($k_1 + k_2$) of α and β were 2.3 times faster in pure water than in 50% (v/v) ethanol solution. Table III also shows that both k_1 and k_2 were less in 50% (v/v) ethanol than in water though k_1 was reduced in ethanol to only 41% of its value in water, k_2 was reduced to 46.5% of its value in water. Therefore it can be concluded that the smaller equilibrium constant for mutarotation of lactose in ethanol is due to a greater decrease in the change of α to β (k_1) than the reverse reaction (k_2). Consequently, less α is changed to β (by a smaller k_1) while more β is changed to α (by a relatively larger k_2), with the resulting increase in α overall, this yielding a smaller equilibrium constant ($K = k_1/k_2$).

ACKNOWLEDGMENT

We gratefully thank E. E. Moore for technical assistance and advice.

LITERATURE CITED

Bleyer, B., Schmidt, H., *Biochem. Z.* **141**, 278 (1923).
Gillis, J., *Recl. Trav. Chim. Pays-Bas* **39**, 88 (1920).
Haase, G., Nickerson, T. A., *J. Dairy Sci.* **49**, 127 (1966).
Herrington, B. L., *J. Dairy Sci.* **17**, 659 (1934a).
Herrington, B. L., *J. Dairy Sci.* **17**, 701 (1934b).
Hudson, C. S., *J. Am. Chem. Soc.* **26**, 1065 (1904).
Hudson, C. S., Yanovsky, E., *J. Am. Chem. Soc.* **39**, 1013 (1917).
Majd, F., Nickerson, T. A., *J. Dairy Sci.* **59**, 1025 (1976).
Nickerson, T. A., *J. Dairy Sci.* **39**, 1342 (1956).
Nickerson, T. A., Lactose. "Fundamentals of Dairy Chemistry", Webb, B. H., Johnson, A. H., Alford, J. A., Ed., Avi Publishing Co., Westport, Conn., 1974.
Parisi, P., *G. Chim. Ind. Appl.* **12**, 225 (1930).
Patel, K. N., Nickerson, T. A., *J. Dairy Sci.* **53**, 1654 (1970).
Richards, E. M., Faulkner, I. J., Lowry, T. M., *J. Chem. Soc.*, 1733 (1927).
Troy, H. C., Sharp, P. F., *J. Dairy Sci.* **13**, 140 (1930).

Received for review May 13, 1976. Accepted August 8, 1977. This investigation was supported in part by a grant-in-aid from Foremost-McKesson Foundation, Inc., San Francisco, Calif.

Fate of [^{14}C]Carbon Monoxide in Cooked or Stored Ground Beef Samples

Daniel A. Watts, Steven K. Wolfe,[1] and W. Duane Brown*

Lean ground beef samples were exposed to a 1% [^{14}C]carbon monoxide atmosphere for 3 days, resulting in about 30% saturation with CO of the total myoglobin content. Following such exposure, the samples were either stored or cooked for varying periods of time. Aqueous and fat extracts were made, and the amounts of radioactivity in these fractions, and in residual precipitate fractions, were determined. Activity in the aqueous fraction could be attributed entirely to carboxymyoglobin, and that in the lipid fraction was at the limit of detection and not significant. Less than 0.09 ppm ^{14}C as CO remained in precipitate fractions following cooking or bacterial spoilage, possibly in the form of denatured globin carbon monoxide hemochrome. In the various experimental samples, CO was lost during storage, with a half-life of about 3 days. The maximum loss from cooked patties was about 85%. The use of low levels of CO may prevent the discoloring noted when red meats are stored in elevated levels of CO_2.

It has long been known that carbon dioxide atmospheres inhibit microbial growth in meat (Haines, 1933). When beef is preserved by carbon dioxide, however, there may occur undesirable surface discoloration due to oxidation of oxymyoglobin (MbO_2) to metmyoglobin (MetMb). While reports vary to some degree, it appears that at levels of CO_2 lower than 20%, discoloration is not a major problem (Clark and Lentz, 1969, 1972, 1973). Others report that samples held in air had better color than those stored in 20 to 25% CO_2 (Huffman et al., 1975). A procedure in which a chemical system for continuous carbon dioxide generation was incorporated inside packages presented no problem with discoloration (Benedict et al., 1975). Elevated levels of oxygen in atmospheres containing high levels of CO_2 help prevent pigment changes (Clark and Lentz, 1973; Taylor and MacDougall, 1973).

However, at higher levels of CO_2 (30 to 60%) there is pronounced discoloration. Some workers have reported development of a greyish tinge which was not attributed to MetMb (Ledward, 1970; Ledward et al., 1971). More recently, the fairly rapid development of severe surface browning as a result of MetMb formation has been reported for beef samples held in elevated levels of CO_2 (Silliker et al., 1977). This undesirable oxidation could effectively be prevented by the incorporation of 1% carbon monoxide, due to the formation of carboxymyoglobin (MbCO), which is more stable in such atmospheres than MbO_2 (Wolfe et al., 1976). The beneficial effect of CO on color of refrigerated beef in different systems has been reported by others (El-Badawi et al., 1964; Besser and Kramer, 1972; Clark et al., 1976).

The present study, using radioactive CO, was designed to determine the extent and stability of the association of CO with beef during storage and cooking.

EXPERIMENTAL SECTION

Meat. Ground beef was chosen as a limiting case of high surface area for maximum CO incorporation, as well as bacterial loading, to enhance possible reactions. The leanest grade of ground beef was purchased at a local retail chain store. After thorough mixing of the meat, 50.0 ± 0.1 g patties (nominally 0.97 cm thick) were formed using a die and piston of 8.0 cm diameter. Avoiding all mutual

Institute of Marine Resources, Department of Food Science and Technology, University of California, Davis, California 95616.
[1] Present address: TransFRESH Corporation, Salinas, Calif. 93901.

0021-8561/78/1426-0210$01.00/0

contact, 15 patties were placed on three stainless steel mesh shelves (21 guage, 10 mesh) which were stacked into a 10 in. vacuum desiccator (8985 mL volume) equipped with a septum outlet. Three additional patties were lyophilized, then extracted with $CHCl_3$ in Soxhlet extractors, to determine moisture and fat content, consecutively.

Gas Exposure. In the nonlabeled experiments, the desiccator was evacuated to about 400 mmHg, immediately after which carbon monoxide (Matheson, C.P. grade) was introduced by syringe to establish a final 1.0% v/v atmosphere. The residual vacuum was then quickly relieved with compressed air (Liquid Carbonic). In the radioactive trial, [^{14}C]carbon monoxide (Amersham/Searle, 500 μCi, 59.3 mCi/mmol) was introduced by repeated air purging and evacuation of the vial containing the labeled gas into the desiccator. Sufficient nonactive CO to yield 1.0% total CO concentration was then added prior to relieving the residual vacuum as above. Accuracy of the atmospheres established in the absence of meat and [^{14}C]carbon monoxide, as well as the headspace composition after the meat exposure period, were determined by triplicate gas chromatographic analysis, using a Carle Model 8000 instrument equipped with a 2 cm^3 sample injection loop and calibrated with air or 2.09% CO in N_2 (Liquid Carbonic, analyzed grade). The gas exposure period was 72 h, in the dark, at 5.0 ± 1.5 °C.

Postexposure Storage. Following removal from the desiccator, all sample handling was done under low level illumination to minimize photodecomposition of MbCO. Three patties were quickly covered on a ceramic plate with one closely sealed layer of Saran film (known for its low gas permeability) and returned to storage, as above, for an additional 72 h before extraction.

Cooking. The remaining 12 samples were immediately cooked, individually, in succession, in 600-mL beakers preheated for 2.0 min on a hotplate maintained at 195 ± 5 °C throughout. Total cooking time was divided equally between the sides. Cooking was terminated quickly by transfer of the patty and exuded fluids to a blender cup containing cold buffer.

Extraction. The extraction method was based on that of Wolfe et al., (1978). Following 60-s blending at high speed in 80 mL of cold extraction buffer (pH 5.9 phosphate, I = 0.2), each sample was completely transferred with buffer to a tared polycarbonate bottle and centrifuged for 30 min at 2 °C and 14 000g. The layer of fatty material formed above the supernatant was collected separately prior to the crude filtration of the first buffer supernatant through glass wool to remove residual fatty material.

The fatty material (2–5 g) from each sample was thoroughly macerated in 10–20 mL of $CHCl_3$ and centrifuged briefly at 2000g to break the resulting emulsion. Aliquots (5 mL) of the clear lower, chloroform layer were dried in tared scintillation vials, under a stream of N_2 at 35 °C, followed by several minutes under high vacuum, at room temperature.

The volume of the filtered buffer extract was noted prior to recentrifugation of a 20-mL aliquot for 10 min at 2 °C and 37 000g to clarify the solution for spectral and scintillation measurements. Four consecutive extractions of two raw samples established by extrapolation that about 84% of the soluble myoglobin was recovered in the first supernatant.

The insoluble precipitate was thoroughly resuspended in buffer and extracted as above four additional times. Fatty material and supernatant were removed at each step and discarded. About 0.8% of the soluble myoglobin remained after this procedure as determined experimentally. Final precipitate weight was recorded.

Spectrophotometry. Aliquots of the buffer extract were scanned from 700 to 450 nm vs. buffer in a Cary Model 11 double-beam spectrophotometer. The sample cell solution was then saturated with CO and rescanned. Data from these two scans allowed estimation of the total myoglobin (Mb) and the fractions metmyoglobin (MetMb), MbCO, and oxymyoglobin (MbO_2) + deoxymyoglobin (Mb) (Wolfe et al., 1978).

Scintillation Counting. Dry fat samples ranging from 0.21 to 0.70 g were dissolved in 10.0 mL of toluene (Fisher, A.R.) containing 6.0 g of 2,5-diphenyloxazole (PPO, Beckman No. 161692) per 1000 mL of solvent.

Supernatant samples (0.5 mL) were injected through Teflon-lined septa (Supelco, Bellefonte, Pa.) deep into scintillation vials totally filled with dioxane (Mallinckrodt, A.R.) containing 6.0 g of PPO and 60.0 g of Cab-O-Sil (Packard No. 6003008) per 1000 mL of solvent. A second needle, just penetrating the septum, was used as a vent. The sample precipitated and dispersed evenly on shaking.

After thorough mixing, precipitate samples (0.24–0.29 g) were incubated with 1.5 mL of NCS tissue solubilizer (Amersham/Searle No. 190620) at 47 °C until dissolution occured (12–24 h) in vials with polyethylene-lined caps (Amershan/Searle No. 003367). After cooling, 18.5 mL of toluene cocktail (vide supra) was added. Cooked samples yielded colored solutions after slower dissolution.

The samples were counted at room temperature in a Beckman Model CPM-100 liquid scintillation counter, using a factory preset energy discriminator for ^{3}H + ^{14}C; that is, a normal ^{14}C upper energy limit and no lower limit. Background was 35.9 ± 1.2 cpm. Standard [^{14}C]toluene was counted with 94.8 ± 0.8% efficiency. All samples were counted (for 20 min or 2 × 10^4 gross counts) repeatedly in sets to access fluorescent drift or gas leakage. During each count, for the least active samples, at least 200 net counts were observed for fat samples, 1300 for precipitate samples, and 10 200 for Mb samples.

Quench and Fluorescence Correction. Internal standards were used for quench correction. [^{14}C]Toluene (Amersham/Searle No. 188270) was used in the fat and precipitate samples. With the aqueous samples, aliquots (0.1 mL) of a large volume (4200 mL) of [^{14}C]carbon monoxide (~0.4% CO in air) were injected into the scintillation vials. The activity of this internal standard CO was approximated by injection of aliquots of the same gas mixture into inverted vials which had been totally filled with helium bubbled toluene cocktail corrected for quench with [^{14}C]toluene. The determined activity per milliliter was found to be independent of the injected volume over the range tested (0.1–0.5 mL).

Only the precipitate samples exhibited elevated or drifting background counts in nonradioactive samples. Cooking worsened both. A correction curve was prepared from the nonradioactive samples' apparent background activities, after stabilization, vs. their external standard readings.

RESULTS AND DISCUSSION

Initially, the ground beef used in the radioactive trial contained 64.8 ± 0.3% w/w water and 16.0 ± 0.3% w/w $CHCl_3$ extractable fat. Density of the patties was determined to be 1.03 g/mL by water displacement.

Gas chromatographic analyses of five initial atmospheres yielded initial CO concentrations of 0.98 to 1.02% v/v after a calibration curve was used to determine the volume of CO to be injected. After 72 h meat exposure, as above, two trials yielded a final oxygen concentration of about 83% of that in air with much variability. Final CO concen-

Table I. Distribution of Labeled CO in Various Tissue Fractions

Cooking time, min	Sample	Activity, dpm/patty[a] $CHCl_3$ soluble[b]	Insoluble	Buffer soluble	Initial activity remaining, %[c]	Immobilized CO, ppb, w/w[d]
0	A	2.6×10^2	2.6×10^3	11.7×10^5	100	7
	B	3.4	0.5	10.8		2
1	A	2.6	1.3	5.8	51	4
	B	2.9	2.2	6.6	58	6
2	A	3.4	5.9	8.7	77	15
	B	2.9	9.8	9.4	84	25
4	A	3.2	9.4	4.2	38	24
	B	3.4	6.7	4.2	37	17
6	A	3.7	13.1	3.4	31	33
	B	4.4	16.7	2.7	26	42
8	A	4.4	24.4	1.5	15	60
	B	3.3	30.8	1.3	14	76
			Stored Samples Uncooked			
0	A	3.4	13.1	6.0	54	33
	B	3.5	5.9	5.6	50	15
	C	2.2	5.3	4.7	42	13

[a] Total radioactivity in each type of fraction is presented for each of two samples per cooking time. Similar data and results for the three stored raw samples are given in the bottom rows. [b] Calculated from the scintillation sample weight and the total determined fat weight per patty. [c] Column 6 presents the total activity in all fractions at each cooking time divided by the average total activity in all fractions before cooking. [d] Column 7 presents the ppb of CO, equivalent to the total activity found in $CHCl_3$ and precipitate fractions. The specific activity of ^{14}CO of 0.104 μCi/μm, as measured from uncooked samples, was used in this calculation rather than the calculated value in the text.

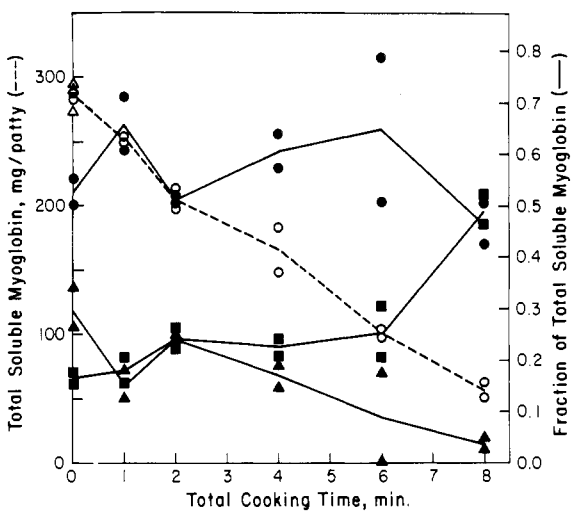

Figure 1. Spectral results showing total soluble myoglobin of all forms in raw and cooked (○) or stored (△) samples and fractions of the total which are metmyoglobin (■), carboxymyoglobin (▲), or oxy- plus deoxymyoglobin (●).

trations in these trials were 0.92 and 0.93 ± 0.02% v/v. Spectral methods showed a total initial amount of MbCO of 73 μmol.

Cooked Samples. Figure 1 displays the spectrophotometric results of the radioactive trial. Visually, 1 min of cooking resulted in lightly browned exteriors and raw interiors, while 8 min cooking heavily browned the exteriors and yielded samples well cooked throughout. The total extractable myoglobin decreased uniformly with cooking, while the MbO_2 plus Mb fraction remained relatively constant. There was a gradual formation of MetMb. Most notable was the decrease in extractable MbCO following cooking. The sum of these two fractions (MetMb and MbCO) remained nearly constant throughout the first 6 min of cooking. Similar behavior has been observed during several days refrigerated storage in air after CO exposure (Gee and Brown, 1978). The uncooked CO saturation of about 30% was approximately half that expected for a similar myoglobin solution, based on reported partition constants of CO and O_2 for several species

Table II. Total Buffer Soluble ^{14}C Activity, dpm/mg of MbCO, in Ground Meat Samples Treated with [^{14}C]CO

Total cooking time, min	Sample	Cooked samples, dpm/mg of MbCO	Stored uncooked samples, dpm/mg of MbCO
0	A	11 900	
	B	14 300	
1	A	17 800	
	B	14 800	
2	A	17 200	
	B	18 300	
4	A	14 900	
	B	15 600	
		Stored Samples	
0	A		8 300
	B		14 500
	C		17 100

(Antonini, 1971). Thus, a 1 cm thickness of ground meat apparently is not totally penetrated in 3 days from a 1% CO atmosphere.

Estimation of the total radioactivity absorbed by the uncooked samples (Table I) yielded 7.6 μCi, or 51 μmol of CO (sp act. of CO was 0.148 μCi/μm, calculated). The discrepancy between this and the spectrophotometrically determined value is believed to be due to inaccuracy in the calculated specific activity. The total ^{14}C associated with the cooked samples decreased about 85% during 8 min of cooking (Table I, column 6), agreeing with the spectral loss of MbCO.

The activity associated with the lipid material showed no systematic change due to cooking. The levels were very low, barely 1 ppb fixation, at or near the experimental error limitation and therefore not significant.

The data in Table II suggest that all remaining water-soluble activity can be attributed to MbCO inasmuch as the ratio of activity to MbCO concentration appears to remain constant. Data are given through 4-min cooking time only as the accuracy of the spectral determination decreases with decreasing myoglobin concentration and later values become erratic.

Larger amounts of activity were found in the precipitate samples. However, fluorescent backgrounds were also high

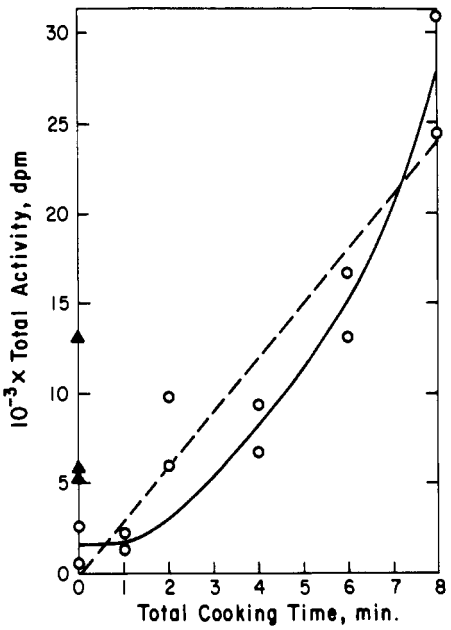

Figure 2. Total radioactivity in the precipitate fraction of each ground beef patty which had been cooked as indicated (O) or stored uncooked after exposure (▲). Curved sample average line ignores the 2-min samples. Straight line is a regression using all points. The data do not distinquish between exponential and linear rates of incorporation.

(up to 4.5 times instrument background) and introduce the largest uncertainty in these results. The extremely low activities found in precipitate and $CHCl_3$ fractions of uncooked samples indicate no significant CO fixation during 3 days of exposure to the gas (zero time samples, Table I, column 7).

In the precipitate samples, the total amounts of CO fixed during 8 min of cooking are below 0.08 ppm (Figure 2). At this point (i.e., 8 min), the total amount of CO remaining that could be fixed is only four to six times that already fixed. Should this happen, it would result in a maximum possible level of fixation on the order of 0.5 ppm as a limiting yield. It has been shown by Tappel (1957) that CO will react with myoglobin in cooked meat, giving a product identified by reflectance spectrophotometry as denatured globin carbon monoxide hemochrome. Ordinarily myoglobin is quite resistant to heat denaturation; however, Bernofsky et al. (1959) have shown that denaturation of myoglobin in meat occurs at lower temperatures than in pure solutions of the pigment. It is also known that fully cooked turkeys will sometimes turn pink, particularly if they have been cooked in gas ovens. One reason for this has been shown to be due to reaction with CO in the atmosphere of the oven (Pool, 1956). It is also known that there can be metabolic production of CO (e.g., Ludwig et al., 1957). Figure 3 shows a linear relationship, within experimental error, between the activity and amount of denatured myoglobin contained in the precipitate samples. Thus, it is reasonable to conclude that the trace amounts of [^{14}C]CO remaining in the precipitate fraction may be attributed to denatured globin carbon monoxide hemochrome.

Stored Samples. The three stored samples lost their characteristic MbCO color after 48 h of storage. When extracted, they were noticeably brown, slimy, and gelatinous on blending, indicating that gross bacterial spoilage had occured. The total soluble myoglobin was unchanged (Figure 1). However, the total activity of CO associated with the meat had dropped by half (Table I, column 6), agreeing with spectral data (fraction MbCO = 16 ± 8%).

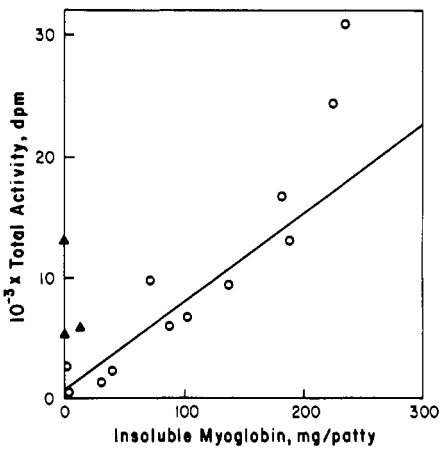

Figure 3. Total radioactivity in the precipitate fraction of each ground beef patty related to the amount of denatured myoglobin in cooked (O) or stored samples (▲). Line is the linear regression excluding the stored and two most active samples.

The values in Table II for the stored samples again indicate no ^{14}C remained in the buffer soluble fraction that was not associated with myoglobin. Table I indicates that levels of ^{14}C in the lipid fraction of stored samples were similar to those in all other samples, i.e., extremely low. In these organoleptically unacceptable samples, ^{14}C remaining in the precipitate fractions was half or less of that found in cooked samples (Table I, Figure 2).

CONCLUSIONS

In summary, less than 0.09 ppm ^{14}C as CO remained in precipitate fractions of samples following cooking or bacterial spoilage of ground meat patties previously exposed to 1.0% [^{14}C]CO for 3 days. Amounts of ^{14}C found in lipid fractions ($CHCl_3$ soluble) were at the level of experimental error and therefore not considered significant. Water-soluble ^{14}C activity could be attributed entirely to MbCO. Carbon monoxide absorbed by ground meat is lost during air storage under the conditions used herein, with a half-life of about 3 days, or during cooking. Maximum loss during cooking was about 85%.

LITERATURE CITED

Antonini, E., Brunori, M., "Hemoglobin and Myoglobin in Their Reactions with Ligands", North-Holland Publishing Co., Amsterdam, 1971, p 225.
Benedict, R. C., Strange, E. D., Palumbo, S., Swift, C. E., *J. Agric. Food Chem.* **23**, 1208 (1975).
Bernofsky, C., Fox, J. B., Jr., Schweigert, B. S., *Food Res.* **24**, 339 (1959).
Besser, T., Kramer, A., *J. Food Sci.* **37**, 820 (1972).
Clark, D. S., Lentz, C. P., *Can. Inst. Food Technol. J.* **2**, 72 (1969).
Clark, D. S., Lentz, C. P., *Can. Inst. Food Sci. Technol. J.* **5**, 175 (1972).
Clark, D. S., Lentz, C. P., *J. Inst. Can. Sci. Technol. Aliment.* **6**, 194 (1973).
Clark, D. S., Lentz, C. P., Roth, L. A., *Can. Inst. Food Sci. Technol J.* **9**, 114 (1976).
El-Badawi, A. A., Cain, R. F., Samuels, C. E., Anglemeier, A. F., *Food Technol.* **18**, 159 (1964).
Gee, D. L., Brown, W. D., *J. Agric. Food Chem.* **26**, 000 (1978).
Haines, R. B., *J. Soc. Chem. Ind., London* **52**, 13T (1933).
Huffman, D. L., Davis, K. A., Marple, D. N., McGuire, J. A., *J. Food Sci.* **40**, 1229 (1975).
Ledward, D. A., *J. Food Sci.* **35**, 33 (1970).
Ledward, D. A., Nicol, D. J., Shaw, M. K., *Food Technol. Aust.* **23**, 30 (1971).
Ludwig, G. D., Blakemore, W. S., Drabkin, D. L., *Biochem. J.* **66**, 38P (1957).
Pool, M. F., *Turkey World* **31**(1), 68 (1956).
Tappel, A. L., *Food Res.* **22**, 404 (1957).

Taylor, A. A., MacDougall, D. B., *J. Food Technol.* 8, 453 (1973).
Silliker, J. H., Woodruff, R. E., Lugg, J. R., Wolfe, S. K., Brown, W. D., *Meat Sci.*, 1, 195 (1977).
Wolfe, S. K., Brown, W. D., Silliker, J. H., Proceedings of the Meat Industry Research Conference, American Meat Institute Foundation, Chicago, Ill., 1976..
Wolfe, S. K., Watts, D. A., Brown, W. D., *J. Agric. Food Chem.* **26**, 000 (1978).

Received for review October 11, 1976. Accepted August 20, 1977. This work was supported by a Grant-in-Aid from the TransFRESH Corporation, Salinas, Calif.

Interactions of Globular Protein with Simple Polyphenols

Navin J. Neucere,* Thomas J. Jacks, and Gene Sumrell

Isolated arachin (the major protein in seeds of the genus *Arachis*) was exposed to concentrations of catechol and pyrogallol ranging from 0.05 to 0.3 M. After removal of these phenols by dialysis, conformational modes of arachin, determined by circular dichroism, indicated that native arachin increased in α-helical content. Exposure to high concentrations of catechol and pyrogallol also changed the antigenic reaction of native arachin with antiarachin from one of identity to one of partial identity. In vitro pepsin hydrolysis showed that arachin exposed to 0.1 M catechol or pyrogallol was more susceptible to hydrolysis than was hydrolysis of native arachin. These data on a model system (arachin) describe the effects of phenols on protein digestibility, solubility, and conformation.

The interactions of phenols or their oxidation products with proteins have long been known. These interactions range from inhibition and activation of enzymes to the formation of certain tastes and flavors in foods (Bate-Smith, 1973; Goldstein and Swain, 1965). As pointed out by Van Sumere et al. (1975), the chemistry and biochemistry involved in these reactions are far from being understood. Some complex polyphenols such as tannins are present in many cereal foods, e.g., grain sorghum, and are reputed to inhibit protein digestibility (Nelson et al., 1975). Tannins are categorized into two groups—condensed and hydrolyzable—and according to Loomis and Battaile (1966), they react differently with polypeptides. The numerous possible reactions between phenols and proteins were reviewed by Van Sumere et al. (1975).

The present study relates to interactions in a model system between a purified protein and simple polyphenols that are basic units of hydrolyzable tannins (Loomis and Battaile, 1966). The analyses are intended to establish a correlation between molecular conformation of protein and susceptibility to enzyme hydrolysis (digestibility). The fairly well characterized peanut globular protein, arachin, was chosen as the model protein (Jacks et al., 1975; Shetty and Rao, 1976). Structural measurements were made with circular dichroic spectra and immunochemistry, and relative digestibilities were determined by pepsin hydrolysis in vitro.

EXPERIMENTAL SECTION

Treatment of Protein. Arachin (50 mg), isolated according to Neucere (1969), was dissolved in 4-mL portions of distilled water, pH 5.5, that contained from 0.05 to 0.3 M catechol or pyrogallol plus a control. Some precipitation was observed for all of the samples including the control. The control and the treated samples were then dialyzed (molecular weight cutoff 6000 to 8000) against phosphate buffer, pH 7.9, ionic strength 0.2, for 24 h at 5 °C to remove the phenols. After dialysis, all samples were centrifuged at 40000*g* for 20 min, yielding clear supernatants; these were used to assess differences in protein solubility and digestibility and for immunochemical determinations. For analysis by circular dichroism, separate samples were treated with 0.2 M aqueous catechol or pyrogallol and then dialyzed against distilled water instead of phosphate buffer because the phosphate ion interferes with the analytical procedure. These samples were subsequently freeze-dried for later spectral determinations with aqueous NaF as the optically clear solvent.

Enzymatic Digestion In vitro. Digestion of treated and untreated arachin was performed in duplicate according to Mauron (1971), with some modifications. Portions of 1.5 mL from the samples dialyzed against phosphate buffer that contained 18.75 mg of protein were made to 0.1 N H_2SO_4. Each sample was then treated with 5 mg of B grade pepsin (Calbiochem) and digested in closed test tubes at 37 °C for 24 h. After precipitation of undigested protein with 70% ethanol followed by centrifugation, the supernatants were analyzed for free amino groups.

Analytical Methods. For immunochemical analyses and assessment of solubility differences, protein contents were determined by the Kjeldahl method or by the method of Lowry et al. (1951). Semiquantitative analyses of treated and untreated arachin by electroimmunodiffusion were performed according to the method of Laurell (1966); sample wells contained 10.0 μg of protein for electrophoresis in agar that contained 1% antiserum against arachin. The antiserum was prepared by Antibodies Incorporated, Davis, Calif. Electrophoresis proceeded for 15 h at 150 V and 7.0 ma. Qualitative analyses of treated and untreated arachin were performed by double diffusion in agar according to Ouchterlony (1949). Free amino groups were determined according to Clark (1964) whereby glycine, expressed in mass units, was used as a standard. Blanks containing catechol or pyrogallol showed only trace absorbances. Circular dichroic spectra of freeze-dried protein samples dissolved in 0.3 M aqueous NaF were obtained with a Cary Model 60 spectropolarimeter equipped with a 6001 CD attachment. Amounts of α-helical, pleated sheet, and unordered conformational modes in each protein sample were calculated by com-

Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana 70179.