Oxidative Stability of Safflower Oil[1]

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Abstract

Oils from a number of varieties of safflower (*Carthamus tinctorius* L.) seeds (achene) were measured for oxidative stability by the gain in weight method. The induction periods of oils containing 75% to 80% linoleic acid ranged from 288 to 715 hr. Safflower oils containing 79% to 80% oleic acid and only 11% to 15% linoleic acid had induction periods ranging from 1274 to 2374 hr. No correlation between induction period and total tocopherol content was observed. However, there were indications that oils from pigmented seeds were less stable than oils from pigmentless seeds. Blending of an oil containing a high amount of linoleic acid with an oil containing a high amount of oleic acid gave a blend with an induction period intermediate between the two. However, the induction period was considerably less than the theoretical average calculated for the mixture.

Introduction

The inclusion of polyunsaturated fats in the diet is known to lower blood levels of cholesterol and consequently is important in the etiology of atherosclerosis (1). This has stimulated interest in edible oils that are rich in linoleic acid such as safflower (*Carthamus tinctorius* L.) oil which may contain up to 80% linoleic acid. This high content of linoleic acid makes these oils highly susceptible to oxidative rancidity. The progress of the oxidative rancidity can be followed in a number of ways. These include measurements of iodine absorption and uptake of oxygen by the oil. A convenient method is a gain in weight technique which has been used from time to time for at least 75 years (2–4). In this report this method was used to determine the oxidative stability of oils from a number of varieties and genetic lines of safflower and the influences of oleic acid and tocopherol contents of these oils on oxidative stability.

Experimental Procedures

The safflower lines investigated in this paper were selected on the basis of seed types and genetic characteristics which might have an influence on the oil stability. The genotypes used were: (a) brown, striped-hull (stpstp) which is associated with an odor and pigment in the oil and is lower in hull percentage than are normal-hull genotypes (StpStp);[2] (b) pigmentless pericarp (pp) which contains no melanin layer in the pericarp as does normal pericarp (PP); (c) light-colored seed coat (ltlt) which is light tan in contrast to normal seed coat (LtLt) which is dark brown; and (d) oleic oil (olol) which contains approximately 80% oleic acid and 10% linoleic acid in contrast to normal (OlOl) which contains approximately 10% oleic acid and 80% linoleic acid.

The seeds were collected from plants grown in Arizona or California. All seed samples were cleaned and inspected for purity, uniformity and external injury.

Percentages of oil and free fatty acids, and peroxide values were determined by AOCS Official Methods (5). The oils were extracted from the seeds as follows.

Approximately 360 ml of petroleum ether (A.R. 30–60 C ACS) were added to 40 g of safflower seeds in a blendor jar and the mixture was blended for 2 min at maximum speed. The contents were poured into a large beaker, the meal and hulls were allowed to settle, and the petroleum ether was decanted into a large separatory funnel containing water. To the residue in the beaker 10 ml of distilled water and 160 ml of 95% ethanol were added, the beaker was swirled and the contents transferred to the blendor jar. The remaining contents of the beaker were washed into the blendor jar with 180 ml of petroleum ether, and the total mixture was blended for 2 min at maximum speed. The extraction was repeated a third time if the residue was still colored. The combined petroleum ether extracts were filtered through a large funnel fitted with a fritted disc. Finally the filtrate was washed with water to remove the ethanol, dried over anhydrous sodium sulfate and the petroleum ether removed under reduced pressure.

The gain in weight technique (4) to measure oxidative stability of the oil consisted of the following steps.

One gram samples of oil replicated five times, were weighed into black painted 30 ml beakers, covered with black painted watch glasses. The covered beakers were placed in a forced draft oven set at a constant temperature of 50 C; all beakers were removed once daily from the oven, allowed to cool to room temperature, weighed rapidly, and returned to the oven. The time required for rapid oxidation to begin is called the induction period of the oil and is a measure of the oxidative stability of the oil (Fig. 1).

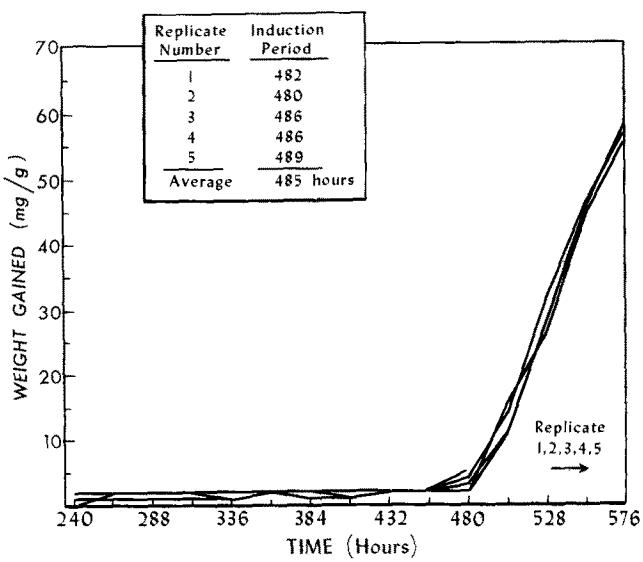

FIG. 1. Induction period of safflower oil.

[1] Arizona Agricultural Experiment Station Technical Paper No. 1389.

[2] Other alleles at the striped locus are $stp^{s}stp^{s}$—gray-striped-hull, and $stp^{p}stp^{p}$—purple-striped-hull which are intermediate in hull percentage between brown-striped-hull and normal hull.

TABLE I

Per Cent of Moisture, Oil and Free Fatty Acids of Safflower Seed

Seeds		% Moisture	Oil	Free fatty acids % of oil
Line or variety	Genotype			
1998-B	P-,stpstp[a]	5.0	46.9	0.04
1998-A	pp,stpstp	5.7	42.3	0.12
2050	pp,stpstp	6.0	40.8	0.02
A0104	PP,stp^{g}stp^g	3.6	39.6	0.08
14154	PP,stp^{p}stp^p	4.4	38.2	0.11
2134-A	pp,StpStp	6.0	38.1	0.10
2134-B	P-,StpStp[a]	5.9	37.2	0.07
A104	PP,StpStp	7.5	35.0	0.11
Gila (65-2-1)	PP,StpStp	3.8	34.2	0.07
VDL-641	PP,StpStp	4.2	33.5	0.05

[a] "P-" consists of two types "PP" and "Pp" both having pigmented melanin layers.

For the determination of fatty acid composition of the oils, the oils were saponified with alcoholic potassium hydroxide and the fatty acid fraction separated and identified by gas chromatography as follows.

To the fatty acids from 1 to 3 g of oil were added 12 ml of 7% boron trifluoride-methanol reagent and the mixture refluxed for 5 min. Then 120 ml of water were added and the total transferred to a separatory funnel and extracted twice with 40 ml of Skellysolve B. The Skellysolve B was evaporated under reduced pressure and the esters stored in a refrigerator.

The fatty esters were identified and the percentage of each ester determined with an Aerograph Hy-Fi Gas Chromatograph (Model 600-C). Good resolution was achieved from columns packed with Ethylene Glycol Succinate (EGS) or Diethylene Glycol Succinate (DEGS). The temperature of the column was 180 C.

For the determination of total tocopherols the method published by the Analytical Methods Committee (6) was modified to fit our conditions. Briefly the method is as follows.

The unsaponified residue from 1 g oil was dissolved in 0.5 ml distilled benzene (A.R.). Ten to 20 μliters of the benzene solution were injected into a low actinic stoppered test tube which contained 1.5 ml of absolute ethanol and 0.25 ml of 6×10^{-3} M bipyridine in absolute ethanol. Exactly 0.25 ml of 1×10^{-3} M ferric chloride in absolute ethanol were added and the test tube was shaken well. Exactly 2 min after adding the ferric chloride, 0.25 ml of 4×10^{-2} M orthophosphoric acid in absolute ethanol were added, the test tube shaken, and the contents transferred to a 1.5 ml spectrophotometric cell. M orthophosphoric acid was added to react with residual ferric ions to stabilize the ratio of ferrous to ferric ions. The absorbance was read at 520 mμ against a reference cell containing absolute ethanol. The same procedure was followed omitting the tocopherol solution for the blank. Net extinction was equal to absorbance of the sample minus the absorbance of blank.

TABLE II

Induction Periods and Total Tocopheral Concentration of Safflower Oils

Seeds		Induction period at 50 C, hr	Total tocopherols μg/g
Line or variety	Genotype		
A0104	PP,stp^{g}stp^g	288	454
2039	pp,stpstp	316	536
2011-B	P-,StpStp[a]	326	450
2129	pp,StpStp	351	.....
VDL-641	PP,StpStp	372	635
2105	PP,stpstp	411	520
A104(1891)	PP,stp^{g}stp^g	413	614
1997-B	P-,stpstp	415	562
2134-B	P-,stpstp	432	.....
A104	PP,StpStp	434	586
2134-A	pp,StpStp	439	.....
1998-B	P-,stpstp	448	.....
1997-A	pp,stpstp	441	560
2050	pp,stpstp	460	.....
US10	PP,StpStp	464	480
Gila (57059)	PP,StpStp	482	646
Gila (3817)	PP,StpStp	486	517
2011-A	pp,StpStp	518	449
12289	PP,stp^{g}stp^g	535	564
Gila (65-2-1)	PP,StpStp	542	514
1998-A	pp,stpstp	560	.....
Gila (1862)	PP,StpStp	576	424
7304	pp,stpstp,ltlt[b]	715	540

[a] "P-" consists of both "PP" and "Pp" genotype, both having pigmented melanin layers.
[b] "ltlt" is light seed coat. All other entries are "LtLt."

TABLE III

Fatty Acid Percentages of Safflower Oils

Seeds		Palmitic acid %	Stearic acid %	Oleic acid %	Lino'eic acid %
Line or variety	Genotype				
US10	PP,StpStp	9	3	13	75
2039	pp,stpstp	8	3	13	76
VDL-641	PP,StpStp	8	2	14	76
Gila (65-2-1)	PP,StpStp	10	3	11	76
2105	PP,stpstp	8	3	12	77
2011-B	P-,StpStp[a]	9	3	11	77
Gila (1862)	PP,StpStp	9	3	11	77
2129	pp,StpStp	8	3	11	78
A104	PP,stp^{g}stp^g	8	2	12	78
A0104	PP,stp^{g}stp^g	8	3	11	78
7304	pp,stpstp,ltlt[b]	6	2	14	78
1998-A	pp,stpstp	8	1	12	79
1998-B	P-,stpstp	8	2	10	80

[a] "P-" consists of both "PP" and "Pp" genotypes.
[b] "ltlt" is light seed coat, all other entries are "LtLt."

Results and Discussion

Table I gives the content of moisture and oil for representative varieties of safflower seed and per cent of free fatty acids in the various oils. The first two seeds are recent crosses and are thin-hulled varieties.

Table II lists induction periods and tocopherol concentrations for several safflower oils. The length of time between the extraction of oil from the seeds and the time the oil is used for investigations is very significant; especially if the oil is not kept tightly closed under nitrogen.

For the oils studied, there was an average weight gain no greater than 5 mg during the induction period. By analysis of variance a difference of 25 hr in induction period between samples was found to be significant at the 5% level. The induction periods of the oils ranged from 288 to 715 hr. Tocopherol contents were somewhat variable but no correlation could be seen between tocopherol content and length of induction period. The tocopherols from several varieties of seed were differentiated by TLC. In all cases the main constituent was α-tocopherol with insignificant amounts of γ-tocopherol. It is reasonably apparent that the differences in stabilities of the various oils are due to other natural antioxidants.

Table III lists the fatty acid percentages for a selected group of safflower oils. The percentages were calculated by triangulation of the peak areas of graphs obtained by gas chromatography. There does not appear to be an appreciable difference in the

TABLE IV

Stabilities of High-Oleic Safflower Oils

Description of oil	Induction period at 50 C, hr	Total tocopherols μg/g	Oleic acid, %	Linoleic acid, %
IIV[a] 143-173, Refined	722	506	46	45
Fls 71C	1274	494	79	15
Fls 71B	1648	583	79	15
Fls 71A	1694	448	79	14
UC-1	1794	575	80	13
	2374	599	81	11

[a] Intermediate iodine value.

TABLE V

Effect of Mixing High Oleic and Normal Safflower Oils on the Induction Period at 50 C

Description of oil	Induction period, hr	Total tocopherols, μg/g	Linoleic acid, %
UC-1	2374	539[a]	11
Gila (65–2–1)	542	514[a]	76
UC-1 + Gila (1:1 mixture)	787	511[a]	45
UC-1 + Gila (3:1 mixture)	830	.....	....
IIV[b]	722	513[a]	45

[a] After column chromatography with Florisil.
[b] Intermediate iodine value.

linoleic acid per cent between the regular genetic lines of safflower oil. Palmitoleic acid was observed in trace amounts in a few oils but it not recorded in the table.

Table IV gives the stability data and contents of oleic acid, linoleic acid and total tocopherols for safflower oil from seeds genetically controlled to give high amounts of oleic acid and small amounts of linoleic acid (7). These oils had long induction periods but their tocopherol contents were in the ranges of those listed in Table II. It is apparent that the stability of these oils is due more to the high contents of oleic acid and low contents of linoleic acid than to their contents of tocopherols.

Table V shows the effect on the induction period of mixing a normal variety of safflower oil with a high-oleic acid variety. It is interesting to compare the values of the 1:1 mixture of UC-1 and Gila with Intermediate Iodine Value (IIV). The induction period of the mixture is closer to the induction period of IIV than the calculated value based on the proportions of the two oils. The total tocopherol concentration and the per cent of linoleic acid in the mixture is also close to the values for IIV. It appears that the overall oxidative stability of UC-1 and the mixture is more dependent on the oleic to linoleic acid ratio than to the concentration of tocopherols. It would be expected, therefore, that the larger this ratio the more stable will be the oil.

TABLE VI

Induction Periods of Oils from Pigmentless (pp) vs. Pigmented (PP) Seeds from Same F_4 Line

Seeds		Induction period, hr	
Line	Hull type	Pigmented (B)	Pigmentless (A)
1997	stpstp	415	441
1998	stpstp	448	560
2134	StpStp	432	439
2011	StpStp	326	518

Table VI compares induction periods for oils from seeds of the same line differing in pigmentation. Not enough lines were available for a statistical treatment of the data. Also, peroxide values for three of the four oils were higher in the pigmented lines than in the nonpigmented lines. However, the data do indicate that the pigments in these lines decrease the oxidative stability of the oil.

ACKNOWLEDGMENTS

This work was supported by contract No. 12-14-100-7763(74) Agricultural Research Service, U.S. Department of Agriculture. Safflower seeds were obtained from the Department of Agronomy, University of Arizona, Tucson, Arizona; P. F. Knowles, Department of Agronomy, University of California, Davis, California; and D. C. Lorance, Anderson Clayton Company.

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[Received September 20, 1968]