JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Heterogeneous Aspects of Lipid Oxidation in Dried Microencapsulated Oils

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This work was aimed at studying lipid oxidation in dried microencapsulated oils (DMOs) during long-term storage. Samples were prepared by freeze-drying of emulsions containing sodium caseinate and lactose as encapsulating components. Evaluation of lipid oxidation was approached by quantitative analysis of nonvolatile lipid oxidation products and tocopherol. Lipid oxidation products were analyzed by separation of polar compounds by adsorption chromatography followed by HPSEC with refraction index detection for quantitation of oxidized triglyceride monomers, dimers, and oligomers. The analytical method applied enabled the detection of different oxidative patterns between the free and encapsulated oil fractions. The free oil fraction of DMOs showed a typical oxidative pattern for oils in continuous phase, which consisted of a clear induction period, in which hydroperoxides (oxidized triglyceride monomers) accumulated, before oxidation accelerated. The end of the induction period was marked by the total loss of tocopherol and the initiation of polymerization. On the contrary, the encapsulated oil showed a pattern characteristic of a mixture of oils with different oxidation status. Thus, high contents of advanced oxidation compounds (polymerization compounds) were detected when the antioxidant (tocopherol) was still present in high amounts. It is concluded that the encapsulated oil was comprised of oil globules with very different oxidation status. The results obtained in this study gave evidence of heterogeneous aspects of lipid oxidation in a dispersed-lipid food system.

KEYWORDS: Microencapsulated oils; lipid oxidation; free oil; encapsulated oil

INTRODUCTION

Microencapsulation of oils in powder particles of carbohydrate and/or proteins is a technological process addressed to protect polyunsaturated oils against oxidation, to mask or preserve flavors and aromas, and to transfer bioactive liquid lipids into easily handled powdery solids for food fortification purposes (*1*).

Evaluation and control of lipid oxidation in microencapsulated oils is of great significance because it results in loss of nutritional value, development of objectionable flavors, and formation of compounds that may be detrimental to health (*2*). In general, the process of lipid oxidation leads to formation of a multitude of compounds of different molecular weight and polarity which make it difficult to evaluate the degree of oxidation (*3*, *4*). This situation becomes even more complex in the case of microencapsulated oils due to additional factors that can be determinant in the rate and pathway of lipid oxidation, essentially those derived from the presence of the encapsulating matrix components and the heterogeneous lipid distribution.

Most of the studies published on microencapsulated oils have focused on the influence of the type and concentration of encapsulating agents, amount of lipids and drying conditions on the encapsulation efficiency, oil globule size, and structural morphology of the solid microparticles (*2*, *5*). However, few are studies reported on lipid oxidation in microencapsulated oils, and furthermore, it is difficult to draw general conclusions due to the great variety of encapsulating components, the different preparation conditions applied, and above all, the diversity of oxidation conditions and analytical methods used to evaluate oxidation.

Although there is a high number of analytical methods available to evaluate lipid oxidation in fats and oils, selection of suitable methods for complex lipid systems such as microencapsulated oils is a difficult task since many factors must be considered. Measurement of hydroperoxides, the primary oxidation products, is in general only meaningful under conditions that favor peroxide formation over decomposition and, hence, may provide insufficient information. Thus, some authors have found it difficult to follow the progress of oxidation in microencapsulated oils on the basis of peroxide values when very high peroxide values were obtained (*6*). Determination of peroxide value in microencapsulated oils has given results difficult to interpret when compared with oxygen uptake (*7*). In general, peroxide value determination is not recommended for low-fat foods or foods containing a noncontinuous lipid phase (*8*).

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10.1021/jf052313p CCC: $33.50
Published on Web 02/07/2006

Loss of lipid substrate is generally used as an oxidation parameter in studies on microencapsulated lipid model systems, such as microencapsulated linoleic acid or methyl linoleate (*9*−*14*). However, it is often not sensitive enough since a significant decrease of polyunsaturated fatty acids is not detected until well within the advanced oxidation stage (*15*, *16*).

One of the main problems in the evaluation of lipid oxidation is that each of the methods commonly used is only applicable to particular stages of the oxidative process, and the information provided depends on the type of method chosen (*3*). During the last years, we have developed and widely applied a methodology based on the combination of adsorption and exclusion chromatography that enables concomitant quantitation of primary and secondary oxidation products (*17*, *18*), thus providing a good measurement for early and advanced stages of oxidation. Application of this procedure has proved to be of great utility for the study of evolution of oxidation in model systems (*19*, *20*), oils (*21*), microencapsulated oils (*5*, *22*, *23*), and oil-in-water emulsions (*24*).

Control of lipid oxidation is essential in functional ingredients consisting of dried microencapsulated fish oils due to their high susceptibility to oxidative degradation. Tocopherol and ascorbic acid constitute an efficient antioxidant pair, the former as the most important natural radical-scavenging antioxidant and the latter as synergist, acting in the regeneration of tocopherol molecules. Lecithin is commonly used to dissolve ascorbic acid in bulk oils (*25*). The resulting antioxidant system ALT (ascorbic acid−lecithin−tocopherol) has proved to be notably efficient for highly unsaturated oils in bulk (*26*, *27*), but the effect of ALT or tocopherol alone in multiphase lipid systems (such as emulsions or dried foods) is far less known (*28*).

The objective of the present work was to study the evolution of oxidation in dried microencapsulated oils during long-term storage through application of an analytical procedure which allows simultaneous quantitation of primary and secondary oxidation products. A milk-based matrix comprised of lactose and sodium caseinate was used, and four oils differing in degree of unsaturation and/or tocopherol content were selected, i.e., fish oil, fish oil with the added antioxidant mixture ALT, sunflower oil, and sunflower oil stripped of natural tocopherols.

MATERIALS AND METHODS

Materials. Refined sunflower oil (SO) was purchased from a local supermarket. Sunflower oil stripped of its natural tocopherols (SO-ST) was obtained by following the method of Yoshida et al. (*29*). Fish oil (refined sand eel oil, *Ammodytes tobianus*) without added antioxidants (FO) and with the added mixture of antioxidants ALT (FO-ALT), that is, ascorbic acid (0.03% w/w), lecithin (0.5% w/w), and δ-tocopherol (0.03% w/w), was supplied by the Danish Institute for Fisheries Research (Lyngby, Denmark). Sodium caseinate from bovine milk and D-lactose monohydrate were obtained from Sigma Chemical Company (St. Louis, MO).

Preparation of Dried Microencapsulated Oils (DMOs). DMOs were prepared with sunflower oil (DMSO), sunflower oil stripped of tocopherols (DMSO-ST), fish oil (DMFO), and fish oil containing ALT (DMFO-ALT). The samples were prepared by freeze-drying of oil-in-water emulsions containing sodium caseinate and D-lactose as encapsulating matrix components The aqueous phase of the emulsions was made by dissolving sodium caseinate and D-lactose in deionized water at 55 °C. The oil was added into the water phase cooled at room temperature, and the emulsions were prepared in a lab mixer at 10000 rpm for 5 min. The weight composition of the emulsions was 10% oil, 10% sodium caseinate, 10% D-lactose and 70% water. The emulsions were frozen at −50 °C for 24 h and freeze-dried for 48 h in a Heto FD3 freeze-drier (Allerød, Denmark). The powdery DMO samples were finally obtained by controlled grinding in a coffee mill.

Preparation of Mixtures. Single mixtures of the DMO components, that is, D-lactose monohydrate, sodium caseinate, and sunflower or fish oil containing ALT were prepared in a 1:1:1 (w/w) ratio. A simple dry-blending of D-lactose and sodium caseinate was prepared thoroughly, and then the oil was added dropwise while adequately blending in a Bosch MFQ 2700 electric mixer (Bosch España S.A., Madrid, Spain).

Storage Conditions. Independent aliquots (4 g) of each DMO sample were placed into stopped 250 mL amber glass jars containing a small beaker with a saturated $MgCl_2$ solution (32% RH at 30 °C). The jars were placed in a temperature-controlled chamber at 30 °C and were not exposed to light during storage. In the case of bulk SO, a 20 g sample was placed into a 125 mL beaker and stored at 40 °C in an oven. One gram aliquots were sampled periodically.

Characterization of the Starting Oils. Fatty acid composition, tocopherol content, unsaponifiable matter content, free fatty acid index, and peroxide value as determined by the iodometric assay were determined according to the IUPAC standard methods (*30*).

Extraction of Oil from DMOs. *Extraction of Total Oil*. The procedure was based on the Rose−Gottlieb method (*31*), widely accepted for quantitative determination of fat in milk and milk powders. An aliquot of 2 g of DMOs was dispersed in 20 mL of water heated at 65 °C. After the mixture was stirred gently, 4 mL of 25% NH_4OH was added, and the solution was heated at 65 °C for 15 min in a shaking water bath. Then, the solution was cooled at room temperature, and the oil was extracted by applying three liquid−liquid extractions as follows: first, 50 mL of diethyl ether and 50 mL of light petroleum ether; second, 10 mL of ethanol, 50 mL of diethyl ether, and 50 mL of light petroleum ether; and third, idem without adding ethanol. After filtration through a filter paper containing anhydrous Na_2SO_4, the solvent was evaporated in a rotary evaporator, and the extracted oil was dried to constant weight using a stream of nitrogen.

Extraction of Free Oil. The free oil fraction, also known as the nonencapsulated oil fraction, was determined according to Sankarikutty et al. (*32*). Thus, 200 mL of light petroleum ether (60−80 °C) was added to 4 g of powder. Stirring was applied at room temperature for 15 min. After filtration through a filter paper, the solvent was evaporated in a rotary evaporator, and the extracted oil was dried to constant weight using a stream of nitrogen.

Extraction of Encapsulated Oil. Starting from DMOs devoid of free oil and dried to constant weight, the encapsulated oil fraction was extracted using the same method as that for the extraction of total oil. Microencapsulation efficiency (ME) was calculated as follows:

$$\text{ME (\%)} = \frac{\text{encapsulated oil (g/100 g DMO)}}{\text{total oil (g/100 g DMO)}} \times 100$$

Extraction of Oil from Mixtures. The oil fraction from mixtures was extracted following the same procedure described above for the extraction of free oil in DMOs.

Quantitative Analysis of Oxidation Compounds. Quantitative analysis of total nonvolatile oxidation compounds was carried out by separation of polar compounds by solid-phase extraction (SPE) and subsequent analysis by high-performance size-exclusion chromatography (HPSEC) (*17*).

Separation of Polar Compounds by SPE. A volume of 2 mL of a hexane solution containing 50 mg of extracted oil and 1 mg of monostearin, used as internal standard, was separated into two fractions by SPE in silica cartridges. A first fraction comprising the unoxidized triglycerides is eluted with 15 mL of hexane:diethyl ether (90:10, v/v). The second fraction is eluted with 25 mL of diethyl ether and comprises the total nonvolatile oxidation compounds, the internal standard, hydrolytic alteration compounds, i.e., diglycerides and free fatty acids, and polar unsaponifiable matter. Thus, the oxidation compounds are separated as compounds with higher polarity than that of the nonoxidized triglyceride molecules. After evaporation of the solvent in a rotary evaporator, the polar fraction was dissolved with 1 mL of diethyl ether. Efficiency of the separation was checked by thin-layer chromatography using hexane/diethyl ether/acetic acid (80:20:1, v/v/v) for development of plates and exposure to iodine vapor to reveal the spots.

Table 1. Characterization of the Starting Oils

	SO[a]	SO-ST[b]	FO[c]	FO-ALT[d]
fatty acids[e] (%)				
C14:0			6.3	6.4
C16:0	6.5	6.6	17.7	17.6
C16:1			6.3	6.3
C18:0	4.5	4.5	1.9	1.9
C18:1	20.0	20.1	8.6	8.5
C18:2	62.5	62.5	2.2	2.2
C18:3			5.4	5.4
C20:1			6.2	6.1
C20:5			11.0	10.8
C22:1			9.7	9.5
C22:6			10.2	10.1
others	6.5	6.3	14.5	15.2
α-Toc[f] (mg/kg of oil)	577	ND[k]	80	82
δ-Toc[g] (mg/kg of oil)	ND	ND	ND	220
UM[h] (wt % on oil)	0.91	nd[l]	1.70	1.99
FFA[i] (g of oleic/100 g of oil)	0.07	0.00	0.37	0.53
PV[j] (mequiv O_2/kg of oil)	7.3	0.0	2.4	2.1

[a] Sunflower oil. [b] Sunflower oil stripped of tocopherols. [c] Fish oil. [d] Fish oil containing the antioxidant mixture ALT. [e] Fatty acid composition. [f] α-Tocopherol. [g] δ-Tocopherol. [h] Unsaponifiable matter. [i] Free fatty acids. [j] Peroxide value. [k] Not detected. [l] Not determined.

Analysis by HPSEC. The fraction of polar compounds was analyzed in an HPSEC chromatograph equipped with a Rheodyne 7725i injector with 10 μL sample loop, a Waters 510 pump (Waters, Milford, MA), and an HP 1037 A refractive index detector (Agilent Technologies, Palo Alto, CA). The separation was performed on two 100 and 500 Å Ultrastyragel columns (25 cm × 0.77 cm i.d.) packed with porous, highly cross-linked styrene−divinylbenzene copolymers (particle size 5 μm) (Agilent Technologies, Palo Alto, CA) connected in series, with tetrahydrofuran (1 mL/min) as the mobile phase. The peaks resolved by HPSEC correspond to TGO (triglyceride oligomers), TGD (triglyceride dimers), oxTGM (oxidized triglyceride monomers), DG (diglycerides), monostearin, and a final peak corresponding to free fatty acids (FFA) and the polar unsaponifiable matter.

According to IUPAC standard method 2.508 (*33*), the direct analysis of oil by HPSEC enables a rapid analysis of the polymer compounds, i.e., TGO and TGD. This rapid determination was used as screening method to select appropriate oil samples for the analysis of total oxidation compounds in the storage studies. Results of polymer compounds by direct HPSEC analysis of extracted oils have also been included in this study when only this determination was applied.

Determination of Tocopherols. Tocopherols were determined by normal-phase HPLC with fluorescence detection according to IUPAC Standard Method 2.411 (*30*).

Detection of Rancidity. Rancidity was determined by six untrained juries. The sample was considered rancid when clearly recognized rancid odor in intact samples was detected by the six juries.

Statistical Analysis. Repeatability of oil extraction methods expressed as coefficient of variation (CV) was obtained by using four determinations. In the case of quantitative analysis of lipid oxidation products, CV was obtained by using duplicate samples. Comparison between means was made by applying Student's *t*-test in Microsoft Excel 2000 (Microsoft corporation, Redmond, WA). Significance was defined at $p < 0.05$. Linear regression analysis between oxidized triglyceride monomers and peroxide value in bulk sunflower oil was made in Microcal Origin 5.0 (Microcal Software, Inc., Northamton, MA).

RESULTS AND DISCUSSION

Characterization of the Starting Oils and DMOs. Table 1 lists results of analytical determinations used in the characterization of the starting oils. The fatty acid composition, tocopherol content, and the unsaponifiable matter content were within normal values. The low free fatty acid index and peroxide value are characteristic of refined oils of high quality. In the case of SO-ST, stripping of tocopherol also resulted in removal of free fatty acids and hydroperoxide compounds.

Table 2. Characterization of the Starting Dried Microencapsulated Oil Samples

	DMSO[a]	DMSO-ST[b]	DMFO[c]	DMFO-ALT[d]
total oil (wt % on sample)	29.7 ± 0.91[f]	29.2 ± 1.53	31.0 ± 0.17	32.1 ± 0.29
ME (wt % on total oil)[e]	72.7 ± 1.88[f]	74.0 ±2.53	71.9 ± 0.90	71.0 ± 1.30

[a] Dried microencapsulated sunflower oil. [b] Dried microencapsulated sunflower oil stripped of tocopherols. [c] Dried microencapsulated fish oil. [d] Dried microencapsulated fish oil containing the antioxidant mixture ALT. [e] Microencapsulation efficiency. [f] Mean ± relative standard deviation ($n = 4$).

Table 3. Polar Compounds (wt % on Oil) in the Starting Oils and Dried Microencapsulated Oils

sample	total	TGO[a]	TGD[b]	oxTGM[c]	DG[d]	FFA + PUM[e]
SO[f]	3.0 ± 0.20[n]	ND[o]	0.6	0.9	1.0	0.5
SO-ST[g]	1.0 ± 0.10	ND	0.7	0.2	0.0	0.1
FO[h]	4.6 ± 0.17	ND	0.5	0.8	2.4	0.9
FO-ALT[i]	4.4 ± 0.17	ND	0.4	0.8	2.3	0.9
DMSO[j]	2.9 ± 0.16	ND	0.6	0.9	0.9	0.5
DMSO-ST[k]	1.5 ± 0.22	ND	0.7	0.7	0.0	0.1
DMFO[l]	4.7 ± 0.24	ND	0.5	0.8	2.5	0.9
DMFO-ALT[m]	4.6 ± 0.28	ND	0.5	0.8	2.4	0.9

[a] Triglyceride oligomers. [b] Triglyceride dimers. [c] Oxidized triglyceride monomers. [d] Diglycerides. [e] Free fatty acids + polar unsaponifiable matter. [f] Sunflower oil. [g] Sunflower oil stripped of tocopherols. [h] Fish oil. [i] Fish oil containing the antioxidant mixture ALT. [j] Dried microencapsulated sunflower oil. [k] Dried microencapsulated sunflower oil stripped of tocopherols. [l] Dried microencapsulated fish oil. [m] Dried microencapsulated fish oil containing the antioxidant mixture ALT. [n] Mean ± relative standard deviation ($n = 2$). [o] Not detected.

Table 2 lists the total oil content and ME of DMOs. The total content of oil was similar for the four samples and close to the theoretical value (33.3 wt % on sample). The content of the encapsulated oil, expressed as ME, was relatively high and very similar for the four DMOs, ranging from 71.0 to 74.0 wt % on total oil. Repeatability of the oil extraction methods was very high, showing coefficients of variation (CV) lower than 2% for the total oil and the encapsulated oil, and lower than 5% for the free oil fraction.

Table 3 shows the results of polar compounds in the starting oils and DMOs. The low values in polar compounds for the starting oils were indicative of high quality. No significant changes in polar compounds were found in DMOs with regard to the starting oils. Hence, the procedures for DMO preparation and oil extraction did not lead to hydrolytic or oxidative degradation. The values of oxTGM, which comprises triglycerides with at least one oxidized fatty acyl chain, were very low (<10 mg/g of oil). The low levels of TGD found in the starting oils can be attributable to nonpolar dimeric triglycerides that originated in the oil refining process (*34*). As for the hydrolytic products in SO-ST, DG and FFA were removed from SO in the tocopherol stripping procedure applied.

Oxidation Pattern in Bulk Oils. Figure 1A shows the oxidative development of sunflower oil at 40 °C in the dark. Values of oxTGM, triglyceride polymers (TGD + TGO), as well as tocopherol content, have been included. As can be observed, oxTGM showed a progressive increase during the early stages of oxidation due to the rise of hydroperoxides. Thus, an excellent linear correlation ($r^2 = 0.996$) was found between the amount of oxTGM and the peroxide value until oxidation accelerated (**Figure 1B**), which is in agreement with results found recently in an extensive kinetic study carried out in our

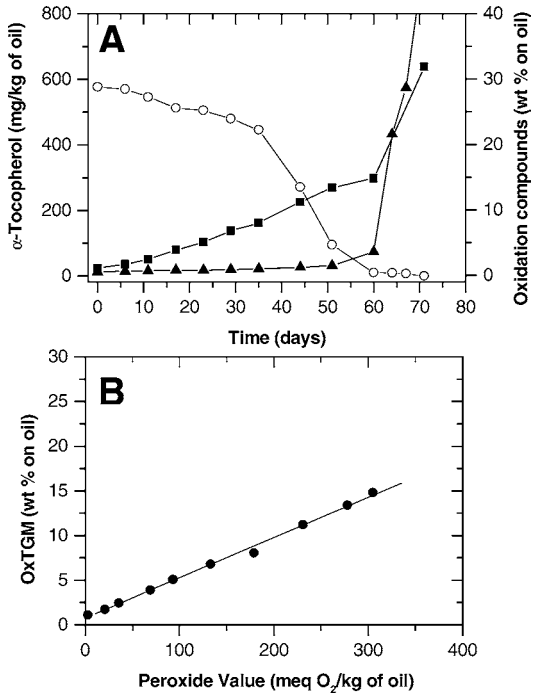

Figure 1. (**A**) Oxidative development in bulk sunflower oil at 40 °C in the dark: analysis of α-tocopherol (○), oxidized triglyceride monomers (■), and triglyceride polymers (dimers + oligomers) (▲). (**B**) Linear correlation between the content of oxidized triglyceride monomers (oxTGM) and peroxide value during the induction period: [oxTGM] (wt % on oil) = 0.77 (±0.16) + 0.045 (±0.001) PV (mequiv O_2/kg of oil), $r = 0.998$.

lab (*21*). The time at which oxidation accelerated (60 days), which is defined as the end of the induction period, was clearly marked by the initiation of polymerization and the exhaustion of tocopherol. During the accelerated oxidation stage, a multitude of secondary oxidation products containing oxygenated functions such as epoxy-, hydroxy-, keto-, and others contributed greatly to enhance the amount of oxTGM. This oxidation pattern has been found repeatedly in numerous experiments carried out in our lab to study the oxidation of bulk oils (*21*) or foods where the oil is in a continuous phase, such as fried foods (*35*). Although the amount of oxTGM found at the end of the induction period depends on the degree of oil unsaturation (*21*) and temperature (*20*), it is commonly observed that formation of polymeric compounds denotes the end of the induction period and that such an event is practically concurrent with the exhaustion of antioxidants.

Evaluation of Oxidation in DMOs. Figure 2 shows the content of oxidation compounds and tocopherol obtained through extraction of the total oil from DMOs during storage. With regard to DG and FFA, their contents remained unchanged during storage, which denoted the absence of hydrolytic degradation. Repeatability of the quantification of oxidation compounds was quite acceptable, showing CV equal to or lower than 8%.

Removal of naturally occurring tocopherol in the sunflower oil and the addition of the antioxidant mixture ALT in the fish oil considerably decreased and increased, as expected, the oxidative stability of DMSO and DMFO, respectively.

In DMSO-ST, rancidity appeared at 8 days, when oxidation accelerated and polymers showed a marked increase. In DMFO, samples were rancid at 49 days, when only residual amounts of tocopherol remained, but polymerization was noted before tocopherols were exhausted. This fact may be attributed to the high tendency of polyunsaturated oils to form polymers at ambient temperatures (*21*). DMSO and DMFO-ALT showed a similar and unexpected oxidation pattern in terms of the relation between oxidation compounds and tocopherol remaining contents, although differing in the more rapid polymerization of DMFO-ALT. For instance, when 50% of tocopherol was still present in DMSO at 162 days of storage, polymers had already reached a significant increase (4.6% on oil). Unlike in the case of bulk sunflower oil (**Figure 1A**), considerably high levels of tocopherol remaining were therefore compatible with high contents of polymers, i.e., with significant advanced oxidation. Furthermore, rancidity was not detected until after 10 months of storage despite the advanced oxidation detected long before.

Similarly, it was surprising that rancidity was not detected in DMFO-ALT until as late as 262 days in samples which had already reached a high level of total polymerization compounds (21.3% on oil). Again, considerable accumulation of oxidation products were concurrent with high levels of tocopherols. As an example, when 50% of total tocopherols were still present (126 days), the polymer quantities were as high as 12.6% on oil. These results reflected the enormous complexity of oxidation in these products and the difficulties to deduce the oxidation status starting from the total oils (*5*).

Independent analysis of the free and encapsulated oil fractions from DMOs made evident some specific facts that were useful to explain the results in **Figure 2**. Thus, oxidation was analyzed in the oil fraction accessible to organic solvents, defined as free oil, and in the fraction nonaccessible to organic solvents, defined as encapsulated oil, and whose extraction requires previous disruption of the matrix structure. Results for the content of oxidation compounds and tocopherol in the free and encapsulated oil fractions are given in **Figures 3** and **4**. Throughout the storage, no significant differences were observed in the amount of the free and encapsulated oil fractions.

As observed in **Figures 3** and **4**, oxidation was unexpectedly more rapid in the encapsulated oil than in the free oil fraction. Only in the sample devoid of antioxidants, i.e., DMSO-ST, oxidation developed faster in the free oil.

The free and encapsulated oil fractions also showed a very different oxidation profile. In the free oil fraction, formation of polymers in relevant amounts and acceleration of oxidation did not occur until tocopherols were almost exhausted. Thus, the free oil exhibited the same oxidation pattern as that described above for bulk oils. In the case of the encapsulated oil, considerable accumulation of polymer compounds was observed when high levels of tocopherols were still remaining (**Figure 4**). Only the free oil fraction showed a clear end of the induction period, as it is commonly observed in bulk oils.

Rancidity was not noted until oxidation accelerated in the free oil despite the high amounts of advanced oxidation compounds accumulated long before in the encapsulated oil fraction. Therefore, rancid odor was related to the course of oxidation in the free oil, and hardly associated with the development of oxidation in the encapsulated fraction. These results explain the inconsistency of high levels of oxidation compounds found in the extracted total oils without detection of objectionable odor in intact samples of DMSO and DMFO-ALT.

As already pointed out by Fritsch, lipid distribution is of paramount importance in food oxidation but still too often ignored (*8*). Only a few researchers have approached the study of oxidation in DMOs by separate extraction of the free and encapsulated oil fractions, and results showed clear differences. Gejl-Hansen and Flink (*36*) carried out separate extraction only in initial samples to study the oxidation of intact samples and

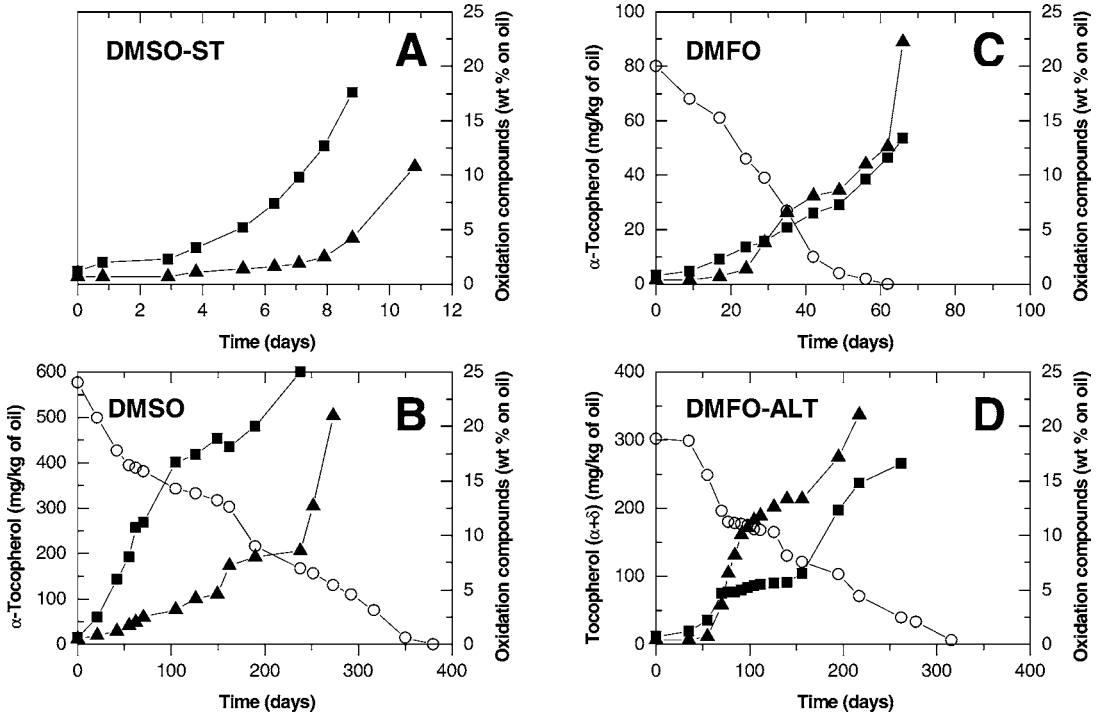

Figure 2. Oxidative development in dried microencapsulated oils at 30 °C in the dark over 32% RH: analysis of tocopherol (○), oxidized triglyceride monomers (■), and triglyceride polymers (dimers + oligomers) (▲) in the total oil. (**A**) Dried microencapsulated sunflower oil stripped of tocopherol (DMSO-ST). (**B**) Dried microencapsulated sunflower oil (DMSO). (**C**) Dried microencapsulated fish oil (DMFO). (**D**) Dried microencapsulated fish oil containing the antioxidant mixture ALT (DMFO-ALT).

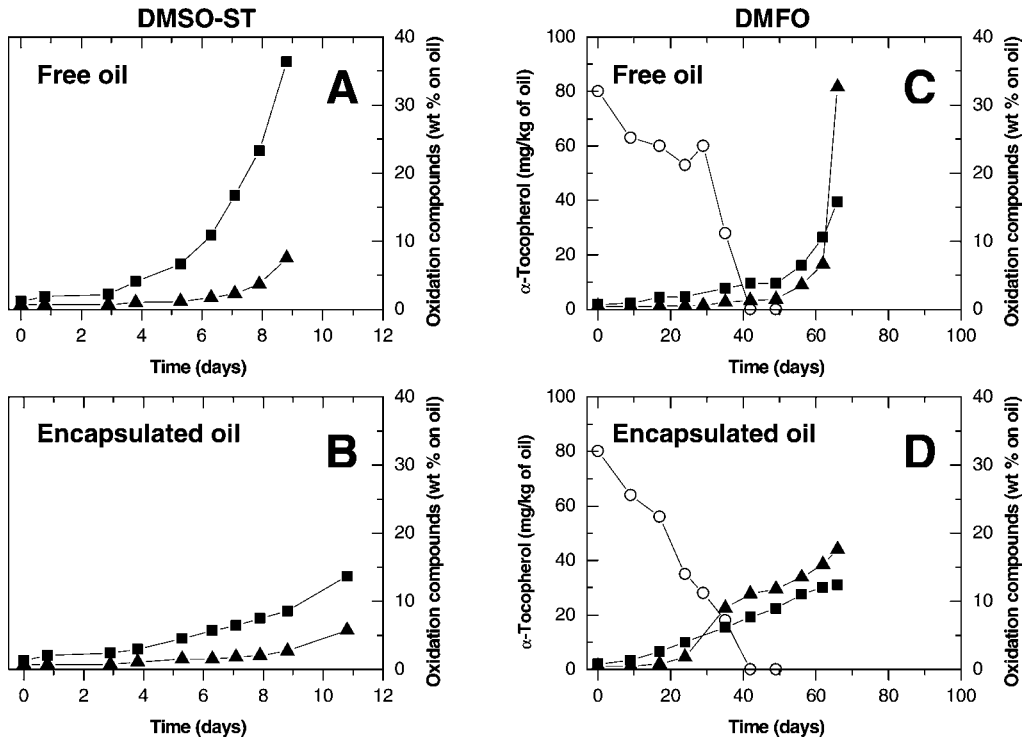

Figure 3. Oxidative development in the free and encapsulated oil fractions of dried microencapsulated oils at 30 °C in the dark over 32% RH: (**A**–**B**) dried microencapsulated sunflower oil stripped of tocopherol (DMSO-ST) and (**C**–**D**) dried microencapsulated fish oil (DMFO). Symbols: (■) oxidized triglyceride monomers, (▲) triglyceride polymers (dimers + oligomers), (○) α-tocopherol.

samples devoid of free oil during storage. Their results clearly showed that the initial extraction of the free oil led to stable samples. Oxidation was detected only in intact samples and attributed therefore to the oxidation of the free oil. Other authors have found that the free oil content increased under certain storage conditions and that this fraction oxidized more rapidly than did the encapsulated oil (*37*). Also, Ponginebbi et al. (*13*) found that oxidation was more significant in the surface fraction under various RH conditions, although some discrepancy can be detected between results obtained through determination of conjugated dienes and residual amount of unoxidized substrate. From previous results obtained in our laboratory, oxidation

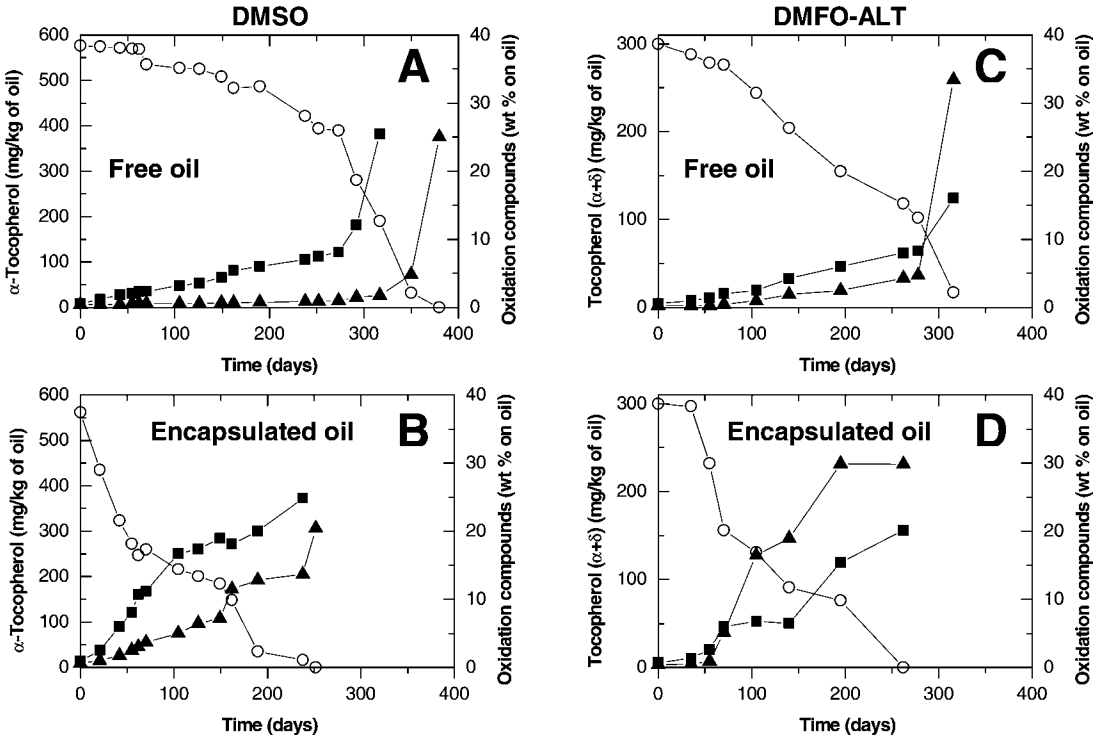

Figure 4. Oxidative development in the free and encapsulated oil fractions of dried microencapsulated oils at 30 °C in the dark over 32% RH: (**A**–**B**) dried microencapsulated sunflower oil (DMSO) and (**C**–**D**) dried microencapsulated fish oil containing the antioxidant mixture ALT (DMFO-ALT). Symbols: (■) oxidized triglyceride monomers, (▲) triglyceride polymers (dimers + oligomers), (○) tocopherol.

proceeded faster in the free oil of DMFO and DMFO-ALT samples exposed to light, while in the dark oxidation was triggered first in the free oil fraction of DMFO but not in DMFO-ALT. It was concluded that the relative oxidation between the free and encapsulated oil fractions may depend on a number of factors (*23*). In spray-dried samples containing a very low amount of free oil, Hardas et al. (*38*, *39*) also found more rapid oxidation of the free oil against the encapsulated oil fraction in microencapsulated milk fat exposed to UV light. Similarly, Baik et al. (*40*) reported that oxidation developed faster in the surface oil in spray-dried microencapsulated fish oil, although the authors did not describe whether protection was taken against daylight exposure.

Unfortunately, it is not possible to clearly establish differences in oxidation in other studies because the separation of the fractions was carried out only in initial samples (*16*, *41*) or because determination of oxidation was exclusively applied to the free oil fraction (*42*) or to the total lipids extracted (*9*, *43*–*46*).

In the present study, simultaneous monitoring of the primary and secondary oxidation products, along with depletion of tocopherol have enabled the detection of different oxidation patterns between the free and encapsulated oil fractions. To gain more insight into such differences, the results were compared with those obtained using model powder systems wherein the oil was just mixed with the DMO components, i.e., lactose and sodium caseinate, and hence forming a free oil phase. In fact, the oil added in mixtures was fully recovered using the method for extraction of the free oil. Results in single mixtures containing SO or FO-ALT are given in **Figure 5**. The oxidation pattern of the mixtures was very similar to that obtained for bulk oils, showing a clear end of the induction period as marked by initiation of polymerization and exhaustion of tocopherols.

In the case of the encapsulated oils, the oxidation proceeded rather unusually, i.e., considerably high polymer values were found in samples containing still high levels of tocopherol remaining. Also, both polymer increase and tocopherol loss showed a "shifting" or "uneven" progress. These results suggest the occurrence of oil globules in a wide range of oxidation status, probably including a part of them at low oxidation stages and

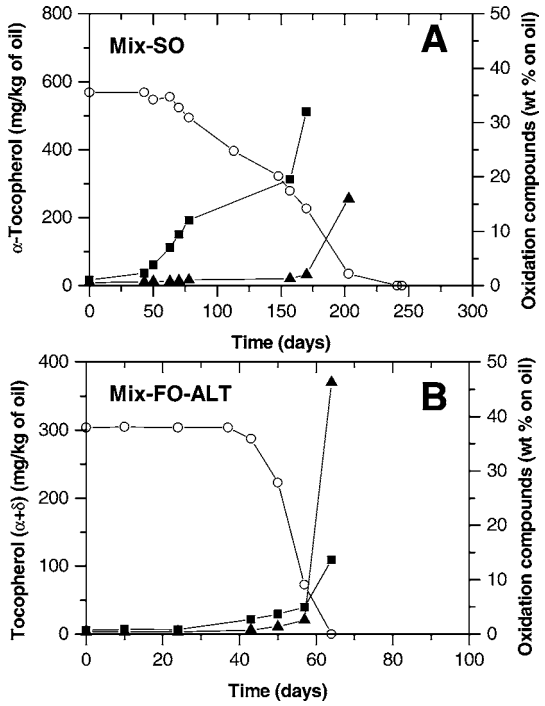

Figure 5. Oxidative development in single mixtures of dried microencapsulated oil components at 30 °C in the dark over 32% RH. (**A**) Mixture containing sunflower oil (Mix-SO). (**B**) Mixture containing fish oil with the added antioxidants ALT (Mix-FO-ALT). Symbols: (■) oxidized triglyceride monomers, (▲) triglyceride polymers (dimers + oligomers), (○) tocopherol.

still protected by the presence of tocopherol and others devoid of antioxidants and well within the advanced oxidation stage. Therefore, analysis of the encapsulated oil fraction provided a profile typical of a heterogeneous mixture of oil droplets showing different oxidation status.

No published information has been found on whether there are different oxidation patterns and/or oxidation rates in the oil droplets immersed in the solid matrix. According to Fritsch, there is no question that, in foods containing a noncontinuous lipid phase, a portion of the lipids will oxidize rapidly and other portions either slowly or not at all (*8*). Differences in oxygen availability due to differences in solid particle size and oil droplet size, the occurrence of pores and air vacuoles in the solid matrix, and the distribution of the oil globules entrapped inside the matrix may be involved (*2*). Thus, oil globules were much more protected by the solid wall of the matrix than others. Compared with systems in continuous phase, the main difference relies on the limitation of oxidation to spread throughout the lipid phase. When oxidation initiates in a single oil globule immersed into the matrix, its propagation to the nearby globules is hampered by the solid barrier of the encapsulating matrix.

This situation is also applicable to other disperse systems where oil droplets may have different susceptibility to oxidation. In a recent study, we also found that some samples of oil-in-water emulsions were markedly oxidized and still contained substantial levels of tocopherol (*24*). Another field of importance for this finding could be oxidation in biological systems. In this regard, it has been found that α-tocopherol and highly oxidized lipids coexist in lipoproteins of advanced human atherosclerotic plaques (*47*), which could also support the notion of oxidation in noncontinuous lipid phase.

The oxidative behavior of the nonencapsulated oil as a continuous lipid phase may be explained in terms of oxygen availability. Thus, no difference in oxygen availability seemed to be present in the oil fraction extractable with petroleum ether, probably due to a high availability.

To summarize, the analytical methods applied in this study enabled the detection of important differences in oxidation between the free and encapsulated oil fractions and for the first time provided evidence of heterogeneous aspects of lipid oxidation in dried microencapsulated oils. The free oil fraction of DMOs showed an oxidative pattern characteristic of oils in continuous phase, such as bulk oil and oil mixed with solid food components, consisting of a clear induction period, in which hydroperoxides (oxidized triglyceride monomers) accumulated, before oxidation accelerated. The end of the induction period was marked by the initiation of polymerization and the total loss of antioxidants. On the contrary, compatibility of antioxidants with high contents of polymerization compounds would indicate the presence of a mixture of oils with heterogeneous oxidation status in the noncontinuous or encapsulated oil phase.

At present, further investigations are being carried out in our laboratory to elucidate the main factors governing oxidation in the two different oil fractions of DMOs under various storage conditions.

ACKNOWLEDGMENT

The authors thank M. Giménez for assistance.

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Received for review September 20, 2005. Revised manuscript received December 28, 2005. Accepted January 4, 2006. This work was supported in part by a research grant from MCYT (AGL 2004-0148) and Junta de Andalucía.

JF052313P