Temperature Effects Upon the Expression of a High Oleic Acid Trait in Soybean

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Soybeans (*Glycine max* L. Merr. cvs. N78-2245 and Dare) were grown to maturity under controlled environments to investigate temperature effects upon the fatty acid composition of developing seed. These genotypes exhibited genetic differences in oleic acid (18:1) content. Mature seed from N78-2245 germplasm normally contained ca. 43 mol% 18:1, and Dare seed contained ca. 18 mol% 18:1. When grown at 30/26 C or 22/18 C, the overall response of these genotypes to temperature resulted in lower 18:1 and higher linoleic (18:2) and linolenic (18:3) acid concentrations in mature seed. However, the genotypic response was much more pronounced in N78-2245 seed than in Dare seed. The basis for these genotypic differences appeared to be related to temperature effects upon the differentiation of the 18:1-synthetic and 18:1-desaturation mechanisms during seed development. Although the high-18:1 trait was expressed during N78-2245 seed development at both temperatures, high-18:1 glycerolipids accumulated during a shorter developmental period at 22/18 C than at 30/26 C. At 30/26 C, glycerolipids containing greater than 50% 18:1 were deposited between 20 and 45 days after flowering (DAF) and accounted for 84% (w/w) of the oil in mature seed. At 22/18 C, glycerolipids with similar fatty acid composition were formed between 30 and 45 DAF and accounted for only 40% (w/w) of the oil. Temperature effects upon 18:1-desaturation also appeared to mediate the overall differences in unsaturated fatty acid composition in these genotypes. The 18:1-desaturation mechanism in N78-2245 seed was more sensitive to temperature than that in Dare seed. These genotype-treatment combinations were ranked by degree of 18:1-desaturation in the order: Dare (22/18 C) = Dare (30/26 C) ≥ N78-2245 (22/18 C) > N78-2245 (30/26 C). It was proposed that the ranking of these genotype-treatment combinations may be attributed, in part, to the tissue levels of the 18:1-desaturase enzymes in soybean seed grown at different temperatures.

The effect of temperature upon the fatty acid composition of glycerolipids in mature seed has been documented for several crop species. These studies usually show a negative correlation between the concentrations of 18:1 and of 18:2 (also 18:3, if present) when seeds are grown at different temperatures. However, the magnitude of such genotype × environment (G × E) interactions attributed to temperature may vary among crop species from slight changes in the unsaturated fatty acid composition of safflower, castor bean, sesame seed and rapeseed (1-3) to more dramatic changes in sunflowerseed, flaxseed, linseed and soybean seed (2-8). Hence, it may be more difficult to determine the intrinsic expression of these traits in seeds of certain plant species. The reason for such differences among various plant species is not understood. In species such as soybean, one can conclude only that the non-heritable component of the phenotypic variability for unsaturated fatty acid composition may be different from other plant species. At this time the same conclusion must be assumed for differences among soybean genotypes. In that regard, the environmentally induced variation in the unsaturated fatty acid composition of soybean seed is significantly greater in high-18:1 genotypes than in typical soybean germplasm with a lower 18:1 concentration (7).

Although several theories have been proposed to explain the relative increase in polyunsaturated fatty acids at lower temperature, little is known about the biochemical mechanism (s) which elicit the response. Most notable among these theories is the concept that oxygen concentration or oxygen solubility in the cell cytoplasm increases at lower temperatures. Because oxygen is required in acyl-desaturation reactions, greater amounts of available oxygen at lower temperature may stimulate 18:1-desaturase activity (9). Another hypothesis contends that decreased membrane fluidity at lower temperature alters the conformational structure of the 18:1 or 18:2-desaturase enzymes (10). Acyldesaturase activities could then increase as a result of greater exposure of the active sites to substrates. Yet another proposal suggests that the 18:1-desaturase has a lower energy of activation (E_a) than the rate limiting reaction in fatty acid synthesis (11). Thus, the 18:1-desaturase may achieve relatively greater activity at lower temperature compared to the overall rate of fatty acid synthesis.

Additional theories might be mentioned. Collectively, the wide range of opinions about the biochemical mechanism by which temperature causes changes in unsaturated fatty acid composition attests to the complexity of this biological phenomenon. Seeking mechanistic explanations through interspecific comparisons only adds to the complexity of this issue. It is doubtful that any single mechanism will fully account for the response of unsaturated fatty acid composition to temperature in all plant species. If several mechanisms are involved and can be identified within a given species, it is also highly probable that there will be genetic variability for those traits. In order to define the conditions which elicit changes in unsaturated fatty acid composition at different temperatures, a research approach should include genotypes within a given plant species that differ in the genetic regulation

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of fatty acid composition. At least the nature of G × E interaction might be addressed in such studies.

Genotypes with genetically altered fatty acid composition, now available within a number of different plant species, might be used to characterize species-dependent effects of temperature upon unsaturated fatty acid metabolism and seed development. In this investigation, two soybean genotypes (Dare and N78-2245) were used which contain genetic differences at loci encoding the 18:1-desaturase (12). Phenotypically, Dare seed represented typical soybean cultivars which normally contained ca. 18 mol% 18:1. N78-2245 germplasm was selected for high-18:1; mature seed from this line normally contained ca. 43 mol% 18:1. The expression of these traits in controlled-temperature environments has not been shown previously. Thus, an objective of this work was to define the genotypic response of unsaturated fatty acid composition to different aerial temperatures during seed development. The arguments advanced in this text have lead to the proposal that the subject genotypic response to temperature may be attributed, in part, to the tissue levels of the 18:1-desaturase enzymes in soybean seed grown at different temperature.

MATERIALS AND METHODS

Plant growth. Soybeans (*Glycine max* [L.] Merr. cv. Dare and N78-2245) were grown to maturity in growth chambers of the North Carolina State University Phytotron (13). The experiment consisted of two replications in identical chambers. A replication consisted of eight plants of each genotype randomly positioned within the chambers. These plants were grown for 30 days at 26/22 ± 0.25 C day/night aerial temperatures and 15/9 hr day/night durations. After 30 days, a 9/15 hr day/night period was imposed to induce anthesis. The plants received a complete nutrient solution (13) and a photosynthetic photon flux density of 700 ± 50 umol/m²/s and photo-morphogenic radiation of 11 ± 1 w/m² between wavelengths of 700 and 850 nm during the day period from a combination of fluorescent and incandescent lamps. Atmospheric CO_2, monitored by IR gas analysis, was maintained at 400 ± 25 μl/l. After pod initiation the temperature regime in the chambers was changed to 30/26 C or 22/18 C and maintained until plant maturity, which was 60 days after flowering (DAF) for both genotypes. All other growth conditions were held constant.

Intact tissue. Three subsamples of seed were harvested from each genotype within a replication of each treatment at 20, 30, 45 and 60 DAF. Portions of each subsample were used to determine seed dry weight, lipid composition and rates of in vivo and in vitro lipid synthesis. Sample means from these respective measurements were calculated from the subsamples of each replication. All data were reported as the means of two replications.

Incorporation of acetate. The seed coat and embryonic axis were removed from seed (5 g fresh weight/subsample) harvested at 20, 30 and 45 DAF. Unsliced cotyledon halves were incubated with 0.02 μmol [2-^{14}C]acetate, 50 Ci/mol, in 0.2 M MES buffer, pH 5.5, at 15 C or 25 C or 35 C in a final volume of 30 ml. Incorporation of [2-^{14}C]acetate into lipid was linear in all samples at all temperatures for at least 1.5 hr. The respective incubations were terminated after 1 hr, extracted, and analyzed as described previously (14, 15). In addition, phenyl-acyl ester derivatives of 16:0, 18:0, 18:1, 18:2 and 18:3 labeled with [2-^{14}C]acetate were prepared from the total lipid extracts, separated by HPLC and collected individually for radiochemical analysis as described by Wood and Lee (16). The radiochemical purity of each HPLC fraction collected was at least 99% as confirmed by GC analysis. Lipid extraction and analysis of mature seed (60 DAF) was performed as described previously (17).

Preparation of homogenates. Unsliced cotyledon halves (2 g fresh weight/subsample) harvested at 20 DAF were chilled to 4 C (the temperature of all subsequent operations) and ground with a mortar and pestle in 10 ml of a medium containing 50 mM HEPES pH 7.5, 0.1% BSA, and 1 mM EDTA. The homogenate was filtered through eight layers of cheesecloth plus one layer of Miracloth, and centrifuged at 20000g for 20 min. The supernatant, made to 20 mM $MgCl_2$, was centrifuged again at 20000g for 20 min. The resultant pellet was resuspended in grinding buffer and used in the desaturase assays.

Enzyme assays. Fatty acid desaturase activity (18) was measured in 0.2 ml 50 mM HEPES pH 7.2 with 10

TABLE 1

Effect of Growth Temperature upon the Glycerolipid Composition of Developing Soybean Seed

Cultivar	Growth Temp[a]	DAF[b]	Lipid[c]			Total Oil	Dry Weight
			TPL	DG	TG		
	C		---μmol/seed[d]---			%(w/w)	g/seed
N78-2245	30/26	20	1.5	N.D.[e]	7.2	15.4	0.016
		30	4.9	0.6	35.2	16.3	0.072
		45	10.5	2.9	148.2	21.2	0.222
		60	9.6	3.3	168.7	21.3	0.248
	22/18	20	1.4	N.D.	6.3	14.1	0.016
		30	4.6	0.3	28.6	15.9	0.061
		45	7.7	1.7	96.1	18.8	0.159
		60	13.0	2.2	165.6	19.0	0.280
Dare	30/26	20	1.5	1.1	10.0	17.6	0.021
		30	5.5	3.8	43.5	21.4	0.072
		45	13.1	1.6	123.1	24.2	0.165
		60	11.6	2.2	144.8	24.4	0.189
	22/18	20	1.4	0.1	6.6	12.2	0.019
		30	6.0	0.6	36.7	18.2	0.069
		45	7.8	0.5	84.0	21.1	0.127
		60	9.6	0.9	136.6	21.0	0.204
	LSD	0.05	2.6	0.7	38.6	2.2	0.021

[a]Day/night temperature throughout reproductive growth.

[b]Days after flowering.

[c]TPL, total polar lipid; DG, diacylglycerol; TG, triacylglycerol.

[d]Based upon μmol fatty acid in each glycerolipid.

[e]ND, not detected.

TABLE 2

Effect of Growth Temperature upon the Unsaturated Fatty Acid Content of Developing Soybean Seed

Cultivar	Growth Temp	DAF	Fatty Acid 18:1	18:2	18:3	18:1	18:2	18:3	Total[a]
	C		------mol %[b]------			--------μmol/seed--------			
N78-2245	30/26	20	40.0	36.3	7.5	3.5	3.2	0.6	8.7
		30	52.3	28.0	4.2	21.3	11.4	1.7	40.7
		45	57.3	26.3	3.3	92.6	42.5	5.3	161.6
		60	54.6	28.2	3.4	99.1	51.2	6.2	181.6
	22/18	20	22.1	42.6	15.9	1.7	3.3	1.2	7.7
		30	26.2	46.3	10.4	8.8	15.5	3.5	33.5
		45	46.4	34.8	5.6	48.9	36.7	5.9	105.5
		60	32.3	45.0	7.3	58.4	81.4	13.2	180.8
Dare	30/26	20	19.3	47.7	12.2	2.4	6.0	1.5	12.6
		30	18.7	53.6	9.0	9.9	28.3	4.7	52.8
		45	19.2	56.5	7.5	26.5	77.9	10.3	137.8
		60	18.9	57.9	7.7	30.0	91.8	12.2	158.6
	22/18	20	19.9	39.5	19.1	1.6	3.2	1.5	8.1
		30	19.4	50.7	12.6	8.4	21.9	5.5	43.3
		45	19.2	53.2	11.3	17.7	49.1	10.4	92.3
		60	17.1	55.4	11.4	25.1	81.5	16.8	147.1
	LSD	0.05	9.2	6.6	2.7	19.2	19.5	3.0	41.5

[a]Summation of μmol (16:0 + 16:1 + 18:0 + 18:1 + 18:2 + 18:3)/seed.

[b]Fatty acid concentration expressed as mol % of the total μmol fatty acid/seed.

mg/ml BSA, 1 mM NADH, 0.5 nM [1-^{14}C] 18:1-CoA (50 Ci/mol) and 100 μl enzyme at temperatures from 15 C to 40 C for one hr. All assays were shaken at 150 strokes/min and terminated by the addition of chloroform-methanol (1:1, v/v). The mixture was partitioned twice against water and methanol to remove nonlipid compounds and nonreacted radioactive substrates. Radiochemical analyses were performed on the chloroform soluble fraction as before, with the exception that methyl ester derivatives of [^{14}C]18:2 and [^{14}C]18:3 were separated by HPLC with methanol-water (92:8, v/v) as the mobile phase, and detected by absorbance at 206 nm. The procedure for the desaturase assay at different oxygen concentrations was the same except that the assay volumes were 3 ml and the reactions were conducted at 25 C. The apparent energies of activation (E_a) in kcal/mol for 18:2 and 18:3 synthesis from [1-^{14}C]18:1-CoA were calculated from the mean respective velocities at 15 and 25 C by the equation: $E_a = [RT_2T_1 \ln (v_2/v_1)]/10^4$; where: R = 1.98, T_1 = 288 K, T_2, = 298 K, v_1 = velocity at 15 C, v_2 = velocity at 25 C. Kcal/mol multiplied by 4.184 gave the apparent E_a in kJ/mol.

RESULTS AND DISCUSSION

Percent oil in soybean seed is thought to be positively correlated with temperature (5,8). No exception to that premise was found in mature seed from N78-2245 and Dare plants continuously exposed throughout reproductive growth to day/night temperatures of 30/26 C or 22/18 C (Table 1). As shown, percent oil was significantly greater in mature seed from N78-2245 or Dare plants grown at 30/26 C than at 22/18 C. In the mature seed, however, the actual amount (μmol/seed) of triacylglycerol (TG), the predominant glycerolipid of soybean oil, was not statistically different between genotypes or treatments. In view of the differences in percent oil of mature seed grown at different temperatures and the known effects of temperature upon metabolic processes, one would expect to find a greater or more rapid accumulation of TG at higher temperature than at lower temperature. Indeed, this anticipated response to temperature was observed in the rate of TG accumulation during early stages of N78-2245 and Dare seed development. Between 20 and 45 DAF, TG accumulated at a rate of 5.8 μmol/seed/day at 30/26 C and 3.7 μmol/seed/day at 22/18 C in N78-2245; in Dare, the rate was 4.6 μmol/seed/day at 30/26 C and 3.1 μmol/seed/day at 22/18 C. Nevertheless, during the final 15 days of seed growth (45 to 60 DAF) there was greater accumulation of TG in these soybean seed at 22/18 C than at 30/26 C. It will be shown later that the fatty acid composition of the glycerolipid deposited within that period tended to contain higher concentrations of 18:2 and 18:3 in all genotype-treatment combinations. Apparently these events resulted from an interaction between the effects of temperature upon seed development and lipid metabolic processes. Normally, TG deposition in soybean seed would be expected to plateau at or near 45 DAF, as seen in the 30/26 C treatment. However, at lower

TABLE 3

Fatty Acid Composition of Glycerolipids Formed Between Specified Stages of Seed Development

Cultivar	Growth Temp	DAF	Fatty Acid 18:1	18:2	18:3	18:1	18:2	18:3	Total
	C		------mol %[a]------			--------μmol/seed[b]--------			
N78-2245	30/26	0-20	40.0	36.3	7.5	3.5	3.2	0.6	8.7
		20-30	55.6	25.6	3.4	17.8	8.2	1.1	32.0
		30-45	59.0	25.7	3.0	71.3	31.1	3.6	120.9
		45-60	32.5	43.5	4.5	6.5	8.7	0.9	20.0
	22/18	0-20	22.1	42.6	15.9	1.7	3.3	1.2	7.7
		20-30	27.5	47.3	8.9	7.1	12.2	2.3	25.8
		30-45	55.7	29.4	3.3	40.1	21.2	2.4	72.0
		45-60	12.6	59.4	9.7	9.5	44.7	7.3	75.3
Dare	30/26	0-20	19.3	47.7	12.2	2.4	6.0	1.5	12.6
		20-30	18.7	55.5	8.0	7.5	22.3	3.2	40.2
		30-45	19.5	58.3	6.6	16.6	49.6	5.6	85.0
		45-60	16.8	66.8	9.1	3.5	13.9	1.9	20.8
	22/18	0-20	19.9	39.5	19.1	1.6	3.2	1.5	8.1
		20-30	19.3	53.1	11.4	6.8	18.7	4.0	35.2
		30-45	19.0	55.5	10.0	9.3	27.2	4.9	49.0
		45-60	13.5	59.1	11.7	7.4	32.4	6.4	54.8
	LSD	0.05	9.8	7.9	2.8	11.2	9.1	1.3	20.2

[a]Fatty acid concentration expressed as mol % of the total μmol fatty acid/seed accumulated between the specified stages of seed development.

[b]Actual amount (μmol/seed) of fatty acid accumulated between the specified stages of seed development.

temperature, TG continued to accumulate at a linear rate until maturity (60 DAF). Although the final amount of TG/seed was not different among these genotype-treatment combinations, the respective weight of the seed was significantly greater at lower temperature. Therefore, the oil percentage in mature seed from plants grown at different temperatures was determined by seed size rather than by gross differences in the actual amount of oil/seed.

With regard to the fatty acid composition of the seed oil from soybeans grown at different temperature, the concentration of 18:1 is thought to be negatively correlated with the concentration of 18:2 and 18:3 (5). no exception to that premise was found in mature seed from the high-18:1 genotype N78-2245 or Dare plants grown at 30/26 C or 22/18 C (Table 2). However, the response of unsaturated fatty acid composition to temperature was much less prominent in Dare than in N78-2245. At maturity the double bond index (DBI), expressed as the summation of mol % [18:1 + 18:2 (2) + 18:3 (3)], was 121.2 at 30/26 C and 144.2 at 22/18 C in N78-2245 seed; in Dare, the DBI was 157.8 at 30/26 C and 162.1 at 22/18 C. During seed development, growth temperature significantly affected the expression of the high-18:1 trait in N78-2245 seed, but had little effect upon mol % 18:1 in Dare seed. In the 30/26 C treatment, the concentration of 18:1 in N78-2245 seed exceeded 50 mol % at 30 DAF; at 22/18 C the high 18:1-trait was not expressed until 45 DAF. Even then the actual amount of 18:1 (μmol/seed) at 45 DAF was ca. 50% of the amount of 18:1 in the 30/26 C treatment. After 45 DAF there was essentially no further increase in the actual amount of 18:1 (μmol/seed) in either treatment.

Developmental effects upon the expression of the high-18:1 trait were shown by computation of the fatty acid composition of that amount of oil which accumulated between specific dates (Table 3). These data demonstrated that glycerolipids with significantly different fatty acid composition were deposited during specific periods of seed development. In N78-2245 seed grown at 30/26 C, glycerolipids with 55 to 59 mol % 18:1, ca. 26 mol % 18:2 and ca. 3 mol % 18:3 accumulated between 20 and 45 DAF. These glycerolipids accounted for ca. 84% (w/w) of the total μmol fatty acid in mature seed. Glycerolipids with similar unsaturated fatty acid composition were formed only between 30 and 45 DAF in N78-2245 seed grown at 22/18 C, and accounted for ca. 40% (w/w) of the total μmol fatty acid in mature seed from that treatment. Although the anticipated response of lower temperature upon unsaturated fatty acid composition of N78-2245 seed grown at 22/18 C was not seen in the glycerolipids formed between 30-45 DAF, a lower amount of high-18:1 oil was deposited in this seed at lower temperature than at higher temperature. As a result there was a greater proportion of highly unsaturated glycerolipids formed in N78-2245 seed at

TABLE 4

Effect of Incubation Temperature upon the Incorporation of [2-^{14}C]Acetate into Unsaturated Fatty Acid

Cultivar	DAF	Incubation Temp.	18:1 + 18:2 +18:3[a]		18:2 + 18:3[b]		Relative[c] 18:1-Desaturation	
			30/26 C[d]	22/18 C	30/26 C	22/18 C	30/26 C	22/18 C
		C	-----nmol[^{14}C]/hr/seed-----				-----%-----	
N78-2245	20	15	0.85	0.81	0.10	0.14	11.8	17.3
		25	1.70	1.80	0.29	0.51	17.1	28.3
		35	2.55	2.63	0.25	0.35	9.8	13.3
	30	15	0.92	1.08	0.11	0.23	12.0	21.3
		25	1.93	2.16	0.31	0.59	16.1	27.3
		35	2.85	2.97	0.20	0.38	7.0	12.8
	45	15	0.51	0.87	0.06	0.12	11.8	13.8
		25	1.17	1.94	0.16	0.36	13.7	18.6
		35	2.41	2.84	0.10	0.24	4.2	8.5
Dare	20	15	0.58	0.57	0.10	0.10	17.2	17.5
		25	1.07	1.02	0.32	0.32	29.9	31.4
		35	1.54	1.58	0.21	0.19	13.6	12.0
	30	15	0.84	0.71	0.15	0.13	17.9	18.3
		25	1.54	1.36	0.48	0.48	31.2	35.3
		35	2.29	2.00	0.32	0.25	14.0	12.5
	45	15	0.57	0.61	0.10	0.11	17.5	18.0
		25	1.26	1.14	0.36	0.33	28.6	28.9
		35	1.61	1.50	0.21	0.21	13.0	14.0
		LSD 0.05	0.29		0.05		3.0	

[a]Total 18:1 radioactivity, expressed as the summation of the radioactivities in [^{14}C]18:1, [^{14}C]18:2 and [^{14}C]18:3.

[b]Total 18:2 radioactivity, expressed as the summation of radioactivities in [^{14}C]18:2 and [^{14}C]18:3.

[c](Total 18:2 radioactivity/total 18:1 radioactivity) × 100.

[d]Diurnal temperature at which seed was grown.

22/18 C than at 30/26 C. Hence, the expression of the high-18:1 trait in N78-2245 seed grown at different temperatures was not simply governed by effects upon 18:1-desaturation per se, but depended upon the differentiation of the 18:1-synthetic and 18:1-desaturation mechanisms during seed development. In Dare seed, however, it appeared that the differentiation of these mechanisms during seed development was not as sensitive to temperature as those in N78-2245 seed.

Although the rates of unsaturated fatty acid synthesis derived from the incorporation of [2-^{14}C]acetate may not be comparable to the in vivo rate of unsaturated fatty acid accumulation, such information does show the relative effect of temperature upon 18:1-synthesis and 18:1-desaturation. Because the amount of radioactivity accrued in 18:2 and 18:3 is obligatorily derived from 18:1, the total radioactivity in 18:1 may be expressed as the sum of [^{14}C]18:1 plus [^{14}C]18:2 plus [^{14}C]18:3. Data presented in Table 4 showed a linear increase in the total-18:1 radioactivities for all genotype-treatment combinations when the incubation temperature of the reactions was increased from 15 C to 35 C. Thus, within that range there appeared to be a positive correlation between temperature and 18:1 synthesis.

A relative estimate of percent 18:1-desaturation in Dare and N78-2245 seed grown at 30/26 C or 22/18 C was determined by expressing the total radioactivity in 18:2, [^{14}C]18:2 plus [^{14}C]18:3, as a percentage of the total radioactivity in 18:1 (Table 4). As found by others (19,20) and verified by the desaturation of [1-^{14}C]18:1-CoA (Fig. 1), these calculations revealed an apparent temperature optimum for 18:1-desaturation near 25 C in both genotypes at all stages of seed development. At comparable stages of seed development, percent 18:1-desaturation was greater in Dare seed than in N78-2245 seed from plants grown at 30/26 C. The differences in the respective percent 18:1-desaturation values, however, were not as great between Dare and N78-2245 seed grown at 22/18 C. In addition, greater percent 18:1-desaturation values were found in N78-2245 seed grown at 22/18 C than in

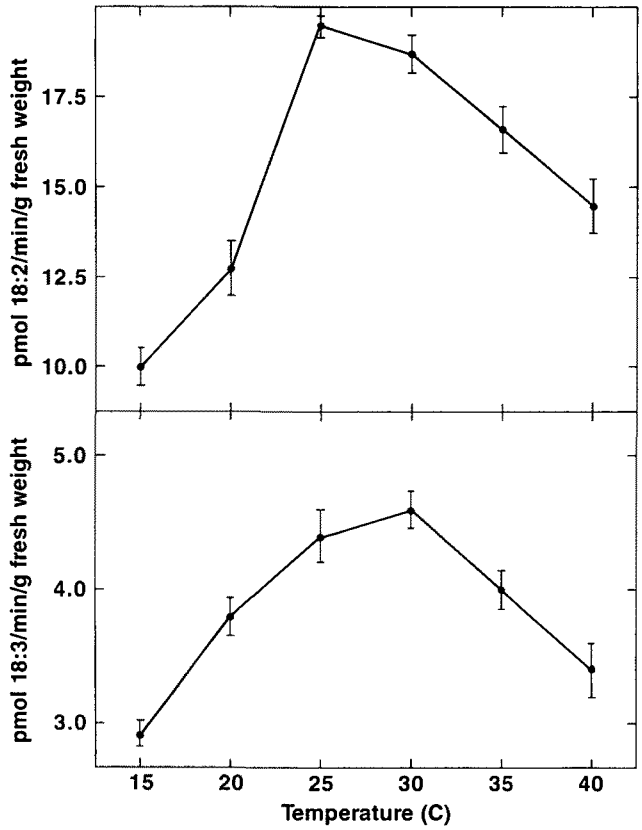

FIG. 1. Effect of incubation temperature upon in vitro assays of 18:2 and 18:3 synthesis. Homogenates of soybean seed, prepared as described in the Methods Section, were incubated with [1-^{14}C]18:1-CoA for 1 hr at 15 to 40 C. Radioactive 18:2 and 18:3 were separated via HPLC.

N78-2245 seed grown at 30/26 C; no differences were found in percent 18:1-desaturation values between Dare seed grown at either temperature. On the basis of these data the genotype-treatment combinations were ranked with respect to apparent 18:1-desaturation ability in the order: Dare (22/18 C) = Dare (30/26 C) ≥ N78-2245 (22/18 C) > N78-2245 (30/26 C). To show a parallel between these data and the in vivo responses in unsaturated fatty acid composition of N78-2245 and Dare seed to growth temperature, the amount (μmol/seed) of 18:2 plus 18:3 was expressed as a percentage of the amount (μmol/seed) of 18:1 plus 18:2 plus 18:3 accumulated in mature seed. The values derived from these calculations were 79.7% (Dare at 22/18 C), 77.6% (Dare at 30/26 C), 61.8% (N78-2245 at 22/18 C) and 36.7% (N78-2245 at 30/26 C). Therefore, by either method the 18:1-desaturation activity in N78-2245 seed was shown to be more sensitive to temperature than that in Dare seed. Hence the rate of 18:1-desaturation was one of the factors which significantly influenced the expression of the high-18:1 trait in N78-2245 seed.

In a previous study, Carver and Wilson (21) demonstrated that the relative rate of 18:1-desaturation in Dare seed was ca. twice that in N78-2445 seed at comparable stages of development. Evidence shown in the present study supported that finding, but only in plants grown at 30/26 C. At lower temperatures the rate of 18:1-desaturation increased in N78-2245, but not in Dare seed. No evidence was found to implicate changes in tissue-oxygen concentration as a cause of the different responses in 18:1-desaturation rates to temperature between these two soybean genotypes. In addition, the apparent E_a for 18:2 synthesis (47.5 ± 1.4 kJ/mol) and for 18:3 synthesis (30.0 ± 1.8 kJ/mol) from [1-^{14}C]18:1-CoA (Fig. 1) did not differ among these genotype-treatment combinations. However, if the apparent E_a for these reactions involved in 18:1-desaturation were constant in N78-2445 and Dare seed grown at different temperatures, the rate of 18:1-desaturation should be directly related to the absolute tissue level of these enzymes. As opposed to conformational changes in a constant tissue level of these enzymes, we might then speculate that there was a lower tissue level of the enzymes which desaturate 18:1 to 18:2 and 18:3 in N78-2245 seed grown at 30/26 C than at 22/18 C; in Dare seed the tissue level of these enzymes was not affected by growth temperature. There is ample evidence for an increase in the levels of certain proteins and enzymes in plant tissues at lower temperature (22), yet little is known about temperature effects upon the tissue levels of acyldesaturases or the genetic regulation of such a response among genotypes. The proof for such a hypothesis will rest ultimately with the purification and quantification of the 18:1 and 18:2-desaturase enzymes from these genotypes grown at different temperatures. Studies have been initiated to achieve that objective. The results of such studies could determine the molecular genetic basis for at least one factor which mediates the expression of the high-18:1 trait in N78-2245 germplasm grown at different temperature.

ACKNOWLEDGMENTS

This work is based on cooperative investigations of the USDA, ARS; the North Carolina Agricultural Research Service, Raleigh, North Carolina, and the Illinois Agricultural Experiment Station, Urbana, Illinois. It was sponsored in part by Grants No. 80455 and 80465 from the Research Foundation of the American Soybean Association. Paper No. 9820 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601.

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[Received May 15, 1985]

Effectiveness of Antioxidants in Refined, Bleached Avocado Oil

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The addition of antioxidants propyl gallate (PG), α-tocopherol and ethoxyquin at a level of 250 ppm to refined, bleached avocado oil resulted in the retardation of the oxidative deterioration of the oil when it was stored in the dark at room temperature, exposed to daylight at room temperature (on the shelf) and at 60 C.

The extent of oxidation was followed by measuring the peroxide and anisidine values and oil color. Ethoxyquin and α-tocopherol were relatively ineffective antioxidants, whereas PG greatly improved the stability of avocado oil stored in the dark at 60 C, but not in oil exposed to daylight.

Avocado oil is derived from the mesocarp of the avocado fruit. There are two major methods for producing avocado oil. One is by drying and pressing the fruit at elevated temperatures and extracting the oil with an organic solvent. The second is by centrifugal force separation. Crude avocado oil undergoes refinement, bleaching and deodorization, yielding the edible oil. Avocado oil is used mainly in the cosmetic industry in its crude form. In the future, avocado oil is expected to enter the food industry. Like other well known edible oils, avocado oil is sensitive to oxidative processes resulting in rancidity, production of undesirable flavors and quality losses during storage (1). The primary products of lipid oxidation are hydroperoxides which generally are referred to as peroxides. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. However, this approach is misleading because of the transitory nature of the peroxides (2). Moreover, the iodometric method for peroxide value has inherent errors due to the absorption of iodine at the unsaturated bonds and the generation of iodine from potassium iodide by oxygen in the solution (3).

The effectiveness of various antioxidants in stabilizing edible oils has been studied previously (3-6). Most of the commonly used antioxidant compounds are referred to as phenolic antioxidants (7). These antioxidants contain an unsaturated aromatic ring containing hydroxyl or amine groups that are hydrogen donors, and thus retard the production of free radicals during the initiation stage of oxidative processes (8).

In view of the lack of data on the retarding effect of antioxidants on autoxidation of avocado oil, the present study was set up to evaluate the effectiveness of propyl gallate, α-tocopherol and ethoxyquin under various conditions.

EXPERIMENTAL PROCEDURES

Preparation of samples. Refined, bleached avocado oil was obtained from Avochem, Santa Paula, California. In accordance with the manufacturer's statement the oil is free of added antioxidants and preservatives. The characteristics of the avocado oil are given in Table 1.

The antioxidants were of analytical grade. DL-α-tocopherol was obtained from Fluka AG, Buches, Switzerland. Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl quinoline) was obtained from ABIC LTD, Ramat-Gan, Israel, and n-propyl gallate from Sigma Chemical Co., St. Louis, Missouri.

Each antioxidant, at a level of 250 ppm, was added directly to the oil, which was stirred at ca. 60 C for one hr to ensure complete dissolution of the antioxidant in the oil. Samples of oil, 10 ml each, were then transferred to a series of transparent bottles of 20 ml volume and 5 cm^2 cross section each. The bottles were loosely closed to enable direct contact between the oil surface and atmospheric air.

The oil samples were exposed to several oxidative conditions. These were (i) complete darkness at room temperature; (ii) "on the shelf," exposed to daylight at ambient temperature, and (iii) opaque laboratory oven at 60 C.

Analysis of samples. Peroxide values were determined periodically in accordance with the AOCS Official Methods (9). Ansidine values were obtained using IUPAC methods(10).

Oil color was evaluated using the Stillman (11)