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ORIGINAL PAPER

Antioxidant Activity of Added Phenolic Compounds in Freeze-Dried Microencapsulated Sunflower Oil

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Abstract This study was aimed at evaluating the effectiveness of phenolic antioxidants with a similar structure but having a different polarity in dried microencapsulated sunflower oil. The antioxidants tested were, on one hand, α-tocopherol and its water soluble analogue, Trolox, and on the other, gallic acid and its ester derivatives, propyl gallate and dodecyl gallate. At a moderate temperature (40 °C), the samples were oxidized under accelerated conditions by using Cu(II) as an oxidation catalyst. The progress of oxidation was followed up over time in the free and encapsulated oil fractions. The peroxide value, the total content of polymers and, when appropriate, the content of α-tocopherol were determined. Quantitative analysis of the total fraction of the non-volatile oxidation products and their distribution in oligomers, dimers and monomers was applied to samples to obtain a complete evaluation of oxidation. Finally, as a complementary measure, the antioxidants were also assessed by direct application of the Rancimat test at 100 °C on the dried microencapsulated oil samples. Results showed that the antioxidants of lower polarity in each series, i.e. tocopherol and dodecyl gallate, were to a great extent the most protective antioxidants. The results obtained by the Rancimat test were consistent with those found during oxidation at moderate temperature. Furthermore, the addition of Cu(II) reduced proportionally the oxidative stability index of the dried microencapsulated samples.

Keywords Antioxidant · Microencapsulated oils · Lipid oxidation · Free oil · Encapsulated oil

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Introduction

Microencapsulation of oils is a technological approach addressed at incorporating nutritionally functional lipids such as ω-3 long-chain polyunsaturated fatty acids (PUFAs) as powdered ingredients into foodstuffs [1]. Microcapsules, generally made of proteins and/or carbohydrates, provide the core material with protection against lipid oxidation by limiting the transport of oxygen through the solid matrix. Nevertheless, the addition of effective antioxidants is necessary to prevent the oil from quality deterioration during the processing and storage of microcapsules.

It has been well documented that the effectiveness of antioxidants in multiphase food systems, such as oil-in-water emulsions, is not predictable from their behavior in bulk oils [2]. Polar primary antioxidants, or amphiphilic with high hydrophilic–lipophilic balance, tend to be more effective in bulk oils than lipophilic antioxidants. However, partition of antioxidants into the different phases of oil-in-water emulsions influences their effectiveness significantly. Thus, hydrophilic antioxidants are generally less effective in oil-in-water emulsions than lipophilic antioxidants because of their higher affinity toward the water phase [3–5].

While numerous reports on the activity of antioxidants in oil-in-water emulsions are found in the literature, very few studies have been published on the effect of

antioxidants in dried microencapsulated oils (DMOs). Recently, the effectiveness of antioxidants with the same structure, but different polarity has been studied in microencapsulated linoleic acid [6, 7] and microencapsulated fish oil [8]. Results showed that the lipophilic antioxidants studied were more effective than their hydrophilic homologues. These studies only focused on the effectiveness of antioxidants on the total fat, whereas the effect on the free and encapsulated lipid fractions separately was not investigated. Lipid oxidation was approached by the loss of lipid substrate [6, 7] or by the peroxide value along with anisidine value [8]. Therefore, information on the antioxidant effect on the formation of oxidation products was not given or was very scant.

In general, one of the main drawbacks to evaluate antioxidants under conditions that are as close as possible to those during the commercialization of the stabilized food product is that time-consuming long-term storage studies are required. With regard to DMOs, the Rancimat method has been applied directly to samples as a rapid test to evaluate the effectiveness of antioxidants and results were consistent with those obtained in storage assays [9].

The objective of this study was to evaluate the effectiveness of phenolic antioxidants, structurally similar but different in polarity, in freeze-dried microencapsulated sunflower oil, previously stripped of naturally occurring antioxidants, under moderate temperature (40 °C) and copper (II) catalyzed oxidation conditions. α-Tocopherol and its more polar homologue, Trolox, and gallic acid and its ester derivatives, propyl and dodecyl gallate, were studied. Microcapsules were made of sodium caseinate and lactose. Effectiveness of antioxidants was evaluated in the free and encapsulated oil fractions. In order to describe the behavior of the antioxidants at different stages of the oxidative process, oxidation was followed up until well-advanced oxidation state. Peroxide value, the total content of polymers and, when appropriate, the content of α-tocopherol were determined over time. A novel analytical methodology that allows the quantitative analysis of the total fraction of the non-volatile oxidation products and their distribution in oligomers, dimers and monomers was applied to samples to obtain a complete evaluation of oxidation. Finally, as a complementary measure, the antioxidants were also assessed by the Rancimat test at 100 °C.

Materials and Methods

Materials

Refined sunflower oil was purchased from a local supermarket. α-Tocopherol (purity 97%), *S*-Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (purity 98%), gallic acid, propyl gallate and dodecyl gallate were acquired from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and used as received, whilst sodium caseinate from bovine milk, D-lactose monohydrate and cupric sulphate pentahydrate ($CuSO_4$ $5H_2O$) were obtained from the Sigma Chemical Co. (St. Louis, MO) and also used as received.

Preparation of Oils

Refined sunflower oil was stripped of its natural tocopherols by following the method of Yoshida et al. [10]. The major fatty acid composition of the stripped oil was as follows: 6.4% C16:0, 4.7% C18:0, 21.0% C18:1 and 67.7% C18:2. Except for α-tocopherol, which was dissolved in hexane, the antioxidants were dissolved in methanol and added to the stripped oil. The concentration of α-tocopherol and Trolox in the oil was 2.32 mmol/kg oil (equivalent to 1,000 ppm for α-tocopherol and 613 ppm for Trolox). The concentration of gallic acid, propyl gallate and dodecyl gallate was 0.59 mmol/kg oil (equivalent to 100 ppm for gallic acid, 125 ppm for propyl gallate and 200 ppm for dodecyl gallate). After the addition of the antioxidant solutions, the solvent was evaporated at room temperature with a stream of nitrogen and then the oils were vigorously shaken for 2 min in a vortex mixer at maximum speed.

Preparation of Dried Microencapsulated Oils (DMOs)

DMOs were prepared by freeze-drying oil-in-water emulsions containing sodium caseinate and D-lactose as encapsulating components. The aqueous phase of the emulsions was made by dissolving first sodium caseinate and then D-lactose in deionized water at 55 °C. The solution was cooled at room temperature. Then the oil was added and the emulsions were prepared in a lab mixer at 10,000 rpm for 5 min. The weight composition of the emulsions was 10% oil, 10% sodium caseinate, 10% D-lactose and 70% water. A $CuSO_4$ aqueous solution (1.66 mmol/L) was added just after the emulsion preparation to obtain a final copper concentration of 0.1 mmol/kg emulsion. Then the emulsions were frozen at −30 °C for 24 h and freeze-dried for 48 h in a Heto FD3 freeze-dryer (Allerød, Denmark). The powdered DMO samples were finally obtained by controlled grinding in a coffee mill.

Oxidation Conditions

Independent aliquots (4 g) of each DMO sample were placed into stoppered 250-mL amber glass jars containing a small beaker with a saturated $MgCl_2$ solution (32% RH). The jars were placed in a temperature-controlled chamber at 40 °C in the dark.

Oil Extraction Procedures

Extraction of Total Oil

The procedure was based on the Rose–Gottlieb method [11], which is widely accepted for quantitative determination of fat in milk and milk powders. A quantity of 4 g of DMO was dispersed in 40 mL of water heated at 65 °C. After stirring gently, 8 mL of 25% NH_4OH was added and the solution was heated at 65 °C for 20 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, 20 mL of ethanol, 50 mL of diethyl ether and 50 mL of *n*-hexane; second, 10 mL of ethanol, 50 mL of diethyl ether and 50 mL of hexane; and, third, idem without adding ethanol. In each extraction operation, the solvents were added successively and shaking was applied after each addition. Then the solvent was filtered through a filter paper containing anhydrous Na_2SO_4 and evaporated in a rotary evaporator. The extracted oil was finally dried to constant weight by using a stream of nitrogen.

Extraction of Free Oil

The free oil fraction was extracted according to Sankarikutty et al. [12]. A volume of 200 mL of *n*-hexane was added to 5 g of DMO. Then, stirring was applied for 15 min at room temperature. After filtration through a filter paper, the solvent was evaporated in a rotary evaporator and the extracted oil was dried to constant weight by 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 described above for the extraction of total oil. Microencapsulation efficiency (ME) was calculated as follows:

$$\mathrm{ME}(\%) = \frac{\text{Encapsulated oil}\ (\mathrm{g}/100\,\mathrm{g\,DMO})}{\text{Total oil}\ (\mathrm{g}/100\,\mathrm{g\,DMO})} \times 100.$$

Peroxide Value

The peroxide value was determined by the iodometric assay following the IUPAC standard method [13].

Direct Analysis of Polymer Compounds by HPSEC

A rapid analysis of polymers was carried out following the IUPAC standard method 2.508 [14]. The chromatographic conditions used were those described below for the analysis of the polar fraction.

Quantitative Analysis of Oxidation Compounds

Quantitative analysis of the total fraction of the non-volatile oxidation compounds and their distribution in triacylglycerol oligomers (TGO), triacylglycerol dimers (TGD) and oxidized triacylglycerol monomers (oxTGM) was carried out by isolation of a polar fraction by SPE and subsequent analysis by HPSEC-RID [15].

Separation of Polar Compounds by SPE

A volume of 2 mL of a hexane solution containing 50 mg of extracted oil was separated into two fractions in a Waters Sep-pak Vac silica cartridge (6 mL volume and 1 g of silica) (Waters, Milford, MA, USA). The first fraction, which comprises the non-oxidized triacylglycerols, was eluted with 15 mL of *n*-hexane:diethyl ether (90:10, v/v). The second fraction was eluted with 25 mL of diethyl ether and it comprises the total non-volatile oxidation compounds, lipid hydrolysis products, i.e. diacylglycerols and free fatty acids, and polar unsaponifiable matter. Thus, the lipid oxidation products are separated as compounds with higher polarity than that of the unoxidized triacylglycerols. For quantitative purposes, 1 mg of monostearin, used as internal standard, was added to the second fraction. After removal 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 a silica plate and *n*-hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as elution solvent. The spots on the plate were revealed by exposure to iodine vapour.

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, USA) 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 (film thickness 10 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, TGD and oxTGM, which constitute the non-volatile lipid oxidation compounds, as well as diacylglycerols, monostearin, and a final peak

corresponding to both free fatty acids and the polar unsaponifiable material.

Determination of Tocopherol

When appropriate, the content of α-tocopherol was determined by normal-phase HPLC with fluorescence detection following IUPAC Standard Method 2.411 [13].

Determination of the Oxidative Stability Index (OSI)

OSI was determined in a 679 Rancimat device (Metrohm, Switzerland). Intact samples of DMOs (5.0 g ± 0.1 g), or samples of bulk oils (2.0 g ± 0.1 g), were placed into Rancimat standard tubes and subjected to the normal operation of the test by heating at 100 °C with a flow of air of 20 L/h. Evaluation mode 1 provided by the test as the intersection point of the two extrapolated straight parts of the conductivity curve was taken as the OSI.

Statistical Analysis

All analytical determinations were carried out at least in duplicate and the results represent the means of the replicates. Comparison between means was made by applying Student's t test in Microsoft Excel 2000 (Microsoft Corporation, Redmond, WA, USA). Significance was defined at $p < 0.05$.

Results and Discussion

The DMOs showed similar contents of total oil and of encapsulated oil. The total content of oil ranged from 29.1 to 30.2 wt.% on sample, i.e. close to the theoretical value (33.3 wt.%), and the encapsulated oil, expressed as ME, ranged from 67.5 to 70.1 wt.% on total oil. Repeatability of the oil extraction methods was high and similar to previously reported [16]. Thus, coefficients of variation were lower than 2% for the encapsulated oil fraction and lower than 5% for the free oil fraction.

Results of PV and content of polymers found in the control sample and the DMOs containing Trolox or tocopherol are presented in Fig. 1. The PV data showed definite induction periods (IPs) in the free oil fraction of the Trolox (10 days) and Tocopherol (56 days) samples. The control did not exhibit an IP because the increase of PV accelerated from Day-0. The encapsulated fractions also showed IPs that were practically the same as those found in the free oils in the control and tocopherol samples, and slightly longer in the sample containing Trolox (14 days), which obviously indicates a protection of the encapsulation matrix. The protection of the matrix was also observed in

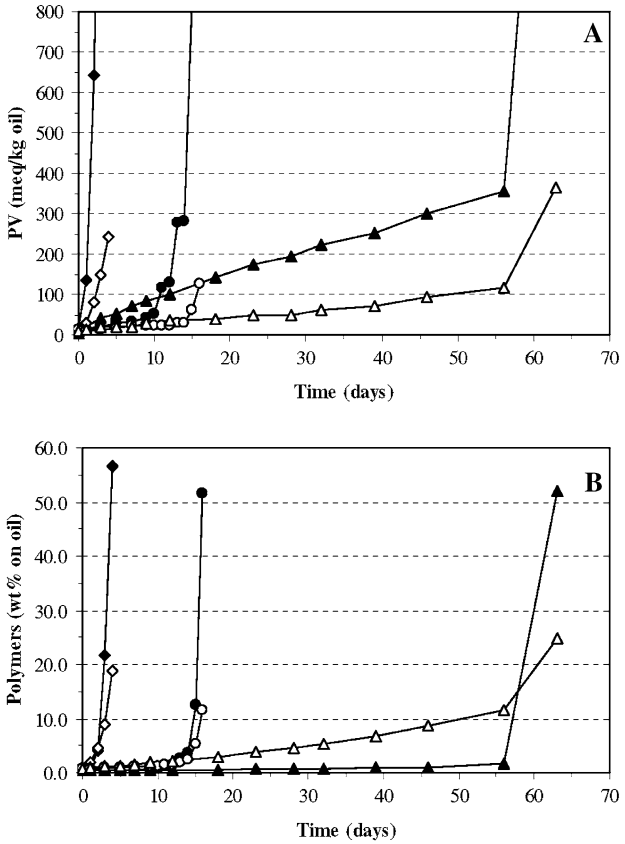

Fig. 1 Peroxide value (**a**) and content of triacylglycerol polymers (dimers + oligomers) (**b**) in the free (*filled symbols*) and encapsulated (*hollow symbols*) oil fractions of the control (*diamonds*), Trolox (*circles*) and tocopherol (*triangles*) DMOs during oxidation

the control and in the sample containing tocopherol, where after the IP, the increase of hydroperoxides was slower in the encapsulated oil as a consequence of a lower availability of oxygen. In addition, the PV increased quite more slowly in the encapsulated oil of the tocopherol sample during the IP and therefore differences in the PV between the two fractions were notable at the end of the IP.

The analysis of polymers showed the same IP for the free and encapsulated oils in the three samples. These were found to be 1, 14 and 56 days, respectively, for the control, Trolox and tocopherol. The content of polymers did not increase significantly in the free oil fractions until a sudden rise in the rate of hydroperoxides took place, i.e. after the IP as detected by the PV. On the other hand, a significant increase of polymers was observed within the IP in the encapsulated fraction of the Trolox sample and, to a greater extent, of the tocopherol sample.

By the joint assessment of both determinations, it was evident that Trolox and tocopherol exerted antioxidant action and that tocopherol was quite more effective than its more polar counterpart. Thus, the protection provided by tocopherol was as much as five times larger than that of

Trolox. These results are in agreement with those found by Hogan et al. [8] in spray-dried microencapsulated fish oil and in turn with the generalized trend of the decreased effectiveness of polar antioxidants in oil-in-water emulsions [3–5].

Figure 2 illustrates the results obtained for gallic acid and its ester derivatives. Only dodecyl gallate, the more lipophilic compound, showed a significant antioxidant effect. The oxidation curves of propyl gallate and the control were quite similar, whilst those of gallic acid seemed to show a pro-oxidant effect. The protected effect of the encapsulation wall was also observed in the DMO containing dodecyl gallate. Both the PV and the content of polymers increased more rapidly in the free oil. Thus, the IP as detected by the PV was 3 days for the free oil and 5 days for the encapsulated fraction. Although the course of polymers showed the same IP (Day-5) for the two oil fractions, a faster increase was observed in the free oil after the IP.

Table 1 lists the results obtained for the total content of the non-volatile oxidation products and their distribution in

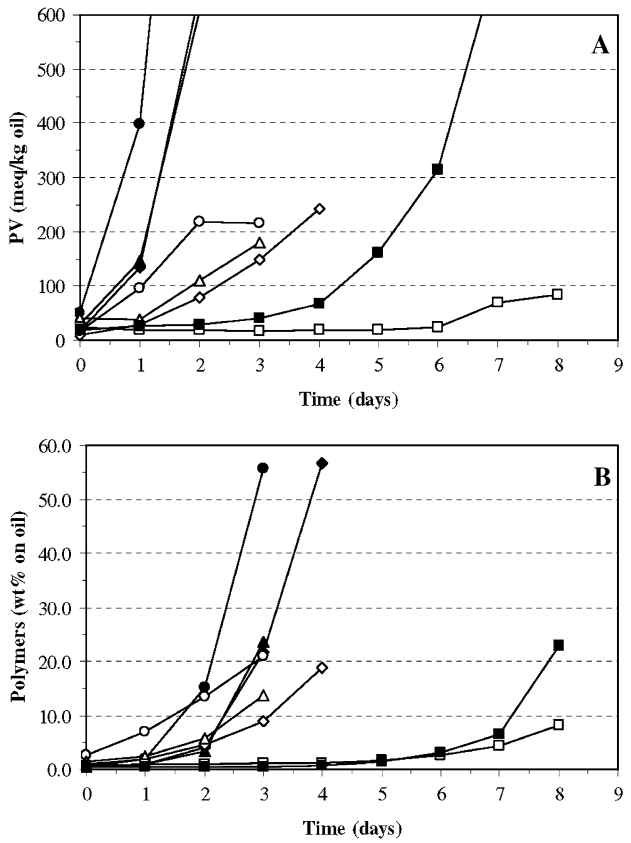

Fig. 2 Peroxide value (**a**) and content of triacylglycerol polymers (dimers + oligomers) (**b**) in the free (*filled symbols*) and encapsulated (*hollow symbols*) oil fractions of the control (*diamonds*), gallic acid (*circles*), propyl gallate (*triangles*) and dodecyl gallate (*squares*) DMOs during oxidation

monomers, dimers and oligomers. Repeatability of the quantitative analysis was similar to that previously reported [16], showing coefficients of variation equal or lower than 8%. Results confirmed the larger antioxidant effectiveness of tocopherol versus Trolox and the antioxidant effect of dodecyl gallate. Gallic acid and propyl gallate showed a pro-oxidant effect. In addition, results also confirmed that oxidation was faster in the free oil.

The pro-oxidant effect found for gallic acid and propyl gallate has also been observed by Schwarz et al. [17] in oil-in-water emulsions. These authors pointed out that the pro-oxidant effect observed may be attributed to the high reactivity of gallic acid and propyl gallate radicals, because of their non-hindered phenol structure, capable of initiating lipid oxidation. More relevant to the present study, the authors also indicated that because of their strong reducing activity, both antioxidants are able to reduce trace of metals to their more active valence state.

Irrespective of the type of antioxidant, the distribution of the oxidation compounds showed that the progress of oxTGM and polymers (TGD plus TGO) was similar to that found in previous investigations [16]. Thus, the free oil exhibited the same oxidative pattern as that found in bulk oils. This consists of a definite IP during which only formation of oxTGM is detected and whose end is marked by a sharp rise in oxTGM and the onset of polymerization.

The encapsulated oil also showed a similar oxidative behavior to that found in previous studies in terms of an early polymerization that occurs when the total content of oxidation products is still relatively low as compared to the free oil. Thus, the relative proportion of polymers (TGD plus TGO) was larger in the encapsulated oil than in the free oil. The early polymerization of the encapsulated oil was attributed to differences in oxidation rate among the population of oil droplets immersed into the solid matrix. While in droplets well protected by the matrix oxidation was still in an early stage, i.e. droplets only containing oxTGM as oxidation products, in others oxidation was in the accelerated phase, i.e. showing significant contents of polymers. Therefore, it was concluded that the encapsulated oil was comprised of droplets with very different oxidation states [16].

In order to examine the influence of the antioxidants on the formation of oxidation products, Fig. 3 shows the progress of oxTGM, TGD and TGO during the IP in the tocopherol sample and the loss of antioxidant. As can be observed, α-tocopherol inhibited completely the polymerization of the free oil fraction (Fig. 3a). Thus, polymers did not significantly increase until tocopherol was completely depleted at the end of the IP (56 days) (Fig. 1b). On the contrary, polymeric compounds, i.e. TGD and TGO, increased gradually from the beginning, that is, in the presence of α-tocopherol, in the encapsulated oil (Fig. 3b).

Table 1 Quantitative analysis of the total fraction of the non-volatile oxidation products and its distribution in triacylglycerol monomers (OxTGM), dimers (TGD) and oligomers (TGO)

Sample	Time (day)	Free oil				Encapsulated oil			
		Total (wt%)	Distribution			Total (wt%)	Distribution		
			TGO[a]	TGD[b]	OxTGM[c]		TGO	TGD	OxTGM
Control	0	2.5	nd[d]	0.7	1.8	2.7	nd	1.1	1.6
	1	6.0	nd	0.9	5.1	3.7	0.3	1.8	1.7
	2	26.8	0.7	3.6	22.6	9.3	1.0	3.6	4.7
Trolox	0	2.0	nd	0.5	1.5	2.3	nd	0.7	1.6
	9	2.3	nd	0.7	1.6	2.4	0.1	1.1	1.2
	12	5.1	0.1	1.0	4.0	3.0	0.2	1.4	1.5
	13	12.5	0.6	2.1	9.9	3.9	0.3	1.6	2.0
	15	41.1	4.1	8.4	28.6	10.0	1.3	3.9	4.8
Tocopherol	0	1.9	nd	0.5	1.4	2.4	nd	0.8	1.5
	3	3.9	nd	0.6	3.3	3.6	nd	1.6	1.9
	5	4.0	nd	0.6	3.4	4.0	0.2	1.5	2.3
	12	6.9	nd	0.7	6.2	5.7	0.4	2.2	3.1
	18	9.3	nd	0.6	8.7	7.5	0.6	3.0	4.0
	32	15.5	nd	0.9	14.6	12.3	1.2	4.9	6.3
	46	18.4	nd	0.9	17.5	17.1	2.0	6.7	8.4
	56	21.8	nd	1.8	20.0	22.7	2.2	9.4	11.1
Gallic acid	0	4.0	nd	0.7	3.3	4.8	0.3	2.4	2.0
	1	17.4	nd	1.9	15.5	14.8	1.3	5.7	7.8
Propyl gallate	0	2.7	nd	0.5	2.1	4.7	nd	1.6	3.2
	1	9.4	nd	1.0	8.4	5.6	0.5	2.2	2.9
Dodecyl gallate	0	2.5	nd	0.7	1.9	2.7	nd	0.8	1.9
	3	3.2	nd	0.7	2.5	3.2	nd	1.3	1.9
	5	8.1	nd	1.5	6.6	3.9	0.3	1.6	2.0
	6	14.9	0.9	2.7	11.3	5.5	0.6	2.2	2.8
	7	28.9	1.7	4.4	22.8	8.8	1.0	3.3	4.5

[a] Triacylglycerol oligomers

[b] Triacylglycerol dimers

[c] Oxidized triacylglycerol monomers

[d] Not detected

As outlined above, these results can be attributed to droplets with very different oxidation states, i.e. droplets in an early stage, with still tocopherol remaining, and droplets in accelerated oxidation phase, without antioxidant and containing polymers [16].

Of interest is that tocopherol was rapidly depleted in the encapsulated oil (Fig. 3b) as compared to the free oil (Fig. 3a). Thus, tocopherol exhausted at Day-12 in the encapsulated oil fraction, when the total content of oxidation products was 5.7%, whereas in the free oil the total content of the oxidation compounds was as high as 21.8% when tocopherol was completely depleted at Day-56. On the other hand, this rapid loss of tocopherol in the encapsulated oil differs from results found in numerous experiments carried out in our laboratory under similar oxidation conditions but in the absence of Cu(II). In fact, in a recent report, large contents of polymers were compatible with considerably high levels of tocopherol remaining in the encapsulated oil [16]. Therefore, the early loss of tocopherol may be related to its direct interaction with Cu(II) to give the tocopheroxyl radical and Cu(I) [18], being for some reason more favored in the encapsulated fraction.

After the complete depletion of tocopherol in the encapsulated oil, a change in the rate of formation of the oxidation compounds was surprisingly not observed (Fig. 3b). This fact seems to indicate that the transport of oxygen through the encapsulation matrix was the rate determining step.

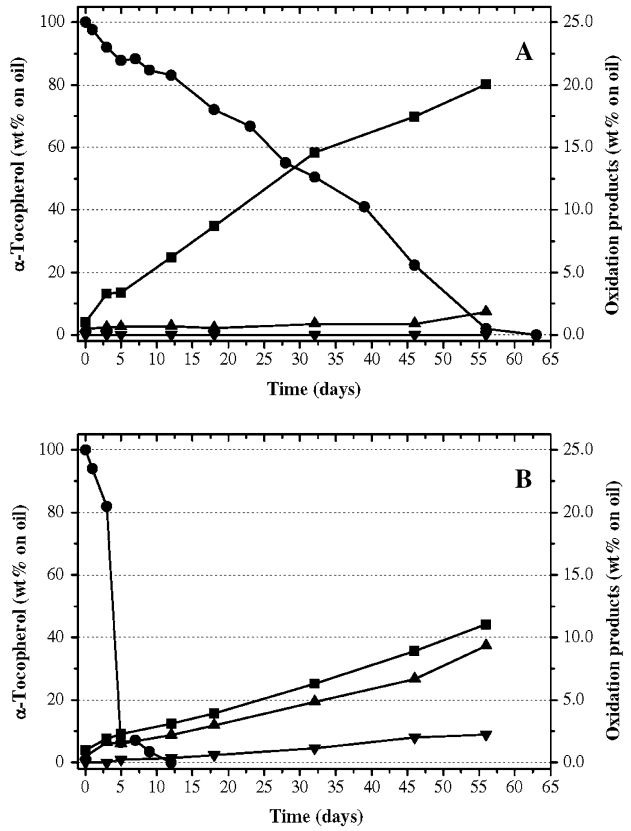

Fig. 3 Content of triacylglycerol monomers (*filled square*), triacylglycerol dimers (*filled triangle*) and oligomers (*inverted filled triangle*), and relative content of α-tocopherol (*filled circle*) in the free (**a**) and encapsulated (**b**) oil fractions of the tocopherol DMO during oxidation

Table 2 lists results of the oxidative stability index (OSI) obtained by application of the Rancimat test to the bulk oils that were used to prepare the DMOs, the DMO samples and the free oil fractions extracted thereof. For comparative purposes, results obtained in DMOs prepared without addition of the Cu(II) catalyst have also been included. As can be observed, the stability provided by Trolox to the bulk oil was as much as three times larger than that by tocopherol. Gallic acid and its ester derivatives showed similar OSI values and slightly higher than that found in the bulk oil containing tocopherol. The OSI values obtained in the DMO samples were consistent with those obtained in the oxidation assay at 40 °C. Thus, tocopherol was a lot more effective than was Trolox; gallic acid and propyl gallate did not show an antioxidant effect; and dodecyl gallate enhanced slightly the stability of the control. In addition, the OSI results obtained in the DMO samples were quite coherent with those found in the DMOs prepared without addition of copper. In fact, the Cu(II) catalyst led to a proportional decrease of the OSI that practically resulted in unaffected effectiveness of each antioxidant with respect to the control. As compared to the bulk oils, it was evident that the antioxidants assayed lost effectiveness in the DMOs and to a greater extent with their hydrophilic nature. Only tocopherol seemed to be as effective as it was in the bulk oil. Results in the free oil fraction seemed to indicate that only tocopherol, the more lipophilic antioxidant, was extracted along with the oil. Surprisingly, the OSI value of the extracted oil was not statistically different from that of the starting oil, suggesting the absence of copper in the extract, probably due to a strong interaction between the metal and the microencapsulation matrix.

Table 2 Oxidative stability index (h) by the Rancimat test at 100 °C

Samples	Bulk oil	DMO[a]	DMO-Cu(II)[b]	Free oil[c]
Control	1.4	1.6	1.4	1.4
Trolox	30.8	5.4	4.4	1.5
Tocopherol	10.7	15.2	10.6	11.2
Gallic acid	13.7	1.6	1.3	1.5
Propyl gallate	15.4	2.0	1.5	1.4
Dodecyl gallate	13.6	3.7	2.2	1.5

[a] Dried microencapsulated oils prepared without addition of Cu(II)

[b] Dried microencapsulated oils prepared with addition of Cu(II)

[c] Free oil extracted from DMO-Cu(II)

The results in this study are consistent with previous results obtained for oil-in-water emulsions, i.e. in the liquid system, containing the same components, sodium caseinate and lactose [19], and with the generalized trend of a decreasing effectiveness of polar antioxidants in oil-in-water emulsions because of partitioning between the phases [3–5]. The use of Cu(II) as an oxidation catalyst has allowed us to study the effectiveness of the antioxidants under accelerated oxidation conditions at a moderate temperature (40 °C). Finally, although the direct interaction of the antioxidants with the oxidation catalyst cannot be ruled out, the conditions applied were appropriate to evaluate and to rank the effectiveness of the antioxidants.

Concluding Remarks

Application of a novel analytical methodology was very useful to evaluate the effect of antioxidants in microencapsulated oils, and to determine the differences in oxidation between the free and encapsulated oil fractions. Irrespective of the type of antioxidant, the oxidative pattern of the former was similar to that found in bulk oils, i.e. only oxidized triacylglycerols (essentially hydroperoxides) increased during the induction period, while polymeric compounds were not formed in significant amounts until the end of the induction period. The pattern observed in the encapsulated oil consisted of a gradual increase in both

hydroperoxides and polymeric compounds from the onset of oxidation, suggesting that this fraction corresponded to a mixture of oil droplets with different oxidation states.

The efficacy of the antioxidants tested in dried microencapsulated oils was similar to that reported in oil-in-water emulsions, thus indicating that polar antioxidants were mostly located in the aqueous phase during the preparation of the emulsion and did not protect the oil after the drying process.

The measures of oxidation applied showed similar induction periods for the free and encapsulated oil fractions in each system studied. However, the encapsulation matrix presented a definite protective effect in terms of the overall rate of oxidation within the induction period as determined by the total content of oxidation products. Furthermore, advanced oxidation products, i.e. polymers, formed since the onset of oxidation in the encapsulated oil and therefore none of the antioxidants assayed was effective in suppressing this formation.

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