Acceleration of the Thermoxidation of Oil by Heme Iron

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ABSTRACT: Transition metals, including iron, occur naturally at significant concentrations in meat. Iron can be extracted from the food into the oil and potentially decrease the stability of the oil during frying by accelerating thermoxidation. The objective was to examine the thermoxidative stability of partially hydrogenated soybean oil after addition of heme iron. Heme iron (2.7 ppm) was added to the oil, and then oil samples were heated continuously at 160, 180, or 200°C for 72 h. Oil samples were removed for analysis every 12 h. The acid values, color, food oil sensor readings, and TAG polymer content of the heated oil samples were compared with oil samples containing no added iron that were held at the same temperatures. Generally, each oxidative index increased with (i) an increase in temperature, (ii) an increase in heating time, and/or (iii) the addition of iron. Generally, the extent of oxidation was greater for samples heated at 200°C than for oil samples heated at 160 or 180°C. The oil samples heated at 200°C reached the target polymer content of 20% after 27 h of heating. If heme iron accumulates in the oil, it will increase the rate of oxidation and thermal degradation and reduce the frying life of the oil.

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KEY WORDS: Deep-fat frying, heme iron, iron, oil stability.

Transition metals such as iron and copper are normal trace components in food materials. Iron is particularly problematic for fried meat products, since meat contains relatively large amounts as heme in myoglobin and hemoglobin (1). The high temperatures involved can denature myoglobin and hemoglobin, releasing heme and increasing the iron content of the oil to as much as 4 ppm (2). Heme iron can act as a catalyst during lipid oxidation through homolytic cleavage of hydroperoxides with formation of alkoxy and hydroxy radicals or by directly attacking the lipid, generating alkyl radicals (3). Apte and Morrissey (4) and Rhee *et al*. (5) reported that heme iron compounds have significant pro-oxidant effects. The release of iron from meat into the oil can increase the rate of oil deterioration during frying. Even if the iron form is altered following transfer to the oil, it can still catalyze oxidation (6). Augustin and Chong (7) found that Fe(III) palmitate increased the rate of secondary oxidation product formation in vegetable oil during storage at 65°C.

Numerous studies have demonstrated that transition metals accelerate food oil oxidation. These studies have included a variety of model systems (emulsions, bulk oils, various food systems), iron forms (iron palmitate, heme iron, and cooking equipment fabricated from iron), and conditions (from room temperature to ~100°C, in the absence/presence of light, etc.). One of the first reviews on heme and lipid oxidation was by Tappel (8) in 1962. There also have been numerous studies examining vegetable oil stability at high temperatures. However, there appear to be very few, if any, studies quantifying the effect of heme iron on oil degradation at the temperatures encountered during frying.

Although a direct measurement of oxidation (e.g., PV) would be best, that is not feasible for monitoring oxidation at frying temperatures. The quantification of the primary oxidation products as a result of lipid oxidation, hydroperoxides, is not possible since hydroperoxides are rapidly destroyed at temperatures near 100°C and above or by low concentrations of transition metals. Likewise, it is not feasible to monitor oxygen absorption, another direct measurement of oxidation, at frying temperatures. An analysis of the substrate concentration (unaltered or unoxidized TAG) as a function of heating time would be an option, although there are no published methods using HPLC for this particular application. Therefore, indirect methods of measurement are required. Several different analyses are carried out to evaluate frying oil stability; the analytes determined reflect the major classes of compounds formed due to the oxidation and hydrolysis reactions occurring during frying and oil degradation (9,10).

The objective of this series of experiments was to determine the effect of heme iron on oil thermoxidative degradation at high temperatures. After addition of heme iron, the thermoxidative stability of partially hydrogenated soybean oil (PHSBO) was evaluated at temperatures that encompass the range used for deep-fat and pan frying (160–200°C).

MATERIALS AND METHODS

A standard solution of heme iron [*hemin,* chloroprotoporphyrin IX iron(II); Merck, Darmstadt, Germany] in oil was prepared by dissolving heme iron containing 1 g of iron in a mixture of xylene and ethylhexanoic acid (AOCS method Ca 15-75) (11), which was then added to a sample of PHSBO (Golden Fry; ADM Packaged Oils, Decatur, IL). The iron concentration in the standard was confirmed with inductively coupled plasma spectrometry (ICP).

To remove iron from the surfaces of all the glassware, plasticware, etc. used in the analyses, all of the glassware and plasticware was immersed in a nitric acid solution (10% vol/vol in

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deionized water) for 4 h and then rinsed with deionized water before use. The iron content of the PHSBO was <0.01 mg/kg before iron addition, which was confirmed with ICP.

Aliquots of the iron standards in oil, which had been prepared as just described, were diluted with the proper amount of PHSBO to prepare three 400.0 g oil samples containing ~2.7 ppm of heme iron (2.65 ± 0.25 mg of added Fe per kg of PHSBO) in 500-mL round-bottomed flasks. The flasks were covered with aluminum foil and heated continuously for 72 h with heating mantles. After heating, the iron content of each oil sample was confirmed with ICP. Preliminary experiments indicated that the oil samples containing added Fe should be heated for an extended period of time to best examine the effect of iron on reducing the stability of the frying oil. Previous studies correlated the percentage of polar compounds in the heated oil with the concentration of polymeric material and determined that 27–28% of the polar material corresponds to a polymer content of approximately 20% (12,13). The percent polymer of the heated oil could be accurately determined and provided an indication of the degree of oxidation in the oil. The oil samples were heated until a target end point of ≥20% polymer was achieved, which was estimated to occur in samples containing added iron after approximately 72 h of heating at 160°C. All the samples were heated therefore for 72 h in this study.

The temperature of all of the oil samples was monitored with copper-constantan thermocouples encased in stainless steel probes and recorded every 10 min with a data logger system (data not shown). A 12-g oil sample was removed every 12 h and placed in a dark brown glass vial. After cooling, the headspace was purged with argon and the sample was stored in a freezer until the physical-chemical and chromatographic analyses were performed.

The stability of PHSBO at different temperatures (160, 180, and 200°C) was studied with samples containing added heme iron. A sample of unmodified PHSBO (no iron added) was heated under the same conditions for comparison. The timing and subsequent sampling was started after the oil samples had reached the desired temperature.

The iodine value was determined according to AOCS Official Method Cd 1d-92 (11).

The FA profile of the unheated oil sample was determined by the analysis of FAME according to AOCS Official Method Ce 2-66 (11). The GLC standard 15a (Nu-Chek-Prep, Inc., Elysian, MN), suitable for soybean and cottonseed oils, was used as the FA reference. The column used was a DB-1701 (J&W Scientific, Agilent Technologies, Folsom, CA; 60 m, 0.25 mm i.d., d_f = 0.25 μm) for the FA separation. The initial column temperature was 190°C (2 min), then the temperature was ramped at 0.5°C/min to a final temperature of 225°C (70 min). Helium was used as the mobile phase (1 mL/min). The injector split ratio was 1:100.

The iron content of the oil samples was determined with an inductively coupled plasma spectrometer (ICP) in aqueous solution after pyrolysis overnight of the oil samples in a muffle furnace at 500°C, using a method adapted from Black (14) and Garrido *et al*. (15). Thirty-gram samples were pyrolyzed, and the ashes were dissolved in an aqueous acidic solution. Next, iron emission at 259.9 nm was determined with an ICP (Model ICAP 61; Thermo Elemental, Franklin, MA). Instrument operation, interelement interference correction, background correction, and data collection were controlled using ThermoSPEC/AE 6.20 software. Blanks, calibration check standards, and reference standards were run with each analysis set. The iron determinations were conducted in triplicate.

The acid value (AV) was determined in triplicate for each sample as described in AOCS Official Method Cd 3d-63 (10).

The Food Oil Sensor (FOS) model NI-21A (Northern Technologies International Corporation, Lino Lakes, MN) was used to measure the dielectric constant of the heated oil samples, after calibration (zero value reference) with unheated PHSBO samples. The method correlates well with the polar content and is useful for estimating oxidation in heated oils (16).

Although the color change alone is not a useful method to monitor oil oxidation, color is an important parameter associated with consumer acceptance (17). The color intensity changes were determined according to AOCS Official Method Cc 13b-45 (11).

High-performance size exclusion chromatography analyses were conducted in duplicate according to the method published by Artz *et al*. (16).

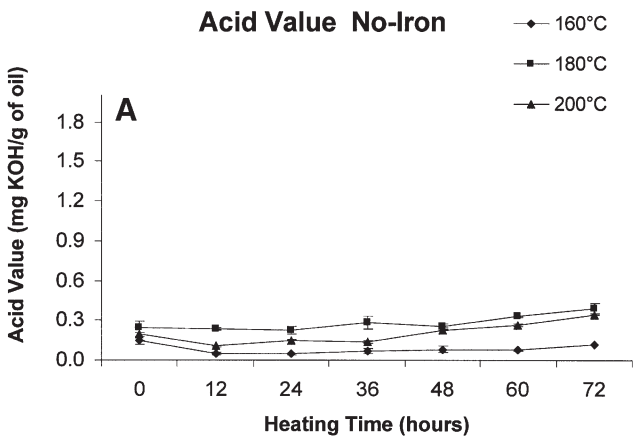
The values in the figures and tables represent an average of three replicate analyses. The averages and their respective SD are represented as the average ± SD. The relationship of mean FOS values and percent polymer vs. time for the samples without iron added and the samples containing heme iron was determined by statistical regression analysis and correlation coefficient analysis. To check on the reproducibility of the experiment, the average of all replicates in the first heating was compared to a second heating experiment as well. Statistical significance was expressed at the 95% confidence level.

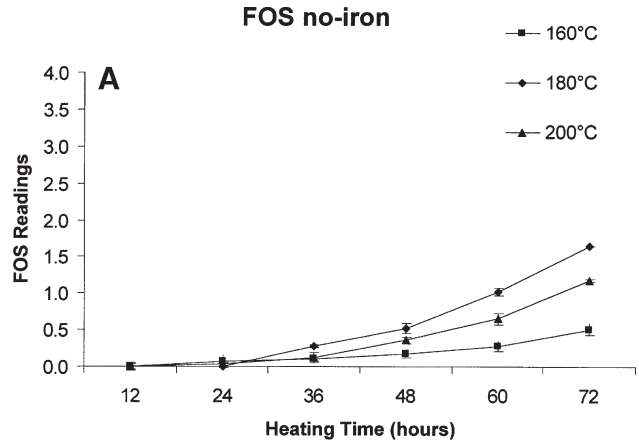
Comparisons of the degradation parameters of PHSBO as a function of heating time were made using Statistical Analysis System software (SAS Institute, 2001; Cary, NC). Data were treated as a 2 (type of samples) by 7 (heating times) by 3 (temperatures) unbalanced factorial design and subjected to ANOVA for main effects. For comparison of treatment means, ANOVA and Duncan's multiple range test with acceptance at a significance level of 95% were used. The comparison was based on the degradation rates for both oil samples (iron and no iron added).

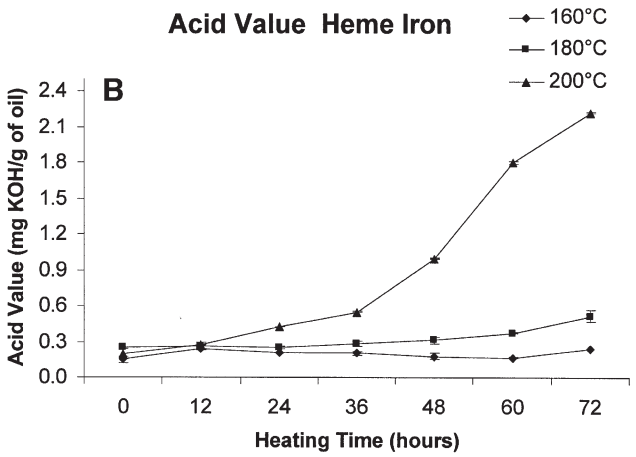
RESULTS AND DISCUSSION

The iodine value of the PHSBO sample was 103. The FA composition for the PHSBO sample prior to frying was 10.2 ± 0.1, 4.8 ± 0.1, 28.0 ± 0.1, 53.3 ± 0.1, and 3.0 ± 0.0% for the FA C16:0, C18:0, C18:1, C18:2, and C18:3, respectively.

Substantial increases in the rate of oxidation were due to the added heme iron. The FOS values, AV, and polymer content values for the oil samples as a function of time at different heating temperatures are shown in Figures 1–3 ("A" panels: no







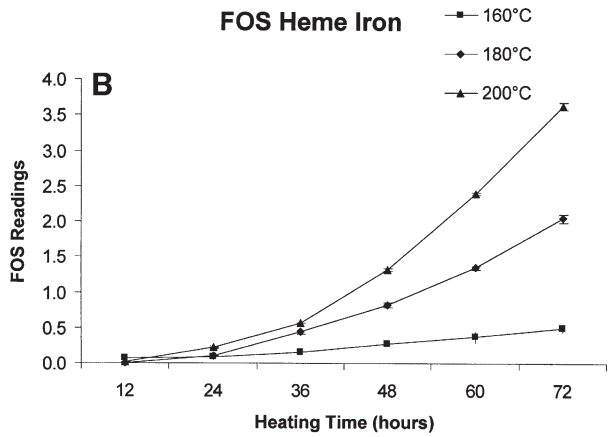


FIG. 1. Acid values (A) of unmodified partially hydrogenated soybean oil (PHSBO) samples (no-iron added) and (B) of PHSBO samples with 2.65 ± 0.25 mg of added Fe per kg of PHSBO, both heated for 72 h at 160, 180, and 200°C. The error bars indicate averages ± SD (n = 3).

FIG. 2. Food oil sensor (FOS) readings (A) in the unmodified PHSBO samples (no-iron added) and (B) in the PHSBO samples with 2.65 ± 0.25 mg of added Fe per kg of PHSBO, both heated for 72 h at 160, 180, and 200°C. The error bars indicate averages ± SD (n = 3). For other abbreviation see Figure 1.

added iron; "B" panels: added heme iron). Generally, there was an increase in the value of each oxidative index with an increase in temperature and heating time for each sample type, i.e., with added iron and without added iron. This was confirmed by ANOVA, with significant differences between different temperatures and heating times.

The concept that a transition metal can initiate a free radical chain reaction by an electron transfer reaction with the substrate was originally introduced by Harber and Willstäter (18) in 1931. Maier and Tappel (19) identified secondary products from the hematin-catalyzed decomposition of linoleate hydroperoxide; these were principally oxirane, hydroxyl, and carbonyl compounds, as well as polymers. Pearson *et al.* (20) also noted that lipid oxidation may be accelerated by a variety of heme compounds.

The results overall indicated that oil samples with added heme iron had a significantly greater amount of oxidative degradation than oil samples without added iron following an increase in either temperature or heating time (Figs. 1–3). The results from different oil sample treatments (samples with or without added heme iron) were statistically different based on FOS results, the polymer content, the AV, and the sample color (both yellow and red) intensity. The means for the analytical results for the samples with iron added were compared with the means from the samples of oil containing no added iron using Duncan's multiple range test.

The AV for oil samples without added iron and with added heme iron (2.7 mg of Fe/kg of PHSBO) are shown in Figures 1A and 1B, respectively. The samples heated at 160, 180, and 200°C were significantly different from each other. There is a much larger increase in the AV with time in the samples with added iron as compared with the samples without added iron at the same temperature, especially for samples at 200°C.

The lightly hydrogenated soybean oil samples of Handel and Guerrieri (21), heated without added heme iron at 200°C, degraded at a rate that was similar to the rate for the samples without added heme iron heated at 200°C that were reported here.

The degradation rates (oil samples heated with added heme iron) were best described by a second-order polynomial regression, with greater correlation coefficients for the samples with

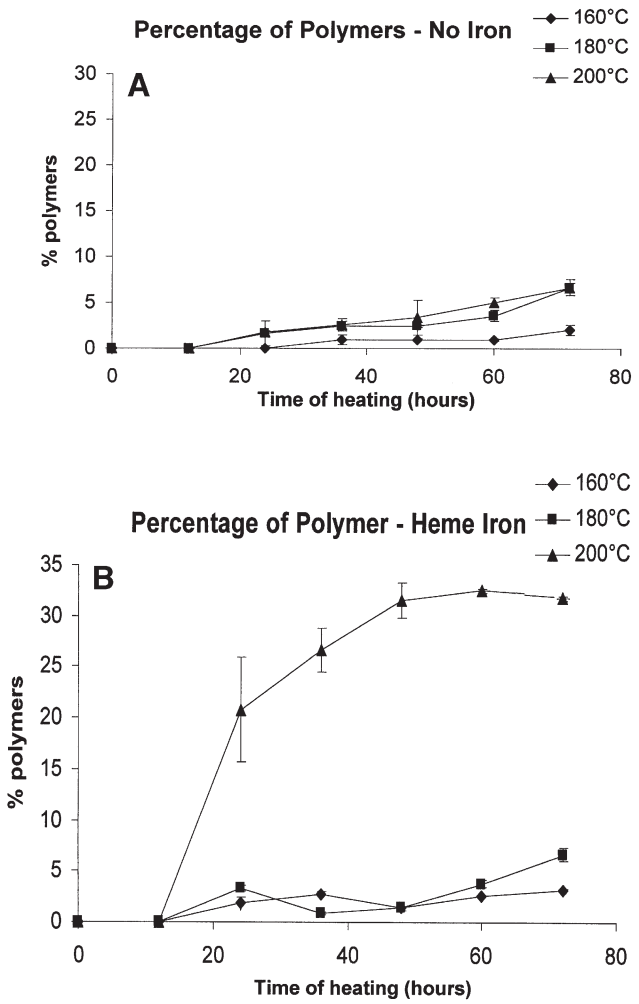


FIG. 3. Polymer content (%) (A) in the unmodified PHSBO samples (no-iron added) and (B) in the PHSBO samples with 2.65 ± 0.25 mg of added Fe per kg of PHSBO, both heated for 72 h at 160, 180, and 200ºC. The error bars indicate averages ± SD (n = 3). For abbreviation see Figure 1.

added heme iron and the samples heated at greater temperatures ($R^2 > 0.96$ for the heme iron-added samples heated at 200°C).

The FOS readings for both oils (without and with added heme) are shown in Figures 2A and 2B, respectively. The trend in the rate of oil degradation for both types of samples was similar, although, as expected, there was a greater rate of increase in the oxidation index (FOS) with an increase in either time or temperature for the samples with added heme iron. The rate of change in the FOS values followed the same pattern as the AV and can best be described by second-order polynomial equations ($R^2 > 0.99$). There were significant differences between the oil samples (no added heme iron and added heme iron) at all three temperatures for each heating interval after 36 h; and the samples heated at 160, 180, and 200°C were also significantly different from each other. A substantial increase in the rate of lipid oxidation was found with the addition of iron to the oil (6).

TAG polymers are secondary oxidative products that form during deep-fat frying in the absence of sufficient oxygen to form hydroperoxides. The results of the polymer analysis are shown in Figures 3A and 3B. The oil samples exhibited significant differences in the TAG polymer contents only after 24 h of heating for all the temperatures and treatments except for the non-iron sample at 160°C. At a sample heating temperature of 160°C, samples heated at 0, 12, and 24 h did not show significant differences from each other.

Both the FOS values and the polymer content correlate well with the total polar compound content in used frying oils. The FOS values and percent polymer contents (for samples containing no added iron) are comparable to values reported for the percentage of total polar material and the polymerized TAG content obtained under similar heating experiments (190 and 204°C) using a soybean oil-based shortening (22).

For the TAG polymer results, the effect of temperature on the increase in the rate of degradation was similar to the effect on the AV and FOS values, although the correlations were lower. The TAG polymer values obtained from samples heated at 200°C were significantly different from the values obtained from samples heated at lower temperatures (160 and 180°C). The samples heated at 160 and 180°C were not significantly different from each other.

The change in color is an important parameter associated with the consumer acceptance of fried food products, although the color change is not a particularly useful method to monitor oil oxidation since it is not a reliable indicator of oil quality (22), even though oils can darken substantially on heating. The results of the color analysis are shown in Table 1. Samples with added heme iron were statistically different from the samples containing no added heme iron for both red and yellow color changes. Moreover, the means comparison test revealed that there were significant differences in oil color for samples heated at 200°C, as compared with samples heated at lower temperatures, as indicated by the larger color changes (yellow and red). The increase in total color intensity was much greater for the samples containing added heme iron than for the samples that were heated without added heme iron. The increase in yellow color intensity with an increase in heating time or temperature was much greater than the increase in red color intensity for all of the samples. Experiments with transesterified soybean oil heated at 190°C (16) also showed large increases in both red and yellow color intensity after heating.

To estimate oil degradation/reaction rates, the data from the polymer analyses for samples heated at 180°C was used to determine polynomial regression curves. The effect of heme iron on oil stability was examined by regression analysis of the data for percent polymer data. This index was chosen since it is closely related to the degradation rate of the primary substrate, TAG monomers (17). The temperature of 180°C is closest to the temperature most commonly used in retail and industrial frying (~175°C). From the percent polymer data presented in Figures 3A and 3B, the equations obtained by polynomial regression were:

TABLE 1
Color of PHSBO Samples, Heated at 160, 180, and 200°C for 72 h, as Determined with an AOCS Tintometer[a]

160°C	No added iron		Heme iron	
Heating time	Yellow	Red	Yellow	Red
0 h	0.3	0.4	0.3	0.4
24 h	1.0	0.5	1.9	1.8
48 h	2.0	0.3	6.5	1.9
72 h	9.0	1.1	12.0	2.4
180°C	No added iron		Heme iron	
Heating time	Yellow	Red	Yellow	Red
0 h	0.3	0.4	0.3	0.4
24 h	1.0	0.3	3.0	1.9
48 h	9.0	0.6	17.5	2.3
72 h	14.0	3.1	20.5	4.6
200ºC	No added iron		Heme iron	
Heating time	Yellow	Red	Yellow	Red
0 h	0.3	0.4	0.3	0.4
24 h	6.0	0.5	11.0	2.2
48 h	10.0	3.0	25.0	6.6
72 h	12.0	2.5	47.0	8.2

[a]Values represent the average and SD ($n = 3$) for two separate heating experiments. The replicates from the two experiments were not significantly different from each other at the 5.0% level. PHSBO, partially hydrogenated soybean oil.

$$y = 0.0006x^2 + 0.0485x - 0.1633 \ (R^2 = 0.9855)$$
for the sample without iron added [1]

$$y = 0.0013x^2 - 0.0164x + 0.4796 \ (R^2 = 0.7351)$$
for the sample with heme iron added [2]

where y = percent polymer and x = heating time.

The heating time needed to achieve the ≥20% polymer discard point for each temperature (160, 180, and 200°C) can be calculated from these equations ($y = 20\%$ and x = time to discard point) (Table 2). Once the discard points are obtained, it is possible to quantify the effect of heating in the presence of iron on the frying life of the oil in comparison with a control sample (no iron added) heated under the same conditions. For examples, 147 h at 180°C would be required to reach the discard point by heating the oil sample without added iron, while less time, 129 h, would be needed to achieve the discard point for samples heated with added heme iron.

In general, it was shown that relatively small amounts of heme iron can substantially increase the rate of oil oxidation upon heating at temperatures in the range encountered during frying (160–200°C). The presence of heme iron created a noticeable increase in the rate of degradation relative to the rate of degradation for the oil samples that did not contain added heme iron. The temperature also represents an important factor that contributes substantially to the rate of degradation of the frying oil, since for all analyses performed, there was a significant difference in the degradation parameters with the increase of heating temperatures, particularly for the samples heated at the highest temperature, 200°C. A possible reason for the large increase in degradation parameters at 200°C could be the release of iron from the heme moiety during thermal degradation.

TABLE 2
Estimated Time for Heated PHSBO to Obtain a Polymer Content of ≥20%, Based on Regression Analysis

Temperature	Discard point No added heme[a]	Added heme[b]
160°C	245.2 h	173.0 h
180°C	147.3 h	129.0 h
200°C	139.5 h	26.7 h

[a]$y = 0.0006x^2 + 0.0485x - 0.1633$ ($R^2 = 0.9855$).
[b]$y = 0.0013x^2 - 0.0164x + 0.4796$ ($R^2 = 0.7351$) where $y = 20\%$ and x = time to discard point. For abbreviation see Table 1.

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