and Agriculture Organization of the United Nations, Rome, 1963.
Scott, J. L., Bulletin, Department of Agriculture Gold Coast Yearbook, pp 58–73 (1928); in "Processing of Raw Cocoa for the Market," Rohan, T. A., Food and Agriculture Organization of the United Nations, Rome, 1963.
Stokoe, W. N., *Biochem. J.* **22,** 80 (1928).
Theimer, O. F., *Int. Choc. Rev.* **13,** 122 (1958).
Wijngaarden van, D., Thyssen, L. A., Osinga, T. D., *Z. Lebensm. Unters. Forsch.* **137,** 171 (1968).
Wilcox, J. C., Nelson, W. O., Wood, W. A., *J. Dairy Sci.* **38,** 775 (1955).

Received for review November 20, 1972. Accepted April 12, 1973. Paper No. 3793 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, N. C. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named nor criticism of similar ones not mentioned.

Several Compounds in Golden Delicious Apples as Possible Parameters of Acceptability

Natalio Gorin

Golden Delicious apples stored in a controlled atmosphere at 4° were subjected to enzymatic analyses of soluble sugars (D-glucose, D-fructose, and sucrose) and L-malic acid, determination of protein patterns by disk electrophoresis, and a palatability test. The content of sucrose and L-malic acid decreased, whereas D-glucose and D-fructose remained constant during storage. The protein pattern varied during storage. The contents of sucrose and L-malic acid and protein patterns could be useful parameters of quality (acceptability for marketing).

The purpose of this work was to determine objective criteria (chemical or biochemical) for acceptability and quality of apples.

Golden Delicious apples stored in a controlled atmosphere have an attractive appearance when they reach the market but their aroma and taste are unacceptable. Therefore an attempt was made to find parameters related to good flavor of fruit.

This study was confined to the natural aging of the apples in storage. Problems related to physiological disorders and deterioration due to infection were ignored. Golden Delicious apple was shown to be a suitable variety for this type of research (Faust *et al.*, 1969; Knee, 1971).

Some parameters were studied during storage in a controlled atmosphere (CA) at 4°. D-Glucose, D-fructose, sucrose, and L-malic acid were estimated enzymatically. The changes in protein patterns were determined.

It is known that sugar and acid content are related to taste (Smock and Neubert, 1950a). The problem is that the content of sugars is strongly influenced by climatic and geographic conditions. This can be seen clearly from the work of Rotstein *et al.* (1969), Kvåle (1969), and Kenworthy and Harris (1963). The present study considers the changes in protein pattern in apples during storage. Apparently they are independent of the said factors (Adriaanse *et al.*, 1969) but are related to the genetic makeup of the apple.

MATERIALS AND METHODS

Golden Delicious apples (200 kg) of uniform color and size (70–75 mm) were purchased from a fruit grower in Puifluik (Netherlands) in 1971. They were stored at 4° in two tanks, each containing 100 kg under an atmosphere of 7–8% CO_2 and 3–4% O_2 (Stenvers, 1969, 1970). The equipment and conditions used (Figure 1) were as follows. Five boxes (length 56.5 cm, width 36.5 cm, height 30.5 cm), each containing 20 kg of apples, were placed in one tank which was hermetically closed with a round cover (RC) surrounded by a bicycle tire (BT). Once the tire had been inflated, nitrogen (10 l./min) was purged for 90 min through stopcock S_2. S_1 and orifices (OR) constituted the outlet. Afterwards S_1, S_2, and orifices OR were closed. When the CO_2, produced by respiration, had increased to the desired level of *ca.* 7–8% (after 1 week), the aquarium pump (P) was switched on in order to scrub CO_2. This gas was measured every 2 days with a Fyriter CO_2 Analyzer (Bacharach Instrument Co., Pittsburgh, Pa., USA). Oxygen was measured every 2 days with a Servomex Oxygen Analyser, Type OA 150 (Servomex Controls Ltd., Crowborough Sussex, England). If the concentration was lower than 3%, some orifices of OR were opened. The required concentration was attained within a day.

After storage for 0, 68, 103, 146, 195, and 216 days, respectively, 1-kg lots of sound samples were removed from the five boxes in each of the two tanks. Rotten fruit was discarded. Rotting had reached serious proportions (30%) after 7 months storage. Of the total mixed sample of 10 kg, 2 kg was used for estimating soluble sugars, L-malic acid, nitrogen, dry matter, and ash, 5 kg was for protein patterns, and 2–3 kg was for a palatability test.

Estimation of Soluble Sugars (D-Glucose, D-Fructose, Sucrose) and of L-Malic Acid. These substances were estimated enzymatically as described by Boehringer Mannheim GmbH (1971) with slight modifications. The estimates made with model systems (*i.e.*, the respective compounds dissolved in distilled water) proved satisfactory (coefficients of variability 2–6%). The recovery factors of these substances, added to apples at the start of the procedure described below, were 97–103% (Gorin, 1970, 1971).

Apples without pedicel (2000 g) were homogenized with distilled water (1000 g) in a large Waring Blendor for *ca.* 3–4 min at room temperature. The suspension was stirred at the lowest and medium blending settings. This constitutes *suspension WB*.

A sample of suspension WB (100 g) was promptly pasteurized at 81–82° for 4 min and immediately cooled by placing it at −12° for 25 min. Pasteurization avoided degradation of glucose (Table I) probably by bacterial contamination or conversion by enzymes naturally present in the apple. This preparation was then poured into a vol-

Sprenger Instituut, Wageningen, The Netherlands.

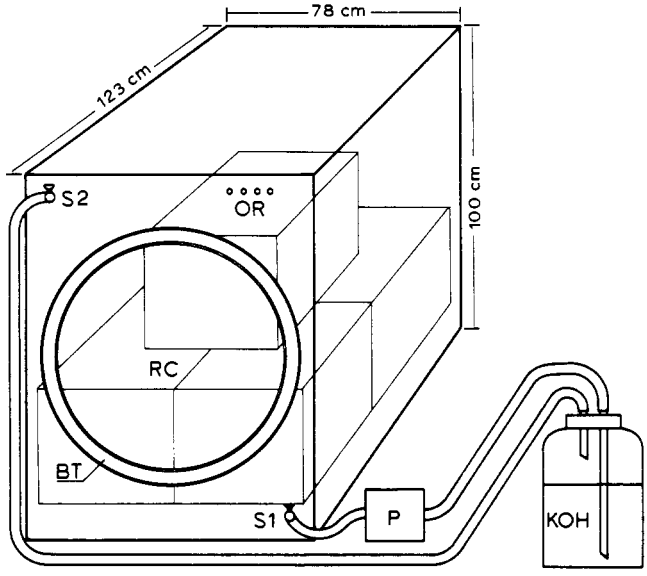

Figure 1. Equipment and conditions for the controlled atmosphere (CA) storage of Golden Delicious (Stenvers, 1972).

umetric flask and distilled water was added to a final volume of 250 ml. This suspension was filtered through double cheesecloth. The filtrate was centrifuged at 1600 × *g* for 30 min at room temperature. The supernatant was distributed between five flasks (capacity *ca.* 30 ml) and kept at −20°. Before analysis, the samples were allowed to reach room temperature. A portion of this solution diluted 50 times with distilled water was poured into a cuvette for spectrophotometric analysis for D-glucose and D-fructose (determined simultaneously) and sucrose. Another portion diluted ten times was used for analysis for L-malic acid.

The content of each flask (30 ml) was used for these analyses. Consequently, there were five replicates for each substance in every sampling.

Preliminary experiments showed that deproteinization (with 1 *M* perchloric acid, 1:1 v/v) of the apple extracts was not necessary. Likewise, homogenization of the apple, giving a homogenate of pH 7 or perfusion of whole apples with 5% $NaHCO_3$ (to attain pH 7), was not necessary to avoid any inversion of sucrose. This observation agrees indirectly with the work of Hanes and Kidd (1936).

Estimation of Dry Matter, Ash, and Nitrogen. Dry matter was determined by drying a sample of suspension WB at 105° for 4 hr. A second sample of suspension WB was ashed at 550° in the presence of $AlCl_3$ for 2–3 hr (Zonneveld and Gersons, 1966). The nitrogen in a third sample was estimated by the micro-Kjeldahl method.

Extraction and Electrophoresis of Proteins (Protein Patterns). Homogenization, preparation of "acetone powders," and extraction of proteins from the powders for disk electrophoresis were performed by Clements' method (1965) with the following modifications—the solids were washed twice (instead of once) with a mixture of acetone–ether (1:1, v/v) at −30° instead of −60°.

The powders were lyophilized after removing most of the mixture of acetone and ether. During this process, the temperature of the powders did not rise above 0°. The residual moisture in the lyophilized powders was 2.1–2.2%, as determined by drying at 40° for 24 hr with the "drying pistol" (under vacuum) containing P_2O_5. The lyophilized powder (168 g) was stored at 5°. The nitrogen content of the powders was between 1.00–1.17%. No corrections were made for the moisture contents.

Phosphate-buffer extracts contained 2.30–3.03 mg of protein/ml, which was estimated by a modified method of Lowry *et al.* (1951) as described by Bailey (1967). For disk

Table I. Content of D-Glucose (%) in Golden Delicious with or without Pasteurization of Its Extract

Storage of extract at 8°, days	Pasteurized extract[a]
0	1.4
10	1.4
	Unpasteurized extract[a]
0	1.8
5	1.3
7	1.0

[a] Average of triplicate determinations, for which values were similar.

electrophoresis 275 μl (*ca.* 0.750 mg of protein) was applied to the tubes. It was found that similar protein patterns were obtained for each sample when applying between 0.275 and 0.325 ml of phosphate solution. However, there was a difference in protein patterns of an extract made as soon as the powder was ready as compared with an extract of the same powder stored for 2.5 months at 5°. Perhaps the powder was not dry enough to prevent enzymatic changes during storage. Protein patterns presented in this article were based on the extracts made directly or 2–3 days after the powder was ready.

Disk electrophoresis was performed according to Ornstein and Davis (1962), Maurer (1968), and Clements (1965). The tubes (75 mm long, internal diameter 5 mm) were coated twice with a warm (50°) solution of 1% dimethyldichlorosilane in benzene. The tubes contained spacer and separating poly(acrylamide) gels. Sample gel was not prepared. The spacer was acrylamide in Tris-HCl buffer pH 6.9 (2 g/100 ml) and the separator in the said buffer was pH 8.9 (7.5 g/100 ml).

Electrophoresis was performed at constant current, 3 mA per tube, at 5° for *ca.* 2 hr. This was long enough for the bromphenol blue band to reach to 3 mm of the bottom.

Proteins were stained at room temperature for 2 hr with a solution of 0.5% Amido Black in 5% trichloracetic acid previously filtered through a fritted-glass filter G2. The excess of Amido Black was removed by diffusion in 5% acetic acid at room temperature for 48 hr.

The electropherograms were measured with the densitometer Vitatron TLD 100 at wavelength 555 nm and a diaphragm of 0.1 mm. Relative mobilities (R_m) of the bands were calculated in relation to bromphenol blue (R_m = 1.00). The results were based not only on the electropherograms but in addition, as a decisive factor, on observation of the gels by eye. This was done because sometimes a band is not registered as a clear peak. Moreover, for the results given here, only the distinct bands were considered.

Palatability Test. This was performed from a consumer's point of view by persons experienced in judging apple quality, which was rated as acceptable, just acceptable, and not acceptable.

Two judges regularly tasted the apples sampled after 0, 68, 103, 146, 195, and 216 days of storage. In addition, after 195 and 216 days, they were examined by four other judges. Each judge received six apples, *i.e.*, *ca.* 900 g.

RESULTS

Table II shows the effect of CA storage at 4° on the nitrogen, dry matter, ash, D-glucose, D-fructose, sucrose, and L-malic acid content of Golden Delicious apples. All values were corrected to initial fresh weight by assuming that after 150 days Golden Delicious lost 3% of its weight (Knee, 1971; Sprenger Instituut, 1972); *i.e.*, 0.6% per 30 days.

Table II. Some Parameters of Golden Delicious, Harvest 1971, during Storage in CA Atmosphere at 4°

Date of sampling	Storage in days	Dry matter,[a] %	Glucose			Fructose			Sucrose			L-Malic acid			Palatability test
			$\bar{x}$	s, ±	CV, ±	$\bar{x}$	s, ±	CV, ±	$\bar{x}$	s, ±	CV, ±	$\bar{x}$	s, ±	CV, ±	
Nov 11, 71	0		2.1	0.08	3.8	7.3	0.06	0.8	3.1	0.21	6.9	0.56	0.01	2.5	Acceptable
Jan 18, 72	68	14.16	2.4	0.14	5.9	7.1	0.18	2.5	1.8	0.15	8.6	0.47	0.01	2.5	Acceptable
Feb 22, 72	103	13.95	2.5	0.07	2.8	7.1	0.17	2.4	1.4	0.20	14.5	0.39	0.01	2.1	Acceptable
Apr 5, 72	146	13.65	2.5	0.04	1.7	7.1	0.15	2.1	1.2	0.12	9.9	0.37	0.01	3.5	Acceptable
May 24, 72	195	13.58	2.6	0.07	2.8	7.5	0.13	1.8	0.7	0.13	18.0	0.28	0.01	2.9	Just acceptable
June 14, 72	216	13.40	2.5	0.09	3.7	7.7	0.13	1.7	0.7	0.11	16.3	0.27	0.02	6.3	Not acceptable

All values have been corrected to initial fresh weight. Nitrogen (0.050–0.055%) and ash (0.30–0.32%) remained constant. [a] Average of duplicate determinations, for which values were similar. $\bar{x}$ = average of five replicates. Expressed as g/100 g apple. $s = (\epsilon d^2/n - 1)^{1/2}$. CV (%) = $s \cdot 100/\bar{x}$.

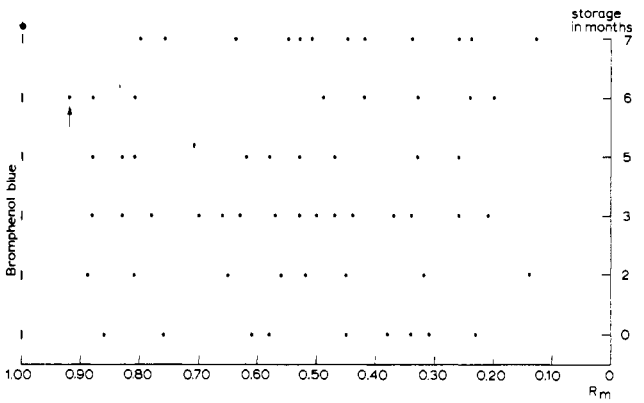

Figure 2. Protein patterns of apple after different times of storage.

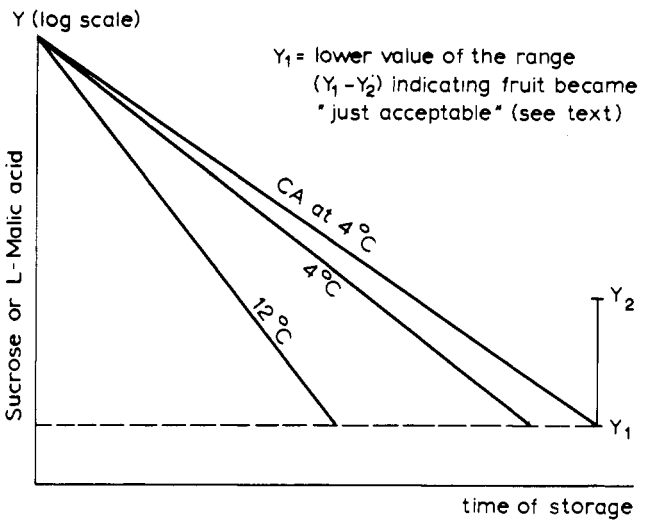

Figure 3. Hypothetical diagram for an index delimiting acceptability of Golden Delicious. If y_1 were known, the slope of the line (conditions of storage) would become unimportant.

The decrease in dry matter indicated the consumption of substances in the apple for production of CO_2 (respiration), ethylene, and other volatiles (Nursten, 1970). Total nitrogen and ash did not change.

The sucrose content decreased during storage, whereas glucose and fructose remained practically constant. L-Malic acid also decreased during storage.

The higher coefficients of variability for sucrose content after 195 and 216 days of storage may be explained by the high relative content of natural glucose to sucrose. Original glucose was not destroyed before the enzymatic analysis because the ratio of glucose to sucrose was lower than 5:1 (Boehringer Mannheim, 1971).

The coefficients of variability recorded in Table II show the reproducibility of the analytical method.

The protein patterns of apples after different times of storage are recorded in Figure 2. The marked points are the average of at least two bands (within 6%) obtained from duplicate experiments. An experiment means the performance of the whole procedure: homogenization, extraction, and electrophoresis.

For the palatability test, the ratings of the judges agreed in all cases.

DISCUSSION

The decrease in sucrose and L-malic acid with time was a straight line (based on data of Table II) when concentrations were plotted on a logarithmic scale: for sucrose $r = -0.992$ and for L-malic acid $r = -0.989$, both at a significance level of 99.9%. The fact that L-malic acid gave a straight line showed that the apples were not injured (Fidler, 1951).

Comparing the present results with those mentioned by Smock and Neubert (1950b), it was concluded that the Golden Delicious apple has a pattern of metabolism for soluble sugars and L-malic acid similar to other varieties. However, the detection of a point indicating the grade "just acceptable" was not easy, since the contents of sucrose and L-malic acid (and their quotient) differed from one harvest to another (Gorin, 1972). Therefore, it is difficult to use contents of sucrose and L-malic acid or their quotient as parameters of quality, even if the metabolites have been analyzed by a more specific method (enzymatic) than the traditional ones of Lane and Eytan (Fehlings solution) or of refractometry for sugars and titratable acidity for L-malic acid (Bergmeyer, 1965). Perhaps the solution would be to screen many samples over a period of several years in order to establish a range of values (maximum and minimum) for sucrose and L-malic acid. The lowest value could indicate the limit of acceptability of the fruit.

This would be comparable to the range of nicotinic acid (0.19–0.41 mg per 100 ml) for orange juices originating from Mediterranean countries (Goddijn, 1970; Sawyer, 1963). A content lower than 0.19 mg/100 ml indicates that the juice has been adulterated (Weits *et al.*, 1971).

Determination of such an index for apples would not necessitate any knowledge of the storage conditions of the apples before reaching the market (Figure 3).

Thiault (1970) established *via* total sugars (refractometry) and L-malic acid (titratable acidity) two limits for grading the Golden Delicious apple in France as "higher" and "favorable" qualities. A rough estimate showed that these limits can not be applied under our conditions.

Concerning the protein patterns, it may be inferred that, since the nitrogen content of the various powders did

not change, a breakdown and resynthesis of proteins occurs during storage. Breakdown and resynthesis of proteins have already been found by Pech *et al.* (1970) during storage of pears at 4°.

The band marked with an arrow (Figure 2, R_m 0.92) could indicate that the apple has reached the condition of "just acceptable." Further investigation is necessary to characterize this protein and to see whether its appearance can be used as a criterion for fruit quality.

ACKNOWLEDGMENT

I wish to thank Gerrit J. Mooi for his skillful work on the protein patterns, Hendrik Zonneveld for enzymatic analysis, Willem Klop for valuable discussions, Ricardus A. Hilhorst for statistical calculations, David Yarrow (Centraal Bureau voor Schimmelcultures, Delft), Gustaaf A. de Zoeten (University of Wisconsin, Madison, on sabbatical leave at the Department of Virology, Agricultural University, Wageningen) for reading the manuscript, and John Christopher Rigg (Pudoc, Wageningen) for English revision.

LITERATURE CITED

Adriaanse, A., Klop, W., Robbers, J. E., *J. Sci. Food Agr.* **20**, 647 (1969).
Bailey, J. L., "Techniques in Protein Chemistry," Second revised and expanded edition, Elsevier Publishing Company, Amsterdam, London, New York, 1967, pp 340–341.
Bergmeyer, H. U., "Methods of Enzymatic Analysis," Verlag-Chemie, GmbH, Weinheim/Bergstr., Academic Press, New York, London, 1965.
Boehringer Mannheim GmbH, Food Analysis, Glucose, Fructose, Sucrose, L-Malate, Western Germany, 1971.
Clements, R. L., *Anal. Biochem.* **13**, 390 (1965).
Faust, M., Shear, C. B., Williams, M. W., *Bot. Rev.* **35**, 168 (1969).
Fidler, J. C., *Exp. Bot.* **2**, 41 (1951).
Goddijn, J. P., *Voedingsmiddelentechnologie* **1**, 1 (1970).
Gorin, N., Annual Report of the Sprenger Instituut, Wageningen, 1970, p 86.
Gorin, N., Annual Report of the Sprenger Instituut, Wageningen, in press, 1971.
Gorin, N., Sprenger Instituut, Wageningen, unpublished data, 1972.
Hanes, C. S., Kidd, F., Report of the Food Investigation Board, Department of Scientific and Industrial Research, London, 1936, p 131.
Kenworthy, A. L., Harris, N., *Mich. Quart. Bull.* **46**, 293 (1963)
Knee, M., *J. Sci. Food Agr.* **22**, 368 (1971).
Kvåle, A., *Acta Agr. Scand.* **19**, 3 (1969).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Maurer, H. R., "Disk-Elektrophorese," Walter de Gruyter Co., Berlin, 1968.
Nursten, H. E., "The Biochemistry of Fruits and their Products," Vol. I, Academic Press, London and New York, 1970, pp 239–264.
Ornstein, L., Davis, B. J., "Disc Electrophoresis", Parts I, II, Preprinted by Distillation Products Industries, Rochester, N. Y., 1962.
Pech, J. C., Bardet, C., Fallot, *J., Physiol. Veg.* **8**, 461 (1970).
Rotstein, E., Namor, M. S. S., Sica, A. M., Aruannd, J., *J. Milk Food Technol.* **32**, 79 (1969).
Sawyer, R., *J. Sci. Food Agr.* **14**, 302 (1963).
Smock, R. M., Neubert, A. M., "Apples and Apple Products," Interscience, New York, London, 1950a, pp 171–172.
Smock, R. M., Neubert, A. M., "Apples and Apple Products," Interscience, New York, London, 1950b, p 106.
Sprenger Instituut, Wageningen, "Produktgegevens Groente en Fruit," mei 1972, 11.06, p 11.
Stenvers, N., Bull. no. 85, 92, Sprenger Instituut, Wageningen, 1969.
Stenvers, N., Bull. no. 102, Sprenger Instituut, Wageningen, 1970.
Stenvers, N., Sprenger Instituut, Wageningen, unpublished data, 1972.
Thiault, J., *Bull. Tech. Inform.* **248**, 191 (1970).
Weits, J., Ten Broeke, R., Goddijn, J. P., Gorin, N., Schütz, G. P., *Z. Lebensm. Unters. Forsch.* **146**, 335 (1971).
Zonneveld, H., Gersons, L., *Z. Lebensm. Unters. Forsch.* **131**, 205 (1966).

Received for review October 10, 1972. Accepted March 12, 1973.

Identification of Volatile Compounds from Heated L-Cysteine·HCl/D-Glucose

Richard A. Scanlan,* Stanley G. Kayser, Leonard M. Libbey, and Max E. Morgan

The volatile compounds produced in a heated L-cysteine·HCl/D-glucose system were collected on Porapak Q and transferred to a capillary column for separation. Identification of compounds was accomplished by tandem gas chromatography–mass spectrometry. The compounds identified included acyclic α-dicarbonyls, furan derivatives, aromatic and aliphatic monocarbonyls, and sulfur-containing heterocyclics.

In spite of the tremendous amount of work on the chemistry of nonenzymatic browning, relatively little has been reported concerning the volatiles produced from sulfur-containing amino acids in heated food systems. Kobayashi and Fujimaki (1965) found that boiling cysteine with α-diketones produced mercaptoacetaldehyde, hydrogen sulfide, and acetaldehyde and they discussed the route of formation of hydrogen sulfide. The pyrolysis of sulfur-containing amino acids was studied by Fujimaki *et al.* (1969) and these workers reported the formation of eight volatile compounds, including 2-methylthiazolidine, from pyrolyzed cysteine and cystine. The pyridoxal-catalyzed elimination of hydrogen sulfide or methyl mercaptan from L-cysteine, *S*-methyl-L-cysteine, and DL-methionine in the presence and absence of metal ions was quantitatively studied by Gruenwedel and Patnaik (1971).

Herz and Shallenberger (1960) described the aromas produced upon heating various amino acids with glucose. Arroyo and Lillard (1970) conducted odor evaluations and chemical analyses, and studied the effect of pH on the nonenzymatic browning reaction products formed from equimolar concentrations of glucose and the sulfur-containing amino acids. They concluded that none of the three mixtures studied emitted an odor associated with meat. The reaction products from the nonenzymatic browning of glucose and methionine were studied by Lindsay and Lau (1972) and they concluded that methional was responsible for the boiled potato-like aroma of the reaction mixture. Stoll *et al.* (1967) identified a number of aliphatic and heterocyclic sulfur-containing compounds in

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331.