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Hydrogenation and Interesterification Effects on the Oxidative Stability and Melting Point of Soybean Oil

ROGER L. DANIELS, HYUN JUNG KIM, AND DAVID B. MIN*

Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Columbus, Ohio 43210

Soybean oil with an iodine value of 136 was hydrogenated to have iodine values of 126 and 117. The soybean oils with iodine values of 136, 126, and 117 were randomly interesterified using sodium methoxide. The oxidative stabilities of the hydrogenated and/or interesterified soybean oils were evaluated by measuring the headspace oxygen content by gas chromatography, and the induction time was measured using Rancimat. The melting points of the oils were evaluated by differential scanning calorimetry. Duncan's multiple range test of the headspace oxygen and induction time showed that hydrogenation increased the headspace oxygen content and induction time at $\alpha = 0.05$. Interesterification decreased the headspace oxygen and the induction time for the soybean oils with iodine values of 136, 126, and 117 at $\alpha = 0.05$. Hydrogenation increased the melting points as the iodine value decreased from 136 and 126 to 117 at $\alpha = 0.05$. The random interesterification increased the melting points of soybean oils with iodine values of 136, 126, and 117 at $\alpha = 0.05$. The combined effects of hydrogenation and interesterification increased the oxidative stability of soybean oil at $\alpha = 0.05$ and the melting point at $\alpha = 0.01$. The optimum combination of hydrogenation and random interesterification can improve the oxidative stability and increase the melting point to expand the application of soybean oil in foods.

KEYWORDS: Hydrogenation; interesterification; soybean oil; oxidative stability; melting point

INTRODUCTION

Soybean oil is the most popular and abundant vegetable oil in the world including the United States. Soybean oil represents 85% of edible vegetable oils in the United States (*1*). Soybean oil has a low oxidative stability during storage and processing and is liquid at room temperatures due to the presence of approximately 55% linoleic acid and 8% linolenic acid. The undesirable rancid flavor makes soybean oil less acceptable to the food industry and less competitive with other vegetable oils economically. Soybean oil has not been effectively used to make shortenings, margarines, and dressings without modifications due to the low melting point. Hydrogenation is used to improve the oxidative stability of oil and to modify the physical characteristics by reducing the double bonds (*2*). Interesterification changes the distribution and positions of fatty acids within and among triglycerides to change the physical properties and behaviors of oil (*2*). The oil industry hydrogenates and/or interesterifies soybean oil to improve the oxidative stability and to modify the physical states of oil to meet the needs of margarine, shortening, coating fat, and deep fat frying oil. However, information on the effects of combined processing of hydrogenation and interesterification on the oxidative stability and melting point of soybean oil is not easily available. The oxidative stability of oil has been studied by measuring headspace oxygen and induction time. The determination of headspace oxygen uptake has been used to evaluate the oxidative stability of oil (*3*, *4*). In addition, the Rancimat test automatically and continuously measures the conductivity change due to the ionic volatile organic acids, mainly formic acid, to evaluate the oxidative stability of oils (*5*).

A differential scanning calorimer (DSC) monitors the changes of physical and chemical properties of a material as a function of temperature by detecting the heat changes associated with phase transition such as crystallization (*6*, *7*). A DSC thermogram is characterized by endothermic and/or exothermic peaks whose area is proportional to the enthalpy gained or lost by the material undergoing phase transition. The phase transition takes place over a specific temperature range depending on the sample composition and physical properties (*6*). DSC has been used in the characterization of melting and crystallization of pure edible oils. The correlation of DSC analysis with the standard method for fat oxidation such as total polar compounds has been reported (*8*, *9*). The objective of this research was to study the effects of hydrogenation and interesterification on the oxidative stability and melting point of soybean oil.

MATERIALS AND METHODS

Materials. Fifty-five gallons of refined, bleached, and deodorized soybean oil with an iodine value of 136 was supplied by Karlshamns Food Ingredients USA, Inc. (Columbus, OH).

* To whom correspondence should be addressed. Tel: 614-292-7801. Fax: 614-292-0218. E-mail: min.2@osu.edu.

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Hydrogenation. Hydrogenations were carried out on a pilot plant scale. Twenty-two kilograms of refined, bleached, and deodorized soybean oil was hydrogenated with 0.30% w/w Nysosel 325 nickel catalyst at 210 °C and a hydrogen pressure of 15 psig for different times to obtain the oils with iodine values of 126 and 117. The hydrogenated oils were filtered to remove the catalyst. The filtered oils were bleached with 1.1% w/w Filtrol 160 (Chicago, IL).

Interesterification. Random interesterification was carried out in a glass apparatus. One thousand grams of soybean oil with an iodine value of 136, 126, or 117 was reacted with 0.4% w/w of sodium methoxide at 100 °C for 2 h under nitrogen atmosphere. The sodium methoxide after interesterification was neutralized with 85% phosphoric acid at the ratio of 10 for sodium methoxide and 7 for phosphoric acid by weight basis. The interesterified oils were cooled to 80 °C and washed with water four times. The washed oil was dried at 110 °C for 1 h under nitrogen and bleached with 1.1% w/w Filtrol 160.

Deodorization. Two thousand grams of hydrogenated oil with an iodine value of 126 or 117 and 900 g of interesterified soybean oil with an iodine value of 136, 126, or 117 was steam stripped for deodorization under a vacuum of 2 mmHg at 252 °C for 6 h in a 4000 mL round-bottom glass flask. The deodorized oil was stored in darkness at −21 °C until use.

Fatty Acid Analysis. Fatty acid methyl esters were prepared with 0.5 N sodium hydroxide in methanol at 100 °C for 20 min and then derivatized with 14% boron trifluoride solution in methanol. The sample was analyzed with a gas chromatograph equipped with a flame ionization detector at 250 °C. The temperature of the gas chromatograph was programmed at 4 °C/min from 140 °C for 5 min to 220 °C for 15 min. The column was a SP-2560 capillary column of 100 m × 0.25 mm with 0.2 μm film thickness from Supelco (Bellefonte, PA). An 1 μL sample was injected into the injection port with the split ratio of 100:1 at 220 °C, and the helium flow rate was 1 mL/min.

Free Fatty Acid and Peroxide Value. Free fatty acid and peroxide values were determined by an AOCS method (*10*).

Headspace Analysis. The headspace oxygen content of the oil bottle content during storage was measured by a Hewlett-Packard gas chromatograph 5890 (Avondale, PA) equipped with a thermal conductivity detector. Fifteen grams of hydrogenated or interesterified soybean oil was transferred into a 60 mL serum bottle. The bottle was sealed airtight with Teflon septa and aluminum caps. Bottles were stored in darkness at 50 °C for 5 days. Samples were analyzed in duplicate daily for headspace oxygen content. Forty microliters of headspace gas was analyzed by gas chromatography with 60/80 molecular sieve 13× (Alltech Associates, Inc., Deerfield, IL) and a 1.8 m × 0.32 cm column at a helium carrier gas flow rate of 22 mL/min. The temperatures for the oven, injector, and detector were 40, 120, and 250 °C, respectively. The oxygen content in the headspace of oil bottle was obtained by a Hewlett-Packard 3390A electronic peak integrator. Parker (*11*) reported that the oxygen content of air was 20.946%.

The oxygen electronic counts of sample bottle headspace were converted to the oxygen concentration in percentage (%) by the following formula:

$$\text{oxygen percentage (\%)} = 20.946\ (\%) \times \frac{\text{(electronic counts of oxygen peak in the 40 } \mu\text{L of headspace gas of sample bottle)}}{\text{(electronic counts of oxygen peak in the 40 } \mu\text{L of room air)}}$$

Induction Time. The induction time of the oil was determined by Metrohm 679 Rancimat (Brinkaman Instruments Co., Des Plains, IL). The six hydrogenated and/or interesterified oils were randomly evaluated at 110 °C according to the statistical procedure of Gomez (*12*). Rancimat analyzed six samples per run. Each sample was analyzed in duplicate. A 2.5 g amount of oil was placed into each Rancimat reaction vessel. Six reaction vessels were placed into the Rancimat heating block and preheated for 15 min at 100 °C. The air supply to the reaction vessel was 10 L/h, and the conductivity curve of the oil was recorded.

DSC. The melting points of oils were determined by a DuPont 9900 DSC. The 20 mg sample was completely melted at 130 °C to destroy crystal nuclei and was cooled to −80 °C. The samples were heated at the rate of 10 °C/min from −80 to 50 °C until melting was completed. The end of the melting range was considered as the melting point where a steady baseline was reached on the thermogram as shown in **Figure 3**.

Table 1. Effects of Hydrogenation on the Fatty Acid Composition of Soybean Oil

fatty acid	SBO[a]-IV[b] 136	HSBO[c]-IV 126	HSBO-IV 117
16:0	10.2	10.2	10.1
18:0	3.9	4.2	4.4
18:1	21.6	30.2	37.2
18:2	54.7	47.4	42.1
18:3	8.1	6.6	4.9

[a] SBO, soybean oil. [b] IV, iodine value. [c] HBSO, hydrogenated soybean oil.

Statistical Analysis. Coefficients of variation were calculated. Significant differences between treatment means were determined using Duncan's multiple range tests at $\alpha = 0.05$ (*13*).

RESULTS AND DISCUSSION

Deodorization. The free fatty acid value and peroxide value of the deodorized oil were 0.03% and 0.0 mequiv/kg, respectively. We considered that the deodorization process to remove the free fatty acids and to destroy the hydroperoxides in oils was carried out successfully.

Fatty Acid Compositions. The effects of hydrogenation on the fatty acid compositions of soybean oil are shown in **Table 1**. With the increase of hydrogenation time, the amounts of stearic and oleic acid increased while those of linoleic and linolenic acid decreased. The result is consistent with a hydrogenation reaction (*14*).

Hydrogenation Effect on the Oxidative Stability. Hydrogenation involves the hydrogen addition to double bonds to improve the oxidative stability of oils and to convert the liquid oils to semisolid fats for specific applications such as shortening and margarines. Hydrogenation changes the fatty acid molecular configuration and modifies the number, geometry, and locations of double bonds to alter the physical and chemical properties of oils (*2*).

Singlet state fatty acids do not react with the atmospheric triplet oxygen due to the electronic spin conservation (*15*). The fatty acid, which is not a radical compound, should be converted to a free radical before it reacts with atmospheric oxygen, which is a diradical compound for oxidation reaction. The energy required to remove hydrogen from fatty acids to be radical compounds is dependent on the hydrogen position in the molecules. The formation of a free radical at C8 or C11 of oleic acid requires 75 and 50 kcal/mol at C11 of linoleic acid in **Figure 1**. The formation of a free radial at C18 of oleic or linoleic acid requires 100 kcal/mol. The initial radical electron at C11 of linoleic acid is delocalized through five carbon systems from C9 to C13 and then forms a stable conjugated diene with a radical at C9 or C13 (*16*). The relative oxidative rates of oleic acid, linoleic acid, and linolenic acid are 1:12:25 (*17*). Because hydrogenation increased oleic acid and decreased linoleic and linolenic acid contents, the oxidation rate and the amount of headspace oxygen that reacted with the hydrogenated oil during storage should be decreased for hydrogenated soybean oil.

The headspace oxygen uptake is the depleted headspace oxygen in the gastight sample bottles, which reacted with oil to form peroxy radical. The reactive peroxy radical eventually forms hydroperoxide as shown in **Figure 1**. The headspace oxygen determination has been used to study the oxidative stability of oil (*3*, *4*). The more headspace oxygen reacted with oil, the less oxidative stability the oil presented. The oxidation

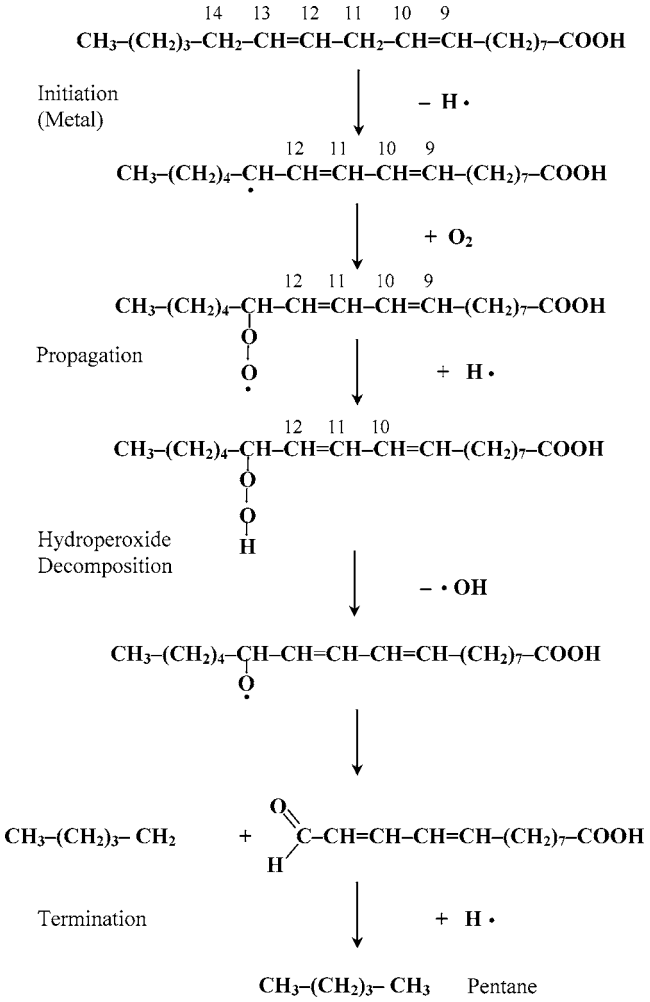

Figure 1. Oxidation mechanism of linoleic acid with triplet oxygen.

Table 2. Duncan's Multiple Range Test for Effects of Hydrogenation and Interesterification on Headspace Oxygen Content

	headspace oxygen (%)						
soybean oil	0	1	2	3	4	5	mean[f]
SBO[a]-IV[e] 136	20.95	18.97	15.42	12.76	10.95	9.20	14.7[b]
ISBO[b]-IV 136	20.95	14.17	13.34	8.31	8.20	7.37	12.1[d]
HSBO[c]-IV 126	20.95	20.44	19.94	18.06	14.43	11.60	17.6[a]
HISBO[d]-IV 126	20.95	17.95	14.47	11.88	8.72	8.46	13.7[c]
HSBO-IV 117	20.95	20.28	20.13	18.55	15.49	12.48	18.0[a]
HISBO-IV 117	20.95	19.55	16.21	13.15	11.13	9.43	15.1[b]

[a] SBO, soybean oil. [b] ISBO, interesterified soybean oil. [c] HBSO, hydrogenated soybean oil. [d] HISBO, hydrogenated and interesterified soybean oil. [e] IV, iodine value. [f] Different superscripts are significantly different at $\alpha = 0.05$.

rate is independent of oxygen concentration at high levels of oxygen, but when oxygen levels are low, the rate of oxidation is almost proportional to the oxygen concentration (*2*).

The effects of hydrogenation on the headspace oxygen in the oil bottle are shown in **Table 2**. The coefficient of variation of headspace oxygen analysis was 1.8%, which is considered to be excellent. The headspace oxygen values of soybean oils with iodine values of 136, 126, and 117 during storage were 14.7, 17.6, and 18.0%, respectively. The values of 14.7 and 17.6% were significantly different at $\alpha = 0.05$ as shown in **Table 2**.

The oxidative stability of oil has been studied by measuring the induction time (*5*). The induction time was determined by the Rancimat method. Rancimat measures the change of the conductivity of sample, which is due to the ionic organic acid.

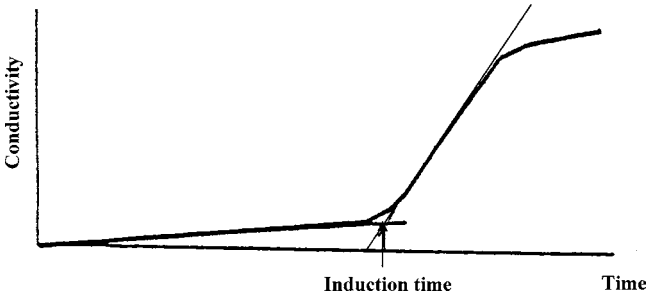

Figure 2. Induction time determination by Rancimat.

Table 3. Duncan's Multiple Range Test for Effects of Hydrogenation and Interesterification on Rancimat Induction Hours

	Rancimat induction hours			
soybean oil	trial 1	trial 2	trial 3	mean[f]
SBO[a]-IV[e] 136	4.2	4.5	4.0	4.2[c]
ISBO[b]-IV 136	1.1	1.7	1.4	1.4[e]
HSBO[c]-IV 126	6.0	5.9	5.7	5.9[b]
HISBO[d]-IV 126	2.1		2.6	2.3[d]
HSBO-IV 117	6.7	6.5	6.9	6.7[a]
HISBO-IV 117	2.9	2.4	2.1	2.5[d]

[a] SBO, soybean oil. [b] ISBO, interesterified soybean oil. [c] HBSO, hydrogenated soybean oil. [d] HISBO, hydrogenated and interesterified soybean oil. [e] IV, iodine value. [f] Means with different superscripts are significantly different at $\alpha = 0.05$.

The ionic organic acid compounds are secondary oxidation volatile compounds, which can be formed from fatty acids as shown in **Figure 2**. The volatile compounds are mainly responsible for the rancid off-flavor of oxidized oil. As the oxidation increases, the amount of volatile compounds increases. The longer the induction time of oil is, the better the oxidative stability of the oil is. The effects of hydrogenation on the induction time of soybean oil are shown in **Table 3**. The induction times of oils with the iodine values of 136, 126, and 117 were 4.2, 5.9, and 6.7 h, respectively and were significantly different at $\alpha = 0.05$. Draquez-DeHault and Demoulin (*18*) reported that the hydrogenation of soybean oil can produce improved oxidative stability liquid soybean oil with nickel catalysts. The headspace oxygen and induction time results showed that hydrogenation significantly improved the oxidative stability of soybean oil at $\alpha = 0.05$.

Interesterification Effect on the Oxidative Stability. The random interesterification involves an exchange of acyl group among triglycerides and changes the physical properties of fats and oils, especially the melting point. Natural oils do not have a random distribution of fatty acids among triglycerides. The tendency of specific fatty acids to be distributed at stereospecific numbering (sn) positions is different among the species of plants and animals (*5*). The physical properties of oils are greatly influenced not only by the length and the number of double bond of fatty acids but also by the distributions of fatty acids on sn positions of fats and oils. Interesterification can be carried out to an equilibrium condition, at which point the fatty acids assume almost random distribution on the sn positions of triglycerides (*5*, *19*).

The effects of random interesterification on the headspace oxygen of the oils are shown in **Table 2**. The headspace oxygen mean values of noninteresterified soybean oils with the iodine values of 136, 126, and 117 were 14.7, 17.6, and 18.0%, respectively. The mean values of headspace oxygen of interesterified oils with iodine values of 136, 126, and 117 were 12.1, 13.7, and 15.1%, respectively, and were significantly different at $\alpha = 0.05$ (**Table 2**). The interesterified oils had

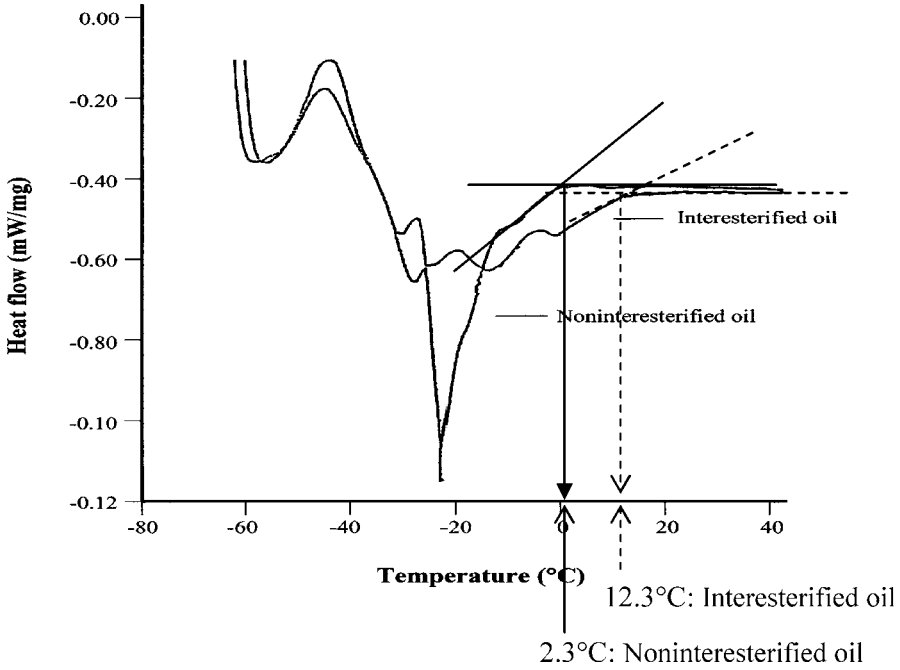

Figure 3. DSC thermal curves for soybean oil before and after the interesterification.

smaller headspace oxygens than the noninteresterified oil when the iodine values were the same as shown in **Table 2**. Interesterification has lowered the oxidative stability determined by headspace oxygen at $\alpha = 0.05$. The effects of random interestifiaction on the induction times of oils are shown in **Table 3**. The induction times of noninteresterified oils with iodine values of 136, 126, and 117 were 4.2, 5.9, and 6.7 h, respectively, and were significantly different at $\alpha = 0.05$. The induction times of interesterified oils with iodine values of 136, 126, and 117 were 1.4, 2.3, and 2.5 h, respectively. The induction time showed that the random interesterification significantly decreased the oxidative stability of oils at $\alpha = 0.05$. The interesterification process requires prolonged exposure time to high temperatures, which could provide sufficient energy for the initiation of oxidation reactions to decrease the oxidative stability of oil (*20*). The interesterification for 2 h at 100 °C could oxidize linoleic acid and linolenic acids of soybean oil. The oxidized fatty acids in oils are prooxidants (*21*), and they could decrease the induction times (*17*). The coefficient of variation for induction time analysis was 10.3%. The headspace oxygen and induction time results showed that interesterificaion lowered significantly at $\alpha = 0.05$ the oxidative stability of nonhydrogenated and hydrogenated soybean oils with iodine values of 136, 126, and 117.

Melting Points. The melting points of hydrogenated and interesterified oils were measured by DSC. The DSC method for melting point determination was essentially the same as that of List et al. (*22*). The thermogram and melting point determination of soybean oil by DSC are shown in **Figure 3**. The effects of hydrogenation and interesterification on the melting points are shown in **Table 4**. The melting points of soybean oils with iodine values of 136, 126, and 117 were 2.3, 3.6, and 6.0 °C, respectively. Hydrogenation increased the melting point of oil and had a significant effect on the melting points at $\alpha = 0.05$ (**Table 4**). The decreases of polyunsaturated fatty acids and isomerization of cis to trans isomers by hydrogenation increase the melting point (*18*). Rossell (*23*) reported that DSC thermogram was due to the increase of high melting glycerides by hydrogenation. The melting points of noninteresterified soybean oils with iodine values of 136, 126, and 117 were 2.3, 3.6, and 6.0 °C, respectively. The melting points of interesterified soybean oils with iodine values of 136, 126, and 117 were 12.3, 20.1, and 22.3 °C, respectively. The interesterification increased the melting points and had a significant effect on the melting points at $\alpha = 0.05$ (**Table 4**). The randomization of fatty acids of soybean oil by interesterification must be responsible for the increase of melting points. The melting points of noninteresterified and interesterified soybean oils with an iodine value of 136 were 2.3 and 12.3 °C, respectively (**Table 4**). Interesterification of soybean oil increased the melting point temperature from 2.3 to 12.3 °C. Norris and Mattil (*24*) reported that melting points for the noninteresterified and interesterified soybean oils were −7 and 5.5 °C, respectively. The interesterification increased the melting point from −7 to 5.5 °C. They used the capillary technique of crystallization to determine the melting points of oils. The melting point of oil is influenced by the type of crystallization process employed (*25*). The differences of melting point for soybean oil by Norris and Mattil (*24*) are mostly due to the differences in the determination methods.

Melting point determination of the oil is an indirect measurement of interesterification. The DSC differences between the interesterified and the noninteresterified oils indicate interesterification of oil. The noninteresterified soybean oil has a sharp endothermic point at −24 °C, which was not observed for the interesterified oil (**Figure 3**). The temperature range from −64

Table 4. Effects of Hydrogenation and Interesterification on the Melting Point of Soybean Oil

	melting points (°C)			
soybean oil	trial 1	trial 2	trial 3	mean[f]
SBO[a]-IV[e] 136	3.0	2.0	2.0	2.3[a]
ISBO[b]-IV 136	13.0	12.0	12.0	12.3[c]
HSBO[c]-IV 126	3.0	3.8	4.0	3.6[a]
HISBO[d]-IV 126	20.5	20.0	19.8	20.1[d]
HSBO-IV 117	5.8	5.9	6.3	6.0[b]
HISBO-IV 117	22.0	23.0	22.0	22.3[d]

[a] SBO, soybean oil. [b] ISBO, interesterified soybean oil. [c] HBSO, hydrogenated soybean oil. [d] HISBO, hydrogenated and interesterified soybean oil. [e] IV, iodine value. [f] Means with different superscripts are significantly different at $\alpha = 0.05$.

to 12.3 °C for the interesterified oil was greater than the temperature range from −58 to 2.3 °C for the noninteresterified oil. The thermogram of oil returned to the baseline at 12.3 °C from the endothermic region for the interesterified soybean oil (**Figure 3**). This suggests that the fatty acid distribution on glycerol for the noninteresterified sample was not randomized.

Natural oils have some degree of order for fatty acid distribution (*26*). The interesterification alters this natural order, which causes a melting point change. The complete random interesterification represents all fatty acid combinations of interesterified glycerols and provides a broader melting range. Similar quantitative and qualitative differences between the interesterified and the noninteresterified oils were observed in **Figure 3**.

Conclusion. The headspace oxygen and induction time showed that hydrogenation increased and random interesterefication decreased the oxidative stability at $\alpha = 0.05$. Hydrogenation or random interesterification increased the melting point of soybean oil at $\alpha = 0.05$. The combined effect of hydrogenation and interesterefication was greater than either hydrogenation or interesterefication alone on the increase of the melting points at $\alpha = 0.05$.

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