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ORIGINAL PAPER

Oxidative Stability of Crude Mid-Oleic Sunflower Oils from Seeds with High γ- and δ-Tocopherol Levels

K. Warner · Jerry Miller · Y. Demurin

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Abstract In this study, mid-oleic and high-oleic sunflower seeds were developed with high levels of γ- and δ-tocopherols by traditional breeding techniques. Sunflower seeds containing various profiles of tocopherols, ranging from traditional high α, low γ, low δ relative to those with high γ, high δ, and low α, were extracted, and the crude oil evaluated for oxidative stability. After aging at 60 °C, oils were measured for peroxide value and hexanal as indicators of oxidation levels. We found that when the γ-tocopherol content of mid-oleic sunflower oil (MOSFO) (NuSun) was increased from its regular level of 20 to 300–700 ppm, the oxidation of the oil was decreased significantly compared to MOSFO with its regular low γ-tocopherol level. The modified oils had α-tocopherol contents of up to 300 ppm without negatively affecting the stability of the oil. An oil with one of the best oxidative stabilities had a tocopherol profile of 470 ppm γ, 100 ppm δ, and 300 ppm α, indicating that MOSFO could be more oxidatively stable and still be a good source of Vitamin E from α-tocopherol.

Keywords α-Tocopherol · δ-Tocopherol · γ-Tocopherol · Oxidative · Oxidative stability · Sunflower oil · Tocopherols

Introduction

Sunflower oil is a good quality liquid salad oil and an excellent source of linoleic acid, an essential fatty acid, and α-tocopherol (vitamin E); however, the levels of linoleic acid in sunflower oil are too high for high-stability applications, such as frying. Over 40 years ago, a variety of sunflower was developed that contained high amounts of oleic acid (>70%) [1] with the aim of increasing the stability of the oil for uses such as frying without the need for hydrogenation. More recently, a new variety of sunflower, known as NuSun, has been produced with moderate levels of oleic acid (approx. 55–70%) [2]. This oil also has increased oxidative stability compared to traditional high linoleic acid sunflower oil [2–4]. Although sunflower oil stability was enhanced by decreasing the linoleic acid levels, a study [5] on the tocopherol content of sunflower oil showed that the oil could be improved by adding γ- and δ-tocopherols. In that research, the typical tocopherol profile of sunflower oil (high α, low γ, and low δ) was substituted with that characteristic of soybean oil (high γ, high δ, and low α); the result was an improved oxidative stability of the sunflower oil. These findings are not unexpected based on the antioxidant potentials of the four tocopherol homologues that have been extensively reported in the literature. Gottstein and Grosch [6] found that oxidation stability tests performed in lard at temperatures

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

K. Warner (✉)
National Center for Agricultural Utilization Research (NCAUR), U.S. Department of Agriculture/Agricultural Research Service (USDA/ARS), 1815 N. University Street, Peoria, IL 61604, USA
e-mail: kathleen.warner@ars.usda.gov; warnerk@ncaur.usda.gov

J. Miller
USDA/ARS, Fargo, ND, USA

Y. Demurin
All-Russia Research Institute of Oil Crops (VNIIMK), Krasnodar, Russia

above 60 °C resulted in a relative antioxidant activity order for tocopherols of δ better than γ and γ better than α. In contrast, the antioxidant activity of the tocopherols in menhaden oil stored at 37 °C was γ equal to δ, with both being better than α [7]. α-Tocopherol is the precursor of vitamin E and an excellent in vivo antioxidant; however, it is not as good an antioxidant in vitro as γ- and δ-tocopherol. Sunflower oil, which has approximately 95% of its tocopherols in the α configuration, could possibly have improved oxidative stability if its tocopherol profile had more γ- and δ-tocopherols.

The objective of the study reported here was to investigate the oxidative stability of mid-oleic (MOSFO) and high-oleic (HOSFO) sunflower oils bred to have increased γ- and δ-tocopherols.

Material and Methods

Sunflower Seed Breeding

Crosses of various sunflower germplasm with LG-24 (a sunflower germplasm line developed by Demurin, VNIIMK, Krasnodar, Russia, with approximately 84% γ-tocopherol and 8% α-tocopherol of the total tocopherols [8]) were made in 2000 at Fargo, ND, USA. Plants with high-oleic and high-linoleic compositions were selected, and seeds were crossed to produce mid-oleic (NuSun) hybrid sunflower seed. The germplasm created a maintainer line, USDA 109, which is an F_6-derived F_7 germplasm with 88% oleic acid developed from the cross HA 341/HA 821/LG-24. HA 341 is a high-oleic maintainer line released by the USDA-ARS and the North Dakota Agricultural Experiment Station in 1986 [9]. HA 821 is a high-linoleic maintainer line released by the USDA-ARS and the North Dakota Agricultural Experiment Station in 1983 [10]. The other sunflower germplasm created were a restorer line, USDA 112 with 35% oleic acid, an F_6-derived F_7 germplasm developed from the cross RHA 344/Krasnodar 917//LG-24; RHA 344, a restorer line released by the USDA-ARS and the North Dakota Agricultural Experiment Station in 1986 [9], and Krasnodar 917, a high-oleic, high γ-tocopherol hybrid obtained from the Office of International Cooperation and Development (OICD) germplasm exchange with VNIIMK, Krasnodar, Russia, in 1998. Half seeds that ranged from 50 to 85% γ-tocopherol of total tocopherol were selected for further advancement. Several generations of seeds were grown from F_3 plants through F_5 plants. An F_6 seed (USDA 109) with 84% γ-tocopherol and 13% δ-tocopherol of total tocopherol and 88% oleic acid composition and an F_6 seed (USDA 112) with 75% γ-tocopherol and 11% δ-tocopherol of total tocopherol composition and 32% oleic acid composition were crossed to produce mid-oleic (NuSun) hybrid sunflower seed for this study.

Tocopherols

Tocopherol standards (α-, β-, γ-, δ-tocopherols, at 95% purity) were purchased from Matreyac (Pleasant Gap, PA). High-performance liquid chromatography (HPLC)-grade solvents, including hexane and 2-propanol (98.5% purity), were purchased from Fisher Scientific (Fair Lawn, NJ).

Oil Extraction Procedures

To obtain oil for oxidative stability determinations, crude oil was extracted from the sunflower seeds using a Soxtec extractor (Model Avanti 2050; Foss, Eden Prairie, MN).

Fatty Acid Compositions

Fatty acid compositions of the oils were determined in duplicate by capillary gas chromatographic (GC) analysis with a Hewlett-Packard 5890 GC (Wilmington, DE) equipped with a SP2330 column (30 m, 0.20 mm i.d., 0.20 μm film thickness) (Supelco, Bellefonte, PA). The carrier gas was He at 1 mL/min. A 5-μl sample was injected in triplicate. The temperature of the column was first held at 190 °C for 5 min and then programmed to 230 °C at 20 °C/min. The injector was held at 250 °C and the detector at 260 °C. Peaks were identified by retention time compared to commercial standards.

Tocopherol Analysis of Seeds

The analysis α-, β-, γ-, and δ-tocopherols by normal-phase HPLC was conducted on a Varian ProStar (Varian Assoc, Walnut Creek, CA) with a Model 363 fluorescence detector. The detector was set at 290 nm for excitation and 330 nm for emission. The HPLC was fitted with a 5-μm Varian Inertsil Si column (250 × 4.6 i.d.). The isocratic solvent system, 0.5% 2-propanol in hexane, was pumped at 0.5 mL/min. The tocopherols were quantified using external standard calibration. Peaks were identified by retention time compared to commercial standards.

Tocopherol Analysis of Extracted Crude Oils

Tocopherols in the oils were measured in duplicate by HPLC with a polar phase column coupled with a fluorescence detector. The HPLC column used was a 3-μm particle size ultra silica HPLC column (25 × 0.49 cm; Phenomenex, Torrance, CA). The solvent system was 2% 2-propanol in hexane. The solvent was pumped at 0.5 mL/min.

The sample size was 10 μL of a 50-mg solute per milliliter of the mobile solvent. The fluorescence detector was a programmable unit (model HP1046 A) with an excitation wavelength set at 298 nm and an emission wave length set at 345 nm, with gain at 6 (Hewlett-Packard, Palo Alto, CA). The tocopherol standards were prepared by appropriate dilutions of the standard tocopherol—50 mg tocopherol, 99.4% pure—in 1 mL hexane.

Oxidation Procedures

Crude oils were used in the oxidation tests because the limited quantity of seeds available did not allow for processing of the oil to the RBD stage. The crude oils were oxidized under accelerated temperature conditions according to AOCS oven storage method Cg 5-97 [11] at 60 °C in the dark, with air in the headspace of the storage container. The MOSFOs were aged for up to 4 days at 60 °C, whereas the HOSFOs were aged up to 5 days at 60 °C. A 4-g sample of each oil was placed in a 4-dram glass vial with a screw cap. Oxidation tests were repeated using the same oils.

Measurement of Oxidation Levels

Oxidation levels of all oils were determined in duplicate by peroxide value (PV) (AOCS method Cd 8-53) [11] and by hexanal analysis using dynamic headspace capillary gas chromatography (AOCS method Cg 4-94) [11].

Statistical Analysis

Data were statistically analyzed by ANOVA, and statistical significance is expressed as $P \leq 0.05$ unless otherwise noted [12].

Results and Discussion

Fatty Acid Composition

Our analyses of the fatty acid compositions of crude oils from ten sets of sunflower seeds with modified tocopherol compositions showed that the oils had a range of high- to mid-oleic acid contents (Table 1). The oils were divided into two sets—one with oleic acid contents in the mid-range (55–70%) and the other with oleic acid levels >70%. Control samples were high-oleic and mid-oleic sunflower oils (MOSFO) with regular tocopherol compositions. The oleic acid contents of oils in set 1 (<70% oleic) ranged from 56.1 to 67.1%, with corresponding linoleic acid levels ranging from 33.2 to 23.2%. The oleic acid contents of oils in set 2 (>70% oleic) ranged from 70.1 to 75.6%, with corresponding linoleic acid levels ranging from 19.4 to 14.7%.

Table 1 Fatty acid compositions (%) of crude sunflower oils

	C16:0	C18:0	C18:1	C18:2
Set 1				
Mid-oleic				
A	4.2	3.6	61.5	28.7
B	4.1	3.6	63.3	26.7
C	4.4	4.0	58.5	30.9
D	5.0	3.0	56.7	33.4
E	4.1	3.4	67.1	23.2
Control 1	4.9	3.8	56.1	33.2
Set 2				
High-oleic				
F	4.0	2.8	72.8	18.2
G	4.1	3.9	73.2	16.7
H	3.6	3.6	75.6	14.7
J	3.8	3.4	73.3	17.1
K	3.8	3.4	73.0	17.3
Control 2	4.8	3.6	70.1	19.4

Tocopherol Composition

Tocopherol levels in the modified oils and in the controls are shown in Table 2. The controls had the typical tocopherol profiles found in sunflower oil with high α- and low γ- and δ-tocopherols and were in agreement with the

Table 2 Tocopherol compositions (ppm) of extracted crude sunflower oils

	Alpha	Beta	Gamma	Delta
Set 1				
Mid-oleic				
A	393 a	82 a	333 a	64 a
B	356 b	78 a	364 b	68 a
C	289 c	56 b	472 c	95 b
D	69 d	24 c	680 d	221 c
E	33 e	12 d	656 d	188 d
Control 1	909 f	34 e	18 e	3 e
Set 2				
High-oleic				
F	365 a	109 a	391 a	120 a
G	512 b	79 b	223 b	42 b
H	44 c	8 c	678 c	140 c
J	52 c	9 c	558 d	135 c
K	308 d	47 d	522 e	121 a
Control 2	779 e	34 e	10 f	2 d

Tocopherol values within each set for each homologue followed by a different letter are significantly different ($P \leq 0.05$)

values reported for crude sunflower oils in the Codex standards [13]. The α-tocopherol levels ranged from 33 to 393 ppm in the modified MOSFO, and from 52 to 512 ppm in the modified high-oleic oils. γ-Tocopherol levels ranged from 333 to 680 ppm in the modified MOSFO, and from 223 to 678 ppm in the modified high-oleic oils. δ-Tocopherol levels ranged from 3 to 221 ppm in the modified MOSFO, and from 2 to 140 ppm in the modified high-oleic oils. We noted that when the amount of α-tocopherol decreased, the level of β-tocopherol decreased correspondingly; whereas the levels of γ- and δ-tocopherol increased. Most of these modified oils had tocopherol compositions similar to the low α- and high γ- and δ-tocopherol profile found in crude soybean oils [13].

Measurement of Oxidation Levels in Oils

The amount of oxidation produced in the various oils was measured by PV as an indicator of primary oxidation products and by hexanal to indicate the levels of secondary oxidation products. The PVs for the MOSFO control were significantly higher at all storage times after 0 days than any of the values for the oils with modified tocopherol profiles (Fig. 1). For most of the samples, the PVs decreased significantly as the amount of γ-, and δ-tocopherol increased. The two MOSFO with the highest amounts of γ-tocopherols, D and E, had significantly lower PVs than the other samples. The hexanal contents of the modified oils showed a pattern similar to that of the PVs; however, the differences between the oils were not as distinct (Fig. 2). Studies have been conducted on antioxidants, such as tocopherols, to determine how and where antioxidants function in order to discover, for example, if a compound is

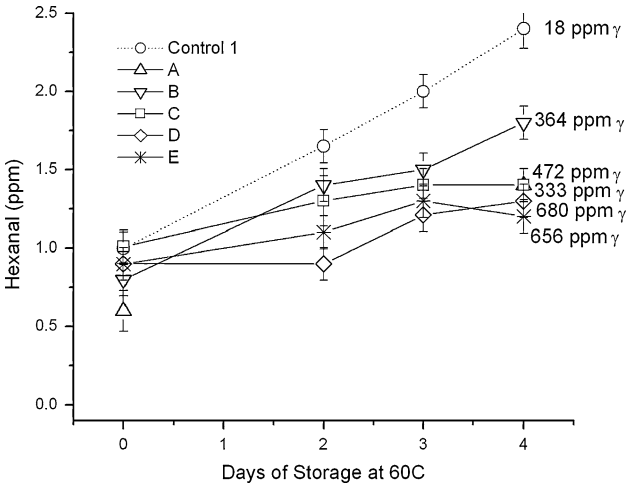

Fig. 2 Hexanal contents of crude MOSFOs (*A–E*) containing various levels of tocopherols and aged up to 4 days at 60 °C

a chain-breaking antioxidant (initiation, propagation, or termination phase) or a preventive antioxidant [14]. It appears that the γ-, and δ-tocopherols had more of an effect on inhibiting the formation of primary oxidation products than on inhibiting secondary oxidation products. Figure 3 presents the correlation curves and coefficients for γ- and δ-tocopherols with the changes in PVs for the MOSFO. Both tocopherols had significant coefficients.

The PVs for the HOSFO control were significantly higher at all storage times after 0 days than any of the values for the oils with modified tocopherol profiles (Fig. 4). As with the mid-oleic oils, the PVs decreased significantly as the amounts of γ- and δ-tocopherol increased. Sample K had the lowest PVs even though it did not have the highest γ- and δ-tocopherol levels plus it even

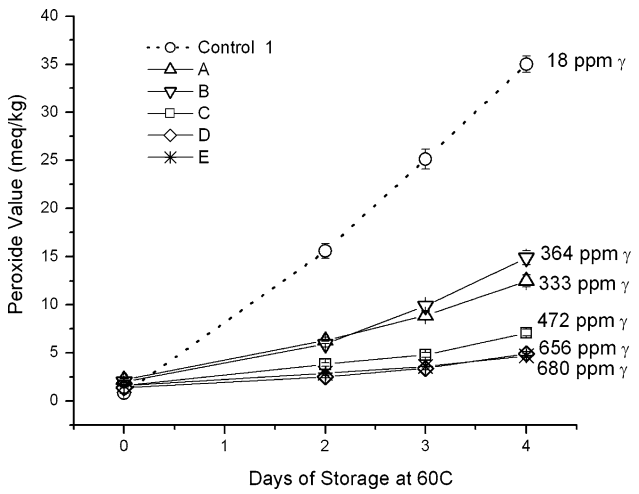

Fig. 1 Peroxide values (*PV*) of crude mid-oleic sunflower oils (*MOSFO*, *A–E*) containing various levels of tocopherols and aged up to 4 days at 60 °C

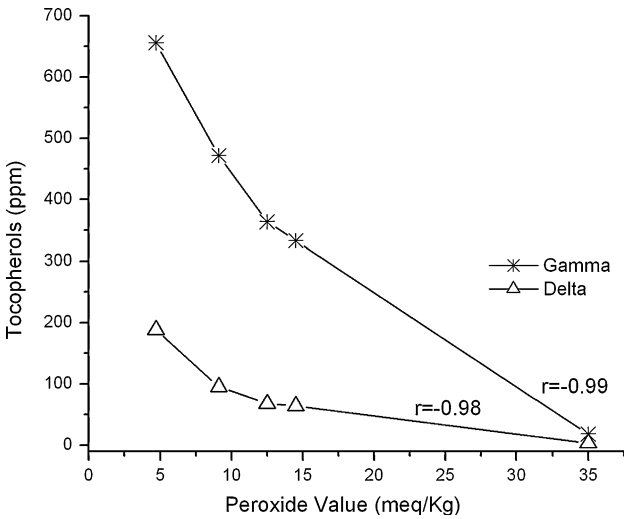

Fig. 3 Correlation coefficients of tocopherol levels and PVs in crude MOSFOs

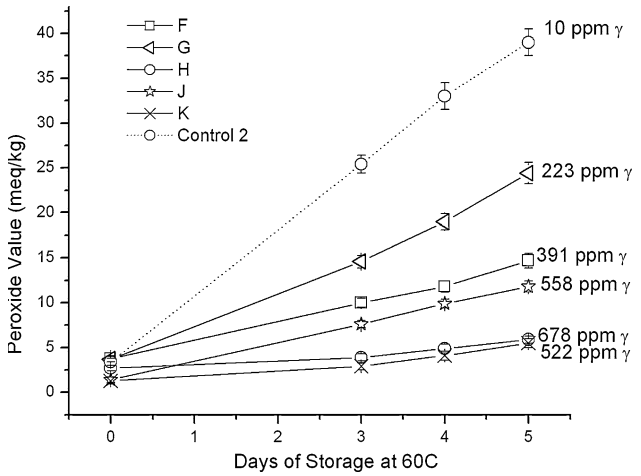

Fig. 4 Peroxide values of crude high-oleic sunflower oils (*HOSFO*, *F–K*) containing various levels of tocopherols and aged up to 5 days at 60 °C

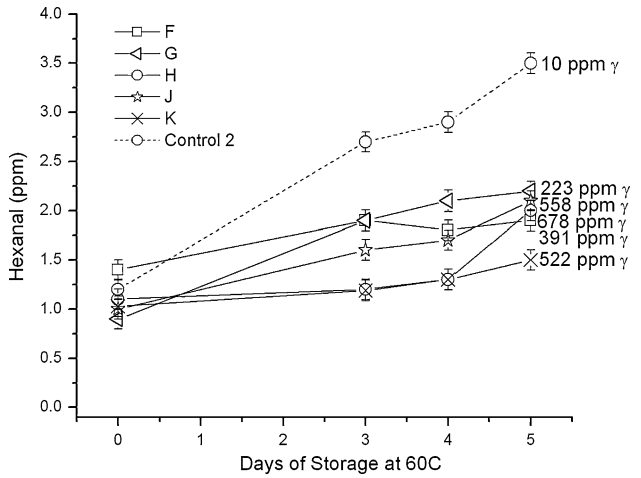

Fig. 5 Hexanal contents of crude HOSFOs (*F–K*) containing various levels of tocopherols and aged up to 5 days at 60 °C

had a moderate rather than a low amount of α, indicating that the level of α does not have to be low for the oil to have good oxidative stability. The hexanal contents of the modified oils showed a pattern similar to that of the PVs; however, the differences between the oils were not as distinct (Fig. 5). As with the MOSFO, the γ- and δ-tocopherols in HOSFO had more of an effect on inhibiting the formation of primary oxidation products than on inhibiting secondary oxidation products. Figure 6 presents the correlation curves and coefficients for γ- and δ-tocopherols with the changes in PVs for the HOSFO oils. Some of the PVs did not increase linearly with increasing or decreasing tocopherols, therefore the correlation coefficients for the HOSFO were lower than those for the MOSFO.

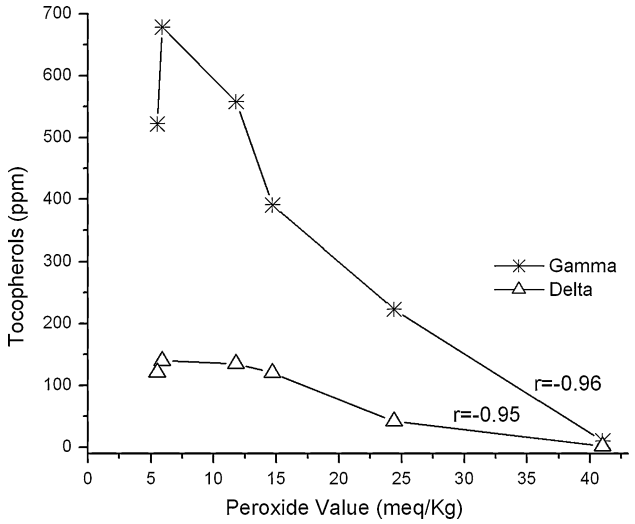

Fig. 6 Correlation coefficients of tocopherol levels and PVs in crude HOSFOs

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