Antioxidants in heme iron fortified palm oil as a model for bakery

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Effectiveness of antioxidants in preventing oxidation of palm oil enriched with heme iron: a model for iron fortification in baked products.

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List of abbreviations used: AP, ascorbyl palmitate; AUC, area under the curve; CA, citric acid; CHP, cumene hydroperoxide; FOX, ferrous oxidation-xylenol orange; LHP, lipid hydroperoxide; MAXLHP, maximum lipid hydroperoxide; *p*-AnV, *p*-Anisidine value; PV, peroxide value; TE, tocopherol extract; TMAX, time to reach the maximum lipid hydroperoxide value

ABSTRACT

Bakery products such as biscuits, cookies, and pastries represent a good medium for iron fortification in food products, since they are consumed by a large proportion of the population at risk of developing anemia, mainly children. The risk, however, is that iron fortification can promote oxidation. To assess the extent of this, this study evaluates the oxidative stability of palm oil after the addition of heme iron and antioxidants. The samples were heated at 220 °C for 10 minutes to mimic the conditions found during a typical baking processing. The selected antioxidants were a free radical scavenger (tocopherol extract, 0 and 500 mg/kg), an oxygen scavenger (ascorbyl palmitate, 0 and 500 mg/kg), and a chelating agent (citric acid, 0 and 300 mg/kg). These antioxidants were combined using a factorial design and were compared to a control sample, which was not supplemented with antioxidants. Primary (peroxide value and lipid hydroperoxide content) and secondary oxidation parameters (*p*-anisidine value) were monitored over a period of 200 days in storage at room temperature. The combination of ascorbyl palmitate and citric acid was the most effective treatment in delaying the onset of oxidation. Tocopherol extract was not effective in preventing oxidation. The *p*-anisidine value did not increase during the storage period, indicating that this oxidation marker was not suitable for monitoring oxidation in the palm oil used.

PRACTICAL APPLICATIONS

Commonly consumed foods, such as baked food products, are often enriched with iron in efforts to address iron deficiencies. Iron deficiency is one of the most prevalent health problems around the world, affecting mainly children. The bioavailability of heme iron is higher than that of inorganic iron. However, as with inorganic iron, heme iron induces oxidation, which affects organoleptic properties and shelf life. Adding antioxidants to fortified products could prevent such undesired changes. Among the antioxidants studied here, ascorbyl palmitate, either alone or in combination with citric acid, prevented oxidation in a palm oil system enriched with heme iron. This is a valid model for iron fortification in baked products. Therefore, these results are applicable in the baking industry and contribute to efforts to increase the nutritional value of different food products.

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1. INTRODUCTION

Iron deficiency is one of the most prevalent health issues in the world [1]. The fortification of foods with iron is a common strategy used to tackle this problem. Among the different iron compounds that can be used, heme iron has a particularly high bioavailability, which is higher than that of inorganic iron mainly because the inhibition of heme iron absorption by food ligands is low [2], and also their absorption process is different [3]. As a result, trials have taken place to fortify different foods such as biscuits [4, 5], cookie fillings [6], weaning foods [7], flour [8], and black beans [9] with heme iron.

It has been reported that heme iron has the ability to act as a prooxidant [10-12], however, only a small number of authors paid attention to this matter. For instance, Asenjo et al. [4] found that fortifying biscuits with 6% bovine heme iron concentrate (a red cell isolate containing the whole molecule of hemoglobin) was acceptable based on a sensory evaluation (although the control ranked better). Asenjo et al. [4] monitored the peroxide value (PV) during storage (at 17 - 20 °C) and found that it increased compared with the non-fortified control sample. However, the authors did not provide any data on secondary oxidation products. In another study, Martínez Graciá et al. [7] used 0.5% (wt/wt) heme concentrate containing the entire molecule of hemoglobin to fortify a commercially available weaning food. After 8 months of storage at 22 °C and 37 °C no rancidity was detected by a sensory evaluation. The low dose of iron used could explain these results, but the authors provided no additional information regarding lipid oxidation. The above examples illustrate the lack of information regarding the evolution of oxidation in products supplemented with heme iron.

A good medium is an important part of a successful iron fortification program [13] as it has to be something that is consumed by a large proportion of the target population. Since anemia is most prevalent children [1] and because cookies are a daily component of children's diets, cookies and/or pastries are a good choice. Furthermore, a dark ingredient such as heme iron could be disguised well in a chocolate cookie or filling.

The objective of this work was to evaluate the oxidative stability of a refined palm oil sample – to which a heme iron ingredient was added – which was subjected to a heating treatment to replicate the baking conditions of cookies and pastries. In addition, we evaluated the oxidative stability of this matrix after various antioxidants were added with different mechanisms of action [tocopherol extract (TE), ascorbyl palmitate (AP) and citric acid (CA)]. A control group to which no antioxidants were added was used for comparison. Primary and secondary oxidation parameters were monitored during

storage at room temperature for a period of up to 200 days. Oxidative stability was also assessed by means of an induced method.

2. MATERIALS AND METHODS

2.1. Materials. Palm oil was obtained from Lípidos Santiga S.A. (Santa Perpètua de Mogoda, Spain). Ascorbyl palmitate (99.5% purity) was purchased from Sigma-Aldrich (Madrid, Spain). CA (99.5% purity) and triacetin (99% purity) were purchased from Scharlab S. L. (Barcelona, Spain), and tocopherol extract Guardian Toco 70TM was donated by Danisco Cultor España S.A. (Barcelona, Spain). The concentration of the tocopherol extract Guardian Toco 70TM was 70% TE and 30% sunflower oil. The following concentrations of the different analogues were determined in our laboratory by HPLC: 10.9% alpha-tocopherol, 1.3% beta-tocopherol, 47.7% gamma-tocopherol and 18.9% delta-tocopherol). The heme iron ingredient (AproFER 1000TM) was from APC Europe S.A. (Granollers, Spain). It consists of a dark powder obtained from the blood of healthy pigs. Hemoglobin was liberated, later enzymatically hydrolyzed, and the heme group was concentrated/purified by ultrafiltration and spray-drying as described by Quintero-Gutierrez et al. [6]. This heme iron ingredient has a total iron concentration of 1.07%, most of which is heme iron.

All chemicals used were ACS grade with the exception of the solvents used in the ferrous oxidation xynol orange (FOX) method and when determining tocol; the chemicals used for the latter two processes were HPLC grade.

2.2. Ingredient selection and Study design. Samples were made up of a palm oil base in which the heme iron ingredient (15%, wt/wt) was dispersed. Refined palm oil was chosen for this study because it is frequently used in the manufacturing of biscuits, cookies, pastries, and fillings. Based on the factorial design ($2 \times 2 \times 2$), we compared different combinations of two concentrations of three types of antioxidants: TE (0 and 500 mg/kg), AP (0 and 500 mg/kg), and CA (0 and 300 mg/kg) (Table 1). These antioxidants were chosen because their main mechanisms of action vary – free radical scavenger (tocopherols), oxygen scavenger (AP), and transition metal chelating agent (CA) – and this favors the possibility of synergistic effects among them. Moreover, these compounds are generally recognized as safe (GRAS) and can be used according to the good manufacturing practices [14, 15].

2.3 Preparation of samples. Palm oil was melted (55 °C), mixed, and divided into eight aliquots. To prepare each treatment, the palm oil was heated at 140 °C under agitation and the corresponding antioxidants were added to it. A 10% (wt/wt) solution of CA in triacetin was prepared before adding it

into the palm oil because of the low solubility of CA in the oil. A 35% (wt/wt) of the commercial TE was dissolved in sunflower oil before it was added into the palm oil to favor the dispersion of the tocopherols in the matrix.

After complete dissolution of the antioxidants, the heme iron ingredient was added to the mixture. The sample was then heated in an oven at 220 °C for 10 minutes to replicate typical baking conditions. The resulting sample was allowed to cool to 70 °C, homogenized, and distributed into 20 and 10 mL capacity vials. Vials were agitated until the content was solid. This was done to ensure the heme iron ingredient was dispersed evenly among the vials.

Vials were stored at room temperature in the dark for 0, 10, 20, 40, 80, 120, and 200 days. Afterwards, the samples were frozen at -20 °C until their analysis.

2.4 Tocol composition. The analysis of the tocol content of the palm oil was adapted from the method described by Hewavitharana et al. [16]. Briefly, 5 mL of absolute ethanol containing 1% pyrogallol (wt/vol), 0.012% butylated hydroxytoluene (wt/vol), and 0.4% anhydrous citric acid (wt/vol) were added to 200-250 mg of palm oil. Then, 10 mL of 1.6 N methanolic KOH were added, and saponification was carried out at 70 °C for 30 minutes. Non-saponifiable fractions were then extracted with petroleum ether and filtered through a 0.45 µm teflon membrane. After solvent evaporation under a nitrogen stream at 30 °C was achieved, the residue was re-dissolved in 99% n-hexane (HPLC grade). For the tocopherol extract, 133 mg were dissolved in 50 mL of 99% n-hexane (HPLC grade) and then 1 mL of this solution was transferred to a 50 mL volumetric flask with 99% n-hexane. In both cases, chromatographic separation of tocopherols was performed using a liquid chromatograph Series 1100 (Hewlett-Packard, Waldbronn, Germany) with a loop volume of 20 µL and a Luna column (4.6 mm i.d. x 1500 mm; Phenomenex, USA) packed with 3 µm – 100 Å silica. Tocopherols were isocratically eluted with n-hexane:1,4-dioxane (95:5, vol/vol) and detected using a Hewlett-Packard 1046A (Waldbronn, Germany) spectrofluorometric detector (excitation and emission wavelengths of 290 and 320 nm, respectively).

2.5 Fatty acid composition. The FAME were prepared from palm oil after a reaction with sodium methoxide followed by boron trifluoride in methanol and were then finally extracted with n-hexane [17]. Fatty acid methyl esters were analyzed on an Agilent (Waldbronn, Germany) 4890D model gas chromatograph, fitted with a flame-ionization detector and split-splitless injector, set at 300 and 270 °C, respectively. The split ratio was 1:30. Chromatographic separation of FAME was performed on a fused-silica capillary column (60 m x 0.25 mm i.d.) coated with 0.2 µm of a stationary phase of 90%

biscyanopropyl plus 10% cyanopropylphenyl-polysiloxane (SP-2380 from Supelco, USA). Helium, at 30 psi, was used as a carrier gas. The oven was programmed as follows: firstly 2 minutes at 150 °C, then the temperature was increased by 1.5 °C/min to 180 °C, then by 7.5 °C/min to 220 °C at which point it was held for 6 minutes, and then finally the temperature was increased by 5 °C/min to 250 °C and held for 20 minutes. The sample volume injected was 1 µL.

2.6 Susceptibility to oxidation. To assess the susceptibility of the samples to oxidation, the induced version of the FOX method [18] was performed with the samples taken after the thermal treatment. This is an induced method that measures the formation of lipid hydroperoxides (LHP) in a sample extract over time [19]. To set up the conditions for this method, preliminary tests were conducted using 500 µL of a dissolution of 1.2 g of the sample in 10 mL of dichloromethane:ethanol (3:2, vol/vol). This was added to a glass-cuvette containing the FOX reaction media, the composition of which was similar to that proposed by Navas et al. [20]: 100 µL of 5 mM aqueous ferrous ammonium sulfate, 200 µL of 0.25 M methanolic H₂SO₄, 200 µL of 1 mM methanolic xylenol orange, and 1000 µL of dichloromethane:ethanol (3:2, vol/vol). Cuvettes were capped with Teflon caps, and were maintained in subdued light. The evolution of the LHP formation was followed until the absorbance at 560 nm was stable (the difference between two consecutive measurements was less than 0.05). Thus the final incubation time of the FOX reaction was 93.75 hours. During the incubation of FOX reaction, absorbance was measured at different times according to Tres et al. [21] to monitor the susceptibility of the samples to oxidation. The LHP formed were expressed as millimole of cumene hydroperoxide (CHP) equivalents/kg of each sample, with reference to a calibration curve prepared using CHP as standard. To better describe the differences observed, the following parameters were calculated: Initial LHP (the initial LHP value was measured after 30 minutes of incubation), MAXLHP (the maximum LHP value), TMAX (the time that the MAXLHP was achieved), Final LHP (the final LHP value measured after 93.75 hours of incubation) and AUC (area under the curve) [21].

2.7 Evolution of oxidation status during storage. The oxidation of samples was assessed using the PV [22], the LHP content measured by a non-induced FOX method [20] after 30 minutes of incubation, and the *p*-Anisidine value (*p*-AnV) [23].

2.8 Statistical analysis. All measurements were replicated twice. The relationship between the PV and the LHP content (non-induced FOX method) was assessed using the Pearson's correlation coefficient. Using the Spearman's correlation coefficients we assessed the relationships between the following parameters: firstly, those calculated in the FOX induced method applied to thermally-treated samples

(MAXLHP, TMAX, Final LHP, and AUC), and secondly the parameters for the areas under the curves representing the evolution of PV and LHP content (non-induced FOX method) in the samples stored for 200 days.

3. RESULTS AND DISCUSSION

3.1 Palm oil characterization. Table 2 shows the concentration of the different fatty acids (FA), which corresponds with the typical FA composition of palm oil [24]: 50.10% saturated FA, 38.94% MUFA, 10.83% PUFA and 0.12% *trans* FA.

The tocol content of the palm oil was determined by HPLC (**Error! Reference source not found.**). The concentration of tocotrienols was high, particularly in alpha- and gamma-tocotrienol, which is characteristic of palm oil [24]. Since palm oil has a total tocol content of 530.9 mg/kg (total tocopherols: 133.8 mg/kg and total tocotrienols: 397.1 mg/kg), all the samples had a certain amount of these antioxidants – including those without added TE.

3.2 Basal content of tocols after the heat treatment. To determine the basal concentration of tocols after the heat treatment, the tocol content of a sample containing 85% palm oil and 15% heme iron ingredient (control) was analyzed. The results are shown in **Error! Reference source not found.** The content of the tocol homologues in the heated control sample was lower than in the non-heated palm oil, multiplied by the dilution factor (0.85). It is important to note that, because the control sample contained only 85% palm oil, the tocols were more diluted than in the palm oil samples. Consequently, some tocol degradation may have occurred in the control sample as a result of the heat treatment. This is in line with the results of a study by Giuffrida et al. [25], in which alpha- and delta- tocopherol began decomposing at 165.1 °C and 179.2 °C, respectively. Therefore, it is possible that some of the naturally occurring palm oil tocols may have been decomposed in the samples exposed to temperatures of 220 °C.

3.3 Susceptibility to oxidation. The induced FOX method was applied to samples of all treatments (Table 1) following the heat treatment. By measuring the evolution of the LHP values during the incubation period, we were able to assess sample’s susceptibility to oxidation rather than its present level of LHP thus providing valuable information on the future oxidative stability [19, 21]. The evolution of the LHP values during the incubation period is shown in **Error! Reference source not found.** To better describe the differences observed, the following parameters were calculated as

explained above in the Materials and Methods section: Initial LHP, MAXLHP, TMAX, Final LHP, and AUC (**Error! Reference source not found.**).

Only in the treatments containing AP were levels of AUC and Initial LHP lower in comparison to other treatments. As Initial LHP is considered to measure the levels of LHP already present in the samples, we can conclude that during the heat treatment the presence of AP provided a certain level of protection against the onset of oxidation.

The AUC and MAXLHP value were higher in samples to which TE and CA were added (CA and TE), whereas in samples with added AP (AP, AP + CA, TE + AP and TE + AP + CA) a lower MAXLHP value was found. It is interesting to note that the time required to reach this peak concentration (TMAX) was different for each sample; treatments containing TE and/or CA (CA, TE and TE + CA) required only 4 hours to reach the TMAX, whereas those containing AP (AP, AP + CA, TE + AP and TE + AP + CA) required more than 20 hours. However, the Final LHP values (at 93.75 hours) were relatively similar in all samples. In conclusion, the samples that contained AP had lower Initial LHP values, reached lower MAXLHP values, at a longer incubation time (TMAX), thus presenting a lower AUC. Therefore, the addition of AP reduced the susceptibility to oxidation of the matrix studied.

3.4 Assessment of primary oxidation during storage. To follow the evolution of primary oxidation in samples during storage, PV and LHP content were measured.

Error! Reference source not found. shows the evolution of PV over storage time. We observed that in the treatments without the addition of AP (Control, CA, TE and TE + CA) PV was generally higher than the rest. In addition, the PV of these samples started to increase from the beginning, whereas the PV of the treatments with added AP (AP, AP + CA, TE + AP and TE + AP + CA) were still below 0.4 mEq O₂/kg during the first 40 days. We therefore conclude that the addition of AP delayed the onset of oxidation, whereas in the rest of the treatments oxidation started to occur relatively quickly. The effectiveness of AP in delaying the onset of oxidation has been previously described by Masson et al. [26]. They observed that the level of PV in potato chips fried in palm oil containing 500 mg of AP/kg was lower than in chips fried in the control group (without addition of AP).

After 80 days in storage the PV of the treatments containing AP and CA (AP + CA and TE + AP + CA) was lower than the PV of the treatments containing AP, but not CA (AP and TE + AP), which showed a synergism between both antioxidants.

After 200 days in storage, the addition of AP alone and its combination with CA inhibited the formation of primary oxidation compounds (as measured by PV) by 38% and 87%, respectively,

1 compared with the control. The addition of TE did not reduce the PV, except when combined with AP
2 + CA.
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5 Despite some differences found after long storage times, the evolution of the LHP content during
6 storage measured using the non-induced FOX method (Fig. 3) corroborates the ability of AP to delay
7 the oxidation in certain samples observed by means of the PV (**Error! Reference source not found.**).
8 In samples containing only AP or AP combined with other compounds (AP, AP + CA, TE + AP and
9 TE + AP + CA) the LHP content was from the beginning lower than in the rest of the samples (Control,
10 CA, TE, and TE + CA). As with the PV, there was a synergism between AP and CA between day 80
11 and 120. For example at around day 120, the addition of AP alone was able to inhibit LHP formation
12 by 38%, whereas the combination of AP and CA resulted in a 70% inhibition.
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14 Moreover, looking at the two analytical parameters (PV and LHP content) in relation to the
15 concentration of LHP in the samples, we found that they were significantly correlated (Pearson's $r =$
16 0.833 , $p < 0.01$, $N = 56$). Therefore any of the two methods could be used to monitor changes in LHP
17 in the samples. Despite this, after a period of 200 days the antioxidant effect of AP was more evident
18 when the hydroperoxides were measured using the PV.
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20 **3.5 Correlation between FOX induced parameters, and PV and LHP content during storage.** The
21 FOX induced method was applied to samples immediately after the thermal treatment to assess their
22 susceptibility to oxidation [21]. As mentioned above, the FOX induced method assesses sample
23 susceptibility to oxidation measuring the formation of LHP during the reaction time. On the other hand,
24 the hydroperoxide content of samples during storage at room temperature was measured using the PV
25 [22] and the non-induced FOX method [20]. By evaluating the results from the FOX induced method
26 with data from the PV and the non-induced FOX method during the storage of the samples, it was
27 possible to evaluate the correlation between results from induced and non-induced methods. To achieve
28 this, the area under the curve given by the PV and by the LHP content obtained by the non-induced
29 FOX method was calculated at each storage time (10, 20, 40, 80, 120, and 200 days). Spearman's
30 correlation coefficients were used to assess the relationship between these areas and the MAXLHP,
31 TMAX, Final LHP value, and the AUC obtained by the induced FOX method in the samples after the
32 heating treatment (Table 5). Highly significant correlations were observed between the TMAX and the
33 areas under the curves of the PV and the LHP content for all storage times. These areas were also
34 significantly correlated with the MAXLHP value and the AUC up until day 40 and day 120,
35 respectively. The TMAX, MAXLHP, and AUC calculated in this induced method are consistent with
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the formation of primary oxidation compounds during the actual storage of samples. Therefore, the FOX induced method is a useful tool that can be used to evaluate the susceptibility to oxidation of these particular palm oil samples (+ heme iron and antioxidants). However, in this case the Final LHP value calculated in the FOX induced method is not a useful parameter since it does not correlate with the PV or LHP content for any of the storage times (Table 5). Parameters such as TMAX, MAXLHP, and AUC have also been useful to assess the oxidability of meat samples [21].

3.6 Assessment of secondary oxidation during storage. Secondary oxidation products were monitored during storage by measuring the *p*-AnV. The results of this analysis revealed low values during all the storage times monitored (**Error! Reference source not found.**). The samples containing AP (AP, AP + CA, TE + AP and TE + AP + CA) had a lower *p*-AnV than the other samples thus indicating the effectiveness of AP as an antioxidant, as it was encountered by primary oxidation measurements. Peroxides decompose over time and lead to the formation of secondary oxidation products. However, the synergistic effect of AP and CA recorded in this study when primary oxidation products were monitored was not observed when *p*-AnV was studied, even though samples were monitored for a long period of time. In addition, no increase in the *p*-AnV was observed during the 200 days of storage.

Similarly, in an experiment performed with olive oil (which has a high content of oleic FA and a low concentration of PUFA), Lee et al. [27] observed very little variation in the *p*-AnV when oxidizing the oil at different temperatures. For example, after 180 days at 25 °C the *p*-AnV varied from 5.8 to 3.7; whereas after 18 days at 60 °C the change in the *p*-AnV was from 5.8 to 6.2.

The formation of secondary oxidation products such as aldehydes depends on the FA composition of the fat. In addition, different aldehydes react differently with *p*-Anisidine. For example the reaction of 2,4- dienals with *p*-anisidine had the highest molar absorbance, followed by 2-alkenals. Alkanals had the lowest molar absorbance [28]. Therefore, the *p*-AnV may not be useful for monitoring oxidation in the present type of sample matrix. It may be necessary to combine it with another secondary oxidation parameter.

Tompkins and Perkins [29] determined the *p*-AnV and headspace volatiles of partially hydrogenated soybean oil used for frying. This oil had a similar oleic content (40.49%) to the palm oil used in the present study (Table 2) but a much higher linoleic acid content (37.9%). The authors found a highly significant correlation between the *p*-AnV and the following volatile aldehydes: 2-alkenals, *tt*-2,4-decadialenal, hexanal, and heptanal. Most of these aldehydes would be primarily formed by the

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breakdown of linoleic acid [30]. However, nonanal, which was the only selected aldehyde that originates primarily from the breakdown of oleic acid [30], did not significantly correlate with the *p*-AnV [29]. As palm oil has a very low concentration of linoleic acid and a high proportion of oleic acid, it is possible that the analysis of nonanal was more effective as a secondary oxidation marker than other volatile aldehydes and *p*-AnV.

4. CONCLUSIONS

The use of AP – alone or in combination with other antioxidants – delayed the onset of oxidation measured by the PV and LHP content (non-induced FOX method) in a palm oil matrix. In addition, there was a synergistic effect between AP and CA after 80 days of storage at room temperature. The TE was not useful to delay the oxidation in the iron fortified palm oil used in this study. This could be related to the high tocopherol and tocotrienol content of the palm oil used. Therefore, the combined use of AP and CA is highly recommended to extend the shelf-life of heme iron-enriched food products containing palm oil (such as biscuits and pastries).

The application of the induced FOX method allowed us to predict the ability of AP to delay lipid oxidation. The advantage of using this method is that the results were obtained in 4 days, instead of the longer period needed to monitor oxidation during storage. However, the synergistic effect between AP and CA mentioned before could not be observed with this induced method.

The *p*-AnV was lower for the samples containing AP. This value did not change much during 200 days of storage at room temperature, showing that this oxidation parameter was not suitable for monitoring the oxidation of palm oil during storage. Alternatively, the measurement of headspace volatiles such as nonanal, might be better for monitoring secondary oxidation of fats rich in oleic acid.

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For Peer Review

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Table 1. Antioxidants used in the different treatments.

Treatments	Tocopherol extract [mg/kg]	Ascorbyl palmitate [mg/kg]	Citric acid [mg/kg]
Control	0	0	0
CA	0	0	300
AP	0	500	0
AP + CA	0	500	300
TE	500	0	0
TE + CA	500	0	300
TE + AP	500	500	0
TE + AP + CA	500	500	300

Table 2. Fatty acid composition of the palm oil (expressed as area normalization in percentage).

Fatty Acid	%
10:0	0.05
12:0	0.31
14:0	1.06
15:0	0.05
16:0	43.64
16:1n-9	0.03
16:1n-7	0.16
17:0	0.11
17:1	0.03
18:0	4.42
18:1t	0.12
18:1n-9	37.90
18:1n-7	0.69
18:2n-6	10.52
18:3n-3	0.19
20:0	0.39
20:1n-9	0.14
20:2n-6	0.05
20:3n-6	0.08
24:0	0.08
Saturated FA	50.10
Monounsaturated FA	38.94
Polyunsaturated FA	10.83
PUFA n-6	10.65
PUFA n-3	0.19
Trans-FA	0.12

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Table 3. Tocol composition of the non-heated palm oil, and of the control sample [palm oil with heme iron added (85% palm oil/15% heme iron ingredient, wt/wt) after being heated at 220 °C for 10 minutes].

	Tocopherol content	Tocotrienol content
	[mg/kg]	[mg/kg]
Palm oil (non-heated)		
Alpha	124.1	173.3
Beta	2.2	24.4
Gamma	6.4	182.1
Delta	1.1	17.3
Total	133.8	397.1
Palm oil (non-heated) x 0.85 (dilution factor)		
Alpha	105.5	147.3
Beta	1.9	20.7
Gamma	5.4	154.8
Delta	0.9	14.7
Total	113.7	337.5
Palm oil with heme iron (after heating)		
Alpha	92.8	126.6
Beta	3.0	18.4
Gamma	5.0	129.6
Delta	ND [†]	16.3
Total	100.8	290.9

[†] ND: not detected

Table 4. Susceptibility to oxidation (FOX induced method): Initial lipid hydroperoxide value (Initial LHP), maximum lipid hydroperoxide value (MAXLHP), time to reach the maximum lipid hydroperoxide value (TMAX), final lipid hydroperoxide value (Final LHP), and the area under the curve (AUC) found in a palm oil matrix to which heme iron and different antioxidants were added.

Treatments	Initial LHP [mmol CHP eq/kg]	MAXLHP [mmol CHP eq/kg]	TMAX [h]	Final LHP [mmol CHP eq/kg]	AUC [(mmol CHP eq/kg)×h]
Control	0.14	0.15	8.67	0.06	10.37
CA	0.21	0.22	4	0.06	12.55
AP	0.02	0.13	21.75	0.06	9.13
AP + CA	0.01	0.14	28	0.06	9.07
TE	0.21	0.23	4	0.07	13.76
TE + CA	0.15	0.16	4	0.06	10.52
TE + AP	0.00	0.12	28	0.06	8.26
TE + AP + CA	0.01	0.14	28	0.07	9.51

Table 5. Spearman correlation coefficients between parameters from the FOX induced method used to assess the susceptibility of samples to oxidation and the area calculated under the curves given by peroxide value and FOX (non-induced) method used to assess sample oxidation during storage.

		Peroxide Value						FOX (non-induced) method						
Days of storage		10	20	40	80	120	200	10	20	40	80	120	200	
Parameters from induced FOX method														
	MAXLHP	<i>r</i>	0.833	0.762	0.714	0.619	0.667	0.690	0.905	0.857	0.786	0.667	0.667	0.619
		(<i>P</i>)	(0.010)	(0.028)	(0.047)	(0.102)	(0.071)	(0.058)	(0.002)	(0.007)	(0.021)	(0.071)	(0.071)	(0.102)
		<i>n</i>	8	8	8	8	8	8	8	8	8	8	8	8
	TMAX	<i>r</i>	−0.901	−0.851	−0.851	−0.851	−0.851	−0.851	−0.751	−0.901	−0.951	−0.851	−0.851	−0.751
		(<i>P</i>)	(0.002)	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)	(0.032)	(0.002)	(0.000)	(0.007)	(0.007)	(0.032)
		<i>n</i>	8	8	8	8	8	8	8	8	8	8	8	8
	Final LHP	<i>r</i>	−0.048	−0.214	−0.357	−0.548	−0.357	−0.262	−0.024	−0.095	−0.214	−0.357	−0.357	−0.405
		(<i>P</i>)	(0.911)	(0.610)	(0.385)	(0.160)	(0.385)	(0.531)	(0.955)	(0.823)	(0.610)	(0.385)	(0.385)	(0.320)
		<i>n</i>	8	8	8	8	8	8	8	8	8	8	8	8
	AUC	<i>r</i>	0.881	0.905	0.857	0.714	0.714	0.667	0.905	0.833	0.786	0.667	0.714	0.714
		(<i>P</i>)	(0.004)	(0.002)	(0.007)	(0.047)	(0.047)	(0.071)	(0.002)	(0.010)	(0.021)	(0.071)	(0.047)	(0.047)
<i>n</i>		8	8	8	8	8	8	8	8	8	8	8	8

Fig. 1. Susceptibility to oxidation (FOX induced method): Evolution of the LHP values during the incubation of FOX reactions containing palm oil in which heme iron and different antioxidants [(Control) without antioxidants, (CA) 300 mg/kg citric acid, (AP) 500 mg/kg ascorbyl palmitate, (AP + CA) 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid, (TE) 500 mg/kg tocopherol extract, (TE + CA) 500 mg/kg tocopherol extract + 300 mg/kg citric acid, (TE + AP) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate, (TE + AP + CA) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid] were added.

79x74mm (150 x 150 DPI)

Fig. 2. Oxidation during storage time (Peroxide Value): Evolution of the PV in a palm oil in which heme iron and different antioxidants [(Control) without antioxidants, (CA) 300 mg/kg citric acid, (AP) 500 mg/kg ascorbyl palmitate, (AP + CA) 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid, (TE) 500 mg/kg tocopherol extract, (TE + CA) 500 mg/kg tocopherol extract + 300 mg/kg citric acid, (TE + AP) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate, (TE + AP + CA) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid] were added.

79x69mm (150 x 144 DPI)

Fig. 3. Oxidation during storage time (non-induced FOX method): Evolution of the LHP value in a palm oil in which heme iron and different antioxidants [(Control) without antioxidants, (CA) 300 mg/kg citric acid, (AP) 500 mg/kg ascorbyl palmitate, (AP + CA) 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid, (TE) 500 mg/kg tocopherol extract, (TE + CA) 500 mg/kg tocopherol extract + 300 mg/kg citric acid, (TE + AP) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate, (TE + AP + CA) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid] were added.

80x70mm (150 x 150 DPI)

Fig. 4. Oxidation during storage time (p-Anisidine Value): Evolution of the p-AnV over storage time in a palm oil in which heme iron and different antioxidants [(Control) without antioxidants, (CA) 300 mg/kg citric acid, (AP) 500 mg/kg ascorbyl palmitate, (AP + CA) 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid, (TE) 500 mg/kg tocopherol extract, (TE + CA) 500 mg/kg tocopherol extract + 300 mg/kg citric acid, (TE + AP) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate, (TE + AP + CA) 500 mg/kg tocopherol extract + 500 mg/kg ascorbyl palmitate + 300 mg/kg citric acid] were added.

79x71mm (150 x 150 DPI)