

VOLATILE COMPONENTS OF MATURING SOYBEANS¹

D. H. HONIG and J. J. RACKIS, Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture², Peoria, IL 61604

ABSTRACT

Soybeans were picked at intervals from 24 to 69 days after flowering and analyzed for major volatiles by vapor phase chromatography. Total volatiles measured as sublimates from whole soybeans decreased from a maximum value of 113 ppm during early maturation to about 0.1 ppm at maturity; yields from aqueous slurries of macerated beans decreased from 695 to 13 ppm during maturation. The major constituent, methanol, accounted for 30 to 90% of total volatile content. Other major

compounds were ethanol and ethanal, together with much smaller amounts of propanal, acetone, pentane, pentanal, and hexanal. Yields of volatiles from maturing soybeans began to decrease rapidly at the yellowing stage. On the other hand, flavor intensity values for bitterness increased twofold after yellowing. Some correlation was found between lipoxxygenase activity and yields of ethanal in early stages of maturation.

In previous studies as Rackis *et al.* (1) followed variations in enzyme activity of maturing soybeans, they found increases in lipoxxygenase activity overall, and corresponding increases in bitter flavor.

Volatile compounds have been studied as possible sources of flavor or as indicators of biochemical activity that might produce flavor in vegetable products. A number of workers have studied volatile compounds in soybean meals and other soybean products (2-6).

Changes in volatile components of green peas have also been studied. Bengtsson and Bosund (7) found that ethanal, ethanol, and hexanal were formed during storage. Shipton *et al.* (8) used low-temperature vacuum distillation to compare the levels of volatile compounds from intact peas with those from macerated peas.

Pattee *et al.* (9) also used low-temperature vacuum distillation of volatiles from maturing peanuts to follow changes in formation of pentane, ethanal,

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methanol, ethanol, and hexanal during maturation and were able to correlate the patterns with variations in lipoxygenase and alcohol dehydrogenase activities.

We determined levels of volatile compounds present in intact maturing soybeans, as well as changes in these levels which occur when samples are macerated for analysis. The contribution of volatile compounds to the organoleptic properties of maturing soybeans is examined. The relationship between lipoxygenase and formation of volatile compounds during maturation is also discussed.

MATERIALS AND METHODS

Preparation of Sample

Amsoy soybeans, 1972 crop, were harvested as described previously by Rackis *et al.* (1) at intervals of 3 to 7 days beginning 24 days after flowering. Sample sizes ranged from 10 g to 78 g. Average fresh weight, percentage of dry matter, and color of soybeans in relation to days after flowering are given in Table I. Dry weights were determined by drying 1 to 2 g of soybeans in a forced-draft oven for 2 hr at 130°C. Per cent dry matter was determined on beans from the same picking and weight range as the samples analyzed for volatile compound content. Range in weight was within $\pm 40\%$. As the soybeans were shelled from the pods, they were cooled with Dry Ice to minimize changes in content of volatiles. At each stage, one portion of soybeans intact was analyzed for volatiles on the day of picking, whereas the other portion was first macerated in a Waring Blender (one speed) with 2 vol of distilled water for 1 min after overnight storage in the deep freeze.

To evaluate the effect of moisture levels on the yields of volatile compounds at maturity, 50-g samples of mature Amsoy beans, 1970 crop, were soaked in deionized distilled water for various lengths of time. These were then analyzed as were the samples of maturing soybeans.

TABLE I
Fresh Weight, Dry Matter, and Color
of Maturing Soybeans^a

| Days after flowering | Dry matter % | Bean color | Average wt. whole beans mg/seed |
|-------------------------|-----------------|----------------------|---------------------------------------|
| 24 | 18.8 | Dark green | 192 |
| 28 | 23.9 | Dark green | 241 |
| 31 | 28.7 | Dark green | 297 |
| 35 | 30.0 | Pale green | 334 |
| 39 | 30.8 | Pale green | 350 |
| 44 | 34.1 | Pale green | 442 |
| 49 | 37.6 | Yellowing pale green | 447 |
| 55 | 38.8 | Yellowing | 456 |
| 62 | 75.6 | Yellow | 267 |
| 69 | 84.2 | Brown | 196 |

^aFlowering date, July 17, 1972; Amsoy variety.

TABLE II
Relative Retention Times of Volatile Standards

| Compound | t_R/t_R Ethanol | |
|----------|-------------------|-----------|
| | Carbowax 20M | Porapak P |
| Pentane | 0.10 | 1.35 |
| Ethanal | 0.27 | 0.57 |
| Propanal | 0.41 | 1.45 |
| Acetone | 0.49 | 1.38 |
| Methanol | 0.80 | 0.42 |
| Ethanol | 1.00 | 1.00 |
| Pentanal | 1.16 | 2.80 |
| Hexanal | 1.72 | 3.50 |
| Water | 1.86 | 0.32 |

TABLE III
Volatiles from Whole Maturing Soybeans

| Days after flowering | Yield, $\mu\text{g/g}$ | | | | |
|----------------------|------------------------|---------|----------|---------|-------|
| | Ethanal | Acetone | Methanol | Ethanol | Total |
| 24 | 0.6 | ... | 100.0 | 12.5 | 113.1 |
| 28 | 4.9 | 0.1 | 57.0 | 18.1 | 80.1 |
| 31 | 3.1 | 0.5 | 41.1 | 27.8 | 72.5 |
| 35 | 4.6 | 0.8 | 14.7 | 7.1 | 27.2 |
| 39 | 3.0 | 0.2 | 17.0 | 42.4 | 62.6 |
| 44 | 1.8 | 0.1 | 11.9 | 4.6 | 18.4 |
| 49 | 2.1 | 1.2 | 20.0 | 12.4 | 35.7 |
| 55 | 3.2 | 0.3 | 2.5 | 2.0 | 8.0 |
| 62 | ... | 0.6 | 0.9 | 0.6 | 2.1 |
| 69 | ... | 0.04 | 0.04 | 0.01 | 0.09 |

TABLE IV
Volatiles from Macerated Maturing Soybeans

| Days after flowering | Yield, $\mu\text{g/g}$ | | | | | | | | |
|----------------------|------------------------|---------|----------|---------|----------|---------|----------|---------|-------|
| | Pentane | Ethanal | Propanal | Acetone | Methanol | Ethanol | Pentanal | Hexanal | Total |
| 24 | 0.6 | 34.2 | 0.2 | 29.7 | 519 | 83.3 | 0.3 | 27.3 | 695 |
| 28 | 3.5 | 31.1 | 1.2 | 12.0 | 409 | 184 | 0.4 | 11.4 | 653 |
| 31 | 4.1 | 61.6 | 2.3 | 2.7 | 140 | 67.2 | 6.5 | 1.8 | 286 |
| 35 | 1.5 | 30.7 | 0.2 | 8.6 | 136 | 130 | 3.0 | 8.2 | 318 |
| 39 | 2.5 | 121 | 1.2 | 2.3 | 159 | 171 | 0.1 | 8.6 | 466 |
| 44 | 2.3 | 77.7 | ... | 1.3 | 123 | 143 | ... | 0.1 | 347 |
| 49 | 3.0 | 63.5 | ... | 21.6 | 92.5 | 86.5 | 0.6 | 1.1 | 269 |
| 55 | 1.2 | 44.0 | 2.4 | 0.4 | 47.5 | 76.3 | 0.3 | 0.1 | 172 |
| 62 | 1.2 | 5.2 | ... | 9.5 | 21.6 | 36.0 | ... | 0.1 | 74 |
| 69 | 0.7 | 3.2 | 0.1 | 1.7 | 4.5 | 2.9 | 0.1 | 0.1 | 13.3 |

Collection of Volatiles

The methods of Pattee *et al.* (9) were adapted for collection of volatiles. Soybean samples were placed in a three-necked distillation flask and frozen with liquid nitrogen. After evaporation of the nitrogen, volatiles were stripped from the beans through a series of condensing traps. A vacuum of approximately 1 Torr was maintained for 6 hr and the beans remained below 0°C once this vacuum level was reached. Approximately 50% of the moisture in the sample was removed during this time. Most of the water was collected in the first two traps cooled with Dry Ice, while the more volatile compounds were effectively concentrated in the third trap cooled with liquid nitrogen.

Analysis of Volatiles

When collection of volatiles ended, stopcocks on each side of the third trap were closed; it was then removed from the system, allowed to warm to room temperature, and the vacuum slowly released. Three-milliliter samples of the vapor were taken through a rubber septum for analysis. After the vapor was sampled, the condensed volatiles in the trap were brought with water to 0.5 to 2 ml, and 2 μ l was then analyzed.

From the first and second traps, 2- μ l aqueous samples were also analyzed and results, if any, added to those from the third trap. Further stripping of the samples in these traps did not isolate more material in significant amounts. All samples of volatiles were analyzed by vapor phase chromatography.

The gas chromatograph was a Packard 7409 equipped with dual flame ionization detectors. Two 6 ft \times 1/4 in. o.d. glass columns were used. One column was packed with 15% Carbowax[®] 20M on 80-100 Chromosorb W/AW; the other with Porapak P 80-100 mesh (Supelco Inc., Bellefonte, Pa.). Chromatographic conditions: inlet—150°, detector—210°, oven programmed from 30° to 180°C at 3°C/min, carrier gas—helium, gas flow readings He-30 and H₂—35 ml/min. Maximum sensitivity 1×10^{-11} . Quantitation was based on response of standards and integration of area under the peaks with an Autolab 6300 digital integrator. Components were identified from retention time of standards (Table II) relative to ethanol on one or both columns.

Carbonyls were further identified by analysis of 2,4-dinitrophenylhydrazine derivatives according to procedures reported by Sessa *et al.* (6).

RESULTS AND DISCUSSION

Analyses for volatiles from whole soybeans (Table III) are reported as μ g of volatiles removed per g of dry matter in the original sample and related to days after flowering. From a high of 113 μ g/g at 19% dry matter 24 days after flowering, the total volatiles declined to 8.0 μ g/g at 39% dry matter 55 days after flowering. Less than 0.1 μ g/g was found in the mature bean with 84% dry matter. The main component of these volatiles was usually methanol, which declined from 100 μ g/g at 24 days to 2.5 μ g/g at 55 days after flowering. The amount of ethanol measured shows a different pattern, increasing from 12.5 μ g/g at 24 days to 42.4 μ g/g at 39 days and then declining to 2.0 μ g/g at 55 days. Other volatiles were detected in small amounts; of these ethanal and acetone were identified in most stages up to maturity but no pattern is evident.

Much larger amounts of volatiles came from macerated maturing soybeans than from intact beans (Table IV). Total volatiles declined from 695 $\mu\text{g/g}$ initially, to 13 $\mu\text{g/g}$ at 69 days after flowering or 84% dry matter. Here, too, methanol is usually the major constituent. It shows a steady decline from 519 $\mu\text{g/g}$ to 4.5 $\mu\text{g/g}$. Ethanol increases to 170 and 180 $\mu\text{g/g}$ at intermediate stages, then declines to 3 $\mu\text{g/g}$. Amounts of hexanal were greatest in early stages while

TABLE V
Effect of Soaking on Volatile Content of Soybeans^a

| Soaking time min | Ethanal | Methanol | Ethanol | Hexanal | Total |
|---|---------|----------|---------|---------|-------|
| Intact Soybeans, $\mu\text{g/g}^b$ | | | | | |
| 0 | 0.01 | 0.04 | 0.01 | | 0.06 |
| 5 | 0.27 | 0.65 | 0.08 | | 1.00 |
| 20 | 3.3 | 6.5 | 9.9 | | 19.7 |
| 60 | 6.8 | 3.7 | 15.6 | | 26.1 |
| 240 | 1.1 | 6.9 | 37.5 | | 45.5 |
| 960 | 1.0 | 9.1 | 79.7 | | 89.8 |
| 1,020 | 1.7 | 6.0 | 61.3 | | 69.0 |
| Macerated Soybeans, $\mu\text{g/g}^c$ | | | | | |
| 0 | 16.6 | 44.1 | 74.9 | 1.1 | 136.7 |
| 5 | 13.4 | 36.7 | 54.8 | 0.6 | 105.5 |
| 30 | 22.4 | 68.1 | 145 | 0.9 | 236.4 |

^aMature Amsoy 1969 crop soybeans.

^bSoaked in 1 vol of water.

^cSoaked in 2 vol of water.

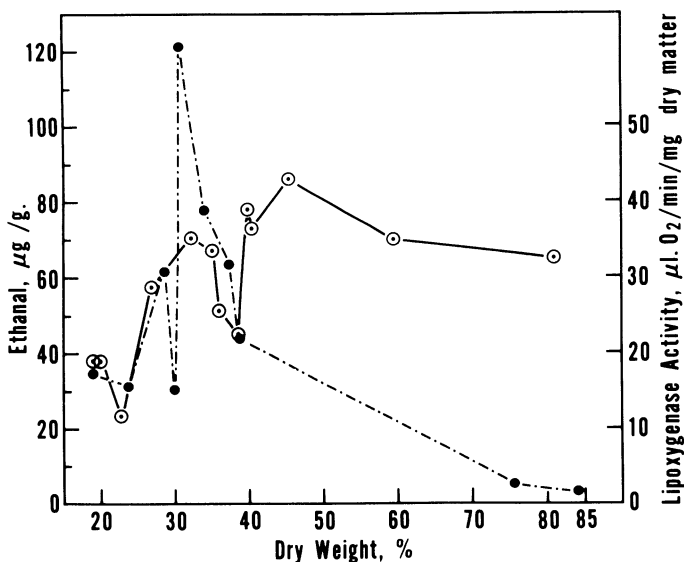


Fig. 1. Ethanal (●) profile of macerated maturing soybeans vs. lipoxygenase activity (○), as per cent dry weight increases during maturation.

ethanal reached a peak at the 31% dry matter stage 39 days after flowering. Smaller amounts of pentane, propanal, acetone, and pentanal were found, but no pattern is readily apparent.

These values should represent minimum values of these compounds present in the intact soybean or formed on maceration of soybeans and since the patterns are different, they likely represent several different biosynthetic processes. Although other compounds were detected, they were not identified. When stripping of macerated beans picked 49 days after flowering was continued overnight, removal of moisture increased from 77 to 95%; at the same time yield of volatiles increased 15%; an increase that indicated the degree of removal of nonaqueous volatiles reported here corresponds roughly to the degree of water removed.

Mature soybeans were soaked to determine whether values at the 84% dry matter stage are less representative than values at higher moisture levels. As shown by H. G. Maier (10), at low-moisture levels some volatiles are bound to carbohydrates and protein and become less available. From Table V, we see that the yield of all three volatiles from intact beans increased when soybeans were soaked for 20 min. This increase can be attributed mainly to greater availability from the soaked bean. The increase in yield of ethanol, however, is several times that of the other two compounds. This large increase suggests that additional ethanol is formed as well as becoming more available. In the macerated soybeans there seems to be little increase in volatiles beyond that which occurs during maceration itself.

Figure 1 compares changes in content of ethanal from the macerated soybeans with changes in lipoxygenase activity as reported by Rackis *et al.* (1). Since these changes were measured in different years, they are compared on the basis of percentage dry matter. Up to the 40% dry matter stage, the maximum amount of ethanal is reached at the same dry matter stage as a peak in lipoxygenase activity. After this period, lipoxygenase activity rises again to maximum while the levels of ethanal continue to decline. Ethanal formation is usually attributed to other causes, but some ethanal, as well as propanal, pentanal, and hexanal, has been found in the products of linolenic or linoleic acid oxidation catalyzed by a crude pea lipoxygenase (11).

The values from other volatiles, especially hexanal, show little direct relation to lipoxygenase activity, but some may reflect increased autoxidation during maceration. Although lipoxygenase is reported to be responsible for most of the hexanal in mature soybeans (4), other mechanisms may predominate during maturation. The higher levels of hexanal initially may also be accounted for by greater percentages of nontriglyceride lipids in early stages of maturity as reported by Roehm and Privett (12). No direct correlation was found between changes in yields of these volatiles and the patterns of beany and bitter flavor previously obtained (1). Improved assays, as well as detection and identification of minor constituents, should further clarify how volatiles are formed and to what extent they are responsible for the flavors of soybean products.

We found significant amounts of ethanal, methanol, and ethanol naturally present in maturing soybeans and noted a large increase in these and other volatiles once soybeans were macerated in water in the presence of air. These values need to be considered when assessing the effects of various processing

steps on volatile content. The amounts of these components naturally present should also be considered when analyzing for substances left as residue after treatment of soybeans or soybean meals with solvents.

Acknowledgment

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