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The Formation of 1-Octen-3-Ol in Soybeans During Soaking

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Abstract

The soaking of soybeans in water as a pretreatment for soy milk manufacture was found to result in the production of significant quantities of 1-octen-3-ol. The amount of this compound formed at a soak temperature of 50 C increased with length of soak time and reached a maximum after approximately 6 hr. The rate of formation had a pH optimum of 6–7. A pure fraction of 1-octen-3-ol, isolated by a preparative GLC technique, was found to be levorotatory ($[\alpha]_D^{17} = -11.7$; $[\alpha]_D^{13} = -11.8$). Based on these findings, the mode of formation of 1-octen-3-ol in this case is presumed to be enzymatic. The flavor threshold of 1-octen-3-ol in soy milk was determined to be between 0.5 and 1.0 ppm.

Introduction

In the production of soy milk, a desirable first step from a physical standpoint involves the soaking of the soybeans for several hours in order to facilitate grinding. For this reason, the effect of the soaking step on the flavor of the resulting milk was investigated.

Wilkens et al. (1) have shown that in the production of soy milk the quantities of volatile off-flavors can be greatly reduced by raising the temperature of the grinding operation through the addition of water at temperatures approaching 100 C. When a high temperature grinding operation was preceded by an initial 30 C soaking step, however, the gas chromatographic profile showed a very prominent peak with a retention time of 16.5 min. The original investigators noted the formation of this peak but did not identify or study it. The identification of this component, its effect on the flavor of soy milk, and the determination of the mode of its formation during the soaking of soybeans were objectives of this research.

Experimental Procedures

Two methods of sample preparation were used in this experimentation. For the qualitative studies a method was followed which would yield enough sample for infrared analysis. For the quantitative studies a method was devised which would give enough precision to compare the samples from the various soak treatments.

Sample Preparation for the Qualitative Studies

Approximately 15 kg of certified soybeans (Clark var.) were soaked overnight at 28 C. The soaked beans were ground with water at 90–100 C in a 9:1 ratio (water-beans) using a Rietz disintegrator. The soy milk was distilled in 10 liter batches by vigorously passing steam through the soy milk at atmospheric pressure. The distillate (1500 ml) was collected from each 10 liter batch by using a series of three water-jacketed condensers. Six 2.5 liter aliquots of the distillate were extracted five times with 25 ml portions of carbon disulfide in a 4 liter separatory funnel. The extract was concentrated in a Kaderna-Danish evaporative concentrator to a final volume of 5 ml. The unknown was then isolated using an Aerograph Autoprep A-700 gas chromatograph employing a 20 ft × 3/8 in. O.D. aluminum column, packed with 30% XF-1150 on 70–80 mesh Gas Chrom A. Pure fractions of the unknown were collected in a 300 × 1 mm I.D. glass capillary tube.

Sample Preparation for the Quantitative Studies

Samples of soybeans (Clark var.) were soaked in 500 ml of soaking solution. After soaking, the beans were drained, and the soaking solution was saved for analysis. For the pH study the beans were soaked in hydrochloric acid or sodium hydroxide solutions of varying pH's for 5 hr at 50 C. After soaking, the beans were drained and rinsed several times with water to remove the excess soaking solution from the surface of the beans. The beans were then ground with 1 liter of boiling distilled water in a large Waring blendor (Model CB-5). An internal standard of 0.5 mg of *n*-octanol was added to all samples. The standard was added prior to the grinding of the bean samples and to the soaking solutions prior to distillation.

Preparation of samples for gas chromatography. A rotary vacuum evaporator system, employing two water-cooled condensers and an ice-water cooled receiving flask, was used for distillation. An 85 C water bath was used as the heat source, and the pressure in the system was maintained between 100 and 120 mm of Hg by means of a water aspirator and vacuum gauge. Two hundred and fifty milliliters of aqueous distillate were collected from each sample. The distillate was then extracted with 12 ml of carbon disulfide. A 10 ml aliquot of the carbon disulfide was separated from the water phase and concentrated under vacuum to approximately 0.5 ml.

Gas chromatographic analysis. Quantitative measurements of the unknown were conducted on an Aerograph Model 204 gas chromatograph with a flame ionization detector, employing an 8 ft × 1/8 in. O.D. stainless steel column, packed with 5% XF-1150 on 100–120 mesh Chromasorb W. All analyses were carried out isothermally at 125 C using helium carrier gas at a flow rate of 20 ml/min.

Mass spectral analysis. Mass spectra were obtained with a Bendix Model 12-101A Time-of-Flight mass spectrometer which was connected to an Aerograph 204 gas chromatograph through a Bendix Model 1076 chromatograph manifold inlet system.

Infrared spectral analysis. Infrared spectra were obtained with a Beckman Model IR-7 infrared spectrophotometer.

Optical rotation measurements. Measurements of the optical activity of the compound were made using a Rudolph Model 80 polarimeter in a controlled temperature cell with a path length of 1 dcm.

Flavor threshold determination. A triangular intensity panel of 13 members familiar with soy milk was trained to detect the presence of 1-octen-3-ol by repeated exposure to samples of soy milk treated with various levels of synthetic 1-octen-3-ol.

Results and Discussion

Preliminary evidence from gas chromatographic retention time data indicated that the unknown was

[1] New York State Agricultural Experiment Station Journal Paper No. 1651.

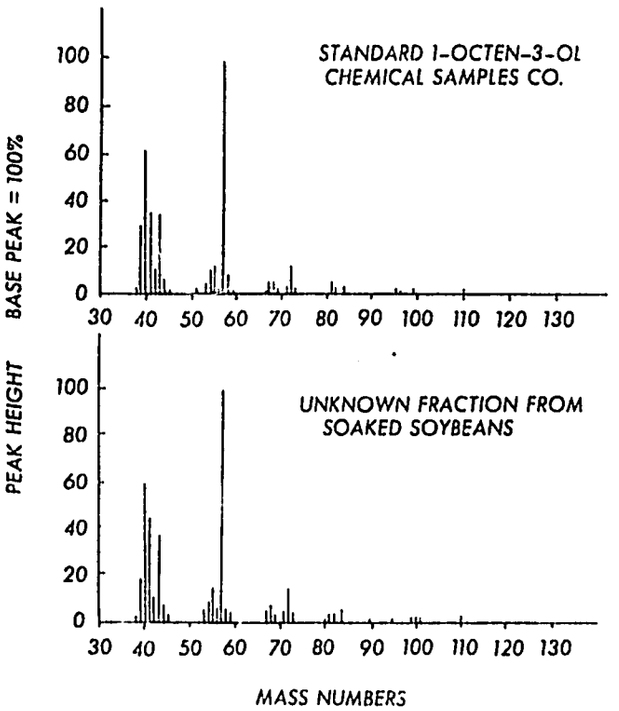

FIG. 1. Mass spectra of standard 1-octen-3-ol and unknown fraction from soaked soybeans (ionizing voltage 70 e.v.).

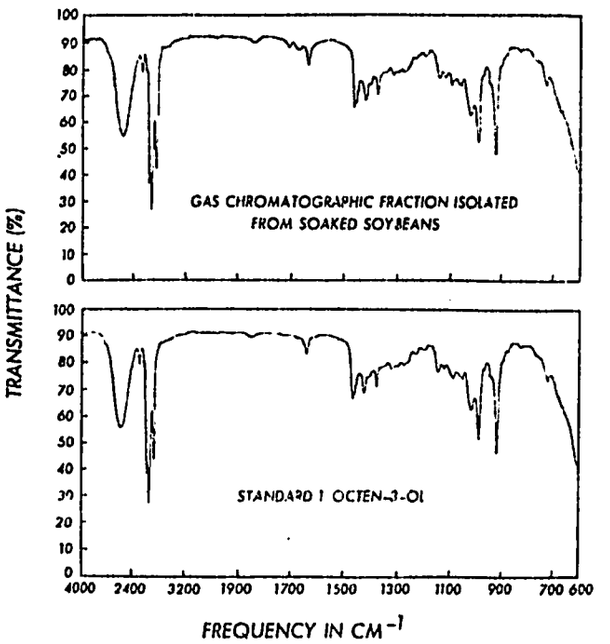

FIG. 2. Infrared spectra of gas chromatographic fraction isolated from soaked soybeans and standard 1-octen-3-ol.

not one of the carbonyl compounds which have been found in reverted soybean oil. Treatment of the unknown with 2,4-dinitrophenylhydrazine produced no observable precipitate, further confirming that it was not a carbonyl. An examination of the mass spectra of non-carbonyl reversion products of soybean oil obtained by Smouse (7) revealed that the mass spectrum of 1-octen-3-ol contained a base peak at mass 57 and a very small molecular ion peak, typical of secondary alcohols, at mass 128. A synthetic standard of 1-octen-3-ol was obtained (Chemical Samples Co.) and the retention times of the standard and the unknown were found to be identical. Furthermore, the odors of the unknown and standard, in the effluent from the column of the gas chromatograph, were similar. Figure 1 shows the mass spectrum of 1-octen-3-ol compared with that of the unknown. Since both spectra were obtained on compounds emerging from the gas chromatograph, the concentration was constantly changing during the scan. This possibly gave rise to the inconsistencies observed in the two spectra, as well as the absence of a molecular ion peak.

For this reason an infrared spectrum of the unknown was obtained. Figure 2 shows the infrared spectrum of the unknown compared with that of authentic 1-octen-3-ol. Both of the spectra were obtained on pure samples using the salt plate film technique. Examination of the two spectra leaves little doubt that the unknown is indeed 1-octen-3-ol. The very slight difference observable in the region between 1600 and 1800 cm^{-1} could be due to the presence of a slight amount of a carbonyl impurity.

1-Octen-3-ol, "Matsutakē alcohol," was first isolated from Japanese mushrooms by Murahashi (2) in 1938. The alcohol has also been found in various kinds of mint and lavender oils and in clover and clover flowers (3–6). It has also been found in reverted soybean oil, oxidized linseed oil and palm oil (7,8). It has been found to be the cause of mushroom flavor in oxidized dairy products (9) and has been produced in model autoxidation of linoleic acid methyl ester (8).

The odor of 1-octen-3-ol has been variously described as mushroom, musty or earthy. In addition to its volatile odor, it also possesses a sweet taste due to the position of the hydroxyl hydrogen between its associated electronegative oxygen atom and the electronegative carbon-to-carbon unsaturated bond (12). This property is of little consequence, however, because the volatile odor would be over-powering at concentrations high enough to produce sweetness.

The volatile flavor threshold of 1-octen-3-ol was determined by Stark and Forss (9). They found that a trained panel could detect it in water at a level of 1 ppb, in skim milk at 1 part in 100 million, and in butter fat at 1 part in 10 million. With a trained panel it was found to be less detectable in soy milk. The threshold here is between 0.5 and 1.0 ppm at the 95% probability level.

The effect of temperature on 1-octen-3-ol formation in soaking soybeans appears to be confounded with the hydration rate of the beans and the completeness of hydration. These factors complicate the problem of determining the true effect of temperature on 1-octen-3-ol formation. Preliminary experience showed that at temperatures below 50 C the rate of hydration was reduced so that it limited the rate of 1-octen-3-ol formation, while at temperatures above 50 C a sharp drop in activity was noted due probably to a direct effect on the chemical or the slowing of possible biological reactions involved in its formation, or both. For these reasons the temperature of 50 C was chosen for the study.

Figure 3 shows the effect of soak time on the amount of 1-octen-3-ol formed per 100 g of beans. The upper curve shows the amount of 1-octen-3-ol contained in the beans after the indicated time of soak. The lower curve shows the amount of 1-octen-3-ol which was measured in the soak water after completion of the soaking step. As can be seen from the figure, the amount of 1-octen-3-ol contained in the bean samples increased from approximately 0.1 mg in 100 g of dry beans to

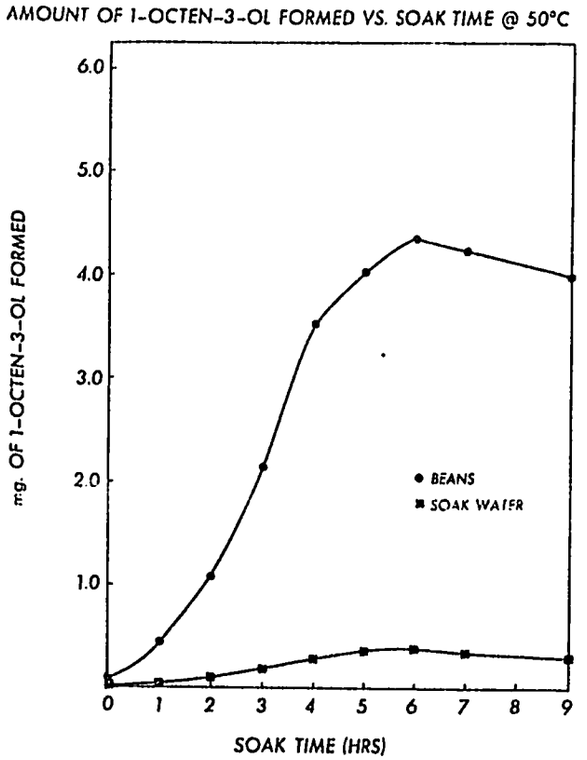

FIG. 3. Amount of 1-octen-3-ol formed vs. soak time at 50C (each point average of three replications).

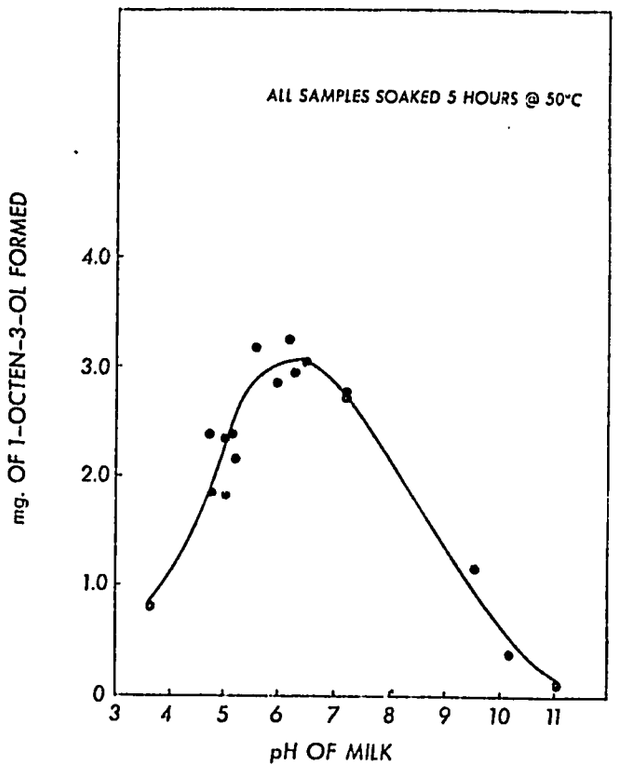

FIG. 4. 1-Octen-3-ol formation vs. pH of resulting milk (each point average of three replications).

approximately 4.4 mg in the samples soaked for 6 hr. The apparent decrease in the amount measured at the longer soak times could be due either to metabolism of the compound by the bean or to slight losses from the sample by evaporation. The amount of 1-octen-3-ol measured in the soak water was found to be approximately 10% of that found in the milk.

The effect of pH on the amount of 1-octen-3-ol formed is shown in Figures 4 and 5.

Since the pH measurements were made on the milk samples after grinding, the graphs show the general effect of pH on the reaction without actually indicating the amount formed at a specific pH, since the pH of the resulting milk would not correspond exactly with the pH at the site of the reaction within the beans. Figure 5 shows the amount of 1-octen-3-ol measured in the soak waters at their respective pH's. Both curves indicate an optimum pH for 1-octen-3-ol formation of between 6 and 7. It is interesting to note that the pH of soy milk made from soybeans soaked in distilled water has a pH of 6.5–6.7 which is within the pH range found to be optimum for 1-octen-3-ol formation.

The effect of reducing the level of molecular oxygen during the soaking step was determined by de-aerating soybeans under vacuum with a slow nitrogen purge for approximately 18 hr. The beans were then soaked in de-aerated water for 5 hr at 50 C under a nitrogen headspace. The amount of 1-octen-3-ol produced per 100 g of beans was found to be 3.5 mg. This compared with 4.1 mg/100 g of beans in a control sample where no de-aeration was attempted. Although absence of molecular oxygen slightly reduced the amount formed, it does not indicate conclusively that it is or is not a factor in the mechanism of formation, since oxygen may be supplied through a biological mechanism under anaerobic conditions.

Several mechanisms of formation for 1-octen-3-ol have been suggested by various workers. Stark and Forss (9) proposed that 1-octen-3-ol could arise in oxidized dairy products by an autoxidative mechanism with either arachidonic or linoleic acids as possible precursors.

Smouse and Chang (7,11), in their work with reverted soybean oil, have also suggested an autoxidative mechanism for the formation of 1-octen-3-ol from linoleic acid. Their mechanism differs slightly from that of Stark and Forss (9). Both mechanisms postulate the initial formation of an 18 carbon hydroperoxide and a concomitant allylic rearrangement followed by chain scission between C_{10} and C_{11}. Stark and Forss postulate that the resulting 8 carbon fragments is a free radical which undergoes allylic rearrangement followed by reaction with a hydroxyl-

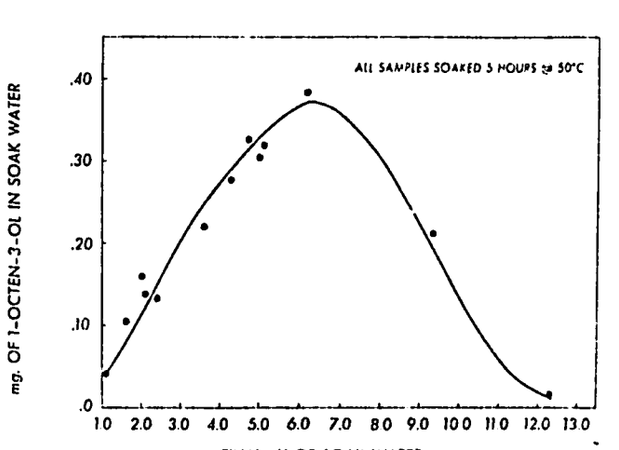

FIG. 5. Amount of 1-octen-3-ol measured in soak water vs. the final pH of the soak water (each point average of three replications).

free radical to produce 1-octen-3-ol. Smouse and Chang, on the other hand, suggest that the resulting 8 carbon fragment is an olefin (2-octene) which then autoxidizes, again with concomitant allylic rearrangement, to 3-hydroperoxy-1-octene. This intermediate then reacts with another molecule of linoleic acid by cleavage of a hydroxyl radical from the hydroperoxide. A subsequent acceptance of a hydrogen ion from the linoleic acid forms 1-octen-3-ol.

Hoffman (8) has also proposed a mechanism for the formation of 1-octen-3-ol from linoleic acid. The mechanism is also autoxidative but is quite different from the other two which have been discussed. He suggested the formation of a free radical at C_{11} which would react with O_2 and another molecule of linoleic acid to yield a hemiacetal via an unstable intermediate. The hemiacetal was then thought to undergo an intermolecular cyclic rearrangement followed by cleavage and allylic rearrangement to yield 1-octen-3-ol.

All of the mechanisms which have been discussed are common in that they are all autoxidative. In each of these cases, an autoxidative mechanism was tenable, due to the conditions under which the compound was observed to form. Based on the conditions under which 1-octen-3-ol was observed to form in this experiment, however, a straightforward autoxidative mechanism did not seem tenable.

Since 1-octen-3-ol possesses an asymetric center, namely the number 3 carbon, two optical isomers are possible. In order to determine the mode of formation of 1-octen-3-ol in soybeans during soaking, a gas chromatographic fraction was collected, and a solution of the compound in chloroform was made up to a concentration of 18 mg/ml. The 1-octen-3-ol was found to be levorotatory with a specific rotation of −11.7 at 17 C and −11.8 at 13 C. These values compare with the literature values of −6.7 at 17 C, as determined by Murahashi (2) for a synthetic sample of 1-octen-3-ol, and −17 at 13 C, as determined by Crabalona (5) for a sample of 1-octen-3-ol isolated from lavender oil.

Since the sample of 1-octen-3-ol which was isolated from soaked soybeans was found to be optically active, the mechanism for its formation is stereospecific. This rules out a straightforward organic autoxidative mechanism and indicates that the compound is formed via a biologically (enzyme)-controlled mechanism, with a pH optimum of 6–7. Based on the suggested autoxidative mechanisms leading to 1-octen-3-ol formation, a substrate of lipid origin is indicated. During the rapid turnover of lipid (10) in the initial stages of the germination of soybeans, 1-octen-3-ol is perhaps one of the first volatile compounds to be formed.

The fact that our rotatory value is lower than one of those reported in the literature does not exclude the possibility, however, that some of the 1-octen-3-ol is formed by non-enzymic autoxidation.

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