Diacetyl as the Buttery Flavor Component in Soybean Oil[1, 2]

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Abstract

Soybean oil often exhibits a buttery flavor in the early stages of autoxidation. Molecular distillates from "buttery" soybean oil consisted of aqueous and oily layers. The aqueous layer contained the buttery flavor. During gas chromatography, the buttery flavor compound had a retention time similar to diacetyl on both polar and nonpolar columns. Diacetyl-bis-2,4-dinitrophenylhydrazone was isolated from the aqueous layer of the distillate. Diacetyl added to fresh soybean oil faithfully reproduced the buttery flavor.

Introduction

BUTTERY FLAVOR is known to be associated with reverted flavor in autoxidized soybean oil. Daubert and O'Connell (1) described buttery flavor as the initial change in fresh autoxidizing soybean oil. Chang et al. (2) noted a buttery fraction among components distilled from autoxidized soybean oil. Moser et al. (3) in their study of linolenate-containing oils, found that buttery flavor occurred in the initial stages of oxidation in all oils studied.

In this study, buttery flavor in soybean oil has been accounted for by the isolation of diacetyl from soybean oil that exhibited a strong buttery flavor.

Methods

SOYBEAN OIL WAS OBTAINED from Swift and Company and had been refined by their usual procedures and stabilized with 0.005% citric acid. Buttery flavor could be induced in this oil in one to three days by contamination with metal ions, either by the addition of traces of copper palmitate or by passing the oil through a molecular still with metal parts. Volatile materials were obtained from the oil by high vacuum distillation in a Rota-Film still at room temperature (4). Gas chromatography was carried out as described previously (5) except 1/4 in. columns 7 ft long were used. Columns packed with butanediol succinate (30% on 45/60 mesh Chromosorb P) were operated at 145C; Apiezon L (20% on 45/60 mesh Chromosorb P) columns at 135C.

Organoleptic evaluations were made by a panel of judges experienced in tasting soybean oil and milk fat. Flavor distillates were examined by adding portions of the distillate to fresh, good-quality corn oil. The amounts of distillate and oil were chosen to give a concentration of the flavor compounds in the tasting oil about twice that in the oil from which they were isolated. This was done to make up for losses of the volatile material, overcome the background flavor of the corn oil and facilitate the detection of compounds present near their flavor thresholds. The aqueous layer of the flavor distillate was emulsified into the corn oil by shaking before organoleptic evaluation. Fractions from the gas chromatograph were examined by bubbling the gas from the tail pipe of the instrument through fresh oil. The amount of distillate injected was chosen so it would give a concentration of the flavor in the tasting oil two or three times higher than in the original oil if there were no losses. Diacetyl and pentanal, and vinylethyl ketone were purified by gas chromatography prior to organoleptic examinations. The flavor threshold for diacetyl was determined by presenting 4-ml samples of deodorized mineral oil containing tenfold increments of diacetyl to a panel of judges. The samples were presented in random order and could be retasted or compared with a control oil at will. The frequency of detection of a difference from the control was corrected for the frequency with which a negative control sample was identified as different. The level at which 50% of the panel detected a difference from the control was defined as the threshold.

2,4-Dinitrophenylhydrazine derivatives (DNPH) were prepared with a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. Alkali spectra were taken immediately after treating the DNPH derivative with 0.5 *N* potassium hydroxide in ethanol (6).

Thin-layer chromatography of the DNPH derivatives was carried out on SeasorbSilica Gel G plates developed in chloroform-tetrahydrofuran-methanol, 15:4:1, according to Cobb (7).

Petroleum ether (Skellysolve B) was purified by the method of Hornstein and Crowe (8).

Results and Discussion

The volatile material obtained by high vacuum distillation of buttery soybean oil gave intense buttery odors. The volatile material consisted of an aqueous layer and an oily material soluble in light petroleum ether. Organoleptic evaluation of appropriate amounts of each layer in fresh corn oil showed that the buttery fraction resided in the aqueous layer. The oily material had no buttery flavor.

Portions of the aqueous layer were reacted with 0.1 *N* hydrochloric acid, 0.1 *N* sodium hydroxide and an aqueous solution of Girard's T reagent. The buttery odor disappeared from the tubes containing sodium hydroxide and Girard's T reagent, but remained in the control and the hydrochloric acid. This indicated that the buttery flavor component was a carbonyl compound.

Fractionation of the aqueous layer by gas chromatography, with organoleptic evaluation of the fractions, showed that the buttery flavor had a retention time close to n-butanal on both butanediol succinate and Apiezon L. Diacetyl, a compound well known for its buttery flavor, was tested and emerged just prior to butanal on the nonpolar column and just after butanal on the polar column. Organoleptic evaluation showed that diacetyl in fresh corn oil or soybean oil at 0.1 ppm reproduced the buttery flavor with fidelity.

The presence of diacetyl in the flavor distillate was demonstrated by the isolation of its DNPH derivative. The aqueous layer of the flavor distillate was reacted with 2,4-dinitrophenylhydrazine, the precipitate was collected by centrifugation, and the aqueous acid layer was decanted. The crystals were washed twice with distilled water and extracted exhaustively with purified petroleum ether to remove the monocarbonyl

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derivatives. The petroleum ether-insoluble material was crystallized from ethyl acetate. The absorption spectrum of the crystals in chloroform revealed a peak at 393 mμ, with a shoulder at 430 mμ, which agreed precisely with the spectrum of authentic diacetyl-bis-DNPH. This spectrum is characteristic of dialkylglyoxals and distinguishes them from other carbonyl compounds (9). Near infrared spectra, taken with a Beckman DK instrument with chloroform as the solvent, showed that the material crystallized from ethyl acetate had a spectrum identical to authentic diacetyl-bis-DNPH, except that the former had an absorption band at 2,250 mμ, which was missing from the known. This band probably represents an impurity. When treated with ethanolic potassium hydroxide, both the isolated and authentic derivatives gave identical spectra with an absorption maximum at 550 mμ.

Thin-layer chromatography of the isolated crystalline compound showed that it migrated with the diacetyl-bis-DNPH derivative rather than with those of glyoxal, methyl glyoxal, or 2-ketobutanal.

Previous results from this laboratory (10) have indicated that vinylethyl ketone plays an important role in the flavor of autoxidized soybean oil and that a mixture of vinylethyl ketone and pentanal at about 10 ppb in fresh oil gives a flavor similar to, but not identical with, the flavor of soybean oil in the early stages of autoxidation. The addition of about 6 ppb of diacetyl to this mixture greatly increases the fidelity with which it reproduces the early stages of autoxidized flavor in soybean oil. The flavor threshold of diacetyl in freshly deodorized mineral oil is about 1 ppb.

Diacetyl is a well-known flavor component of fermented dairy products. Pipen et al. (11) isolated diacetyl from heated chicken, but it was not established that the diacetyl arose from the autoxidation of the chicken fat. Hammond and Bird (9) isolated diacetyl as well as glyoxal, methyl glyoxal, and 2-ketobutanal from autoxidized milk fat, but since they found some diacetyl in the milk fat at the beginning of their experiment, it is not certain that it was a product of autoxidation. The precursor of diacetyl in soybean oil is not known. Citric acid is known as a precursor of diacetyl in fermentations (12), and the degradation of the citric acid synergist in the soybean oil during deodorization might give rise to compounds that could later be oxidized to diacetyl. Moser et al. (3), however, reported that a number of oils containing linolenic acid produced buttery flavors, with and without citric acid as an additive. This points to linolenic acid as a precursor of diacetyl, but it is difficult to account for this with the fatty acyl hydroperoxide decomposition mechanisms that have been proposed. Any route that might be proposed would seem to require multiple oxygen attack and the accumulation of oxidized intermediates, and this seems inconsistent with the appearance of buttery flavor in the very early stages of autoxidation of the oils. Commercial vegetable oils always contain small amounts of polymers that are known to decrease the stability of the oil (13). It may be that the autoxidation of these polymers initiates autoxidation in deodorized oils and that this reaction also gives rise to diacetyl.

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