonenzymatic browning of dried foods at ambient temperatures above their self-heating temperature, proteins and ascorbic acid/carbohydrates in admixture appear to decompose by air oxidation without reacting together chemically. In these circumstances amino–carbonyl interactions in the Maillard reaction would not account for browning. Sodium ascorbate alters the mechanism of protein breakdown.

Finally, Table VI shows a wide variation in the destruction of individual amino acids in baked gluten, depending apparently on the presence of functional groups in the side chain in the order threonine > serine > arginine > lysine > methionine > histidine. Thus, the amino acid composition might be a good indication of the stability of proteins to be added in food processing.

ACKNOWLEDGMENT

We gratefully acknowledge the able assistance of Amy T. Noma and William C. Montague, Jr., with the amino acid analyses and of Gary M. McDonald and Sylvia B. DeMesa with the chemical analyses.

Registry No. Ascorbic acid, 50-81-7; sodium ascorbate, 134-03-2; dehydroascorbic acid, 490-83-5; oxalate, 144-62-7; carbonate, 3812-32-6; bicarbonate, 71-52-3.

LITERATURE CITED

Bauernfeind, J. C.; Pinkert, D. M. Food Processing with Added Ascorbic Acid. *Adv. Food Res.* **1970**, *18*, 220–315.

Bilbao, R.; Aranzo, J.; Millera, A. Kinetics of Thermal Decomposition of Cellulose. Part II. Temperature Differences Between Gas and Solid at High Heating Rates. *Thermochim. Acta* **1987**, *120*, 133–141.

Biliarderis, C. G. Differential Scanning Calorimetry in Food Research—A Review. *Food Chem.* **1983**, *10*, 239–265.

Dollimore, D. The Thermal Decomposition of Oxalates. A Review. *Thermochim. Acta* **1987**, *177*, 331–363.

Emmerich, W.-D.; Hadrich, W.; Kaisersberger, E. Thermoanalytical Studies of Heat and Enzyme Treated Cereal Products. *Thermochim. Acta* **1985**, *72*, 45–60.

Fardell, P. J.; Lukas, C. Understanding Fire. *Chem. Brit.* **1987**, *23*, 221–224, 240.

Friedman, M. Chemicall Reactive and Unreactive Lysine as an Indes of Browning. *Diabetes* **1982**, *31* (Supplement 3), 5–14.

Friedman, M.; Gumbmann, M. R.; Ziderman, I. I. Nutritional Value and Safety in Mice of Proteins and Their Admixtures with Carbohydrates and Vitamin C After Heating. *J. Nutr.* **1987**, *117*, 508–518.

Friedman, M.; Gumbmann, M. R.; Ziderman, I. I. Nutritional and Toxicological Consequences of Browning During Simulated Crust-Baking. In *Protein Quality and the Effects of Processing*; Philips, M., Finley, J. W., Eds.; Dekker: New York, 1988; pp 189–217.

Gumbmann, M. R.; Friedman, M.; Smith G. A. The Nutritional Value and Digestibilities of Heat-damaged Casein and Casein-carbohydrate Mixtures. *Nutr. Rep. Int.* **1983**, *28*, 355–362.

Hargraves, W. A.; Pariza, M. W. Mutagens in Cooked Foods. *J. Environ. Sci. Health* **1984**, *C2*, 1–49.

Hodge, J. E. Chemistry of Browning Reactions in Model Systems. *J. Agric. Food Chem.* **1953**, *1*, 929–943.

Kirk-Othmer *Encyclopedia of Chemical Technology*, 3rd ed.; Wiley: New York, 1984; Vol. 24, p 14.

Mauron, J. The Maillard Reaction in Food: A Critical Review from the Nutritional Standpoint. *Prog. Food Nutr. Sci.* **1981**, *5*, 5–35.

Pearce, K. N.; Friedman, M. Binding of Copper(II) and Other Metal Ions by Lysinoalanine and Related Compounds and its Significance for Food Safety. *J. Agric. Food Chem.* **1988**, *36*, 707–717.

Raemy, A.; Lambelet, P. A Calorimetric Study of Self-Heating in Coffee and Chicory. *J. Food Technol.* **1982**, *17*, 451–460.

Raemy, A.; Hurrell, R. F.; Loliger, J. Thermal Behaviour of Milk Powders Studied by Differential Thermal Analysis and Heat Flow Calorimetry. *Thermochim. Acta* **1983**, *65*, 81–92.

Rendleman, J. A. Carbohydrate-Mineral Complexes in Foods. In *Interactions of Food Components*; Birch, G. G., Lindley, M. G., Eds.; Elsevier: London, 1986; pp 63–83.

Semenov, N. N. *Some Problems of Chemical Kinetics and Reactivity*; Pergamon Press: London, 1959; Vol. 2.

Smith, G. A.; Friedman, M. Effect of Carbohydrates and Heat on the Amino Acid Composition and Chemically Available Lysine Content of Casein. *J. Food Sci.* **1984**, *49*, 817–820, 843.

Taylor, R. T.; Fultz, E.; Shore, V. Mutagen Formation in a Model Beef Boiling System I. Conditions with a Soluble Beef-Derived Fraction. *J. Environ. Sci. Health* **1984**, *A19*, 791–817.

Taylor, R. T.; Fultz, E.; Knize, M. Mutagen Formation in a Model Beef Supernatant Fraction. IV. Properties of the System. *EHP, Environ. Health Perspect.* **1986**, *67*, 59–74.

Ziderman, I. I.; Friedman, M. Thermal and Compositional Changes of Dry Wheat Gluten-Carbohydrate Mixtures During Simulated Crust Baking. *J. Agric. Food Chem.* **1985**, *33*, 1096–1102.

Ziderman, I. I.; Gregorski, K. S.; Friedman, M. Thermal Analysis of Protein-Carbohydrate Mixtures in Oxygen. *Thermochim. Acta* **1987**, *114*, 109–114.

Ziderman, I. I.; Gumbmann, M. R.; Friedman, M. Thermally Induced Toxicity of Proteins and Their Non-Maillardian Browning with Carbohydrates. In *Progress in Diet and Nutrition*; Horowitz, C., Rosen, P., Eds.; Krager: Basel, 1988; pp 91–97.

Received for review October 4, 1988. Revised manuscript received May 5, 1989. Accepted May 31, 1989.