Changes in the Volatile Profile of Peanuts and Their Relationship to Enzyme Activity Levels During Maturation

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The profiles of volatiles from uncured peanut kernels, sampled weekly from the 6th to the 13th after pegging, were determined using gas-liquid chromatography and mass spectrometry. Total volatile production of the kernels reached a maximum at 8 weeks, decreased rapidly to a near minimum value at 11 weeks, and showed a slight increase at 12 weeks. Observations beyond 12 weeks indicate that total production of volatiles gradually decreases. Five major volatile components were identified: acetaldehyde, methanol, pentane, ethanol, and hexanal. Traces of acetone and pentanal also were detected. Except for hexanal, which first appeared at 8 weeks, all major components were present throughout maturation. Possible relationships between alcohol dehydrogenase and lipoxidase activities and the fluctuations in acetaldehyde, ethanol, pentane, and hexanal during maturation are discussed.

The volatile profiles of mature fruits (Creveling *et al.*, 1968; Sevenants and Jennings, 1966; Jennings *et al.*, 1960; Murray *et al.*, 1968; Schultz *et al.*, 1967; Tang and Jennings, 1967) and seeds (Pattee *et al.*, 1969; Murray *et al.*, 1968; Bailey *et al.*, 1962; Rohan, 1967), and the effects of various processing and storage treatments on such volatiles (Luh *et al.*, 1955; Bengtsson and Bosund, 1964; Norman *et al.*, 1967) have been studied in great detail. However, there have been very few studies of volatile profiles during development and maturation of fruits and seeds (Bengtsson and Bosund, 1964; Heinz *et al.*, 1965).

Characterization of the volatile profile of uncured peanuts during maturation should aid in explaining the various physiological and biochemical changes that occur during this period. Many of these compounds are intermediates in the major metabolic pathways occurring in plants (Creveling *et al.*, 1968). Because bulk-harvested peanuts represent a cross section of several stages of maturity, the volatile profiles of uncured peanuts at different stages of maturity could provide important information which might be of value in defining the quality and flavor potential of raw peanuts.

This study was undertaken to determine the volatile compounds which are produced in peanuts during maturation and to begin elucidation of mechanisms which might account for the origin of some of these volatiles.

EXPERIMENTAL

Plant Material. Peanuts (Variety-NC-2) were grown during the summer of 1968 at Clayton, N.C. A sample was collected weekly commencing 6 weeks after the initial pegging date (estimated time the first group of pegs entered the soil) and continuing until the peanuts were mature at 12 or 13 weeks. A sample was also collected at 15 weeks to show the effects, if any, of over-maturation. Peanuts were hand-shelled and kernels were selected for uniformity of size and color of inner shell and seed cost as indicated (Table I). The time between harvest and placing the uncured peanuts under vacuum for volatile distillation was approximately 3 hr.

Preparation of Sample for Gas-Liquid Chromatography. Volatile components produced by peanuts during maturation

Table I. Peanut Characteristics Used to Estimate Kernel Age

Week	Shell	Kernel
4	Very watery, soft	Very small, flattened, completely white, mostly seed coat
5	Still soft, not as watery, inner shell fleshy—no cracks	Larger than 4 weeks, flat; white or maybe just turning pink at one end
6	Inner shell tissue beginning to show cracks	Torpedo shaped; generally pink at embryonic-axis end of kernel
7	Inner shell beginning "cottony" appearance	Torpedo to round shaped; embryonic axis end of kernel pink; other end white to light pink
8	Inner shell beginning to dry out—cracks more numerous	Round, light pink all over
9	Inner shell white but beginning to show brown splotches	Dark pink at embryonic axis end, light to dark pink elsewhere
10	Many dark brown splotches on inner shell	Large, generally dark pink all over; seed coat beginning to dry out
11	Inner shell almost completely brown	Dark pink, may show imprint of shell on seed coat in places; seed coat drying out
12	Black splotches appearing on inner shell	Same as 11
13	Black splotches over at least $\frac{1}{2}$ the shell	Seed coat beginning to turn brown
15	Black splotches throughout shell	Seed coat almost all brown, imprint of shell seen over large part of kernel

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were isolated by using a high-vacuum distillation technique with differential cryogenic trapping according to Pattee *et al.* (1969). The design of the liquid nitrogen traps was modified by adding vacuum stopcocks on both sides of the distillation head. This design of the traps prevented contamination or loss of volatile components, since the distillation head was not removed for further analysis. The coolant for the reflux condenser attached to the distilling pot was changed from tap water to ethylene glycol held at −20° C and was recirculated via a refrigerated bath. A mercury diffusion pump was added to the vacuum system.

Prior to introduction of the sample into the vacuum system, the system was evacuated and the vacuum removed by induction of nitrogen. This was done to prevent autoxidation and oxidative enzymes from influencing the peanut profile between the time of sample introduction and completion of distillation.

Blank runs using only nitrogen and distilled water indicated that there was no contamination or carry-over of volatiles between runs.

To prepare the sample for vacuum distillation, 200 g of peanuts of known kernel count were separated into 100 g lots and blended with two volumes of distilled water for 1 min. The slurry was then placed in a triple-neck distilling flask and subjected to a vacuum of 5×10^{-3} Torr for 3 hr. The temperature of the distilling flask was held at 25° C and constantly monitored.

The volatile components from the peanut slurry were condensed in a trap (−196° C) fitted with stopcocks on both sides and a rubber septum for sampling. After removal of the trap from the vacuum system, it was equilibrated in a water bath held at 70° C. The volatile components were separated on a Micro-Tek 2000 research gas chromatograph equipped with dual-flame ionization detectors. A 5-ml vapor sample taken from the equilibrated trap was used for the analysis. Peak areas were integrated using an Infotronics CRS-100 digital readout system, and identities of the compounds were confirmed by combined GLC-mass spectrometry on representative samples from this study.

The columns used to separate the volatile components were selected on the basis of differences in their degrees of polarity. The columns and conditions were as follows: A $^1/_8$-in. × 10 ft stainless steel column packed with 10% Carbowax 20M on 60–80 mesh Chromosorb G and operated isothermally at 60° C; a $^1/_4$-in. × 6 ft stainless steel column packed with 60–80 mesh Chromosorb 102 and operated isothermally at 125° C for 30 min and then programmed to 175° C at 2°/min.

Alcohol Dehydrogenase (ADH) Assay. The extraction and assay techniques were described previously (Pattee and Swaisgood, 1968).

One unit of enzymatic activity was defined as the amount of enzyme resulting in the production of one μmole of NADH per min under the conditions of the assay.

Lipoxidase Assay. From 20 to 50 g of kernels were found in three volumes of 8% sucrose in a Waring Blendor (10 sec high and 10 sec low speed) and squeezed through four layers of cheesecloth before centrifuging at 2700 × g for 10 min. The resulting supernatant was used as a crude enzyme source. The substrate was prepared according to Surrey (1964) and enzyme activity was measured polarographically (Mitsuda *et al.*, 1967) with a Clark Oxygen Electrode at −0.8v. The enzymatic reaction was started by adding 0.05–0.10 ml of enzyme solution to 1.40 to 1.45 ml of the substrate solution. Oxygen uptake was proportional to enzyme concentration within the range used. Enzyme activity per kernel was calculated using the known number of kernels and the volume of supernatant.

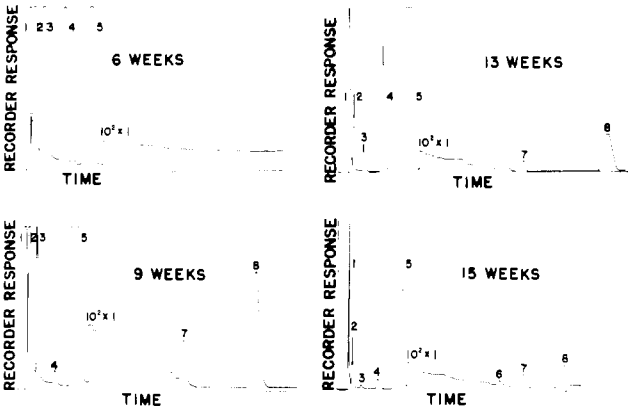

Figure 1. Influence of maturation on the volatile profile of peanuts

RESULTS AND DISCUSSION

Chromatograms from selected maturation levels illustrate the changes found in the volatile profile of peanuts with increasing maturity (Figure 1). The identification assigned to the components by comparison to known standards on two columns and by mass spectral analysis is given in Table II. The pattern of volatile production during maturation (Figure 2) shows that the concentration of volatiles per kernel reached a maximum at about 8 to 9 weeks, dropped rapidly, and then rose slightly at 12 weeks, after which it continued to decrease. It is not known if the rise at 12 weeks is biologically significant, but this general pattern has been shown in ribonucleic acid and protein determinations made during peanut maturation (Aldana, 1968). This pattern is also noted for ADH (Figure 3) and lipoxidase (Figure 4) activities. Possibly the drop and subsequent increase represents a transition point between maturation and preparation for germination.

Five major volatile compounds were responsible for this total volatile pattern: acetaldehyde, methanol, pentane, ethanol, and hexanal. Traces of acetone and pentanal were also found. The pattern for total volatile production and the pattern for the individual compounds suggest that the peanut kernel reaches its highest level of metabolic activity between the seventh and tenth week of maturation. ADH and lipoxidase in the peanut kernel are capable of producing four of these five volatile compounds. Therefore, relationships between the enzyme activity level and substrate, or product, might be expected. Pattee and Swaisgood (1968) characterized an ADH from peanuts. We have determined the changes in its activity level during maturation (Figure 3). ADH

Table II. Identification of Volatile Components Isolated from Peanuts during Maturation

Peak No.	Compound
1	Methanol
2	Acetaldehyde
3	Ethanol
4	Acetone
5	Pentane
6	Unknown
7	Pentanal
8	Hexanal

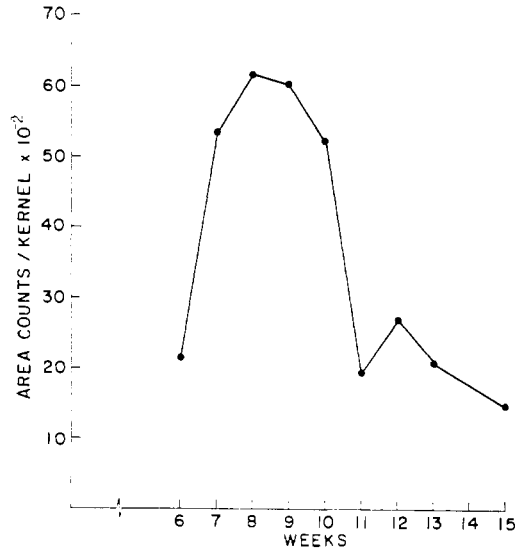

Figure 2. Changes in total volatile content of peanut kernels during maturation

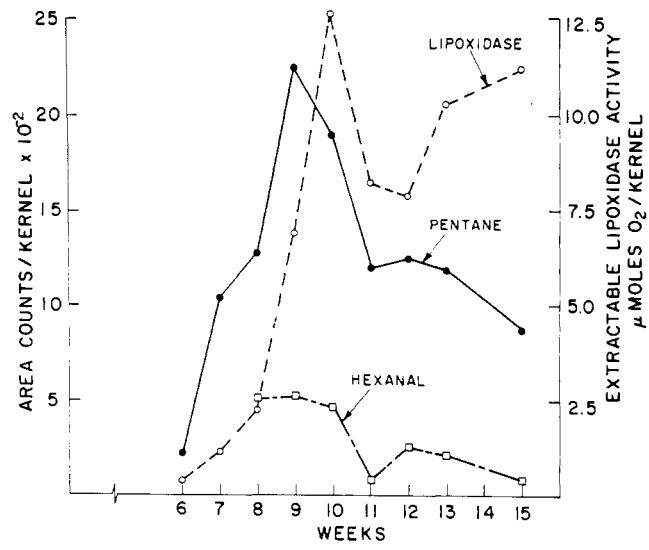

Figure 4. Lipoxidase activity and the content of pentane and hexanal in peanut kernels during maturation

from higher plant sources shows a considerable degree of specificity for ethanol but will utilize higher molecular weight alcohols to a lesser extent (Swaisgood and Pattee, 1968; Eriksson, 1968). Eriksson (1968) also showed that in most alcohol-aldehyde systems the alcohol partner predominates.

Ethanol and ADH peaked at 8 weeks (Figure 3) while acetaldehyde peaked at 7 weeks. The ethanol and acetaldehyde concentrations then decreased somewhat uniformly during the remainder of maturation, while the ADH activity peaked again at 11 weeks. These data indicate that ADH is present in the peanut kernel in maximum amounts at the same time that acetaldehyde and ethanol are maximal. Thus enzyme activity appears to be related to the production of these compounds. A simple relationship need not be expected, since both are common metabolites and could be utilized in the formation of other compounds. For example, Liu *et al.* (1965) showed that ethanol can be conjugated to yield glycosidic derivatives in pea seedling roots. They also found an NAD-dependent dehydrogenase in germinating peanuts and peas which could oxidize acetaldehyde to acetate and speculated that these two processes might help explain the drop in endogenous ethanol as tissues mature. This acetaldehyde dehydrogenase is not known to be present in maturing peanuts.

Methanol also was found during maturation (Fig. 5). Luh *et al.* (1955) postulated that methanol was formed by the demethylation of the ester group in pectin molecules by the enzyme, pectin esterase. Roberts *et al.* (1967) showed that exogenous methanol can be utilized in the biosynthesis of pectin methyl esters in parsley. They also suggest that before utilization, methanol is probably first oxidized to formate and then reduced back to the methyl level; all of these are in the form of tetrahydrofolate intermediates. This and other recent work by Coussins and coworkers (Coussins and Sinha, 1965; Wong and Coussins, 1966) suggest that methanol metabolism in plants, as well as in animals, might be intimately associated with tetrahydrofolate intermediates.

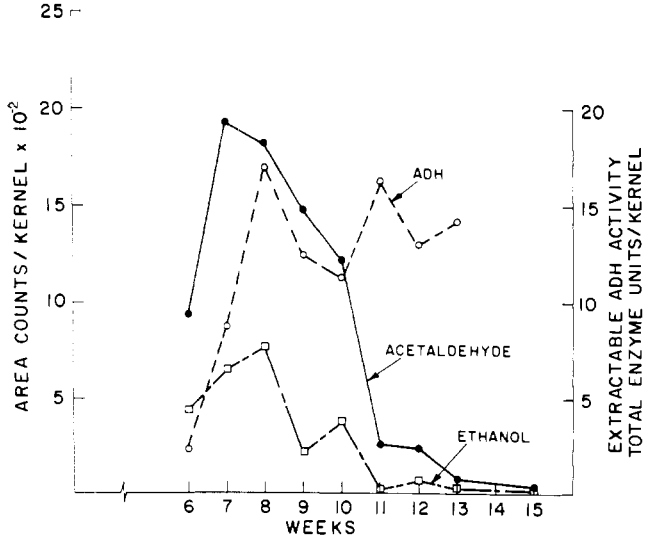

Figure 3. Alcohol dehydrogenase activity and the content of acetaldehyde and ethanol in peanut kernels during maturation

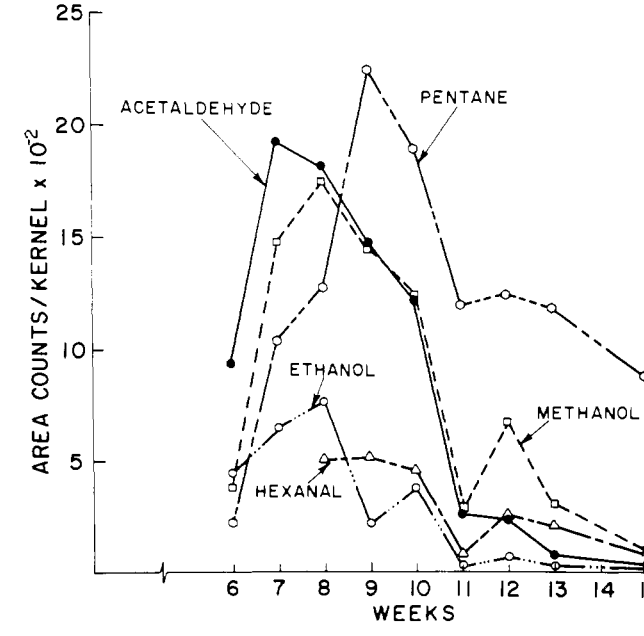

Figure 5. Changes in the major volatiles of peanut kernels during maturation

Siddiqui and Tappel (1957) reported that mature peanut kernels contain lipoxidase. We measured the changes in lipoxidase activity during maturation of the peanut kernel (Figure 4). The enzyme required unsaturated fatty acids which contain the cis,cis-1,4 pentadiene system; linoleic acid, arachidonic acid, and linolenic acid are known to be the most commonly occurring substrates (Tappel, 1963). There is also some evidence for at least two lipoxidases, one specific for triglycerides and another specific for free fatty acids (Dillard *et al.*, 1960).

Linoleic acid has been reported in mature peanuts of various varieties by several investigators (Sreenivasan, 1968; Fedeli *et al.*, 1968; Worthington and Holley, 1967). Linolenic acid has also been reported in peanuts (Worthington and Holley, 1967), but it accounts for only 0.04% of the total fatty acids. Eriksson (1967) showed that in fresh, intact, pea cotyledons the oxygen supply and the fatty acid content should be sufficient for the lipoxidase to function if the necessary *in vivo* contact exists between these substrates and the enzyme.

Further, Grosch (1968) showed that a crude preparation of pea lipoxidase could produce pentanal and hexanal using linoleic acid as a substrate. He was studying the volatile monocarbonyls and hence did not look for pentane. Evans *et al.* (1967) produced pentane by thermal decomposition of 13-hydroperoxyoctadeca-9,11-dienoic acid, which is the primary product after the reaction of crystalline soybean lipoxidase with linoleic acid. Only minimum amounts of pentane resulted unless the oil was heated to temperatures above the decomposition point of the hydroperoxide (200° C).

Lipoxidase activity during maturation appears to be related to concentrations of pentane and hexanal (Figure 4). The relationship between lipoxidase activity and pentane might be metabolic. The minimum amount of pentane found by Evans *et al.* (1967) is not comparable with the pentane concentration shown in Figure 4. The pentane formed during maturation cannot be due to a thermal decomposition but must have some biological mechanism—perhaps similar to the thermal mechanism.

The level of hexanal, although much lower than that of pentane, also demonstrates some relationship to the lipoxidase activity. Hexanal could be formed by mechanisms postulated for lipoxidase activity (Tappel, 1963; Dolev *et al.*, 1967). While hexanal has been shown to be a component of off-flavor in certain instances (Bengtsson and Bosund, 1964; Buttery and Teranishi, 1963) present and previous work, reported elsewhere (Pattee *et al.*, 1969), indicate that hexanal is a normal flavor constituent of peanuts.

The evidence presented suggests that both lipoxidase and ADH are associated with the formation of four of the five major volatiles produced during maturation of the peanut kernel; perhaps these enzymes influence the quantitative changes which occur in the volatile profile during this phase of seed development.

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LITERATURE CITED

Aldana, Angeline B., Ph.D. Thesis, N.C. State University, Raleigh, N.C. (1968).
Bailey, S. D., Mitchell, D. G., Bazinet, M. L., Weurman, C., *J. Food Sci.* **27,** 165 (1962).
Bengtsson, B., Bosund, I., *Food Technol.* **18,** 179 (1964).
Buttery, R. G., Teranishi, R., J. AGR. FOOD CHEM. **11,** 504 (1963).
Coussins, E. A., Sinha, S. K., *Can. J. Biochem.* **43,** 685 (1965).
Creveling, R. K., Silverstein, R. M., Jennings, W. G., *J. Food Sci.* **33,** 284 (1968).
Dillard, M. A., Henick, A. S., Koch, R. B., *Food Res.* **25,** 544 (1960).
Dolev, Ami, Rohwedder, W. K., Dutton, H. J., *Lipids* **2,** 28 (1967).
Eriksson, C. E., *J. Food Sci.* **32,** 438 (1967).
Eriksson, C. E., *J. Food Sci.* **33,** 525 (1968).
Evans, C. E., List, G. R., Dolev, Ami, McConnell, D. G., Hoffman, R. L., *Lipids* **2,** 432 (1967).
Fedeli, E., Favini, G., Camurati, V. F., Jacini, G., *J. Amer. Oil Chem. Soc.* **45,** 676 (1968).
Grosch, W., *Z. Lebensm. Unters. Forsch.* **137,** 216 (1968).
Heinz, D. E., Creveling, R. K., Jennings, W. G., *J. Food Sci.* **30,** 641 (1965).
Jennings, W. G., Leonard, G. S., Pangborn, R. M., *J. Food Technol.* **14,** 587 (1960).
Liu, Tin-yin, Oppenheim, Ariella, Castelfranco, P., *Plant Physiol.* **40,** 1261 (1965).
Luh, B. S., Leonard, S. J., Pattel, D. S., Claypool, L. L., *Food Technol.* **9,** 939 (1955).
Mitsuda, Hisateru, Yasumoto, Kyoden, Yamamoto, Aijiro, Kusano, Takanoir, *Agr. Biol. Chem.* **31,** 115 (1967).
Murray, K. E., Palmer, J. K., Whitfield, F. B., Kennet, B. H., Stanley, G., *J. Food Sci.* **33,** 632 (1968).
Murray, K. E., Shipton, J., Whitfield, R. B., Kennett, B. H., Stanley, G., *J. Food Sci.* **33,** 290 (1968).
Norman, Shirley, Craft, C. C., Davis, P. L., *J. Food Sci.* **32,** 656 (1967).
Pattee, H. E., Swaisgood, H. E., *J. Food Sci.* **33,** 250 (1968).
Pattee, H. E., Singleton, J. A., Cobb, W. Y., *J. Food Sci.* **34,** 625 (1969).
Roberts, R. M., Shah, R. H., Golebiewski, Anne, Loewus, F., *Plant Physiol.* **42,** 1737 (1967).
Rohan, T. A., *J. Food Sci.* **32,** 402 (1967).
Schultz, T. H., Flath, R. A., Black, D. R., Guadagni, D. C., Schultz, W. G., Teranishi, R., *J. Food Sci.* **32,** 279 (1967).
Sevenants, M. R., Jennings, W. G., *J. Food Sci.* **31,** 81 (1966).
Siddiqui, A. M., Tappel, A. L., *J. Amer. Oil Chem. Soc.* **34,** 529 (1957).
Sreenivasan, B., *J. Amer. Oil Chem. Soc.* **45,** 259 (1968).
Surrey, K., *Plant Physiol.* **39,** 65 (1964).
Swaisgood, H. E., Pattee, H. E., *J. Food Sci.* **33,** 400 (1968).
Tang, C. S., Jennings, W. G., J. AGR. FOOD CHEM. **15,** 24 (1967).
Tappel, A. L., "The Enzymes," 2nd Edition, Vol. 8, pp. 275–283, Academic Press, New York (1963).
Wong, K. F., Coussins, E. A., *Can. J. Biochem.* **44,** 1400 (1966).
Worthington, R. E., Holley, K. T., *J. Amer. Oil Chem. Soc.* **44,** 515 (1967).

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