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Identification of a Volatile Component in Soybeans That
Contributes to the Raw Bean Flavor

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A volatile component which develops in soybeans and contributes to the raw bean odor and flavor has been isolated and identified as ethyl vinyl ketone by gas chromatography and mass spectrometry.

The green bean odor and flavor have been attributed to this compound. This component was isolated by distillation at 40° C. into cold traps of dry ice and ethanol or liquid nitrogen.

The raw bean flavor of soybeans has been recognized as a major flavor defect when soybeans are used as food for human consumption. The major objectionable flavor has been described as "green bean-like." This flavor is not present in the original intact raw, whole soybean but develops immediately after maceration of the bean. The formation of the compound probably results from enzymatic action, since its appearance is rapid and only in the unblanched product (Wilkens *et al.*, 1967). Other food products are known to form characteristic flavors by enzymatic action following maceration—i.e., horseradish and onions.

The characterization of the component responsible for the flavor defect and an insight into its mechanism of formation should aid the development of soybean products more palatable for human consumption.

MATERIALS AND METHODS

Sample Preparation. A sample of 350 grams of soybeans (var. Clark) was ground in a Waring Blender (Model CB-4) with 2800 ml. of distilled water. The initial grinding was accomplished at the low speed for 30 seconds, immediately followed with the high speed for 1.5 minutes. The initial temperature of the mixture was 25° C.; however, because of the heat generated during the grind, the final temperature was between 34° and 35° C. This slurry was transferred to a 12-liter distilling flask. A distillation train (Figure 1) was devised employing gradient cooling to condense the volatiles. A vacuum of 5 mm. of Hg was maintained throughout the distillation, as measured between the liquid nitrogen trap and the manostat before the vacuum pump. Heat applied to the distillation flask never exceeded 40° C. A stream of nitrogen was employed to sweep the volatiles from the distillate through the gradient temperature traps and to prevent bumping during the distillation. The sample was subjected to vacuum distillation for 2 hours after the boiling point of the slurry under reduced pressure was reached.

Gas Chromatographic Analysis and Odor Evaluation. Following distillation, the trap, immersed in liquid nitrogen, was sealed with a rubber septum. This tube was

placed in a water bath at 60° C. for 5 minutes. A 10-ml. aliquot of the head space was removed using a 10-ml. gas-tight syringe (Hamilton No. 1010). This sample was introduced into an on-column injection system of a Beckman Model GC-4 gas chromatographic instrument equipped with flame ionization detectors. Since the flame ionization detector will burn the effluent as it emerges from the column, the odoriferous material as it is eluted from the column cannot be detected without modification of the detection system. An effluent splitter (10 to 1) was placed immediately after the gas chromatographic column and before the detector. The single portion was diverted to the detector, while the tenfold portion was expelled to the atmosphere through a heated line. Employing this modification, the retention time of the compound imparting the raw bean odor was determined and the odors of the other components were evaluated.

The carrier gas was helium with a flow rate of 40 ml. per minute. The column was 6-feet X 1/8-inch o.d. stainless steel, with 20% FFAP on Chromosorb W 100- to 120-mesh as the packing. The temperature parameters were: injector, 220° C.; column, 60° C.; and detector oven, 250° C.

Mass Spectrometer Analysis. The outlet gas chromatographic instrument was connected to the inlet of a Bendix Time-of-Flight mass spectrometer (Model 12-101A) through a helium separator (Watson and Biemann, 1964) which was heated and maintained at 200° C.

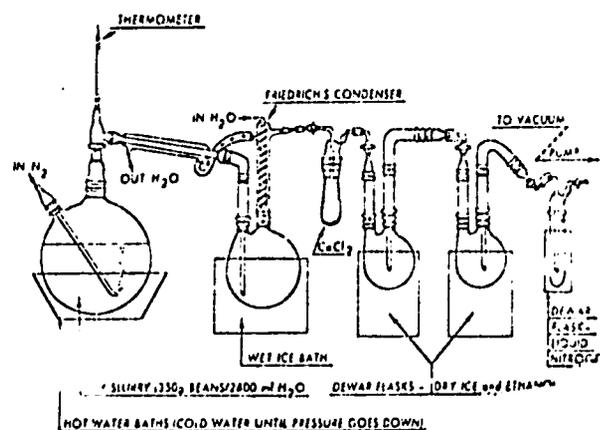


Figure 1. Distillation train for separation of green bean volatile flavor of soybeans

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the analysis. The mass spectrometer was equipped with a Bendix total output integrator. The mass spectrometer was the gas chromatographic detector, and any time lag due to holdup volume of the connecting tubing was eliminated. The scan controls of the mass spectrometer were set to give a 5-second scan between m/e 40 and 150. The gas chromatographic conditions previously described were employed in the separation of the components to be presented to the mass spectrometer. The gas chromatographic system was used to separate the mixture of components, and the mass spectrum of a single component was obtained by direct elution into the mass spectrometer.

RESULTS AND DISCUSSION

The chromatogram of the head space sample (Figure 2) shows the peak which exhibited the green bean odor. This peak, the only peak which exhibited the characteristic odor, was located using the "sniffer" previously described. The odor imparted by the remaining peaks was bland and could be described as "aldehyde-like." The area of the peak exhibiting the raw bean odor was small in relation to the other components eluted. However, the odor was very strong and penetrating.

When this peak was subjected to mass spectrometer analysis, a parent peak of m/e 84 was observed, while the base peak was present at m/e 55. The base peak suggests a vinyl ketone ($\text{CH}_2=\text{CH}-\dot{\text{C}}=\text{O}$). The difference between the base and parent peaks indicates that the remainder of the molecule was an ethyl group (m/e 29). The mass spectrum for ethyl vinyl ketone was obtained with an authentic sample. A comparison of the spectra of the

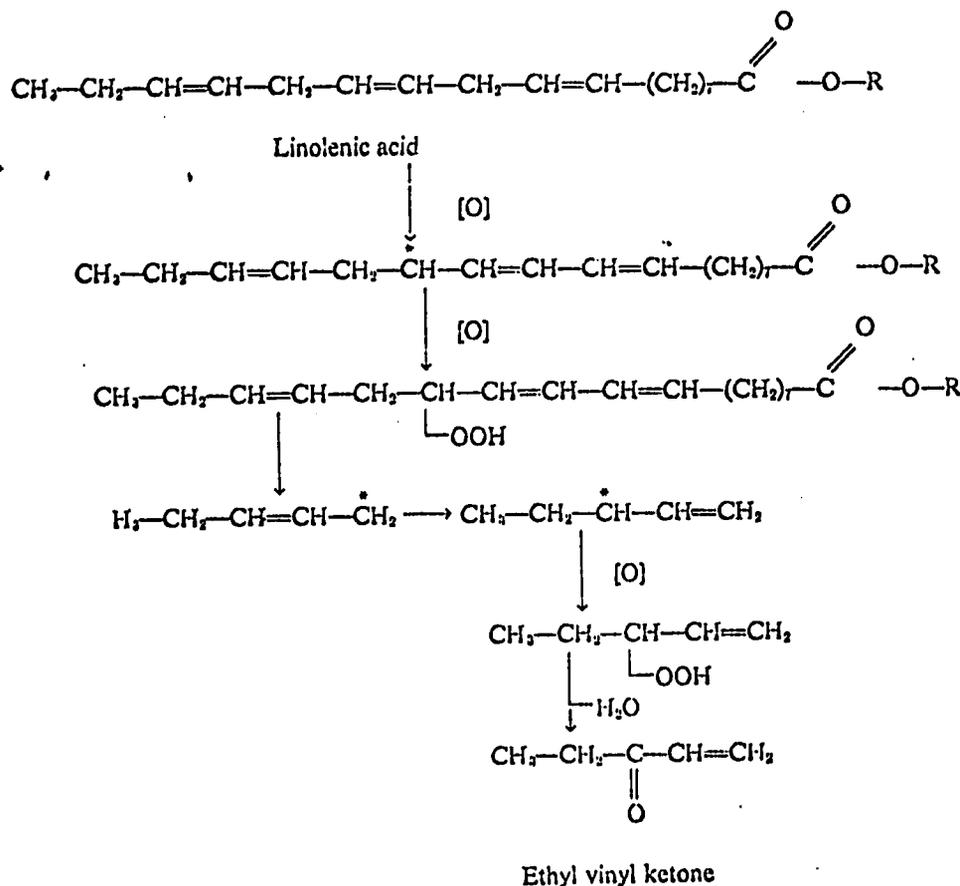
unknown and the known compounds indicates that the compounds are identical (Figure 3). The retention times of the unknown and the authentic ethyl vinyl ketone further substantiate the identity of the compound (Figure 4).

The large peak preceding the ethyl vinyl ketone was identified by the procedure previously described. This compound was identified as 1-pentanal.

Soybeans were ground as described above, but the temperature of the water was 90°C . The results of Wilkens *et al.* (1967) were duplicated. The raw bean odor was not observed during grinding or in the final slurry. When this slurry was subjected to vacuum distillation and gas chromatographic analysis, the ethyl vinyl ketone peak was eliminated. Soybean milk prepared in this manner had no raw bean odor. When ethyl vinyl ketone was added to this milk to give a concentration of 5 p.p.m., the raw, green bean odor was judged to be present.

The green bean flavor is not the only flavor which contributes to the total raw bean flavor of soybean milk. Other components formed during the oxidation of lipoxidase (Wilkens *et al.*, 1967) have an effect on the over-all total raw bean flavor. However, they are relatively mild in odor intensity, compared to the green bean flavor, ethyl vinyl ketone. These data coincide with the results of Hill and Hammond (1965), which attribute the flavor imparted in the early stages of the autoxidation of soybean oil to mixtures of pentanal and ethyl vinyl ketone.

The theories of autoxidation of unsaturated fatty acids, together with the action of lipoxidase in soybean milk, could advance a possible mechanism of formation by the oxidation of linolenic acid:



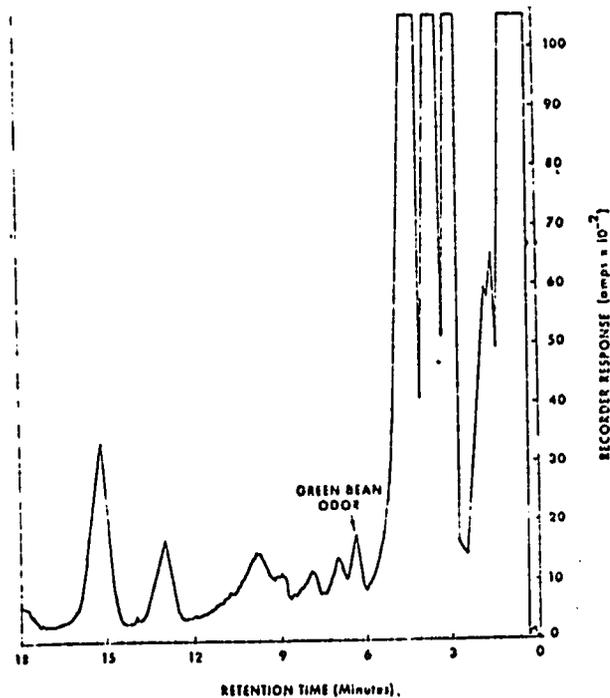


Figure 2. Gas chromatogram of components in liquid nitrogen trap

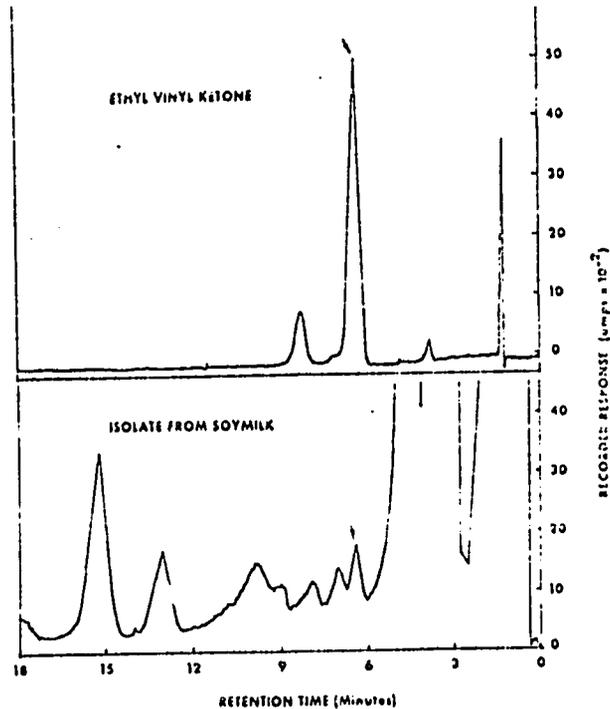


Figure 4. Retention times of ethyl vinyl ketone and component exhibiting green bean odor isolated from soybean milk

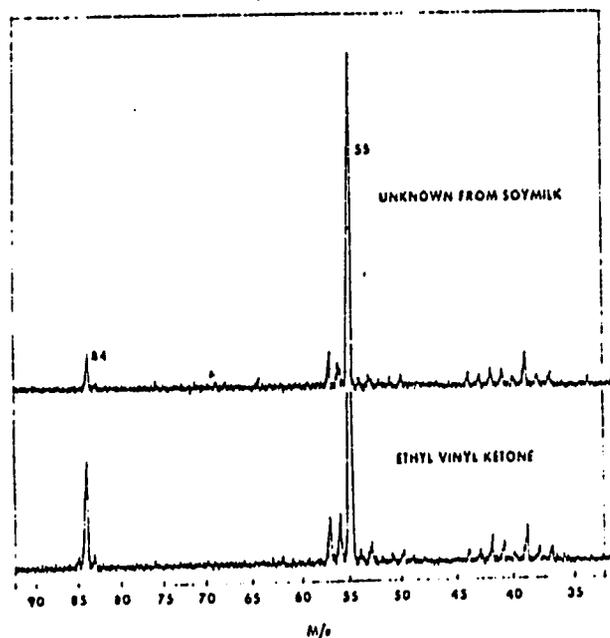


Figure 3. Mass spectra of ethyl vinyl ketone and component exhibiting green bean odor isolated from soybean milk

If this hypothesis is valid, amyl vinyl ketone would also be formed by autoxidation of linoleic acid. Keeney and Doan (1951) have demonstrated the presence of this compound in oxidized milk fat.

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