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ORIGINAL PAPER

Interaction Between Polar Components and the Degree of Unsaturation of Fatty Acids on the Oxidative Stability of Emulsions

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Abstract Minor components (polar components) and the degree of unsaturation of the fatty acids are the main factors responsible for the oxidative stability of bulk oils and emulsions. The isolated effects of these two factors and their interaction were evaluated in oil–in-water emulsions stored at 32 °C. Samples of coconut, olive, soybean, linseed and fish oils, both full and stripped of their polar components, were used to prepare the emulsions (1% w/w). The maximum concentration of hydroperoxide ($LOOH_{max}$) and the rate of formation of hydroperoxides (μmol L^{-1} h^{-1}) were used to measure the primary products. Hexanal, propanal and malondialdehyde were used to determine the secondary products of the oxidized emulsions containing polyunsaturated fatty acids. $LOOH_{max}$ varied from 0.16 to 12.75 mmol/kg among the samples. The interaction between the polar components and the degree of unsaturation of the fatty acids was significant ($p < 0.001$) when the hydroperoxides were evaluated. In general, the degree of unsaturation (β_1) and the absence of polar components (β_2), respectively, represented 30 and 20% of the contribution to increase the mean oxidation, with the interaction (β_{12}) contribution being more sensitive to the rate of formation of hydroperoxides (16%) than to the $LOOH_{max}$ (5%). The significance of this interaction suggests that both strategies present synergism and should be applied to improve the oxidative stability of food emulsions.

Keywords Emulsion · Oxidation · Hydroperoxide · MDA · Hexanal · Linseed · Fish · Olive · Fatty acids · Polar components · Minor components

Abbreviations

LOOH	Hydroperoxide
TBARS	Thiobarbituric acid reactive substances
$LOOH_{max}$	Maximum concentration of hydroperoxide

Introduction

Oxidation is one of the main factors that limit the shelf life of oils and food products with high lipid content. Reactive species naturally present or formed inside the oils can easily abstract hydrogen from the fatty acid chain, initiating the autoxidation process and eventually fatty acid scission, which has an adverse effect on product quality, affecting flavor, color, texture and nutritional value [1, 2]. Autoxidation as well as photooxidation involve three classic stages: initiation, propagation and termination, differing only in their initiation process [3]. In the *initiation phase*, reactive species or initiators promote the homolytic breakdown of the hydrogen–carbon bond in the fatty acid (LH) chain double bond, with the abstraction of an allylic hydrogen from the unsaturated fatty acid ester side chain ($LH \rightarrow L\cdot + H\cdot$), to form an alkyl radical [4]. Under aerobic conditions, oxygen directly reacts with fatty acid alkyl radicals (L·), producing peroxyl radicals (LOO·) and hydroperoxides (LOOH) by abstracting hydrogen from

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another unsaturated lipid (LH). Besides LOO·, the alkoxyl radical LO· can also abstract hydrogen from lipids [4–6]. Hydroperoxides, the primary products of oxidation, are highly unstable and, under elevated temperature and light conditions and in the presence of prooxidants, tend to decompose, via β-scission reaction, giving rise to secondary products such as aldehydes, ketones, lactones, alcohols, keto acids, hydroxy acids, epidioxides and other volatile compounds [6–8]. Some of these secondary products can be toxic to humans and are responsible for the undesirable rancid odor typical of oxidized oils [9, 10].

Factors affecting the oxidative stability include the fatty-acid composition of the oil, the presence of antioxidants, oxygen levels, light, the temperature of storage and packing conditions [11, 12]. It is well known that a high number of double bonds present in the fatty acid chains increases the susceptibility to oxidation [11, 13, 14]. The electronic representation of a double bond consists of two components: a strong symmetry sigma (σ) bond and a pi (π) bond. The π-bond is weaker and has a lower energy and, as a result, is responsible for the greater reactivity of unsaturated compounds [13]. Thus, the tendency of unsaturated lipids to undergo oxidation is related to the number of double bonds in the fatty acid molecule. From a chemical point of view, there is agreement over this statement [15–17], but there is no scientific evidence of an increase of oxidative stress in vivo after a high intake of polyunsaturated fatty acids (PUFA) [14, 18].

Some polar components present in oils can influence their oxidative stability, affecting the shape of the peroxide curve [19]. Compounds such as tocopherols, phenols, sterols, carotenoids and phospholipids, exert a protective effect against reactive species. They can scavenge free radicals, quench singlet oxygen, chelate metal ions and inhibit the decomposition of hydroperoxides. On the other hand, some polar components, such as free fatty acids, chlorophyll, peroxides and metal ions, can act as prooxidants [1, 3]. The balance between the anti- or prooxidant action of the polar components will depend on their chemical composition in relation to the characteristics of the system in which the oil is present.

Oxidation in bulk oils and emulsions is affected by different mechanisms. When the oil is present in an emulsion, the polarity of the free fatty acids and hydroperoxides drives them to the surface of an emulsion droplet, making the emulsion more susceptible to interactions with aqueous-phase oxidation catalysts [8, 20, 21] and thus more oxidizable than bulk oils. Thus, in addition to the factors reported above, oxidation in emulsions also depends on the type and concentration of the emulsifier, the droplet size and stability, the pH, the chemical composition and the physical state of the dispersed phase, lipid phases and interfacial layer [22]. The polar components naturally found or even added to oils can present a different antioxidant behavior when the system is an emulsion. The ability by which these compounds are placed in the interface, where the oxidation is prevalent, will determine their antioxidant action. This ability is greatly dependent on the polarity of these molecules in relation to the oil polarity [20].

Many efforts have been made to improve the oxidative stability of emulsions and thus extend the shelf life of food products. Most have focused on package technology (avoiding oxygen permeability and light incident on the product), the addition of compounds with antioxidant activity and the reduction of the degree of unsaturation of fatty acids by hydrogenation or by genetic modification of the original fatty acid synthesis (GM oils) [6, 7, 11]. Taking into account the antioxidants' addition and the reduction of the degree of unsaturation, there is no information about how these two strategies could interact to improve the stability of oil–in-water (O/W) emulsions. For this reason, our objective was to investigate the interaction between the polar components and the degree of unsaturation of fatty acids on the oxidative stability of emulsions.

Materials and Methods

Materials

Fish oil without added antioxidants was kindly donated by OmegaPure® (Omega Protein Inc., Reedville, VA, USA). Linseed, soybean and olive oils were purchased from a local supermarket and stored in the dark at 4 °C. Coconut oil was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Table 1 presents the major fatty acid composition of the oils evaluated in this study, which was estimated using the USDA composition table [23]. Iso-octane, 2-propanol, methanol and 1-butanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Imidazole, barium chloride dihydrate, ammonium thiocyanate, iron(II) sulfate heptahydrate, silicic acid, activated charcoal, Tween 20, 1,1,3,3-tetraethoxypropane (TEP), cumene hydroperoxide, hexanal and propanal were also purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents were HPLC grade. All other chemicals were of analytical grade.

Preparation of Polar Components-Stripped Oils

Oils were stripped of their polar components, such as tocopherols, polyphenols, free fatty acids and pigments according to the methodology proposed by Khan and Shahidi [24] and modified by Waraho et al. [21]. Briefly, samples were prepared by dissolving 30 g of oil in 270 mL of hexane (sequentially 100 + 100 + 70 mL). Then, the

Table 1 Major fatty acid composition of the oils used to prepare the emulsions, according to USDA [23]

Fatty acids (%)	Coconut	Olive	Soybean	Linseed	Fish
C10:0	20	0	0	0	0
C12:0	50	0	0	0	0
C14:0	20	0	0	0	0
C16:0	10	15	12	5	20
C18:0	0	0	5	0	0
C18:1 n-9	0	75	24	20	30
C18:2 n-6	0	10	52	25	0
C18:3 n-3	0	0	7	50	0
C20:5 n-3	0	0	0	0	25
C22:6 n-3	0	0	0	0	25

mixture was passed through a chromatographic column (29.5-mm ID, 350 mm in length; Wilmad Labglass No.: LG-4567T-130 w/Fritted Disc and PTFE Stopcock). The bottom layer of the column was packed with 22.5 g of silicic acid that had been washed three times with distilled water, filtered and activated at 110 °C for 24 h, according to Waraho et al. [21]. A middle layer of 5.625 g of activated charcoal was used, as was a top layer of 22.5 g of the washed, filtered and activated silicic acid. The solvent was removed in a Rotavapor RE 111 (Büchi, Flawil, Switzerland) at 38 °C. Traces of hexane were removed by flushing with nitrogen. The stripped and non-stripped oils were stored at −80 °C until use. All procedures were carried out keeping the oil as cool as possible with an ice bath and protecting the samples from light.

Preparation, Storage and Sampling of Emulsions

Oil-in-water emulsions (1 wt% oil phase) were prepared using a sodium acetate-imidazole buffer solution (10 mM each, pH 7.0) containing 0.1% Tween 20, a non-ionic low molecular weight surfactant. Coarse emulsions were prepared by blending the oil and aqueous phases for 2 min using a two-speed hand-held homogenizer at the highest speed setting (M 133/1281-0, Biospec Products Inc., Bartlesville, OK, USA), followed by an additional step of sonication for 10 min (0.5 s pulses) at 70% amplitude using a Fisher Scientific Sonic Dismembrator 500. During each step in the preparation of the emulsion, the samples were covered as much as possible to reduce light exposure, and except for the coconut oil, the samples were kept in a crushed ice bath. The emulsion prepared with coconut oil was kept at room temperature. Particle size distributions of the emulsion droplets were measured using a laser diffraction instrument (Malvern Mastersizer, Malvern Instruments Ltd, Worcestershire, UK). The mean particle diameters ($D_{[3,\ 4]}$) were measured just after emulsion preparation and periodically until the end of the experiment.

Lipid Oxidation Studies

Emulsions (1 mL) were transferred to vials sealed with poly (tetrafluoroethylene) butyl rubber septa and metallic caps and were stored in the dark in a temperature-controlled chamber at 32 °C. Sampling was periodically carried out for up to 420 h with attention paid to the primary and secondary products of oxidation. Closer to the maximum hydroperoxide concentration (peak), sampling was done more often (<1 h), while for more stable emulsions, sampling was less frequent (>2 h). Lipid hydroperoxide concentrations were determined according to Shanta and Decker [25] with some modifications. Emulsion samples (0.3 mL) were mixed with 1.5 mL of iso-octane/2-propanol (3:1, v/v) by vortexing (10 s, 3 times). This mixture was then centrifuged at 2,000*g* for 2 min. A 200-μL volume of the resulting organic solvent phase was added to 2.8 mL of methanol/1-butanol (2:1, v/v). A thiocyanate/ferrous solution was prepared by mixing one part 3.94 M thiocyanate solution with one part 0.072 M Fe^{2+} solution (obtained from the supernatant of a mixture of one part 0.144 M $FeSO_4$ and one part 0.132 M $BaCl_2$ in 0.4 M HCl). The thiocyanate/ferrous solution (30 μL) was added to the methanol/1-butanol mixture, vortexed and incubated at room temperature for 20 min. Following the incubation period, the samples' absorbances were read at 510 nm using a Genesys 20 spectrophotometer (ThermoSpectronic, Waltham, MA, USA). The hydroperoxide content was determined using a standard curve developed using known concentrations of cumene hydroperoxide and was expressed as mmol L^{-1}. The primary products of oxidation were evaluated in terms of the rate of formation of hydroperoxides and the maximum concentration of hydroperoxide ($LOOH_{max}$). The formation of hydroperoxides was calculated using the following equation: $y = a \times \exp(b \times x)$, where y represents the hydroperoxide concentration (μmol L^{-1}), a and b are the intercept and the slope of the line generated by the exponential regression, and x is time (h). Thus, b (slope) can be designated as the rate of hydroperoxide formation excluding the lag-phase and is expressed in μmol L^{-1} h^{-1}.

Thiobarbituric acid reactive substances (TBARS) values of the emulsions were determined using a modified method of McDonald and Hultin [26]. The emulsion (1 mL) was mixed with 2.0 mL of TBA reagent (15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl mixed with 2% BHT in ethanol solution) in test tubes and placed in a boiling water bath for 15 min. The tubes were cooled to room temperature for 10 min and then centrifuged (1,000*g*) for 15 min. The absorbance was read

spectrophotometrically at 532 nm using a Genesys 20 spectrophotometer (ThermoSpectronic, Waltham, MA, USA). Concentrations of TBARS were determined from a standard curve prepared using TEP and are expressed in mmol L^{-1} emulsion.

Headspace hexanal and propanal were determined using a GC-17A Shimadzu gas chromatographer equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan), Shimadzu 17A Gas Chromatograph. Emulsions (1 mL) in 10-mL glass vials capped with aluminium caps with PTFE/silicone septa were shaken and heated at 55 °C in an autosampler heating block for 17 min. A 50/30 μm DVB/Carboxen/PDMS solid-phase microextraction (SPME) fiber needle (Supelco, Bellefonte, PA, USA) was injected into the sample vial for 1 min to adsorb volatiles and was then injected into the 250 °C injector port for 3 min. The volatiles were separated at 65 °C after 10 min on a Supleco 30 m × 0.32 mm Equity DB-1 column (Supelco, Bellefonte, PA, USA) with a 1 μm film thickness, according to the method proposed by Nuchi et al. [27]. Helium was used as the carrier gas, with a total flow rate of 15.0 mL/min. A flame ionization detector at a temperature of 250 °C was used. Concentrations were determined from peak areas using a standard curve from authentic propanal and hexanal and are expressed in mmol L^{-1}.

Statistical Analysis

All of the results were initially submitted to a variance homogeneity evaluation using the Hartley test. The values reported for the rate of hydroperoxide formation (slope) and $LOOH_{max}$ were treated by one-way ANOVA followed by the Tukey HSD test, considering p values <0.05 to be significant. A factorial design (2^2) was applied for the samples divided in two groups, where factor 1 was designated as the unsaturation degree and factor 2 was designated as the presence of the polar components. To compose factor 1, samples were divided into two groups (-1 or low: coconut and olive oil and $+1$ or high: soybean, linseed and fish oil). Samples were divided into two other groups to compose factor 2 ($+1$ or stripped, and -1 or full). The effect of each factor on oxidative markers was evaluated by regression analysis. All statistical analyses and graphs were produced using the statistical software package Statistica (version 7.1, Statsoft Inc., Tulsa, OK, USA).

Results and Discussion

The formation of hydroperoxides and degradation curves for all samples are shown in Fig. 1. Differences were observed in the rate of formation of hydroperoxides (slope) and in the maximum concentration of hydroperoxides ($LOOH_{max}$) among the samples prepared with different oils; differences were also observed between the full and stripped samples of the same oil. The statistical significance of these differences is presented in Table 2, including the initial values of the concentration of hydroperoxides, evaluated in the emulsions just after their preparation. Although samples rich in PUFA stripped of their polar components showed higher hydroperoxide levels than the non-stripped samples, probably due to some oxidation during the stripping process, the initial values were low (<0.63 mmol L^{-1}). Linear correlation confirmed that the degree of unsaturation, expressed as the peroxidability index, is directly proportional to the slope ($r = +0.77$, $p < 0.001$) and to the $LOOH_{max}$ ($r = +0.78$, $p < 0.001$). More elevated values of hydroperoxides were initially observed for the emulsions prepared with stripped soybean and linseed oils. Although the droplet size of the emulsions have varied, no difference was observed during the course of the experiment ($p = 0.073$), suggesting that the emulsions were stable against aggregation, flocculation or coalescence.

When the samples were separated into low (coconut and olive) and high (soybean, linseed and fish) degrees of unsaturation (factor 1; β_1), and into full and stripped oils (factor 2; β_2), a significant interaction (β_{12}) was observed (Fig. 2) between these two factors for both primary product markers: slope (Fig. 2a) and $LOOH_{max}$ (Fig. 2b). Table 3 presents the significance of the coefficients to the regression model fitted to the experimental data. It means that there is an increase in the rate of formation of hydroperoxides and in the $LOOH_{max}$ when the oil becomes more unsaturated (from -1 to $+1$), but this behavior is completely changed by the presence of polar components (from $+1$ to -1), independent of the type of oil used to prepare the emulsion. In general, based on the regression coefficient values shown in Table 3, the degree of unsaturation (β_1) and the polar components (β_2), respectively, contributed about 30 and 20% of the increase in the mean oxidation, and the interaction (β_{12}) contribution was more effective for the rate of hydroperoxide formation (16%) than for the $LOOH_{max}$ (5%), as can be seen in Fig. 2. Our study suggests that the effect of these two factors is not additive, but more studies are necessary to explain the chemical mechanisms involved in this interaction.

Polyunsaturated fatty acids are about 10–40 times more susceptible to oxidation than monounsaturated ones [13]. In addition, *cis*/*trans* isomerization, the location of the double bond and the regio-isomerization also exert an influence on the oils' oxidative stability [29, 30]. The degree of unsaturation of the fatty acids is without doubt a principal factor of the oxidative stability of O/W emulsified systems. However, some authors have reported that when individual fatty acids are oxidized in an emulsion system,

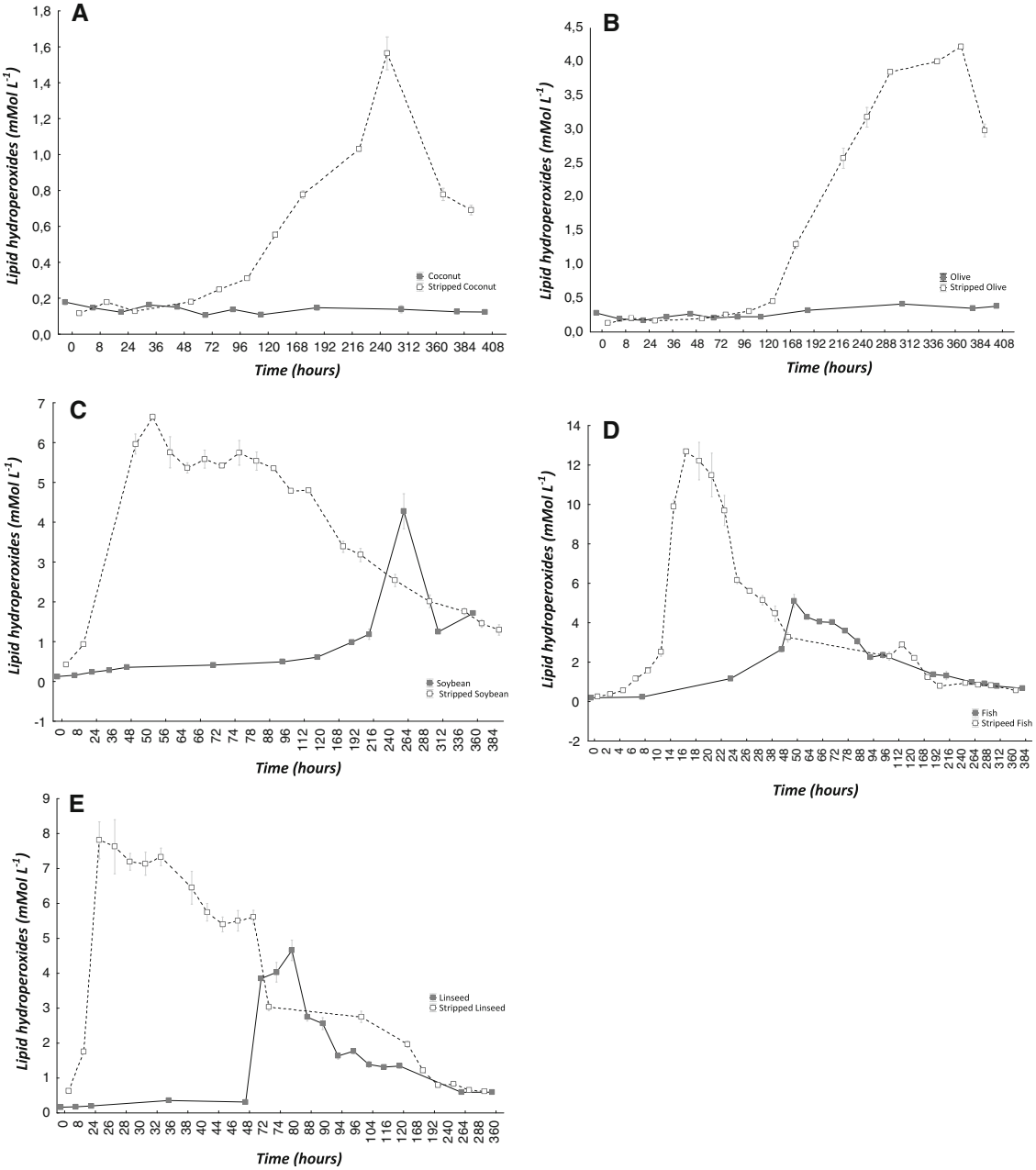

Fig. 1 Hydroperoxide formation and degradation curves obtained for each emulsion at 32 °C. **a** Coconut, **b** olive, **c** soybean, **d** fish and **e** linseed oil. *Error bars* represent the standard deviation ($n = 2$)

the generation of peroxides cannot reflect the degree of their unsaturation [31]. The results shown in Fig. 2, quantified by the β coefficients presented in Table 3, demonstrate that the oxidation of our samples, measured as hydroperoxides, perfectly reflected their peroxidability index. However, although lower than β_1, β_2 coefficient was highly significant, indicating the polar components' action as antioxidants. The chemical stability of the oil phase in dispersed systems is also strictly correlated to its fatty acid composition and the presence of antioxidants, prooxidants and catalysts [22]. Merril et al. [12] analyzed the oxidation of oils with different peroxidability indices. They observed that the fatty acid composition of the oils could explain their oxidative stability, but when oils containing similar fatty acid profiles are compared, their susceptibility to oxidation is explained by their concentration and proportion of the different isomers of tocopherols. The ability of the polar components to impact oxidative reactions depends on their chemical reactivity and physical properties [1]. In fact, the importance of the polar components for the oxidative stability of oils rich in saturated fatty acids has been neglected, as they are very stable products. The

Table 2 Peroxidability index, rate of hydroperoxide formation, hydroperoxide concentration, induction time and droplet size obtained for the emulsion

Oil sample[A]	PI[B]	Slope[C] (μmol L^{-1} h^{-1})	Initial LOOH[D] (mmol L^{-1})	$LOOH_{max}$ [D] (mmol L^{-1})	Induction time[E] (h)	$D^{F}_{[3,\ 2]}$ T_0 (μm)	$D^{F}_{[3,\ 2]}$ $T_{420\ h}$ (μm)
Coconut	0	$-0.35^{a} \pm 0.07$	$0.18^{bc} \pm 0.02$	$0.16^{b} \pm 0.00$	>408	$0.29^{a} \pm 0.00$	0.28 ± 0.01^{a}
Strip-coconut	0	$10.55^{a} \pm 0.07$	$0.11^{a} \pm 0.00$	$1.56^{c} \pm 0.10$	216–264	$0.31^{a} \pm 0.03$	0.28 ± 0.02^{a}
Olive	10	$2.05^{a} \pm 0.07$	$0.28^{e} \pm 0.02$	$0.41^{b} \pm 0.01$	>408	$0.39^{ab} \pm 0.01$	0.37 ± 0.06^{ab}
Strip-olive	10	$10.75^{a} \pm 0.07$	$0.13^{abc} \pm 0.01$	$4.23^{a} \pm 0.04$	312–360	$0.52^{b} \pm 0.01$	0.52 ± 0.04^{b}
Soy	76	$10.85^{a} \pm 0.07$	$0.12^{ab} \pm 0.01$	$4.27^{a} \pm 0.46$	216–300	$0.40^{ab} \pm 0.01$	0.35 ± 0.03^{a}
Strip-soy	76	$52.00^{bc} \pm 0.07$	$0.42^{f} \pm 0.01$	$6.65^{d} \pm 0.08$	0–30	$0.43^{ab} \pm 0.01$	0.42 ± 0.07^{ab}
Fish	225	$62.45^{c} \pm 1.06$	$0.19^{cd} \pm 0.01$	$5.11^{a} \pm 0.34$	30–60	$0.35^{a} \pm 0.00$	0.34 ± 0.01^{a}
Strip-fish	225	$247.05^{e} \pm 7.99$	$0.25^{de} \pm 0.02$	$12.75^{e} \pm 0.22$	0–15	$0.37^{ab} \pm 0.03$	0.36 ± 0.03^{ab}
Linseed	125	$46.10^{b} \pm 0.28$	$0.17^{abc} \pm 0.01$	$4.66^{a} \pm 0.31$	60–90	$0.32^{a} \pm 0.01$	0.30 ± 0.00^{a}
Strip-linseed	125	$101.30^{d} \pm 5.09$	$0.63^{g} \pm 0.01$	$8.20^{f} \pm 0.00$	0–30	$0.37^{ab} \pm 0.01$	0.36 ± 0.01^{ab}

[A] Values are expressed as means ± SD. Values in the same column followed by the same lowercase superscript letter do not differ significantly($p < 0.05$)

[B] Peroxidability index calculated according to Hsu et al. [28]

[C] Rate of LOOH formation expressed as exponential regression slope value

[D] Hydroperoxide concentration expressed as mmol L^{-1}. Milliequivalents/kg = ½ mmol/kg

[E] Lag phase for hydroperoxide formation

[F] Droplet size

lack of double bonds confers to these oils better oxidative stability when compared with several other edible oils [32]. However, when applied in emulsions, this chemical stability can change. In our study, the unsaturated fraction of the coconut oil was oxidized at 32 °C when the polar components were removed. Seneviratne et al. [32] observed that both radical scavenger activity and inhibition of the deoxyribose degradation of coconut oil increase with the concentration of phenolic substances. It is important to consider that the temperature of 32 °C applied in this study regularly occurs in many tropical countries. In addition, more saturated fatty acids have been used to replace *trans*-fatty acids in food emulsions [33].

In general, edible oils contain about 5% non-triacylglycerols or unsaponifiable matter [11]. The main minor components naturally present in oils consist of phenols, sterols, carbohydrates, pesticides, proteins, trace metals, glycolipids, phospholipids, diacylglycerols, monoacylglycerols, free fatty acids, tocopherols and pigments [6, 24]. Other compounds can be added to the oil after bleaching, such as TBHQ, ascorbyl palmitate, EDTA or antichaotropic anionic species, depending on their further application [6, 19]. The minor components can exert an essential role in delaying the initiation and progression phases, and their antioxidant action in O/W emulsions will depend on many factors, including polarity, surface activity and hydrogen donation capacity [6]. In emulsions, the oxidation occurs in the interfacial layer, a narrow region surrounding each lipid droplet, which consists of a mixture of oil, water and other surface-active compounds. Because hydroperoxides are more polar than the lipids from which they are formed, they are able to migrate to the interface, interacting with catalysts present in the aqueous phase, increasing the oxidation [6]. We hypothesized that, independent of the minor component composition, its more polar fraction would able to migrate to the interface between the oil droplet and the aqueous phase and change the oxidation behavior of the samples, even if the emulsion was prepared with oils rich in saturated and monounsaturated fatty acids. Once the polarity of the oil increases with an increase in the degree of unsaturation [34], this fact can contribute to observing the effect of polar components in the oxidation reduction of emulsions containing oils with a lower peroxidability index.

The secondary products of oxidation of the emulsions prepared with full and stripped oils rich in PUFA, expressed as TBARS (Fig. 3) appeared after 0, 72 and 168 h at 32 °C, suggest that this chemical marker can be useful in evaluating fish and linseed oils but appears not to be recommended for soybean oil, as a result of the lower amount of fatty acids containing more than two double bonds in the soybean fat composition (Table 1). The results shown in Fig. 3 also imply that the polar components had little effect on TBARS formation, as no significant differences were observed between the full and stripped samples. The decrease in the TBARS concentration after 72 h might be due to volatilization or further breakdown, as reported by Abuzaytoun and Shahidi [11]. Figure 4 presents the

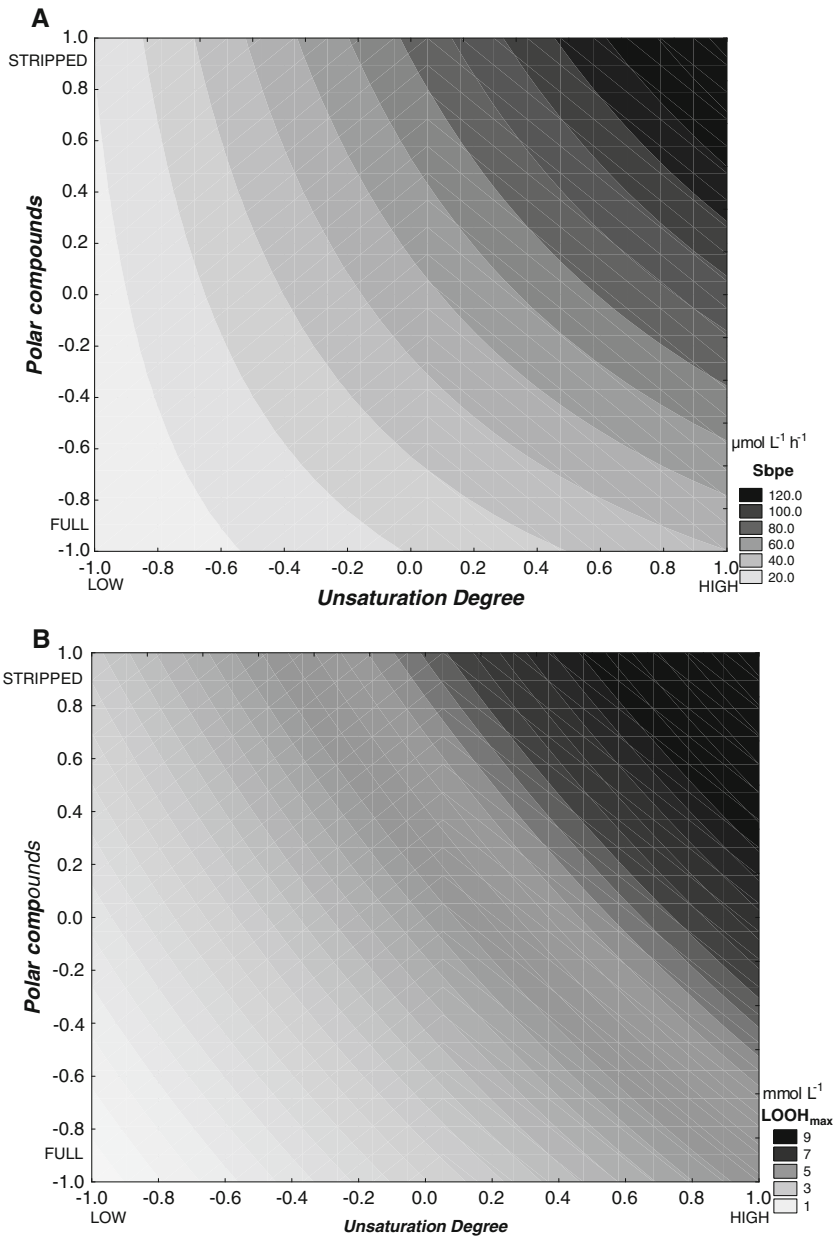

Fig. 2 Fitted response surface of the oxidative stability measured as **a** the rate of formation of hydroperoxides as a function of the degree of unsaturation of fatty acids (-1 = low and $+1$ = high) and the presence of polar components (-1 = full and $+1$ = stripped) and **b** maximum concentration of hydroperoxide ($LOOH_{max}$)

hexanal concentration observed for the unsaturated samples after 0, 72, 120 and 240 h under storage at 32 °C. It is possible to observe the great effect of the polar components on the oxidative stability of the emulsions containing soybean and linseed oils rich in n-6 PUFA. On the other hand, the effect of the polar components in fish and linseed oils can be better observed when propanal is applied as an oxidation marker (Fig. 5).

The ability of the polar components to reduce secondary products was not the same for all three emulsions. In our study, the compounds present in the fraction of polar components within these oils delayed the formation of hydroperoxides, hexanal and propanal, but they were not able to reduce TBARS. To understand the mechanisms involved in lipid oxidation, it is necessary to combine the evaluations of the primary and the secondary products because the latter are formed from the decomposition of the former [19]. Abuzaytoun and Shahidi [11] observed that stripped flaxseed oil oxidized at 60 °C produces fewer hydroperoxides but more TBARS than non-stripped flaxseed oil and attributed this result to the presence of tocopherol in the full oil sample. We observed fewer hydroperoxides but found no effect of the polar components on the TBARS concentration. Di Mattia et al. [22] reported an opposite situation, in which some phenolic antioxidants do not exert a protective effect towards the accumulation of hydroperoxides, instead slowing down the formation of secondary products by

Table 3 Linear and interaction coefficients of the polynomial models

Factors[a]	Slope (μmol L^{-1} h^{-1})	$LOOH_{max}$ (mmol L^{-1})
Constant (β_0)	46.0 ± 0.0	4.26 ± 0.02
p Value (β_0)	<0.001	<0.001
Unsaturation degree (β_1)	40.0 ± 0.0	2.67 ± 0.02
p Value (β_1)	<0.001	<0.001
Contribution of (β_1)to the mean value (%)[b]	30.0	29.1
Polar compounds (β_2)	26.0 ± 0.0	1.78 ± 0.02
p Value (β_2)	<0.001	<0.001
Contribution of (β_2) to the mean value (%)	19.5	19.4
Unsaturation degree × polar compounds (β_{12})	21.0 ± 0.0	0.48 ± 0.02
p Value (β_{12})	<0.001	<0.001
Contribution of (β_{12}) to the mean value (%)	15.8	5.2
Coefficient of determination (R^2)[c]	0.9983	0.9996

[a] Coefficient values ± standard error

[b] Example of the estimate contribution of (β_1)to the slope value: $y_{slope} = 0.05 + 0.04x_1 + 0.03x_2 + 0.02x_1x_2$. Thus, when $x_1 = 1$ and $x_2 = 1$: $y_{slope} = 0.133$ and β_1 contribution is 0.04/0.133 or 30%

[c] Coefficient of determination (R^2) represent the proportion of the variation explained by the fitted model

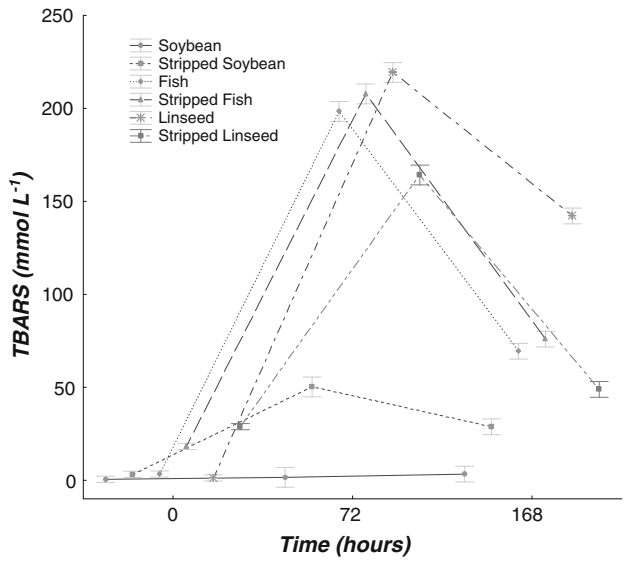

Fig. 3 MDA concentration expressed as TBARS of the emulsions measured until 168 h at 32 °C. *Vertical bars* denote 0.95 confidence intervals

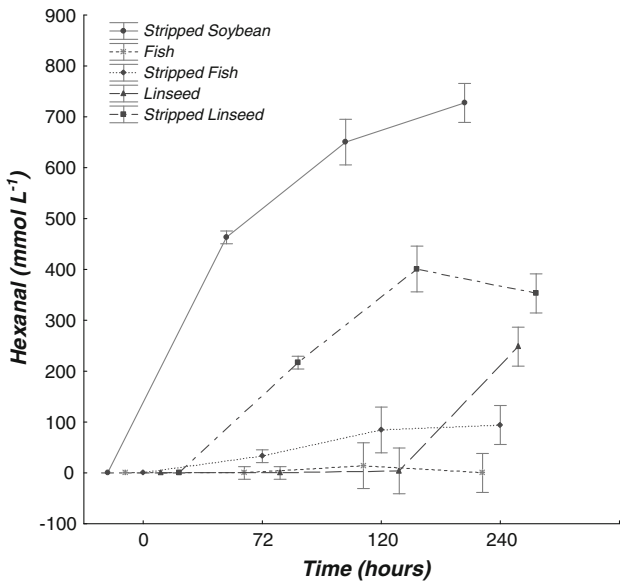

Fig. 4 Hexanal concentration of the emulsions measured until 240 h at 32 °C. *Vertical bars* denote 0.95 confidence intervals

donating a hydrogen atom to the peroxyl radicals, avoiding their further degradation. The presence of some polar components or water at low concentration can also form hydrogen bonds with hydroperoxides, retarding their decomposition to acid [19]. In addition in our study, no propanal was observed for soybean oil, and very little hexanal was measured in fish oil. Propanal and hexanal were the major volatile products formed in the oxidation of n-3 and n-6 PUFA, respectively [15], while malonaldehyde is produced by the decomposition of fatty acids containing more than two double bonds [13]. Giet et al. [7] observed an exponential increase of both hexanal and propanal among other *n*-alkenals and *n*-alkanals during the oxidation of linseed oil rich in n-6 PUFA. We suggested that different mechanisms by which the polar components stabilize hydroperoxides can promote the prevalence of one type of secondary product formation over others. Other than in our study, the reduced number of samples in each assay limited the variation range of unsaturation, and did not allow interactions to be investigated using the secondary products as chemical markers of oxidation.

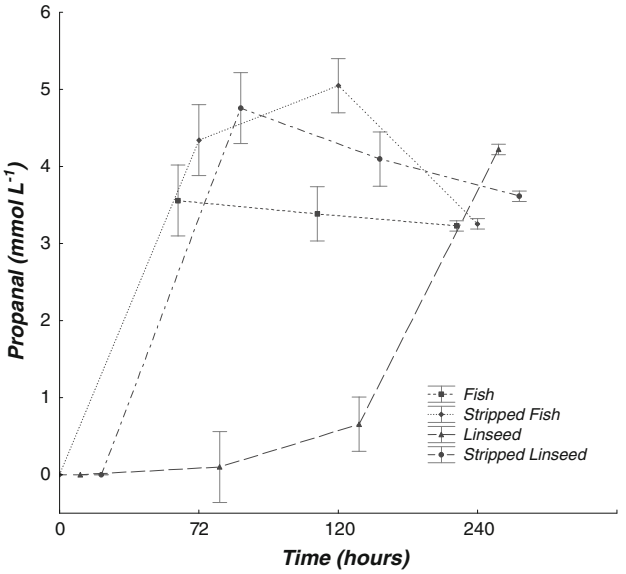

Fig. 5 Propanal concentration of the emulsions measured until 240 h at 32 °C. *Vertical bars* denote 0.95 confidence intervals

Many strategies have been suggested to extend the oxidative stability of oils and emulsions. Some of them involve a reduction of the degree of unsaturation of fatty acids by chemical or enzymatic interesterification or by genetic manipulation of the seeds. Giet et al. [7] reported that the ternary blends obtained by interesterification, containing less highly unsaturated linseed oil, present better oxidative stability. Hayati et al. [8] suggested that the detection of rancidity in emulsions can be delayed with the incorporation of more saturated oils, such as palm kernel olein, to soybean oil. The reduction of the degree of unsaturation has also been proposed for soybean oil to improve the oxidative stability of biodiesel [35]. However, as was discussed by Chaiyasit et al. [6], a decrease in the degree of unsaturation by hydrogenation can lead to higher a formation of *trans*-fatty acids, while the incorporation of saturated fatty acids in foods is not recommended from a nutritional standpoint [2]. Another strategy widely employed to improve oxidative stability is based on the addition of natural or synthetic antioxidants to refined oils. In this case, natural antioxidants are expensive, and synthetic ones are not well perceived by consumers due to their potential mutagenic effects [6].

It is known that both the reduction of the degree of unsaturation and the addition of polar components produce different results, involve different costs and present different claims to the consumers, bringing positive and negative consequences to the nutritional quality, safety and stability of different food products. Our study showed that a combination of techniques are capable of reducing the degree of unsaturation associated with the addition of antioxidants as minor components presents synergism in the oxidation susceptibility reduction, and for this reason could be an efficient balanced strategy to improve the stability of food emulsions.

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