Establishment of a Model Substrate Oil for Antioxidant Activity Assessment by Oil Stability Index Method

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ABSTRACT: A model substrate oil using methyl linoleate was established for the determination of the antioxidant activity by Oil Stability Index (OSI) method. OSI values for methyl linoleate with different concentrations (5–100%) in silicone oil were measured at different temperatures (70–120°C). As the temperature increased, the OSI value decreased in each concentration of methyl linoleate. Optimal temperature and concentration of methyl linoleate were established. The effect of concentration of antioxidants, α-tocopherol, and butylated hydroxytoluene on OSI values for 10% methyl linoleate model oil was measured at 90, 100, 110, and 120°C. The logarithmic relationship between temperature and OSI using model substrate oil was similar to that of soybean oil. Furthermore, application of some spice extracts to this model oil system was carried out to give results that compared well with those available in the literature. Thus, the procedure using methyl linoleate–silicone oil as a model substrate oil is available for evaluating the antioxidant activity by the OSI method.

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KEY WORDS: Allspice, antioxidative activity, cinnamon, ginger, model oil, nutmeg, oil stability index, OSI, oxidative stability, rosemary.

The Oil Stability Index analysis method (OSI; AOCS Official Method Cd 12b- 92) (1) is one of several methods used to evaluate the stability of fats and oils. It determines stability by measuring conductivity due to volatile organic acids evolved during the oxidation of fats and oils. OSI value is defined as the point of maximal change of the rate of oxidation (1,2). A recently developed oxidative stability instrument allows automatic OSI determinations with a simultaneous analysis of up to 24 samples (3). Although several reports (4,5) showed that there is a close correlation between OSI and the Active Oxygen Method (AOM; AOCS Official Method Cd 12-57) (6), the OSI method has several advantages over the conventional AOM with respect to accuracy and repeatability, and it requires less labor, time, and reagents (2,5). Frankel (7) pointed out the limitations of high-temperature stability tests, such as OSI and AOM in evaluating antioxidants, owing to rapid oxidation, polymerization and cyclization of fats and oils, and decomposition of antioxidants at high temperature. However, antioxidants are commonly used under conditions of high temperature in food processing and cooking. Therefore, it seemed worthwhile to evaluate antioxidative activity at high temperatures by OSI.

The OSI method has been used in many studies to evaluate the activity of antioxidants measured in various oils, such as soybean oil (8), lard (9), corn oil (9), whale oil (8), and sardine oil (8). However, the fatty acid compositions of these natural oils vary, which can affect OSI values. Furthermore, these natural oils contain natural antioxidants such as tocopherols which interfere in the determination of the activity of the antioxidants being evaluated. Kajimoto and Murakami (10) revealed that the OSI value of soybean oil stripped of tocopherols decreased as compared with the value of untreated oil. So tocopherols in natural oil had a great influence on the OSI value. Such complications prompted us to establish a model substrate oil that would yield accurate measurement of antioxidative activity by OSI.

Unsaturated fatty acid composition had a great influence on OSI value, and the high degree of unsaturation gave low OSI values. We attempted to establish the model substrate oil using methyl ester of unsaturated fatty acid for measuring OSI. Generally oleic and linoleic acids are the most abundant unsaturated fatty acids in fats and oils. For example, lard, which is commonly used for measuring OSI, as a substrate contains 44% oleic acid and 9% linoleic acid. In a preliminary investigation we compared the OSI values of model oils using methyl oleate and methyl linoleate in silicone oil. The model oil using methyl oleate was very stable, and the OSI value could not obtained (up to 200 h), while OSI in the case of methyl linoleate was 4.5 h. Thus, a mixture of methyl linoleate and silicone oil, which has high thermal and chemical stability, was used as simplified standard model oil.

To test the validity of the model substrate oil established, the antioxidative activities of several spices were examined. We now report on the establishment of a standard procedure using a standardized model oil system for the determination of antioxidative activity by the OSI method.

EXPERIMENTAL PROCEDURES

Materials. Methyl linoleate (99 and 95% grades) was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Butylated hydroxytoluene (BHT), α-tocopherol, methylene chloride (CH_2Cl_2), and methanol were purchased from Wako Pure

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Chemical Industries Ltd. (Osaka, Japan). Silicone oil (TSF451-10, -50, and -100) was purchased from Toshiba Silicone Co. Ltd. (Tokyo, Japan). Allspice, cinnamon, ginger, nutmeg, and rosemary were purchased from a Japanese market.

Procedure. The oxidative stability instrument (Omnion Inc., Rockland, MA) attached to an IBM-compatible computer was used for the OSI measurement.

Extraction of spices (11). Ground allspice, cinnamon, ginger, nutmeg, and rosemary (10 g of each) were extracted with CH_2Cl_2 (50 mL). The solvent was evaporated *in vacuo* to give the CH_2Cl_2 extracts, the amounts of which were 0.93, 0.36, 0.58, 3.80, and 1.10 g, respectively.

Preparation of the model oil. Methyl linoleate was added to silicone oil in the percentages stated below. The mixture was stirred with a vortex mixer for 10 min under nitrogen and then dispensed (5 g) into individual reaction tubes.

OSI measurement. A stream of air was bubbled into 5 g of model oil contained in a reaction tube placed in an electric heating chamber. The effluent air that contained volatile organic acids from the sample oil was collected in another tube containing distilled water (50 mL). The conductivity of the water as oxidation proceeded was measured automatically. Air flow rate was set at 2.5 mL/s for all determinations.

(i) Effects of temperature and concentration of methyl linoleate in model oil on OSI values. Methyl linoleate was added to silicone oil with a concentration of 5, 10, 20, 50, and 100% (w/w). Three replicates of OSI values were measured at 70, 80, 90, 100, 110, and 120°C.

(ii) Effects of temperature and concentration of antioxidants in model oil system on OSI values. Methyl linoleate (10%) in silicone oil was used as the model substrate oil for the measurement of the antioxidative effect of α-tocopherol and BHT. To the model oil was added 100 μL of each solution (2.5, 5, 10, 20, and 40 mM in methanol: final concentration of 0.25, 0.5, 1, 2, and 4 μmol/5 g substrate oil). A control was prepared by adding the same volume of methanol to the model oil (9). The mixtures were shaken on a vortex mixer for 30 s under nitrogen. These were then preheated for 30 min without linking the conductivity measurement tubes, after which time connections were made and OSI values were measured. Three replicates of OSI values were carried out at 90, 100, 110, and 120°C.

(iii) Measurement of antioxidative activity of spices. To the model oil (10% methyl linoleate in silicone oil) was added 100 μL methanol solution of the CH_2Cl_2 extracts of five spices, α-tocopherol, and BHT (final concentration: 0.02% of model oil). OSI measurements of the mixtures were carried out at 90°C in triplicate.

Measurement of conjugated polyunsaturated fatty acids. The absorbance of methyl linoleate dissolved in isooctane was measured at 233, 262, 268, 274, 308, 315, 322, and 346 nm. The contents of conjugated diene, triene, tetraene, and pentaene were calculated by Official method 2.4.3.1 of the Japan Oil Chemists' Society (12).

*Statistical analysis***.** A factorial analysis of variance (ANOVA) with multiple comparisons and linear regression were calculated using the Stat View program package (SAS Institute, Inc., Cary, NC). Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Effects of temperature and concentration of methyl linoleate on OSI values. Table 1 summarizes the OSI values for model oils with different concentrations (5–100%) of 95% methyl linoleate in silicone oil at different temperatures (70–120°C). As expected, as the temperature increased, the OSI value decreased in each concentration of the model oil (Table 1). The curves of OSI appeared to be exponential functions. When the logarithms of OSI values of each model oil were plotted against temperature, they varied linearly with temperature, and a linear equation for each model oil was obtained (Fig. 1). All regression coefficients exceeded 0.97. A similar relationship between the log OSI and temperature was found in studies of fish oils by Mendez *et al*. (13), vegetable oils by Hasenhuettl and Wan (14), and soybean oil by Reynhout (15). In particular, the temperature coefficient value for the model oil (–0.028 to –0.031 per °C) was very close to that for vegetable oil (–0.028 to –0.032 per °C) or soybean oil [–0.03 per °C, calculated by Mendez *et al*. (13)]. Therefore, the model oil composed of methyl linoleate and silicone oil appears to have properties similar to those of vegetable oils.

Table 1 also shows that OSI values decreased at any temperature as the concentration of methyl linoleate increased. No significant difference was observed between the OSI values for 100% methyl linoleate and those for 50% methyl linoleate oil at each temperature. On the other hand, significant differences in OSI values were found between 5% methyl linoleate model oil at 70 and 80, 90, 100, 110, and

TABLE 1
Effects of Temperature and Concentration of Methyl Linoleate on Oxidative Stability Index (OSI) (h)

Concentration of methyl linoleate[a]	70°C	80°C	90°C	100°C	110°C	120°C
100	$9.28 \pm 2.37^{a}{}_{A}$	$3.63 \pm 0.32^{a}{}_{B}$	$1.90 \pm 0.14^{a}{}_{B}$	$1.08 \pm 0.04^{a}{}_{B}$	$0.58 \pm 0.03^{a}{}_{B}$	$0.42 \pm 0.08^{a}{}_{B}$
50	$9.03 \pm 3.78^{a}{}_{A}$	$4.03 \pm 0.04^{a}{}_{A,B}$	$1.70 \pm 0.07^{a}{}_{B}$	$1.03 \pm 0.18^{a}{}_{B}$	$0.58 \pm 0.03^{a}{}_{B}$	$0.40 \pm 0.00^{a}{}_{B}$
20	$15.70 \pm 5.66^{a}{}_{A}$	$7.20 \pm 1.13^{ab}{}_{A,B}$	$2.50 \pm 0.14^{a}{}_{B}$	$1.90 \pm 0.07^{ab}{}_{B}$	$0.95 \pm 0.00^{b}{}_{B}$	$0.65 \pm 0.07^{a}{}_{B}$
10	$33.37 \pm 5.00^{ab}{}_{A}$	$15.37 \pm 2.80^{b}{}_{B}$	$5.47 \pm 0.41^{b}{}_{C}$	$3.08 \pm 0.20^{b}{}_{C}$	$1.58 \pm 0.08^{c}{}_{C}$	$1.03 \pm 0.08^{b}{}_{C}$
5	$51.55 \pm 11.14^{b}{}_{A}$	$25.68 \pm 4.57^{c}{}_{B}$	$10.55 \pm 0.45^{c}{}_{B,C}$	$6.52 \pm 0.78^{c}{}_{C}$	$3.25 \pm 0.21^{d}{}_{C}$	$1.98 \pm 0.08^{c}{}_{C}$

[a]Concentration (% w/w) of methyl linoleate in silicone oil. Values are mean ± standard deviation of three replicates. [a–d]Values in each column with the different superscripts are significantly ($P < 0.05$) different by analysis of variance (ANOVA) with multiple comparisons. [A-C]Values in each row with the different subscripts are significantly ($P < 0.05$) different by ANOVA with multiple comparisons.

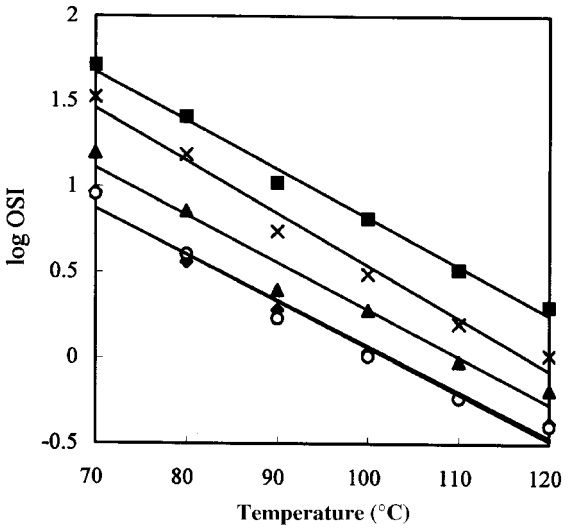

FIG.1. Log oxidative stability index (OSI) of model oil as a function of temperature. Data for 100% methyl linoleate (w/w) in silicone oil (◆) are fitted by the regression line $Y = -0.027X + 2.746$, with a correlation coefficient of $R^2 = 0.98$. Similarly, for 50% methyl linoleate (○), $Y = -0.027X + 2.774$ ($R^2 = 0.99$); for 20% methyl linoleate (▲), $Y = -0.028X + 3.046$ ($R^2 = 0.97$); for 10% methyl linoleate (×), $Y = -0.031X + 3.611$ ($R^2 = 0.98$); and for 5% methyl linoleate (■), $Y = -0.029X + 3.669$ ($R^2 = 0.99$).

120°C. The OSI values for 10% methyl linoleate model oil at 70°C differed significantly from any other model oils at 80, 90, 110, and 120°C. Table 2 shows the coefficient of variation associated with OSI values for each model oil at different temperatures. At 70°C, there was large variation in OSI values for 5, 10, 20, 50, and 100% methyl linoleate model oils. The coefficient of variation of OSI values tended to be greater at lower (70°C) and higher (120°C) temperatures. In addition, it has been proposed that OSI values of less than 2 h or more than 20 h (Japan Oil Chemists' Society) (16) and of less than 4 h (AOCS) (1) are outside the capability of OSI measurement. Therefore, the measurements in the range of 4 to 20 h had a coefficient of variation less than 10%. Desirable conditions of the OSI measurement obtained in this study were as follows: 50% methyl linoleate at 80°C, 10% methyl linoleate at 90°C, and 5% methyl linoleate at 90 and 100°C.

Effect of quality of methyl linoleate. The effect of methyl linoleate quality on OSI value was examined by comparing with different qualities (99 and 95%) of methyl linoleate. Methyl linoleate (10%) in silicone oil was used as a model oil substrate at 90°C. Ninety-nine percent grade methyl linoleate contained 0.99% conjugated diene, and 95% grade one contained 0.30% conjugated diene and 0.03% conjugated triene. When 99% grade methyl linoleate was used, the OSI value was 4.5 ± 1.2 h, and in the case of 95% grade methyl linoleate, the value was 4.3 ± 2.0 h. No significant difference was observed between the OSI values of both grades at 90°C. Therefore, both grades of methyl linoleate are available as a substrate for OSI measurement. In the following experiment, 10% methyl linoleate (95% grade) with silicone oil was used as a model substrate oil.

Effect of viscosity of silicone oil. The effect of viscosity of silicone oil on OSI values was measured for 10% methyl linoleate model oil. The viscosities of silicone oils (TSF451-10, -50, and -100) were 10, 50 and 100 cSt, respectively. The OSI values were 5.1 ± 0.4 h for silicone oil with the viscosity of 10 cSt (TSF451-10), 6.1 ± 1.2 h for TSF451-50, and 4.3 ± 2.0 h for TSF451-100. There was no influence of viscosity of silicone oil on the OSI. The flashpoint of TSF451-10 is 190°C, and this oil would not be good for measuring OSI at high temperatures. Therefore, only TSF451-50 and -100 were suitable for use in the OSI analysis, and for this study the TSF451-100 was used.

Effects of concentration of antioxidants and temperature on OSI values. The effect of concentration of antioxidants on OSI values for 10% methyl linoleate model oil was measured at 90, 100, 110, and 120°C. As shown in Table 3, the OSI values rose sigmoidally with increasing amounts of antioxidants (0.25 to 4.0 μmol/5 g model oil). The OSI values of the model oil with both α-tocopherol and BHT increased linearly with concentration in the range of 0.25 to 1 μmol/5 g model oil at 90 and 100°C, 0.25 to 2 μmol/5 g model oil at 110 and 120°C.

When the log OSI values of the model oil containing α-tocopherol and BHT were plotted as a function of temperature, linear plots were obtained (Figs. 2A and 2B). A similar logarithmic relationship was reported for soybean oil containing BHT, BHA, *tert*-butylhydroquine, tocopherol, and rosemary extract (15). This indicated that the model substrate oil consisting of methyl linoleate and silicone oil could be used as substrate oil in place of natural oil for antioxidant assay by the OSI method. In general, refined, bleached, and deodorized vegetable oils include tocopherols (3). Therefore, when these vegetable oils are used as substrates, there is a need to

TABLE 2
Coefficient of Variation (%)[a] of OSI on Model Oils

Concentration of methyl linoleate[b]	70°C	80°C	90°C	100°C	110°C	120°C
100	25.54	8.78	7.44	3.29	4.95	18.33
50	41.92	0.88	4.16	17.25	4.95	0.00
20	36.03	15.71	5.66	3.72	0.00	10.88
10	14.97	18.25	7.45	6.55	4.82	7.39
5	21.61	17.81	4.27	11.89	6.53	3.85

[a]Coefficient of variation of three replicates.
[b]Concentration (% w/w) of methyl linoleate in silicone oil. For abbreviation see Table 1.

TABLE 3
Effects of Temperature and Concentration of Antioxidants on OSI[a]

Concentration[b]	α-Tocopherol				Butylated hydroxytoluene (BHT)			
(μmol/5 g)	90°C	100°C	110°C	120°C	90°C	100°C	110°C	120°C
0	3.30 ± 0.09	1.87 ± 0.38	0.8 ± 0.05	0.45 ± 0.05	3.03 ± 0.10	1.73 ± 0.10	0.80 ± 0.09	0.27 ± 0.03
0.25	7.60 ± 0.31	4.2 ± 0.87	2.08 ± 0.58	0.77 ± 0.18	4.25 ± 0.39	3.75 ± 0.63	1.47 ± 0.08	0.48 ± 0.03
0.5	15.42 ± 1.63	7.08 ± 0.60	3.45 ± 0.35	1.32 ± 0.39	7.08 ± 0.90	5.67 ± 0.28	1.73 ± 0.34	0.97 ± 0.03
1	22.22 ± 3.24	12.57 ± 1.49	4.82 ± 0.35	2.27 ± 0.25	16.08 ± 2.43	8.68 ± 0.14	2.90 ± 0.09	1.52 ± 0.08
2	33.10 ± 1.33	14.07 ± 1.14	8.15 ± 0.87	3.88 ± 0.42	21.85 ± 3.44	10.87 ± 0.24	4.80 ± 0.31	2.28 ± 0.20
4	39.75 ± 2.11	18.08 ± 2.01	9.53 ± 0.33	3.85 ± 1.25	26.87 ± 2.79	15.62 ± 1.40	5.77 ± 0.80	3.58 ± 0.34

[a]Values are mean ± standard deviation of three replicates.
[b]Concentration of antioxidants in model substrate oil. For abbreviations see Table 1.

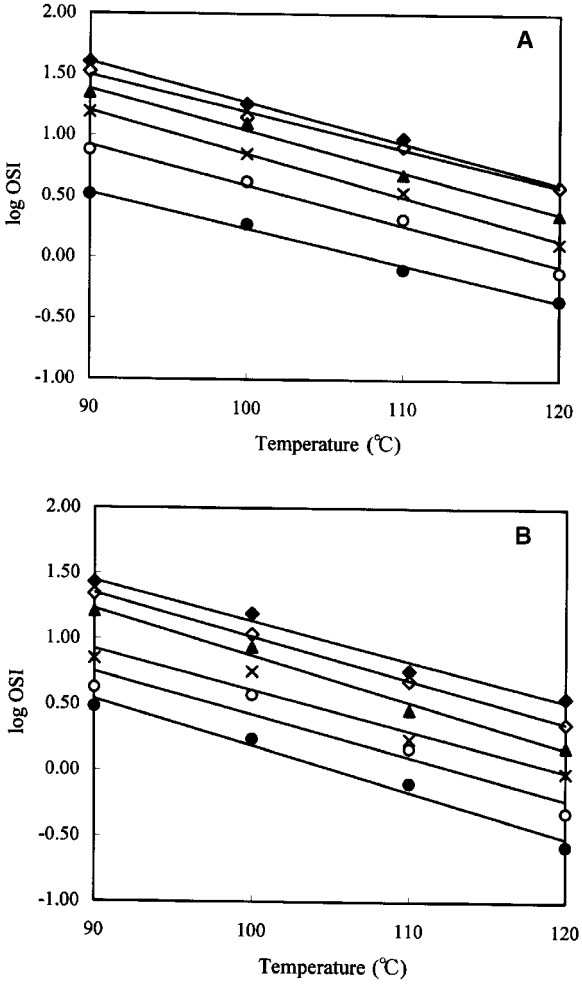

FIG. 2. Log OSI of model oil with antioxidants as a function of temperature. (A) Model substrate oil containing α-tocopherol; (B) model substrate oil containing butylated hydroxytoluene (BHT). For 4 μmol antioxidant (α-tocopherol or BHT)/5 g model substrate oil (◆), the regression line for α-tocopherol is $Y = -0.033X + 4.591$; for BHT it is $Y = -0.031X + 4.195$; similar values for lower concentrations are as follows: 2 μmol/5 g model substrate oil (◇), $Y = -0.030X + 4.222$; $Y = -0.033X + 4.316$. 1 μmol/5 g model substrate oil (▲), $Y = -0.034X + 4.431$; $Y = -0.036X + 4.427$. 0.5 μmol/5 g model substrate oil (×), $Y = -0.035X + 4.368$; $Y = -0.031X + 3.722$. 0.25 μmol/5 g model substrate oil (○), $Y = -0.033X + 3.885$; $Y = -0.032X + 3.665$; control (●), $Y = -0.030X + 3.199$; $Y = -0.035X + 3.691$.

TABLE 4
Antioxidative Activity of Spice Extracts[a], α-Tocopherol, and BHT[b]

Sample	OSI (h)
Control	5.47 ± 0.10[a]
Cinnamon	6.45 ± 0.18[a]
Allspice	9.27 ± 0.73[a]
Nutmeg	9.57 ± 0.51[a]
Ginger	25.25 ± 2.37[b]
Rosemary	98.60 ± 5.02[c]
α-Tocopherol	42.33 ± 3.20[d]
BHT	40.03 ± 2.66[d]

[a]Methylene chloride extract of materials, redissolved in methanol, and added at 0.02% of the oil weight.
[b]Values are mean ± standard deviation of three replicates. Values in each column with the different superscripts are significantly ($P < 0.05$) different by analysis of variance with multiple comparisons.

remove tocopherols in order to avoid antioxidant interactions. Thus, the model oil in this study might be superior to natural vegetable oils as a substrate because it not only eliminates this problem but also yields constant results.

Antioxidant assay of some spice extracts by the OSI method. To make sure that the OSI measurement using the model substrate oil consisting of methyl linoleate and silicone oil is useful for practical assessment of antioxidant activity, the antioxidant activity of some spice extracts was measured by the OSI method. Table 4 shows the OSI values of model substrate oils containing the CH_2Cl_2 extracts of allspice, cinnamon, ginger, nutmeg, and rosemary, and α-tocopherol and BHT at a concentration of 0.02% each at 90°C. Control data are the OSI values of the model oil without any additives. The extracts of ginger and rosemary significantly prolonged the OSI values compared to a control. The activity decreased in the order rosemary > α-tocopherol ≅ BHT > ginger > allspice, cinnamon, nutmeg, and control. These data were in good agreement with the previous results measured by AOM using lard as a substrate (17). Thus the model substrate oil established in this study might be available as a standard procedure for the determination of antioxidative activity by the OSI method.

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