# Oxidative Stability of Fat Substitutes and Vegetable Oils by the Oxidative Stability Index Method

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**Oxidative Stability Index (OSI) of carbohydrate fatty acid polyesters, fat substitutes and vegetable oils were measured with the Omnion Oxidative Stability Instrument according to the new AOCS Standard Method Cd 12 B-92 (*The Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by D. Firestone, AOCS, Champaign, 1991). The stability of crude and refined, bleached and deodorized (RBD) vegetable oils (soybean, hydrogenated soybean and peanut) were determined at 110°C. In addition, OSI times for sucrose polyesters of soybean oil, butterfat, oleate:stearate and methyl glucoside polyester of soybean oil were determined in the absence and in the presence of 0.02 wt% antioxidants, [Tenox TBHQ (tertiary butylhydroquinone, Tenox GT-2 (from Eastman Chemical Products (Kingsport, TN); and vitamin E (from BASF, Wyandotte, MI)], and the results were compared with those of vegetable oils. Crude oils were most stable (20.4–25.9 h), followed by RBD oils (9.3–10.4 h) for soybean and peanut oils, respectively, and fat substitutes (3.8–6.8 h). Overall, Tenox TBHQ was the best antioxidant for improving the oxidative stability of both vegetable oils and fat substitutes. The sucrose polyester made with oleic and stearic acid was more stable than fat substitutes containing more polyunsaturated fatty acids, such as those from soybean oil, or from short-chain fatty acids, such as from butterfat. Antioxidants enhanced the stability of RBD oils (222% increase) and synthetic fat substitutes (421–424% increase) more than that of crude oils (33% increase). The shapes of the induction curves, not the actual OSI times for fat substitutes and vegetable oils, were similar and sharply defined.**

KEY WORDS: Antioxidants effect, fat substitute stability, oil stability, Omnion instrument, oxidative stability index, tocopherol contents, vegetable oils.

Carbohydrate fatty acid polyesters as fat substitutes are lipophilic, nondigestible, nonabsorbable fat-like molecules with the physical and chemical properties of conventional fats and oils (1–7). These polyesters, like vegetable oils and fats, contain various fatty acids, and their functionality in foods and stability are dependent on: (i) the fatty acid composition and degree of unsaturation; (ii) processing conditions; (iii) the presence or absence of residual natural antioxidants; and (iv) storage conditions and nature of abuse. Oxygen reacts rapidly with unsaturated lipids leading to the formation of hydroperoxides, which subsequently break down to form undesirable secondary compounds such as acids, aldehydes and ketones, some of which lead to instability of the oil or fat.

The conventional active oxygen method (AOM) for the determination of the oxidative stability of oils and fats (8) is time-consuming and expensive to run because of the requirement of repeated titrations for peroxide values. Recent innovative methods are based on the 617 Metrohm Ranci-mat (Brinkmann Instruments, Inc., Westbury, NY) and the Omnion Oxidative Stability Instrument (Omnion, Inc., Rockland, MA). Both operate on the same principle to measure oil stability. Both eliminate the time, expense and error associated with repeated manual titrations for the determination of oxidative stability of fats and oils by the AOM method. The added advantage of the Omnion Instrument is that up to 24 samples can be run simultaneously, while the Rancimat can only handle six samples at a time. The Omnion Instrument allows automatic induction period determination and dual temperature operation (9). The induction period is the length of time before the start of rapid acceleration of oxidation, and it indicates the stability or resistance to oxidation of the fat or oil. The Omnion Instrument is computer-controlled and allows conversions of data from oxidative stability index (OSI) at one temperature to OSI at another temperature, and from OSI to 97.8°C for AOM (9). The operation principle is based on the fact that the effluent air from the oil or fat going into the deionized water contains volatile organic acids (predominantly formic) swept from the oxidizing oil, which increase the conductivity of the water. The OSI is defined as the point of maximum change of the rate of oxidation. Indeed, the OSI determination with the Omnion Instrument is an automated replacement for the AOM method for fat stability (AOCS Method Cd 12–57) (8).

If fat substitutes are to be used in food alone or as a partial replacement for vegetable oils and fats, then it is imperative that their oxidative stability and ways to minimize deterioration be investigated. Natural or synthetic antioxidants are frequently used to extend the shelf life of lipids and lipid-containing food formulations. When used in low concentrations, they are able to stabilize food products against oxidative deterioration. Selection of the appropriate antioxidant and level of application for a particular oil, fat or food product can only be done by experimentation. However, for the antioxidant to be effective, it must be soluble or be able to mix into the fat phase sufficiently well to ensure uniform distribution (10).

The author is not aware of any report on the determination of oxidative stability of fat substitutes with the Omnion Instrument. The objective of the present investigation is to determine the OSI of carbohydrate fatty acid polyesters, and vegetable oils with the Omnion Instrument, and to determine the effect of selected antioxidants on their stability.

## MATERIALS AND METHODS

The Omnion Oxidative Stability Instrument, equipped with an IBM-compatible computer and printer, two electric heating chambers capable of heating 24 samples, two digital temperature controllers, pressure gauge, conductivity probes and sample tubes, was used. Crude, refined, bleached and deodorized (RBD) and partially hydrogenated soybean and peanut oils were provided by Archer Daniels Midland Company (Decatur, IL). The free fatty acid content of the RBD oils was about 0.02% and that

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of the crude oils varied from 0.5–1.7%, as determined by the supplier. No additives (including citric acid), antioxidants or antifoams were added to the original oils by the supplier or before analysis. The fat substitutes sucrose polyesters of soybean oil, butterfat and high oleate:stearate and methyl glucoside polyester of soybean oil were synthesized according to published methods (1–3,7). The fat substitutes were prepared with RBD oils and subsequently bleached and distilled, but not deodorized. They did not contain antioxidants or additives. Food-grade Tenox TBHQ (tertiary butylhydroquinone), and Tenox GT-2 (70% tocopherols, > 80% γ- and δ-tocopherols) were supplied by Eastman Chemical Products, Inc. (Kingsport, TN), and the vitamin E (> 96% dL-α-tocopherol) was from BASF Corporation (Wyandotte, MI).

*Gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) analyses.* The fatty acid composition of the vegetable oils and fat substitutes were determined by GLC after methylation according to published procedure (11). An HP 5890A Series II gas chromatograph (Hewlett-Packard, Avondale, PA) was used. A DB 225 fused-silica capillary column of 30 m × 0.25 mm i.d. (J & W Scientific, Folsom, CA) was used and operated isothermally at 205°C. Injector and detector temperatures were set at 250 and 260°C, respectively, and helium was the carrier gas. The relative content of fatty acid methyl esters (FAME) as mol% was quantitated by an on-line computer with 17:0 as internal standard. The natural antioxidant (tocopherols) contents of the vegetable oils and fat substitutes were determined by HPLC with a 250 × 4 mm i.d. 5μ Lichrosorb Si60 normal-phase column (Hibar Fertigsaule RT, Darmstadt, Germany) according to the method of Yao *et al.* (12). A Shimadzu LC-6A HPLC System (Shimadzu Corp., Kyoto, Japan) was used. The mobile phase was 1% isopropanol in hexane at a flow rate of 1.0 mL/min. The mobile phase was filtered through a 0.45-μm Nylon 66$^{R}$ membrane filter and de-gassed by stirring under vacuum before use. The wavelengths of the Perkin-Elmer 650-15 Fluorescence Spectrophotometer (Hitachi, Norwork, CT) were set at 290 nm for excitation and 330 nm for emission. For identification, authentic tocopherol standards were prepared and analyzed by HPLC as described above. The amounts of tocopherols as mg/100 g of oil were evaluated by an on-line computer.

*Oxidative stability measurement.* Five grams of the vegetable oil or fat substitute was weighed and transferred into the disposable borosilicate glass reaction tubes with disposable pipets (to avoid spilling oil on the side of the tubes). Nonliquid samples were melted at 10°C above their melting point prior to introduction into the tubes. The digital heating block temperature was set at 110°C. The polycarbonate conductivity tubes were filled with 50 mL deionized water of conductivity below 950 (25 μS-cm$^{-1}$). The samples were preheated at the operating temperature of 110°C for 10 min to equilibrate before the air supply and the probe were connected. Pasteur pipets from the manifolds were set close to the bottom (≈5 mm) of the oil and the water. The air flow was set to 2.5 mL/s. OSI time was determined with an on-line computer, which monitored the conductivity *vs.* time and automatically plotted the inflection point or induction period in hours by a microprocessor-computed slope/change algorithm according to AOCS Method Cd 12 B-92 (9).

Experiments on the effects of various antioxidants on the oil and fat substitutes followed the above procedure, except that the antioxidant, 0.02% by weight of the sample, was added to the heated oil at 60°C with additional stirring for uniform distribution before transferring to the reaction tube. All the reactions were carried out in duplicate.

The reaction tubes were discarded after each run, but the conductivity tubes and probes were cleaned with hot 1% Alconox (Fisher Scientific, Norcross, GA) solution by first rinsing with acetone and subsequent soaking in the detergent for 1 h. The probes were scrubbed with a brush, and both the tubes and probes were rinsed several times with deionized water. They were ready for use when the water conductivity in the tube was less than 950 (25 μS-cm$^{-1}$).

*Statistical analysis.* A factorial analysis of variance (ANOVA) with multiple comparisons was used to determine the significance of differences among treatments and groups. The upper level of significance was $P < 0.05$.

## RESULTS AND DISCUSSION

The OSI values at 110°C of vegetable oils and fat substitutes without antioxidant addition are shown in Table 1. The crude soybean and peanut oils had OSI values that were significantly ($P < 0.05$) greater than their refined RBD counterparts. For example, the crude soybean oil

**TABLE 1**

**Induction Periods of Vegetable Oils and Fat Substitutes Determined with the Omnion Oxidative Stability Instrument**

| Sample | OSI values (h) at 110°C ± SD[a] | OSI conversion to AOM @ 97.8°C (h) |
|---|---|---|
| RBD soybean oil | 9.4 ± 0.2$^{d}$ | 22.2 |
| Crude soybean oil | 20.4 ± 0.7$^{g}$ | 49.0 |
| RBD peanut oil | 10.3 ± 0.2$^{e}$ | 24.5 |
| Crude peanut oil | 25.9 ± 0.2$^{h}$ | 62.3 |
| Partially hydrogenated soybean oil | 15.3 ± 0.3$^{f}$ | 36.5 |
| Sucrose polyester of soybean oil | 4.3 ± 0.2$^{b}$ | 9.8 |
| Sucrose polyester of butterfat | 4.0 ± 0.4$^{b}$ | 9.3 |
| Sucrose polyester with high oleic:stearic acid | 6.8 ± 0.4$^{c}$ | 16.0 |
| Methyl glucoside polyester of soybean oil | 3.8 ± 0.5$^{b}$ | 8.8 |

[a]Mean ± SD of duplicate analyses. Means with the same letter in a column are not significantly different at $P < 0.05$. OSI, oxidative stability index; AOM, active oxygen method; RBD, refined, bleached and deodorized.

TABLE 2

**Tocopherol Content of Vegetable Oils and Fat Substitutes**

| Oil sample | Tocopherol content (mg/100 g) | | | | Total ± SD[a] |
|---|---|---|---|---|---|
| | α | β | γ | δ | |
| Crude soybean | 9.14 | 1.74 | 79.09 | 3.60 | 93.57 ± 0.01[i] |
| RBD soybean | 8.53 | 1.52 | 66.20 | 2.64 | 78.88 ± 0.03[g] |
| Hydrogenated soybean | 7.10 | 1.57 | 68.80 | 3.21 | 80.54 ± 0.03[b] |
| Crude peanut | 20.64 | 1.09 | 10.92 | 0.66 | 33.32 ± 0.01[e] |
| RBD peanut | 18.87 | 0.45 | 14.08 | 1.09 | 34.49 ± 0.01[f] |
| SPE soybean | ND** | 0.04 | 0.87 | 0.60 | 1.50 ± 0.15[d] |
| SPE with high oleate:stearate | ND | ND | 0.95 | 0.39 | 1.34 ± 0.14[c] |
| SPE butterfat | ND | ND | ND | ND | ND ± 0.00[b] |
| MGPE soybean | ND | ND | ND | ND | ND ± 0.00[b] |

[a]Mean ± SD of duplicate analyses. Means with the same letter in a column are not significantly different at $P < 0.05$. See Table 1 for abbreviations; SPE, sucrose polyester; MGPE, methyl glucoside polyester; ND, not detectable under the analysis conditions.

had an OSI value of 20.4 h whereas the RBD soybean oil was 9.4 h. A possible explanation is that compared to RBD oils, crude vegetable oils contain more natural antioxidants, such as tocopherols, which tend to protect them against oxidative deterioration (10,13). The RBD oils may have lost some of the protective effects of natural antioxidants (*e.g.*, α- and γ-tocopherols) due to loss during refining and processing (see Table 2) or by thermal degradation (10,13). Peanut oils were more stable than soybean oils because they contain more 18:1*n*-9 (49.7–51.9%) than do soybean oils (21.1–23.2%), as determined by GLC (Table 3). In addition, soybean oils contain about 53% 18:2n-6 and 8.7–10.9% 18:3n-3 polyunsaturated fatty acids. Apparently, the high degree of unsaturation in RBD soybean oil (63.8% total polyunsaturated fatty acids) contributed to the low OSI value (Table 3). The relative rate of oxidation of 18:3 is much faster than those of 18:2 and 18:1 (14,15). The OSI value for RBD partially hydrogenated soybean oil was 15.3 h, indicating greater stability than that of RBD soybean oil. Hydrogenation increases the degree of saturation by the addition of hydrogen atoms across the double bonds, and, hence, it improves the shelf life of oils and fats. Soybean oils with 18:3 content below 3% (Table 3) after hydrogenation have improved stability and flavor as compared to unhydrogenated oils (16).

Table 4 shows the fatty acid composition of the fat substitutes. Sucrose polyester (SPE) of soybean oil and methyl glucoside polyester (MGPE) of soybean oil had more polyunsaturated fatty acids (59.7 and 56.8%, respectively) compared to SPE of butterfat (3.0%) and SPE with high oleic and stearic acid (11%). All the carbohydrate fatty acid polyester fat substitutes had lower OSI values than the RBD vegetable oils. The reason is that the oils used in their syntheses were already refined, bleached and deodorized, and thus have lost almost all the natural antioxidants (Table 2). The fat substitutes were further washed, bleached and distilled, and, therefore, the OSI values are expected to be low unless stabilized with an antioxidant (Figs. 1 and 2). However, the amount of polyunsaturated fatty acids in the fat substitute, as in vegetable oils, tends to determine its stability. Another possible explanation for the low OSI values for the fat substitutes is that they were not deodorized. The SPE synthesized with high oleic acid content (53.2%), as determined by gas chromatography (Table 4), was more stable (OSI 6.8 h) compared to SPE of soybean oil (4.3 h), butterfat (4.0 h) and MGPE of soybean oil (3.8 h). It appears that the higher the degree of substitution of fatty acids on the sugar molecule and the greater the amount of 18:1n-9, the more stable it tends to become. SPE has eight fatty acids on the sucrose molecule, compared to four fatty acids on the methyl glucoside. Thus, one would predict that the order of stability, assuming all the samples contain the same fatty acids and were similarly processed, would be:

TABLE 3

**Fatty Acid Composition of the Vegetable Oils[a]**

| Major fatty acid | mol% | | | | |
|---|---|---|---|---|---|
| | Crude SBO | RBD SBO | H-SBO | Crude PO | RBD PO |
| 14:0 | 0.1 | 0.1 | 0.3 | 0.1 | 0.1 |
| 16:0 | 11.5 | 11.5 | 10.5 | 12.1 | 12.1 |
| 18:0 | 3.7 | 3.5 | 1.7 | 2.3 | 2.4 |
| 18:1n-9 | 23.2 | 21.1 | 48.6 | 51.9 | 49.7 |
| 18:2n-6 | 52.8 | 52.9 | 30.3 | 31.6 | 31.0 |
| 18:3n-3 | 8.7 | 10.9 | 1.6 | 0.5 | 0.4 |
| 20:0 | | | | | 1.0 |
| 20:1n-9 | | | | | 1.2 |
| 22:0 | | | | 0.7 | 1.7 |
| Others | | | 7.0 | 0.8 | 0.4 |
| Polyunsaturated | 61.5 | 63.8 | 31.9 | 32.1 | 31.4 |

[a]SBO, soybean oil; RBD, refined, bleached and deodorized; H-SBO, hydrogenated soybean oil; and PO, peanut oil.

TABLE 4

**Fatty Acid Composition of the Fat Substitutes[a]**

| Major fatty acid | mol% | | | |
|---|---|---|---|---|
| | SPE of SBO | SPE of hi-OL:ST | SPE of Butterfat | MGPE of SBO |
| 10:0 | | | 2.1 | |
| 12:0 | | | 3.6 | |
| 14:0 | 0.4 | 3.0 | 13.0 | 0.4 |
| 16:0 | 11.0 | 11.2 | 35.2 | 11.3 |
| 16:1n-7 | | 3.8 | | |
| 18:0 | 4.2 | 14.6 | 15.0 | 4.5 |
| 18:1n-9 | 24.6 | 53.2 | 28.1 | 26.1 |
| 18:1n-7 | | 3.2 | | |
| 18:2n-6 | 49.4 | 10.4 | 3.0 | 51.3 |
| 18:3n-3 | 10.4 | 0.6 | | 5.5 |
| Others | | | | 0.9 |
| Polyunsaturated | 59.7 | 11.0 | 3.0 | 56.8 |

[a]SPE, sucrose polyester; hi-OL:ST, sucrose polyester containing high oleic and stearic acid; MGPE, methyl glucoside polyester; SBO, soybean oil. Note the absence of 4:0, 6:0 and 8:0 in the SPE of butterfat, even though the gas–liquid chromatography temperature program started at 70°C for this specific analysis. They were probably lost during the SPE synthesis or during methylation.

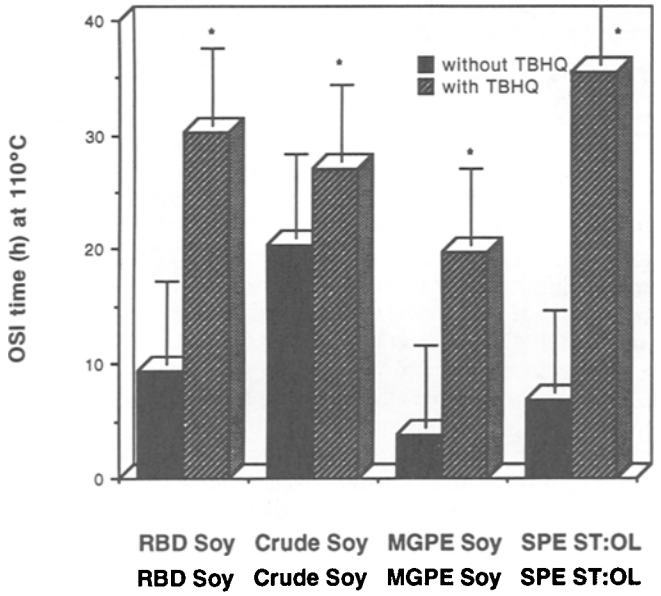


**FIG. 1. Effect of 0.02% tertiary butylhydroquinone (TBHQ) by weight on the oxidative stability of crude and refined, bleached and deodorized (RBD) soybean oil as compared to the fat substitutes. Results are expressed as mean ± SD (n = 2). *Indicates significant difference ($P < 0.05$). OSI, oxidative stability index; MGPE, methyl glucoside polyester; SPE ST:OL, sucrose polyester with high levels of stearic:oleic acid.**

SPE > MGPE > vegetable oil > methyl ester > free fatty acid. Standard deviations for duplicate analysis of the samples were comparable, except at low OSI values. Generally, the deviations seem to be greater than at high OSI values. The theoretical computer conversion of OSI values at 110°C to AOM values at 97.8°C by the automated Omnion Instrument are also shown in Table 1, for comparative purposes. However, the actual experimental OSI values may vary slightly from the predicted AOM values. The AOM values tend to be higher (though not at the same temperature) than the OSI values, suggesting that the Omnion Oxidative Stability Instrument may be a faster predictor of oil stability. For example, the predicted induction time for RBD peanut oil (24.5 h) with the AOM method is approximately double that obtained with the new OSI method (10.3 h). OSI values can also be run at higher temperatures (130°C) for highly stable oils and at lower temperatures (80°C) for highly unstable oils, such as fish oils (9). Fish oils and linseed oils have a long, sloping induction curve, whereas vegetable oils exhibit a sharply defined rapid induction curve (9). The nature of the induction curves, not the OSI time, was similar for both vegetable oils and fat substitutes (Fig. 3). Both RBD soybean oil and its SPE without antioxidants were less stable than the SPE with TBHQ.

The effect of TBHQ on the oxidative stability of RBD and crude soybean oil, MGPE of soybean oil and SPE of high oleate:stearate were compared to those without TBHQ (Fig. 1). In all cases, addition of the antioxidant TBHQ (0.02% by weight) appeared to increase the stability of the oils significantly ($P < 0.05$). The degree of added stability was greater in the fat substitutes and the RBD soybean oil than in the crude soybean oil. TBHQ improved the stability of high-oleic SPE from 6.8 to 35.6 h (representing a 424% increase), and that of RBD soybean oil from 9.4 to 30.3 h (222%). For the crude soybean oil, the improvement in stability was from 20.4 to 27.2 h (33%). The stability for MGPE of soybean oil was significantly ($P < 0.05$) improved, from 3.8 to 19.8 h (421%) by TBHQ. Therefore, the benefit of adding antioxidants in crude oils appears to be small. Obviously, the reason for enhanced stability of the processed oils and fat substitutes was because the natural antioxidants were lost during refining and further processing (Table 2). The total tocopherol contents of crude oils were significantly ($P < 0.05$) greater than those of the RBD oils and fat substitutes (Table 2). Indeed, all the tocopherols were lost in the SPE of butterfat and MGPE of soybean oil, and little was detected in the SPE of soybean oil (1.50 mg/100 g) and SPE with high oleate:stearate (1.34 mg/100 g). The total tocopherol contents of crude and RBD soybean oils were 93.6 mg/100 g and 78.9 mg/100 g, respectively. γ-Tocopherol was the predominant antioxidant detected in the oil samples followed by α-, δ- and β-tocopherols. The same order of tocopherol abundance in soybean oil has been reported by

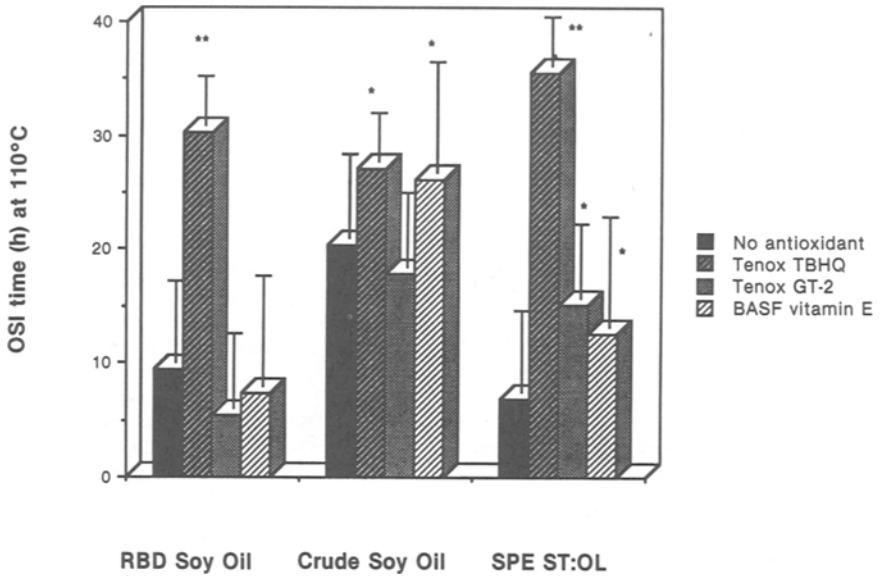


**FIG. 2. Comparison of the efficacy of Tenox TBHQ, Tenox GT-2 (Eastman Chemical Co., Kingsport, TN) and vitamin E (BASF, Wyandotte, MI) on the oxidative stability of crude soybean oil and sucrose polyester fat substitute. Results are expressed as mean ± SD (n = 2). *,**Indicate significant differences (*$P < 0.05$ and **$P < 0.001$), compared to the no antioxidant treatment group. Abbreviations as in Figure 1.**

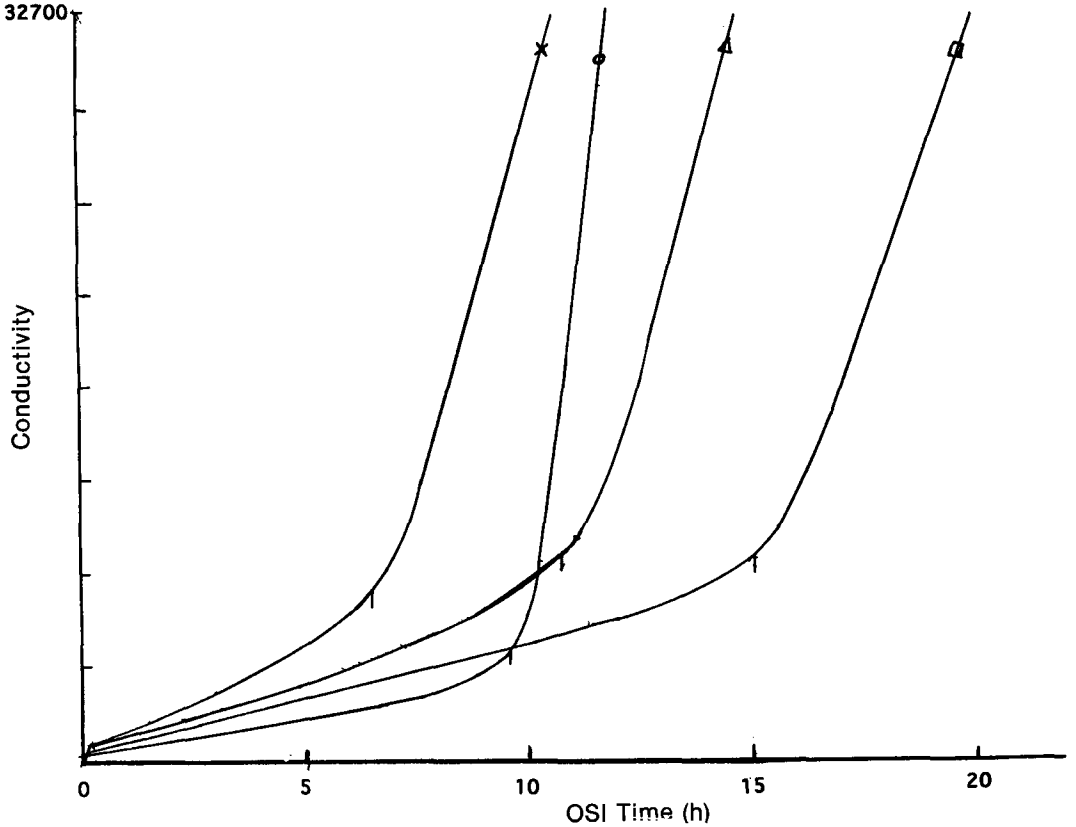


**FIG. 3. Graphic determination of the induction period pattern of fat substitutes and vegetable oils as plotted by a microprocessor-computed slope/change algorithm method; o = sucrose polyester without antioxidant, x = refined, bleached and deodorized soybean oil without antioxidant, △ = sucrose polyester with tertiary butylhydroquinone, □ = partially hydrogenated soybean oil without antioxidant. OSI, oxidative stability index.**

others (13,17). The enhanced stability of MGPE and SPE with high oleate:stearate by the addition of TBHQ can be partially explained by loss of the natural antioxidants (Table 2).

It was also desirable to determine the best antioxidant for preventing oxidation in aged fat substitutes. Figure 2 shows the comparison between controls (no antioxidant) and Tenox TBHQ, Tenox GT-2 and BASF vitamin E as antioxidants on the stability of RBD soybean oil, crude soybean oil, and SPE of high oleate:stearate. TBHQ was the best antioxidant for SPE fat substitutes (OSI 35.6 h) as compared to Tenox GT-2 (> 80% γ- and δ-tocopherols) with an OSI of 15.1 h, and vitamin E with an OSI of 12.6 h. TBHQ was the best antioxidant for crude and RBD soybean oil, (27.2 and 30.3 h, respectively). TBHQ was significantly ($P < 0.05$) better in preventing oxidation in both RBD soybean oil and SPE with high oleate:stearate. The next best antioxidant for crude soybean oil was vitamin E, with an OSI of 26.2 h. TBHQ is most effective in stabilizing highly unsaturated vegetable oils, such as soybean and fish oils (10). Indeed, Tenox GT-2 appeared to have served as a prooxidant for crude and RBD soybean oil, indicating that a combination of the natural tocopherols in the oils, plus the added tocopherol from GT-2, may have exceeded the concentration required to protect the oil from oxidation. Antioxidants may act as prooxidants at high concentrations and as antioxidants at low concentrations (10,18).

The new AOCS automated standard method is a good and reliable quick method for assessing the oxidative stability of fats, oils and fat substitutes. It has been demonstrated for the first time that the shape of the induction curves, not the actual OSI times of the carbohydrate polyester fat substitutes, were sharply defined with rapid rise, and similar to those of vegetable oils. Fat substitutes can be stabilized by food-grade antioxidants. The type of suitable antioxidant will have to be determined by experimentation.

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