Sterol Additives as Polymerization Inhibitors for Frying Oils

R.J. SIMS, J.A. FIORITI and M.J. KANUK, General Foods Corporation, Technical Center, White Plains, New York 10625

ABSTRACT

Addition of certain vegetable oil unsaponifiables to safflower oil protects it from oxidative polymerization during heating at frying temperature. The unsaponifiables isolated from olive, corn, wheat germ and *Vernonia anthelmintica* oils were found to be effective. The fraction responsible for this effect is largely sterol in nature. Although the common plant sterols show no antioxidant activity, the 4-α-methyl sterols function well. The sterols from *Vernonia* oil, which contain no 4-α-methyl group, are also active. It appears that an isofucosterol side chain may be the structural feature required to obtain this protective effect.

INTRODUCTION

The need to protect polyunsaturated oils from oxidative polymerization during heating in air at frying temperature is well known. Oils such as safflower are not suitable for deep fat frying because of their methylene interrupted double bonds which are prone to rapid oxidation at high temperatures.

Of the various additives which have been used to minimize oxidative deterioration during heating, the methyl polysiloxanes are the most effective (1). Apparently the silicones form a film at the air-oil interface which acts as an oxygen barrier. More recently the "alpha-sitosterols," isolated from corn and wheat germ oils, were found to inhibit destruction of linoleic acid in frying oils (2). To our knowledge the mechanism by which these sterols function is unknown.

In connection with another study (3), we analyzed the unsaponifiable fraction from *Vernonia anthelmintica* oil. Since it contains relatively large quantities of sterols, this fraction was tested as an additive to safflower oil and was found to inhibit oxidation of the oil at 180 C. We therefore became interested in the reasons for this "alpha-sitosterol-like" effect and have attempted to determine its mechanism.

EXPERIMENTAL PROCEDURES

When unsaturated oils are heated in air at frying temperatures, there is a loss in unsaturation which continues and accelerates as heating progresses. Among others, Waltking and Zmachinski have recently reported that the decrease in iodine value parallels the loss of polyunsaturates in heated vegetable oils (4). Iodine value is a relatively rapid and simple method for monitoring the rate of destruction of polyunsaturates by oxidative polymerization. Most of the oxidation rate data presented here is based on iodine value determinations. In some instances index of refraction, viscosity measurements and fatty acid composition analyses by gas liquid chromatography (GLC) have been used to supplement and confirm the findings.

The additives to be tested were dispersed in 100 g safflower oil, held at 180 ± 5 C in 150 ml beakers on electric hot plates. Samples were heated intermittently for 7 hr periods with overnight cooling to room temperature. At the end of each heating period, 1-2 g aliquots were

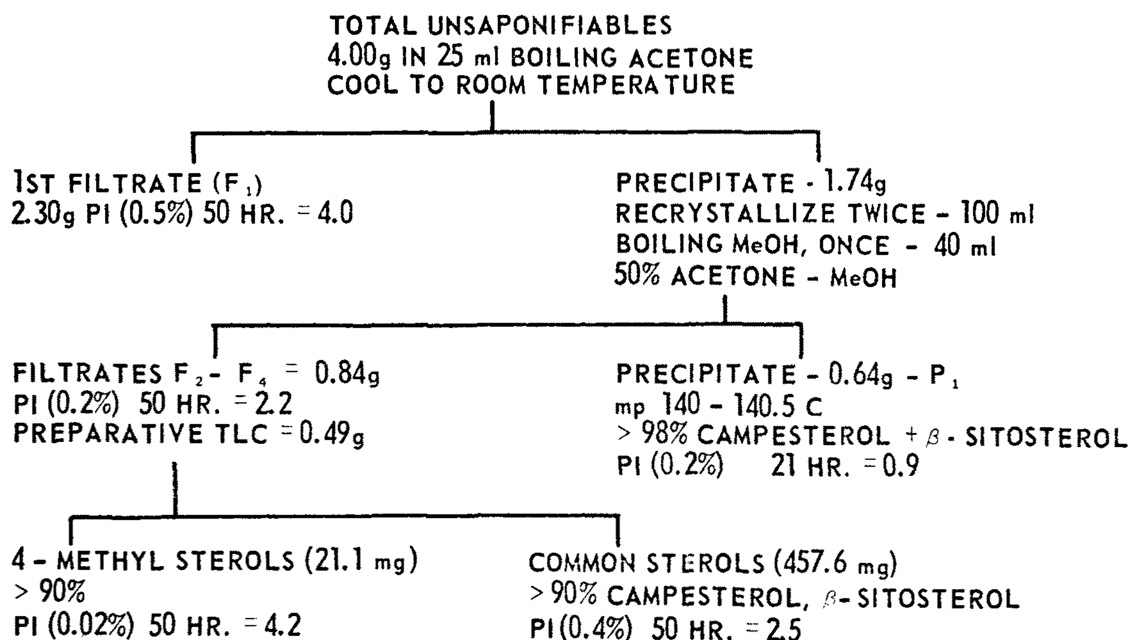

FIG. 1. Fractionation of wheat germ oil unsaponifiables by recrystallization and thin layer chromatography. Protective index (PI) is defined in the text.

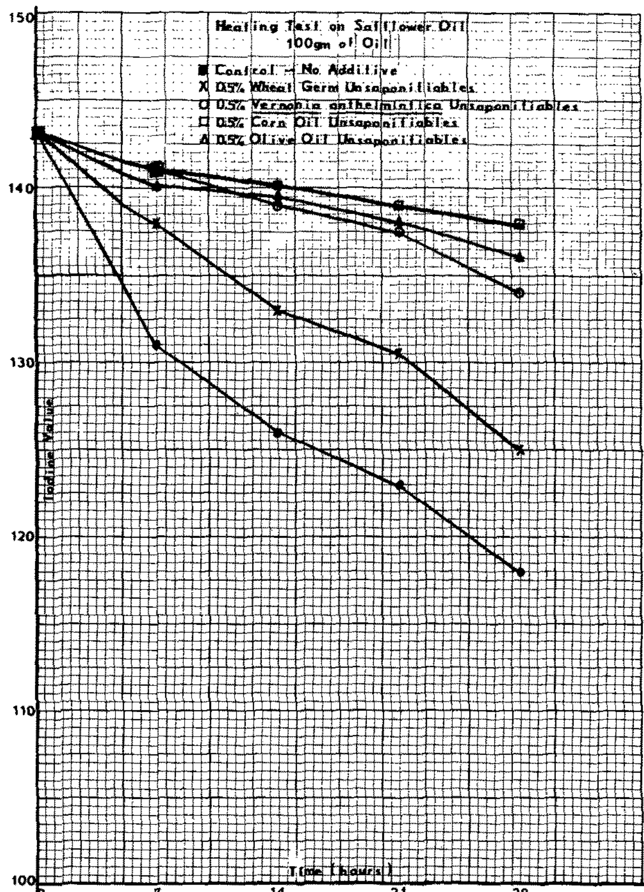

FIG. 2. Thermal oxidation of safflower oil: effect of unsaponifiables.

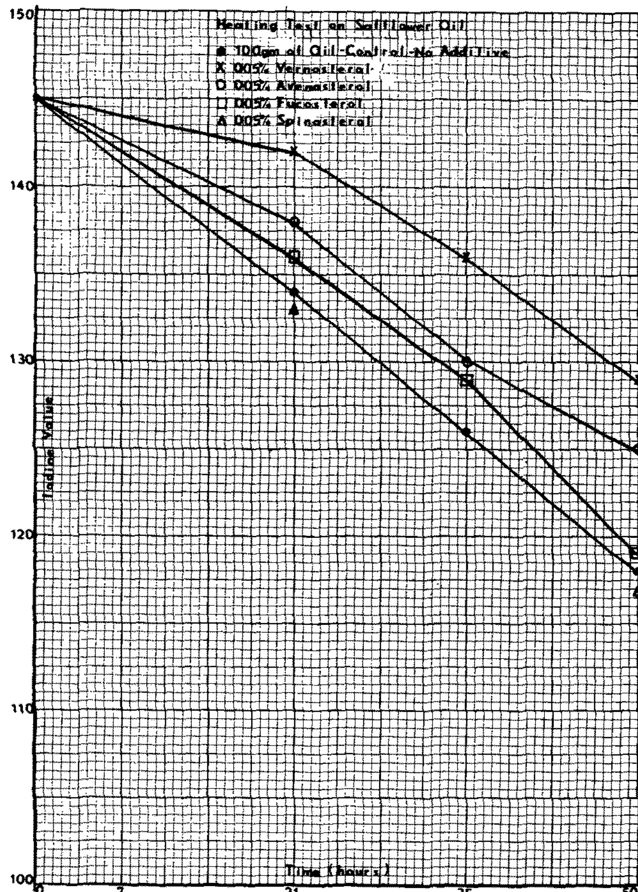

FIG. 4. Thermal oxidation of safflower oil: effect of pure sterols.

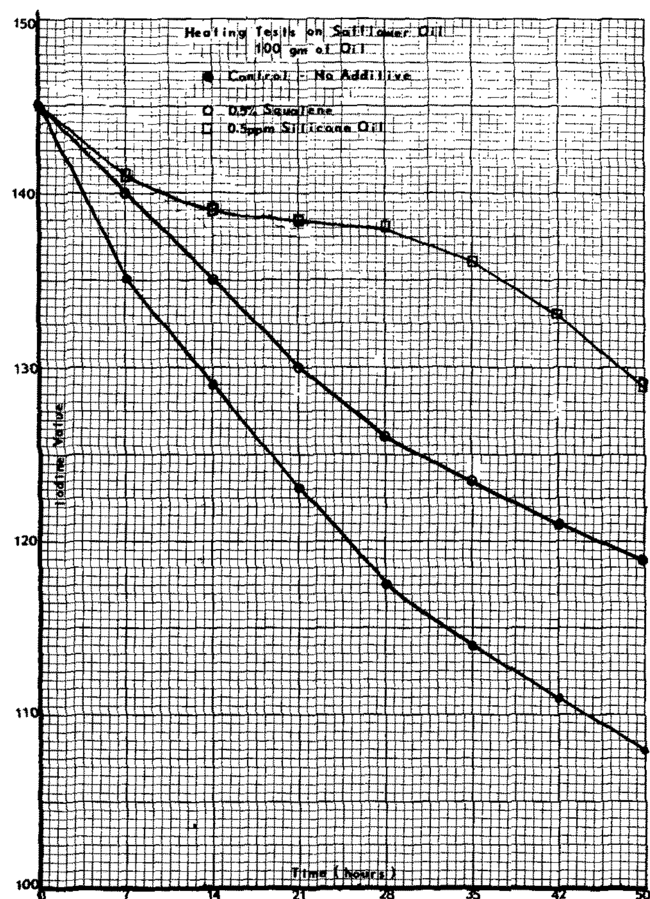

FIG. 3. Thermal oxidation of safflower oil: effect of squalene and silicone oil.

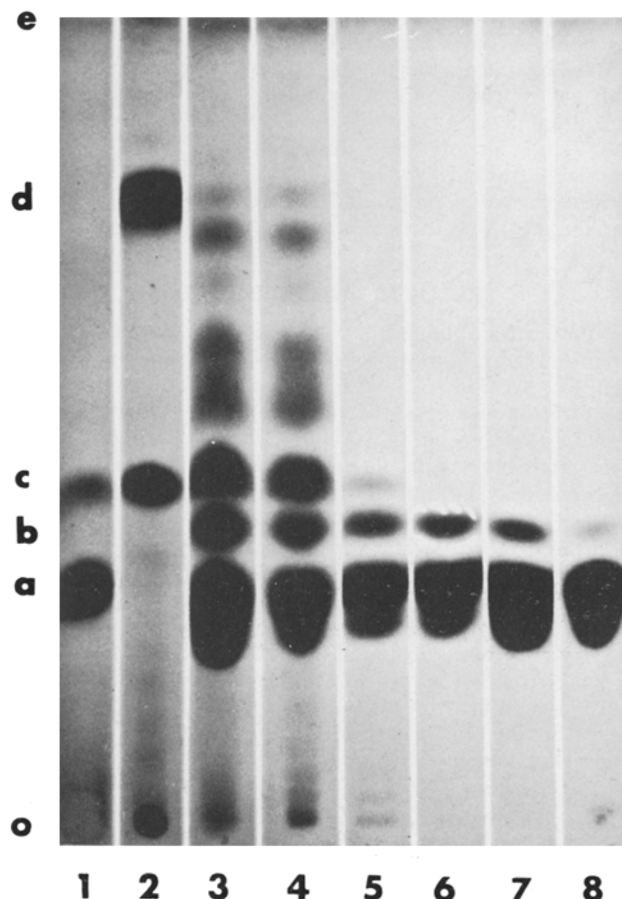

FIG. 5. Thin layer chromatography of wheat germ oil unsaponifiables. See text for details.

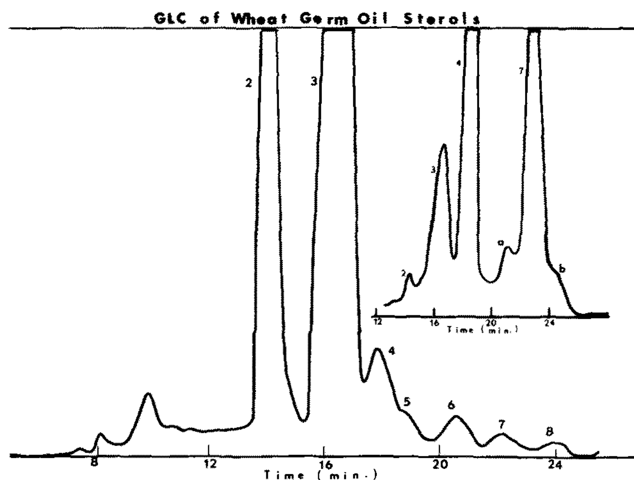

FIG. 6. Gas liquid chromatography of wheat germ oil sterols.

removed for analysis.

The methyl silicone was a Dow Corning product which contained 10% active antifoam material. The unsaponifiable fractions from the various oils were obtained by saponification followed by solvent extraction of the soaps (5). The common sterols were obtained from Steraloids, Inc., and the α-spinasterol, fucosterol, Δ 7-Avenasterol and vernosterol were isolated as described in the literature (3). Fractionation of the unsaponifiables from wheat germ oil was carried out as illustrated in Figure 1.

Commercially available Silica G plates (Analtech, Inc.) were used for thin layer chromatography (TLC). The developing solvent was petroleum ether-ethyl ether-acetic acid 80:20:1; after development the plate was sprayed with dichromate-sulfuric acid solution and charred by heating 15 min at 180 C. For preparative work the repetitive technique described by Sawicki and Mordret was used (6). Gas liquid chromatography was performed by using a Perkin Elmer 900 gas chromatograph equipped with a dual flame ionization detector. The column (10 ft x 1/8 in. OD; 10% OV-17) has already been described elsewhere (7). Except for isolated cases where trimethylsilyl ether or acetate derivatives were prepared, the samples were chromatographed mostly as the free sterols.

RESULTS AND DISCUSSION

The results obtained in a typical heating experiment are given in Figure 2. During the four 7 hr heating periods the safflower oil control lost a substantial amount of its total unsaturation, whereas the oils containing added unsaponifiables from corn oil, olive oil or *Vernonia anthelmintica* oil were relatively stable. The wheat germ oil unsaponifiables appeared only moderately effective in retarding oxidation.

Corn oil, wheat germ oil and olive oil have each been reported to contain "α-sitosterols," but little or none is found in *Vernonia* oil. Since the *Vernonia* unsaponifiables do contain a relatively large amount of squalene (10-25%), and this hydrocarbon has been reported to have antioxidant activity (8), it was tested as a possible oxidation inhibitor. From Figure 3 it is evident that although the squalene does make a significant contribution, it is probably not responsible for the entire effect. Included in this figure is a demonstration of how methyl silicone retards oxidation even at levels as low as 0.5 ppm. For this reason there is a need for carefully excluding silicones when making these evaluations.

In addition to squalene there are a number of other materials which might be found as integral components or artifacts in isolated vegetable oil unsaponifiables. Tests were run using glycerol, mono- and diglycerides, hydroxyoleic

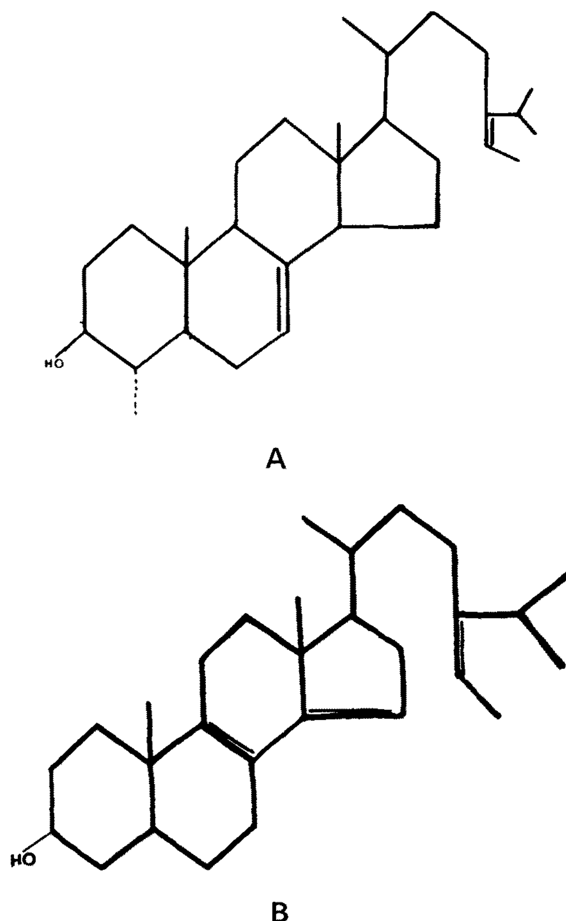

FIG. 7. A: Citrostadienol; B: Vernosterol.

acid, *n*-octadecanol, stearic acid and dihydroxystearic acid as additives to safflower oil. These materials all showed prooxidant activity. Sodium stearate, α-tocopherol, lecithin and cephalin each behaved as oxidation inhibitors. At the 0.2% level sodium stearate has a marked effect in retarding oxidation even after 50 hr of heating at 180 C, while lecithin or cephalin functioned well for only 21 hr. These additives caused development of dark colors and objectionable odors.

Figure 4 illustrates the antioxidant activity of purified vernosterol and Δ 7-avenasterol which together constitute over 50% of the total unsaponifiables in *Vernonia anthelmintica* oil (3). These sterols have considerable inhibiting activity and are responsible for the results observed with the unsaponifiables from this oil. Fucosterol also has some activity but spinasterol is slightly prooxidant. Additional sterols which were tested include ergosterol, lanosterol, β-sitosterol, stigmasterol and cholesterol. These were each either ineffective or slightly prooxidant.

Since wheat germ oil unsaponifiables were reported to contain a relatively high level of "α-sitosterols" (2), they were selected for the fractionation experiments. The scheme, as outlined in Figure 1, was followed using crystallization from acetone to separate common from 4-methyl sterols and preparative TLC as the final purification step for the 4-methyl sterols. The progress of this fractionation was followed by heating experiments which confirmed that the common sterols have little stabilizing effect, whereas the 4-methyl sterols function well even at the 0.02% level in safflower oil. In this instance the data has been expressed as "protective indices" (PI) which represent the ratio: $\frac{\Delta \text{ Iodine value safflower control.}}{\Delta \text{ Iodine value sample tested}}$ Thus a protective index of >1 indicates antioxidant activity. Both

the level tested and the time at which the PI was computed are shown in this figure.

Figure 5 illustrates the TLC of the unsaponifiables of wheat germ oil and the fractionation of the sterol-rich material obtained by acetone recrystallization (Fig. 1). Lane 1 is, in ascending order, common sterols and lanosterol; lane 2 is aliphatic alcohols and α-tocopherol; lane 3 is 800 μg total wheat germ oil unsaponifiables; lane 4 is 500 μg Filtrate F_1; lanes 5-7 ca. 200 μg F_2-F_5, respectively; and lane 8, 400 μg precipitate P_1. The letters on the left hand side of the figure represent: *O*, origin; *a*, common sterols; *b*, 4-methyl sterols; *c*, alcohols (both aliphatic and triterpenic); and *d*, tocopherols. At the solvent front, *e* is squalene and any other hydrocarbon present in wheat germ oil unsaponifiables. The 4-methyl sterols remain in the filtrate so that P_1 is virtually free of methyl sterols.

The sterol fractions were also analyzed by GLC. As is shown in Figure 6, campesterol and β-sitosterol (peaks 2 and 3, respectively) are predominant with at least five other minor sterols visible. The inset chromatogram indicates that at least four 4-methyl sterols are present among the minor components. The larger peaks (4 and 7) are thought to be 24-methylene lophenol and citrostadienol, but this has not yet been confirmed. It is believed that the latter is the compound largely responsible for the antioxidant activity of wheat germ oil unsaponifiables.

Citrostadienol, 4-α methyl-$\Delta^{7,24(28)}$-stigmastadiene-3β-ol, originally called α_1-sitosterol, is shown in Figure 7A. It has the 4-α-methyl group, a double bond in the 7 position and most important an ethylidene group in the 24(28) position. This latter group is commonly referred to as the "isofucosterol" structure. Figure 7B shows vernosterol which contains this same isofucosterol side chain. Since this is the single structural feature which citrostadienol and vernosterol have in common, it may be an important one for antioxidant activity. Of course there may well be other reasons why these two sterols function as oxidation inhibitors, but as yet we have no other clues to this behavior.

REFERENCES

1. Martin, J.B., U.S. Patent No. 2,634,213 (1953).
2. Chang, S.S., and P.E. Mone, U.S. Patent No. 2,966,413 (1960).
3. Fioriti, J.A., M.J. Kanuk and R.J. Sims, JAOCS 48:240 (1971).
4. Waltking, A.E., and H. Zmachinski, Ibid. 47:530 (1970).
5. "Official and Tentative Methods of the American Oil Chemists' Society," Vol. I, Third Edition, AOCS, Champaign, Ill., 1964, Ca 6a-40.
6. Sawicki, J., and F. Mordret, Rev. Franc. Corps Gras 17:685 (1970).
7. Fioriti, J.A., M.J. Kanuk, M. George and R.J. Sims, Lipids 5:71 (1970).
8. Rao, M.K. Govind, and K.T. Achaya, JAOCS 45:296 (1968).

[Received December 21, 1971]