Furanoid Fatty Acids in Oils from Soybeans Lacking Lipoxygenase Isoenzymes

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ABSTRACT: The concentrations of 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid (F20) and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F22) were determined by a stable isotope dilution assay in oils extracted from the soybean cultivar Century and from five soybean genotypes that lacked one or two of the three lipoxygenase isoenzymes. The concentrations of F20 and F22 ranged between 190–225 mg/kg oil and 91–132 mg/kg oil, respectively. The concentration differences were not correlated to the differences in lipoxygenase activities of the soybeans.
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KEY WORDS: Autoxidation, furanoid fatty acids, isotope dilution assay, lipoxygenase, mass spectrometry, rancidity, soybean oil.

Soybean oil (SBO) contains small amounts of furanoid fatty acids, of which those denoted as F20 and F22 (Fig. 1) are the major compounds (1). F20 and F22 are easily photooxidized with formation of the potent odorant 3-methylnonane-2,4-dione (1), which contributes strongly to the light-induced off-flavor of SBO (2,3).

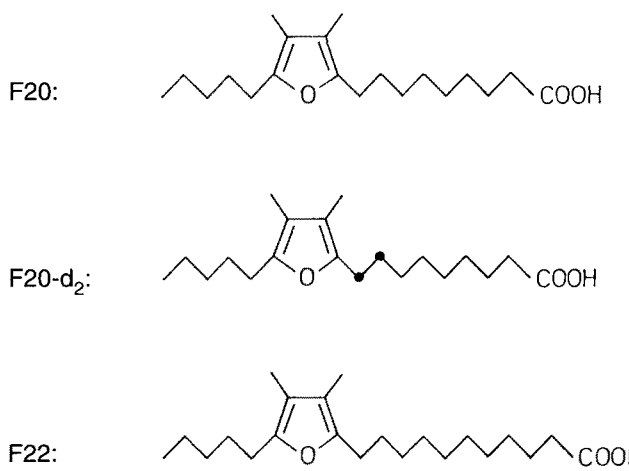

FIG. 1. Numerical key and structures of the furan fatty acids. F20, 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid; F20-d_2, 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid-8,9-d_2; F-22, 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid.

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Three lipoxygenase isoenzymes (linoleate: oxygen oxidoreductase, EC 1.13.11.12) are present in soybean seeds (4). They differ from one another in reaction products, pH optima and substrate specificities (5,6). On the basis that formation of the furan ring system can be explained by a lipoxygenase-catalyzed reaction (7–9), it has been speculated that this enzyme is involved in the biosynthesis of furanoid fatty acids. As soybean varieties lacking one or two isoenzymes have been developed (10–12), it was of interest to investigate whether the lack of a particular lipoxygenase isoenzyme affects the concentrations of F20 and F22 in SBO.

EXPERIMENTAL PROCEDURES

Materials. Mature soybean seeds of the backcrosses of lipoxygenase mutant lines (12) and the wild-type recurrent parent Century were kindly provided by D.F. Hildebrand, University of Kentucky (Lexington, KY). The genotypes designated –L2L3, –L1L3, –L2, –L3, and –L1 are lines backcrossed to Century that are homozygous recessive for the null (or very low) alleles indicated (12). For example, –L2L3 lacks lipoxygenase 2 and 3, but has the normal wild-type level of lipoxygenase-1. Century seeds contain lipoxygenases 1, 2, and 3. F20 and F20-d_2 were synthesized (1,13,14).

Lipoxygenase assay. Ground soybeans (0.5 g) were suspended in a cooled (4°C) aqueous solution of NaCl (10 mmol/L, 20 mL). After stirring for 30 min, the suspension was centrifuged (63,000 × *g*, 4°C, 15 min). In the supernatant the activities of the lipoxygenase isoenzymes were polarographically measured at pH 9.5 (L1) and pH 6.5 (L2, L3), with linoleic acid as substrate (15,16). The lipoxygenase activity is expressed as the consumption of μmol O_2/min per gram of ground soybeans.

Extraction of SBO. Ground soybeans (20 g) were extracted with hexane (100 mL) in a Soxhlet apparatus for 4 h. Hexane was removed by distillation *in vacuo*, and the remaining SBO was weighed.

Determination of furanoid fatty acids. As described previously (1), F20 and F22 were quantitated in SBO on the basis

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of F20-d_2 as the internal standard. The procedure consisted of the following steps: After transesterification of the SBO sample (1 g) with sodium methoxide in methanol, the internal standard F20-d_2 (*ca.* 100 μg) was added in the form of its methyl ester derivative. Then, the methyl esters of F20, F20-d_2 and F22 were enriched by urea fractionation and finally determined by mass chromatography of the protonated molecular ions, which were obtained at *m/z* 337 (F20), *m/z* 339 (F20-d_2) and *m/z* 365 (F22) after chemical ionization.

RESULTS AND DISCUSSION

The lipoxygenase activities of the soybean genotypes are shown in Table 1. As expected, the line –L1 was free of activity at pH 9.5, whereas the activity caused by –L2 and –L3 at pH 6.5 was quite high. The lack of L2 reduced the activity at pH 6.5 only by 7%, which is within the limit of error (±10%) of the lipoxygenase assay. The absence of L3 greatly reduced the activity at pH 6.5, and genotypes lacking both L1 and L3 had greatly reduced activity at pH 9.5. The genotype –L2L3 was only active at pH 9.5, which confirmed the absence of L2 and L3.

The concentrations of F20 and F22 in SBO obtained from the cultivar Century were compared with those of F20 and F22 in the SBO extracted from the genotypes lacking lipoxygenase isoenzymes (Table 2). The SBO from Century contained 190 and 91 mg/kg of F20 and F22, respectively. These data were somewhat different from those obtained from five samples of unprocessed and refined SBO that had also been analyzed (1) for F20 (121–170 mg/kg), and isoenzymes contained similar concentrations of F20 and F22 (Table 2). With exception of the genotype –L2L3, the concentrations of both furanoid fatty acids were up to 18% (F20) and 45% (F22) higher in the SBO from the genotypes than in SBO from Century.

The small concentration differences of F20 and F22 in the SBO of the genotypes did not reflect those of the lipoxygenase activities in the seeds, e.g., the genotype –L1, which shows only lipoxygenase activity of pH 6.5, and genotype –L2L3, which has lipoxygenase activity at pH 9.5, contain equal amounts of F20 and F22, respectively. Also, the large reduction of the total lipoxygenase activity in the genotype –L1L3 (Table 1) was not accompanied by corresponding decreases in concentrations of F20 and F22.

The finding that there was no correlation between lipoxygenase activity in soybeans and the concentrations of F20 and F22 in the oil extracted from these seeds does not rule out the involvement of other lipoxygenase enzymes than those occurring in the ripe seed in the biosynthesis of furanoid fatty acids during growth.

TABLE 1
Lipoxygenase Activities of Soybean Genotypes Lacking Lipoxygenase Isoenzymes[a]

Genotype	Activity (μmol O_2/min • g meal) pH 6.5	pH 9.5
–L1	560	0
–L2	520	240
–L3	108	252
–L1L3	148	2
–L2L3	9	184

[a]The data are means of two assays, maximum SD: ±10%.

TABLE 2
Concentrations (mg/kg SBO) of Furanoid Fatty Acids F20 and F22 in SBOs from Cultivar Century and from Genotypes Lacking Lipoxygenase Isoenzymes[a]

Cultivar	F20	F22
Century	190	91
–L1	203	121
–L2	225	132
–L3	208	118
–L2L3	183	116
–L1L3	202	124

[a]The data are means of two assays, maximum SD: ±10%, SBO, soybean oil.

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