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Oxidation of free and encapsulated oil fractions in dried microencapsulated fish oils

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RESUMEN

Oxidación de fracciones de aceite libre y encapsulado en aceites de pescado microencapsulados.

El objetivo de este trabajo es la evaluación de la oxidación de aceites de pescado microencapsulados en matriz seca (DMFO) durante su almacenamiento a temperatura ambiente, y examinar la influencia de la distribución del aceite (aceite libre frente a aceite encapsulado) en estos sistemas lipídicos complejos. Las muestras se prepararon mediante liofilización de emulsiones constituidas por caseinato sódico, lactosa y aceite de pescado, con o sin la mezcla antioxidante ALT (ácido ascórbico, lecitina y tocoferol); y se almacenaron a 25 o 30°C a la luz o a la oscuridad, con aire limitado, accesible o al vacío. Para el seguimiento del desarrollo oxidativo se aplicó la cuantificación de los polímeros de triglicéridos, y se diferenciaron los niveles de oxidación de las fracciones de aceite libre y encapsulada. Los resultados mostraron que la oxidación se desarrolló rápidamente en ambas fracciones en las muestras expuestas a la luz. A la oscuridad, la oxidación se disparó primero en la fracción de aceite libre de las muestras no protegidas con ALT pero, en contraste, las muestras con ALT mostraron niveles de oxidación significativamente más altos en la fracción encapsulada que en la libre, independientemente de la limitación o libre acceso de aire. Estos resultados indican que la adición del sistema antioxidante ALT fue más efectiva en la fracción de aceite libre, y por tanto reflejan la gran influencia que puede ejercer la partición y/u orientación de los antioxidantes en su eficacia en sistemas lipídicos complejos.

PALABRAS-CLAVE: Aceite de pescado - Almacenamiento - Antioxidantes - Distribución del aceite - Microencapsulación - Oxidación - Polímeros.

SUMMARY

Oxidation of free and encapsulated oil fractions in dried microencapsulated fish oils.

The objective of this work was to evaluate oxidation of dried microencapsulated fish oils (DMFO) during storage at ambient temperature, and to examine the influence of oils distribution (free *vs.* encapsulated oil) in these complex lipidic systems. DMFO were prepared by freeze-drying emulsions containing sodium caseinate, lactose and fish oil, with and without adding the antioxidant mixture ALT (ascorbic acid, lecithin and tocopherol). Samples were stored at 25 or 30°C either in the dark or light with limited, accesible air or under vacuum. The progress of oxidation was followed through quantitation of triglyceride polymers, and oxidation levels of free and encapsulated oil fractions were differentiated. Results showed that oxidation was very rapid both in free and encapsulated oil fractions in all DMFOs exposed to light. In the dark, oxidation was triggered first in the free oil fraction of samples not protected with ALT but, in contrast, samples with ALT showed significantly higher oxidation levels in encapsulated than in free oil fractions, regardless of the limited or free availability of air. These results indicated that addition of the antioxidant system ALT was more effective in the free oil fraction, thus reflecting the great influence of partitioning and/or orientation of antioxidants on their efficacy in complex lipid systems.

KEY-WORDS: Antioxidants - Fish oil - Microencapsulation - Oil distribution – Oxidation - Poymers - Storage.

1. INTRODUCTION

The oxidative deterioration of unsaturated fatty acids is of paramount importance in the food industry because it results in loss of nutritional value and development of flavors that are unacceptable to consumers. The usual approach to minimizing oxidation is by addition of antioxidants and moreover, a technical procedure recently introduced to protect sensitive oils is microencapsulation. In this context, fish oils are probably the substrates more difficult to handle given their high susceptibility to oxidation. Still, dietary supplementation with fish oils is in growing demand because they posses high content of long chain n-3 polyunsaturated fatty acids (PUFAs) with important physiological functions and health benefits (Newton, 1996; Haumann, 1997). Through microencapsulation techniques, powdery ingredients can be obtained consisting on fish oil droplets coated by a matrix of saccharides and/or proteins in order to gain a satisfactory shelf-life and to be suitable for a range of foods, including milk and bakery products, salad dressings and juice drinks (Dziezak, 1988; Matsuno and Adachi, 1993).

The effect of unsaturation, surface area, prooxidants, antioxidants, oxygen, light and temperature on lipid oxidation have been extensively studied and apply equally to lipids alone as well as to lipids in foods, where they are mixed with water, carbohydrates, proteins and minerals. In foods, however, some additional factors are of great influence, and one of the most relevant ones is the distribution of lipids in the food (Fritsch, 1994). On the basis that the fraction of oil which remains free or nonencapsulated after preparation of dried microencapsulates is highly susceptible to oxidation while the fraction embedded in the matrix is resistant to oxidation, the main point of interest regarding

sensitive microencapsulated lipids has been the effectiveness of microencapsulating agents, such as proteins, disaccharides or gums, on oxidative stability (Sims *et al.,* 1979; Anandaraman and Reineccius, 1986; Imagi *et al.,* 1992; Lin *et al.* 1995; Kim and Morr, 1996; Moreau and Rosenberg, 1996; Reichenbach and Min, 1997; Yoshii *et al.,* 1997), although most experimental designs did not include quantitative data on retention capability. This parameter, known as microencapsulation efficiency, can be evaluated indirectly by measuring the oil fraction accesible to extraction simply by washing with an organic solvent, usually hexane, under well-established conditions (Buma 1971). This part of the oil, extracted without disruption of the matrix structure, is known as free, surface or nonencapsulated oil. Using this approach, some researchers have observed that an increase in nonencapsulated lipids due to high relative humidity conditions (Minemoto *et al.,* 1997), poorer encapsulation (Lin *et al.,* 1995) or temperatures over the glass transition temperature (Labrousse *et al.,* 1992) was concurrent with higher oxidation. However, only three papers have been found which provide quantitative determinations of lipid distribution using differential extraction procedures, both showing, as expected, that free oil underwent more rapid oxidation than that of the encapsulated oil (Gejl-Hansen and Flink, 1977; Shimada *et al.,* 1991, Ponginebbi *et al.,* in press).

Specifically focused on oxidation of long-chain PUFA or fish oil-based microencapsulates at ambient temperatures, scarce papers have been published (Thompkinson and Mathur, 1989, 1990; Imagi *et al.,* 1992; Taguchi *et al.,* 1992), in spite of the growing utilization of such ingredients to fortify foods and thereby great interest to assure an acceptable shelf-life. Recently, we have dedicated our efforts to improve evaluation of oxidation in dried microencapsulated fish oils subjected to ambient storage conditions (Márquez-Ruiz *et al.,* 2000, Heinzelman *et al.,* (2000) and on the application of rapid, accelerated oxidative tests which enable to predict shelf-life and efficiency of antioxidants (Velasco *et al.,* 2000).

In this paper, oxidation of dried microencapsulated fish oils (DMFO), with and without antioxidants added, has been evaluated during storage at ambient temperature in the dark or in light and under different conditions of air availability. Further, differentiation of oxidation levels in free and encapsulated oil fractions was approached to study the influence of oil distribution on shelf-life.

2. EXPERIMENTAL

2.1. Samples

Fish oil (refined sandeel oil) without and with the mixture of antioxidants ALT added, i.e., 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). Tocopherols were quantitated by normal-phase HPLC with fluorescence detection (IUPAC, 1992a) and initial contents of α-*tocopherol* were 81 mg/kg for fish oil. Only trace amounts were found for the other tocopherol isomers.

Microencapsulated oils were prepared starting from a mixture of fish or sunflower oil, D-lactose monohydrate (Sigma, St. Louis, MO, USA) and sodium caseinate from bovine milk (Sigma, St. Louis, MO, USA), each at 10% w/w in deionized water. Emulsions were obtained using a Omnimixer (Sorvall, Newton, PA) at 10,000 rpm for 5 min. Following freezing at -50°C for 24 h, samples were freeze-dried for 48 h and after milling, samples of powdered DMFO were obtained.

2.2. Determination of oil globule size

Oil globule size distribution was measured in emulsions using a laser diffraction spectrometer (Malvern Mastersizer, Malvern Co., UK). Samples were tested right after preparation of emulsions. The mean oil globule size ($d_{V,0.5}$) expressed the diameter where 50% of the total droplet volume was created by smaller droplets.

2.3. Extraction of oil from DMFO

a) Extraction of free oil

The free oil fraction, also known as accessible, surface or nonencapsulated oil, was determined according to Sankarikutty *et al.* (1988). Thus, 8 g of powder were added 200 mL light petroleum (60-80°C) and stirred for 15 min at 25°C in a magnetic stirrer. After filtration through anhydrous Na_2SO_4, solvent was evaporated under reduced pressure and sample dried to constant weight using a stream of nitrogen. Determination of free oil gave relative standard deviations lower than 5% for duplicate analyses.

b) Extraction of encapsulated oil

The procedure was based on the Rose-Gottlieb method (Richardson, 1985), widely accepted for quantitative determination of fat in milk and milk powders. One gram of DMFO, devoid of free oil and dried under nitrogen, was weighed and dispersed in 10 mL of water at 65°C. After shaking, 2 mL NH_4OH 25% were added and the solution was heated at 65°C for 15 min in a stirring water bath. Then, the solution was cooled, transferred to a separatory funnel and the flask rinsed with 10 mL ethanol. Oil was extracted three times with, first, 25 mL diethyl

ether and 25 mL light petroleum, second, 5 mL ethanol, 15 mL diethyl ether and 15 mL light petroleum and third, *idem* without adding ethanol. After filtration through anhydrous Na_2SO_4, solvents were evaporated under reduced pressure and sample dried to constant weight using a stream of nitrogen. Determination of encapsulated oil gave relative standard deviations lower than 2% for duplicate analyses.

c) Extraction of total oil

Starting from intact DMFO samples, total oil was extracted using the same method as for extraction of encapsulated oil.

d) Calculation of microencapsulation efficiency

From the quantitative determinations above detailed, microencapsulation efficiency was calculated as follows:

$$\frac{\text{Encapsulated oil (g/100 g DMO)}}{\text{Total oil (g/ 100 g DMO)}} \times 100$$

where DMO is dried microencapsulated oils.

2.4. Quantitation of total content and distribution of polar compounds

Quality evaluation of initial DMFOs was approached by applying a methodology recently developed for quantitative analysis of different groups of oxidation compounds in small samples (Márquez-Ruiz *et al.*, 1996). Briefly, 2 mL of a sample solution containing 50 mg of extracted oil and 1 mg monostearin, used as internal standard, was separated by solid-phase extraction (silica cartridge) into a first fraction (essentially unoxidized triglycerides) eluted with 15 mL hexane:diethyl ether 90:10 and a second fraction, containing polar compounds and the internal standard, eluted with 15 mL of diethyl ether. Subsequently, fractions of polar compounds were analyzed by high-performance size-exclusion chromatography (HPSEC) (IUPAC, 1992b) using a Rheodyne 7725y injector with a 10 µm sample loop, a Waters 510 HPLC pump (Waters Associates, Milford, MA, USA), two 100 and 500 Å Ultrastyragel columns 25 cm x 0.77 cm inner diameter, packed with a porous, highly cross-linked styrenedivinylbenzene copolymer (<10 pm) connected in series, and a refractive index detector (Hewlett Packard, CA, USA). HPLC-grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min. The peaks resolved corresponded to triglyceride oligomers, triglyceride dimers, oxidized triglyceride monomers, diglycerides, monostearin, and a final peak constituted by free fatty acids and polar unsaponifiables.

2.5. Storage conditions

a) Limited air

Samples of DMFO (2 g), with and without added ALT, were placed in 10-mL glass vials, screw-capped and stored at 30°C in the dark or in light (1500 lux). Since air contains about 21% oxygen, each vial contained approximately 1.7 mL oxygen considering that density of the mixture of components of DMFO is about 1 g/mL. At room temperature (29°C) and one atmosphere pressure, 1.7 mL oxygen contains 6.9 x 10^{-5} moles oxygen according to the ideal gas law, PV = nRT (R = 0.080205 L atm/mol K). Taking 900 as average molecular weight of *triglycerides*, 0.66 g of oil (included in 2 g DMFO) would correspond to 7.3 x 10^{-4} moles expressed as triglyceride. That means that the molar ratio for oxygen to triglyceride is only about 1:10, which can be considered oxygen-limiting conditions if we assume that there are two double bonds per fatty acid, on average, in these highly unsaturated oils.

b) Accesible air

Samples of DMFO (20 g), with and without added ALT, were placed in open plastic Petri dishes (10 cm diameter) and stored at 25°C in the dark.

c) Vacuum

Samples of DMFO (10 g) with added ALT, were *vacuum* packed in 20x15 cm *aluminium* foil bags, then inmediately heat-sealed, and stored at 25°C in the dark.

2.6. Evaluation of oxidation during storage

Total oils and separate free and encapsulated oil fractions extracted from DMFO were analyzed for polymerization compounds by HPSEC (IUPAC, 1992b) using the same chromatographic conditions described above in detail for HPSEC analysis of polar compounds. Sample solutions of 50 mg/mL tetrahydrofuran were used. This analysis enabled rapid quantitation of triglyceride dimers and higher oligomers, here quantitated globally and referred to as polymers.

3. RESULTS AND DISCUSSION

Table I shows general characteristics of initial DMFO samples, including the results obtained for total polar compounds and distribution in different

Table I
Characterization of initial dried microencapsulated fish oils (DMFO), without (control) and with added ascorbic acid, lecithin and δ-tocopherol (ALT)

Sample		Extracted oil						Mean oil globule (μm)	Microencapsulation efficiency (%)
		Amount (g/100 g of DMFO)	Total polar compounds (% of oil)	Distribution of polar compounds (mg/g of oil)					
				TGD	oxTGM	DG	FFA+PU		
CONTROL	Total oil	31.0 ' 0.17	4.0 ' 0.24	4.7	9.7	15.5	9.7	1.45	71.9
	Free oil	8.7 ' 0.19	3.8 ' 0.20	4.5	8.5	16.4	9.0		
ALT	Total oil	32.1 ' 0.29	4.5 ' 0.25	5.4	9.8	16.4	13.2	1.53	71.0
	Free oil	9.3 ' 0.23	4.1 ' 0.28	4.7	8.3	15.6	12.1		

Abbreviations: TGD, triglyceride dimers; oxTGM, oxidized triglyceride monomers; DG, diglycerides; FFA; free fatty acids; PU, polar unsaponifiable.

groups, in oils extracted from DMFOs. Mean oil globule size and microencapsulation efficiency were very similar for DMFO control and DMFO ALT. Regarding initial quality of DMFO, no significant changes in polar compounds were found with respect to the starting oil, thus showing that procedures for preparation and extraction of DMFO did not lead to hydrolytic or oxidative degradation. OxTGM, i. e., triglyceride monomers with at least one oxidized fatty acid group, including hydroperoxides and non-volatile secondary oxidation compounds such as hydroxides, ketones and epoxides, showed values always below 10 mg/g, even lower than those normally found in refined vegetable oils (Pérez-Camino *et al.,* 1990). TGD levels were attributable to thermal dimeric triglycerides originated during the deodorization step of the refining process (Ruiz-Méndez *et al.,* 1997). As to hydrolytic products, the level of DG is indicative of the initial hydrolytic alteration of the crude fish oil, since free fatty acids are normally eliminated during refining.

Initial analyses of DMFO consisted of the extraction of total oil and determination of polymers by HPSEC. Figure 1 shows the evolution of oxidation for DMFO samples with and without the antioxidant mixture ALT, stored in vials at 30°C in light. Quantitation of polymers was selected to follow oxidation because it has been recently shown that such compounds are useful markers of the end of the induction period in these highly polyunsaturated oils due to the rapid polymerization occurring at ambient temperature (Márquez-Ruiz *et al.,* 2000). In this former study, a detailed evaluation of the groups of oxidation compounds showed that oxTGM increased during the early phase of oxidation up to a certain point wherein a sharp rise was observed. That point was close to the appearance of oxidized flavor and concurrent with a significant increase of polymerization compounds. In the present study, initiation of polymerization occurred at around 6 and 12 days, and oxidized flavor was clearly detected at 6 and 14 days, for DMFO control and ALT, respectively. Therefore, ALT prolonged shelf-life of DMFO about two fold.

Figure 2 presents the evolution of polymers in DMFOs stored in vials at 30°C in the dark. Polymers showed a sharp increase at around 18-20 days and 35-40 days, for DMFO control and DMFO ALT, respectively. These results indicate that the protection factor of ALT in the dark was also about 2 and that, moreover, exclusion of light was more effective than addition of ALT. Surprisingly though was that oxidized flavor was not detected in DMFO ALT until as late as 70 days, in samples which had already reached a high level of total polymerization compounds (12.6%). In fact, these samples only

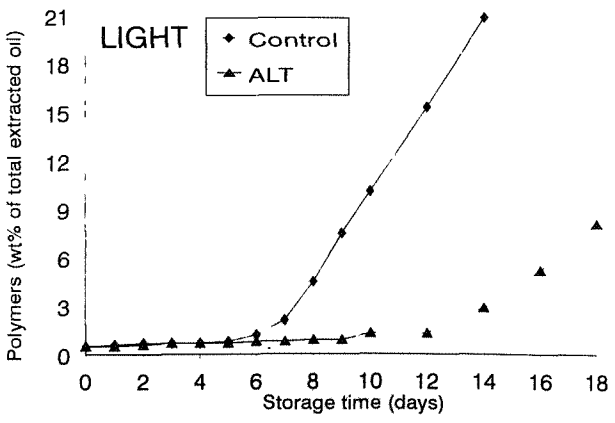

Figure 1
Evolution of polymers in total oils extracted from dried microencapsulated fish oils without (Control) and with added ascorbic acid, lecithin and δ-tocopherol (ALT), stored in vials at 30°C in light.

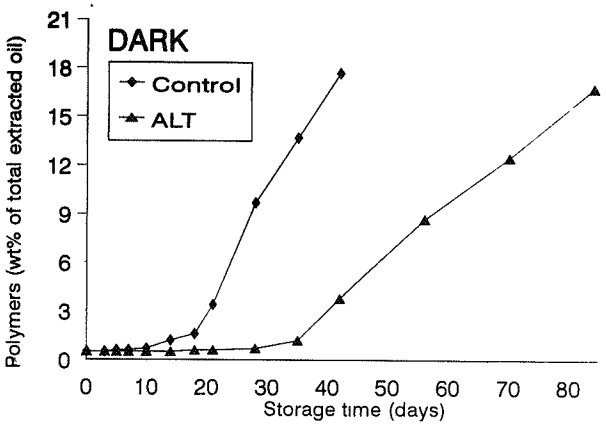

Figure 2
Evolution of polymers in total oils extracted from dried microencapsulated fish oils without (Control) and with added ascorbic acid, lecithin and δ-tocopherol (ALT), stored in vials at 30°C in the dark.

smelled rancid when the powder was rubbed in a mortar and the encapsulated oil was thus released. Therefore, the free oil fraction seemed to be much less oxidized than the encapsulated oil fraction and hence that oxidized smell could not be detected in the intact sample. Our previous results using Rancimat as accelerated oxidative method showed that the protection factor of ALT (induction period of samples with ALT/induction period of samples without ALT) tended to be lower in dried microencapsulated oils devoid of free oil, thus only containing encapsulated oil, as compared to intact dried microencapsulated oils (Velasco *et al.*, 2000). In view of all these observations, it is clear that separate evaluation of the free and encapsulated oil fractions is required to examine the effect of ALT.

Table II lists polymer values for free and encapsulated oil fractions in all DMFO samples stored in vials. For samples under the most unfavorable conditions, i. e., light exposure and absence of ALT, oxidation of free oil was always higher than that of encapsulated oil throughout storage. When ALT was present, however, polymerization was slightly superior in the encapsulated fraction during the early stage of oxidation but, around the end of the induction period, between 14 and 16 days, oxidation was much more accelerated in the free oil fraction. This pattern, although delayed, was similarly observed in samples control in the dark, where oxidation was more rapid in the free oil fraction beyond 18 days. These results indicate that, for all these samples, the end of the

Table II

Evolution of polymers (% of oil) in free and encapsulated oil fractions of dried microencapsulated fish oils, without (control) and with added ascorbic acid, lecithin and δ-tocopherol (ALT), stored in vials, in light or in the dark, at 30°C

Storage time (days)	Light				Dark			
	CONTROL		ALT		CONTROL		ALT	
	Free	Encapsulated	Free	Encapsulated	Free	Encapsulated	Free	Encapsulated
0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
1	0.6	0.6	0.5	0.5				
2	0.9	0.6	0.5	0.6				
3	0.9	0.6	0.5	0.6	0.5	0.5	0.5	0.5
4	1.0	0.6	0.6	0.6				
5	1.1	0.7	0.6	0.6	0.6	0.6	0.5	0.5
6	1.9	0.9	0.6	1.0				
7	4.6	2.3	0.6	0.9	0.6	0.6	0.5	0.5
8	7.8	3.0	0.6	1.0				
9	15.4	4.1	0.6	1.0				
10	19.3	7.4	1.0	1.4	0.6	0.8	0.5	0.5
12	31.5	8.4	0.9	1.5				
14	41.8	12.2	1.9	3.2	0.9	1.3	0.5	0.5
16			8.8	3.6				
18			13.4	5.8	2.2	1.4	0.5	0.6
21					5.9	2.3	0.5	0.6
28					12.6	8.4	0.5	0.8
35					17.1	12.3	0.7	1.4
42					24.1	14.9	0.8	5.1
56					39.7	18.7	0.8	12.1
70							1.4	17.2
84							2.9	22.8

induction period was reflected in both appearance of oxidized flavor, consequence of the free oil oxidation, and the increase of polymers in both fractions. The level of polymers present in the free oil fraction when oxidized flavor appeared was similar (about 2%) for control-light, ALT-light and control-dark, at 6, 14 and 18 days, respectively.

In contrast, DMFO ALT samples in the dark showed an unexpected progress of oxidation up to well within advanced oxidation stages. Up to 28 days, samples did not practically change but beyond that storage point, all samples had markedly higher polymers in the encapsulated *vs.* the free oil fraction. Under these conditions, it is now evident that detection of oxidized flavor in the whole sample could not reflect the end of the induction period. When oxidized flavor was detected (10 weeks), the increase of oxidation in the free oil fraction (1.4% polymers) was responsible for appearance of unacceptable flavor but by then an amount of polymers as high as 17.2% had accumulated in the encapsulated oil fraction. The shift of polymerization rate in the total oil (Figure 2) was, however, useful for determining the end of the induction time, between 35-40 days. This seems to indicate that under the most protective conditions tested, ALT was more effective in the free oil fraction. In this case, these results are of enormous importance since they could be misinterpreted as the «paradox» that encapsulation is not useful.

Table III shows results of polymer evolution in additional storage experiments designed to further investigate the differentitation of oxidation in free and encapsulated oil in DMFO at 25°C in the dark under conditions differing in air availability, since samples were either exposed to ambient air in open Petri dishes or subjected to total *vacuum.* Sampling was done every 2 or 4 weeks, respectively. As expected, oxidation was more accelerated in plates as compared to vials although differences were not as marked as expected, probably because vials were not sealed and hence entrance of air to some extent could not be disregarded. Consistent results were obtained in that free oil oxidation was retarded as compared to that of encapsulated oil in DMFO ALT. As to *vacuum* conditions, the ALT samples tested did not change substantially after more than a year, indicating an acceptable shelf-life for these products under the conditions widely used for powdery food products, such as milk and egg powders.

The results here obtained for DMFO without antioxidants agree with those reporting more rapid oxidation in the free oil than that in the encapsulated oil fraction. Thus, Geijl-Hansen and Flink (1977) tested triolein and oleic acid in maltodextrin matrix and showed that removal of surface oil with hexane gave a powder which did not consume oxygen, while addition of water released encapsulated oil which consumed it. Shimada *et al.* (1991) used methyl linoleate encapsulated in an amorphous lactose-gelatine matrix and found that the oil released as a consequence of lactose crystallization underwent more rapid oxidation than that of the encapsulated oil. Recently, Ponginebbi *et al.* (2000) reported that sucrose crystallization at high moisture reduced surface oil and may be responsible for decreased

Table III

Evolution of polymers (% of oil) in free and encapsulated oil fractions of dried microencapsulated fish oils without (control) and with added ascorbic acid, lecithin and δ-tocopherol (ALT), stored in open Petri dishes or bag in *vacuum,* in the dark, at 25°C.

Storage time (days)	Open Petri dishes				*Vacuum* bags	
	CONTROL		ALT		ALT	
	Free	Encapsulated	Free	Encapsulated	Free	Encapsulated
0	0.5	0.5	0.5	0.5	0.5	0.5
2	24.8	3.1	0.5	0.6		
4	44.2	23.8	0.5	1.7	0.5	0.5
6			0.7	12.0		
8			1.0	26.4	0.5	0.5
10					0.5	0.5
12					0.5	0.5
16					0.5	0.5
20					0.5	0.5
24					0.5	0.5
28					0.5	0.5
32					0.5	0.5
36					0.5	0.5
40					0.5	0.5
44					0.5	0.6
48					0.5	0.6
52					0.5	0.6
56					0.6	0.7

oxidation of linoleic acid in sucrose-maltodextrin matrix. However, no information has been found relating oxidative stability to lipid distribution in dried microencapsulated oils added antioxidants.

Tocopherol and ascorbic acid constitute an efficient antioxidant pair, the former as the most important radical-scavenging natural antioxidant and the latter as synergistic, through regeneration of tocopherol molecules (Coupland and McClements, 1996). Combination with lecithin was introduced to help solubilization in bulk oils (Han *et al,* 1990). The resulting antioxidant system ALT has proved to be notably efficient for highly unsaturated oils, extending the induction period of fish oils at 30°C (Han *et al,* 1991) as well as in Rancimat at 80°C (Yi et al, 1991). However, the effect of ALT or tocopherol alone in homogeneous (bulk oils) *vs.* heterogeneous (such as emulsions or dried foods) systems, is far less known. In our preliminary study (Velasco *et al.,* 2000), oxidative stability in Rancimat at 100°C showed that ALT or tocopherol alone was much more protective in bulk oils than in dried microencapsulated oils and even lower antioxidative effect was found for samples devoid of free oil, indicative of the encapsulated oil. An interesting study (Katusin-Razem and Razem, 1994) showed that efficiency of some common antioxidants (α-tocopherol, butylated hydroxyanisole and butylated hydroxytoluene) was reversed and the range of reactions narrowed, on transition from a homogeneous solution to a model system consisting on oleic acid coated on a solid egg white support.

As to heterogeneous systems other than dried foods, and in support of the Porter theory (Porter, 1993; Porter *et al.,* 1989), Frankel and coworkers found that predominantly non-polar antioxidants (α-tocopherol and ascorbyl palmitate) were more effective in oil-in-water emulsions than in bulk oils because they would be expected to accumulate in the oil-water interface and form a protective membrane around the droplets. In contrast, predominantly polar antioxidants («trolox» and ascorbic acid) were more effective in bulk oils, presumably reducing the accesibility of the lipid substrate to oxygen at the air-oil interface. (Frankel *et al.,* 1994). Related to these are the previous findings that the antioxidant activity of α-tocopherol was markedly reduced in micelle systems (Castle and Perkins, 1986, Pryor *et al.,* 1988,) and liposomal membranes (Barclay *et al.,* 1990) compared with homogeneous solutions. Therefore, it seems clear that the effectiveness of antioxidants depends on their location and orientation in a structured system.

From the present study, results showed that the mixture ALT can be more effective in the free oil than in the encapsulated oil fraction. The differences found in oxidation profiles of free *vs.* encapsulated oil fractions showed the complexity of oxidation in heterogeneous systems. The free oil fraction followed a pattern quite similar to that normally found in bulk (continuous lipid phase) while the encapsulated oil may reflect the oxidation profile typical of a mixture of samples with different level of oxidation, indicating that variables influencing susceptibility to oxidation (i. e., surface-to-oil volume ratio, accesibility of air, etc.) could differ greatly in different portions of the noncontinuous encapsulated lipid phase. The results obtained support that separate evaluation of oxidation of both fractions is essential to gain insight into the antioxidant efficacy in these complex heterophasic lipid systems. Further studies are now underway to determine the influence of polarity of antioxidants on their action in the free and encapsulated oil fractions.

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