Influence of oil droplet size on the oxidative stability of the free and encapsulated fractions of freeze-dried microencapsulated sunflower oil

Francisca Holgado,[1] Gloria Márquez-Ruiz,[1] M. Victoria Ruiz-Méndez,[2] and Joaquín Velasco[2,*]

[1]*Instituto de Ciencia y Tecnología de Alimentos y Nutrición, Consejo Superior de Investigaciones Científicas (CSIC), c/ José Antonio Novais, 10, E-28040 Madrid, Spain*

[2]*Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), Campus Universidad Pablo de Olavide, Ctra. de Utrera km 1, E-41013 Sevilla, Spain*

*To whom correspondence should be addressed:
Telephone: +34 954 61 15 50
Fax: +34 954 61 67 90
E-mail: jvelasco@ig.csic.es

Summary

The effect of oil droplet size (ODS) on the oxidative stability (OS) of dried microencapsulated oils has been scarcely studied and results are contradictory. A few studies have shown increased OS when the ODS was reduced and this was attributed to a decrease in the surface oil content (SOC). Yet, in such studies only the total oil fraction was evaluated. In the present work, the free (FO) and encapsulated oil (EO) fractions of freeze-dried microencapsulated sunflower oil were analysed to study the effect of changes in the ODS by using different homogenisation pressure (15 or 70 MPa) in the emulsification step. The OS of both the free and encapsulated fractions increased when the ODS was significantly reduced in two samples with different encapsulation matrix, namely, caseinate/lactose and maltodextrin/sucrose/gelatine. A reduction in the SOC would explain the increased stability of the FO, but not that of the EO. An additional protective role of the interfacial film could have been involved. In conclusion, if the encapsulation matrix and the interfacial region are effective as oxygen barriers, a reduction of the ODS of the parent emulsion by an increase in the homogenisation pressure will result in capsules more stable against lipid oxidation.

Keywords

Oil microencapsulation, high-pressure homogenisation, oil droplet size, freeze-drying, lipid oxidation.

Introduction

Oil microencapsulation is mainly aimed at protecting oils from lipid oxidation. Basically, the process consists of two steps. First, the oil is emulsified in an aqueous phase containing complex carbohydrates or a combination of proteins and carbohydrates. Then, the emulsion is dried, normally by spray-drying, to obtain a powder product where the oil in small droplets is entrapped in the inner of solid particles (Ghnimi *et al.*, 2017; Ruiz-Ruiz *et al.*, 2017). Although spray-drying is the drying method normally used in the industry because of its relatively low cost, freeze-drying is one of the methods more recommended for oils highly susceptible to oxidation because it is performed at low temperature and vacuum conditions (Bakry et al, 2016).

The non-encapsulated or free oil fraction, mainly located on the particle surface (Drusch & Berg, 2008), is theoretically more susceptible to lipid oxidation than the encapsulated fraction because it is more exposed to oxygen. Therefore, the higher the microencapsulation efficiency (ME), the greater will be the oxidative stability. It is widely accepted that ME is influenced by the oil droplet size because the smaller and more uniform the droplets, the greater amount of oil will be covered and entrapped by the encapsulation matrix. Large droplets poorly stabilized tend to form part of the free oil (Drusch & Berg, 2008; Anwar & Kunz, 2011). In fact, smaller and more monodisperse emulsions have shown higher ME (Hogan *et al.*, 2001; Keogh et al., 2001; Soottitantawat *et al.*, 2003; 2005; Rusli et al., 2006; Jafari *et al.*, 2008; Holgado *et al.*, 2013; Ramakrishnan *et al.*, 2014).

Studies dealing with the effect of oil droplet size on the oxidative stability of dried microencapsulated oils (DMOs) are however scarce and confusing. According to Shiga *et al.* (2017) there are no clear reports on the effect of oil droplet size on the oxidative stability of DMO. While a few studies have shown increased oxidative stability when the oil droplet size was reduced (Ishido *et al.*, 2002; Minemoto *et al.*, 2002; Nakazawa *et al.*, 2008;

Ramakrishnan *et al.*, 2014; Abd Ghani *et al.*, 2016; Shiga *et al.*, 2017), in other studies an increase in ME as a result of decreasing the oil droplet size did not produce longer shelf-life (Risch & Reineccius, 1988) or even parent emulsions containing larger oil droplets provided DMOs with longer shelf-life (Soottitantawat *et al.*, 2005; Ixtaina *et al.*, 2015). The enhancement in the oxidative stability when the oil droplet size was reduced has been attributed to a decrease in the surface oil content. In contrast, a high proportion of free oil does not necessarily result in low oxidative stability (Drusch *et al.*, 2007). According to Drusch and Berg (2008), the extractable or free oil fraction cannot be used to predict the shelf-life of microencapsulated oils.

In all these studies only the total oil fraction was analysed to evaluate DMO oxidation, mainly due to the low amount of the free oil fraction. Previous studies carried out in our lab have however demonstrated the importance of evaluating both fractions separately. In this regard, separate evaluation of the free and encapsulated oil fractions enabled to elucidate why rancidity was detected when the levels of oxidation compounds were globally very low (Márquez-Ruiz et al., 2003), to get to know that these two fractions had different oxidative patterns (Velasco *et al.*, 2006), that relative humidity mainly affected the oxidative stability of the free oil (Velasco *et al.*, 2009a) and that oxidation of the free oil was responsible for the response of the Rancimat test when this was applied directly to DMOs (Velasco *et al.*, 2009b).

The aim of this work was to study the influence of the oil droplet size on the oxidative stability of DMOs by applying an analytical approach that differs from most of the oxidation studies in these products. In this regard, lipid oxidation was evaluated in the free and encapsulated oil fractions separately rather than in the total oil fraction. Towards this end, freeze-drying was the drying method chosen instead of spray-drying because freeze-dried capsules normally contain relatively greater amount of free oil. To produce DMOs with

different oil droplet size, different homogenisation pressures (15 or 70 MPa) were applied in the emulsification step. Two DMO samples containing caseinate/lactose or maltodextrin/sucrose/gelatine were studied. Both matrices were used in a previous physicochemical characterisation study and were chosen due to their relatively high powder physical stability (Holgado *et al.*, 2013).

Materials and methods

Materials

Refined conventional sunflower oil was used as a study model and purchased from a local supermarket. Sodium caseinate, D-lactose monohydrate, gelatine from bovine skin and sucrose (99%) were acquired from Sigma Chemical Co. (St. Louis, MO). Maltodextrin 10 (dextrose equivalent of 10) was purchased from Fluka (Sigma-Aldrich Chemie Steinheim, Germany). All other chemicals used were of analytical grade.

Dried microencapsulated oil samples

The samples were prepared in triplicate by freeze-drying of o/w emulsions. The sample containing sodium caseinate (CAS sample) was made from an emulsion constituted by 10% sunflower oil, 10% sodium caseinate and 10% lactose (w/w). The one containing maltodextrin (MD sample) was obtained from an emulsion that comprised 10% sunflower oil, 12% sucrose, 7% maltodextrin (DE 10) and 1% gelatine (w/w). Both formulated emulsions have already used in a previous work, whose aim was to evaluate how microencapsulation conditions affected the physicochemical characteristics of microcapsules (Holgado *et al.*, 2013). A coarse emulsion was first prepared in a DI-25 Ultraturrax (IKA, Germany) by applying 454 ***g*** (8,000 rpm) for 2 min, 641 ***g*** (9,500 rpm) for 2 min and 1294 ***g*** (13,500 rpm) for 1 min. Then, the coarse emulsion was homogenised in an EmulsiFlex-C5 (Avestin Inc., Canada) high pressure homogeniser by applying 15 or 70 MPa and one pass. Each emulsion was frozen at -32 ºC for 24 h and freeze-dried in a lab-scale Heto FD3 freeze-dryer (Allerød,

Denmark) for 48 h. Finally, the dried samples were ground in batches of 20 g in a domestic electronic coffee grinder of 400 mL volume for 10 s at interval of 5 s.

Physicochemical properties of the DMOs

Microencapsulation efficiency (ME)

ME was determined according to a previous study (Holgado *et al.*, 2013).

Oil droplet size

Analysis of oil droplet size was performed in reconstituted emulsions obtained by dispersing the DMO samples in water at a weight proportion of 1:7 according to Holgado *et al.* (2019). A Malvern Mastersizer X (Malvern Instruments, Malvern, UK) was also used at the same conditions as described elsewhere (Holgado et al., 2019). Ultrasound was applied in the sample dispersion unit to evaluate whether the changes observed were due to changes in the droplet size or to droplet flocculation. The application of ultrasound resulted in unchanged results in all the samples analysed, so flocculation was discarded. The effect of the drying method on the oil droplet size was studied in a previous work (Holgado et al., 2013).

Water activity

Water activity was measured with a PawKit hygrometer (DECAGON, USA).

Glass transition temperature

The glass transition temperature (Tg) was determined with a DSC Q2000 calorimeter (TA Instruments, New Castle, DE, USA) as described elsewhere (Holgado *et al*., 2013).

Storage conditions

DMO samples (10 g) were placed on Petri dishes and stored in desiccators at 30 ºC in the dark and 0.0 % relative humidity (silica gel). Periodic samplings were performed in a period of 170 days. The samples were kept at -25ºC until analysis.

Oil extraction

The free or non-encapsulated oil was extracted from 10 g of DMO, with 100 mL n-hexane and stirring for 15 min at room temperature, as previously reported (Holgado *et al.*, 2013). After the extraction of the free oil from 10 g of DMO, the encapsulated oil was extracted by a procedure based upon a mortar and pestle detailed elsewhere (Holgado et al., 2019).

Evaluation of oxidation

The oxidation state of the oil extracts from initial DMO samples and the oil used in the DMO preparation was evaluated by a combination of solid-phase extraction (SPE) and high-performance size-exclusion chromatography (HPSEC) analysis, according to a previous report (Holgado et al., 2019). The total fraction of polar compounds and the individual groups that form it, i.e. triacylglycerol oligomers (TGO), triacylglycerol dimers (TGD), oxidised triacylglycerol monomers (oxTGM), diacylglycerols (DG) and free fatty acids (FFA), were determined quantitatively. In addition, the peroxide value (PV) and the content of tocopherol were also analysed.

The oxidised DMO samples were evaluated applying the PV, the levels of triacylglycerol polymers by HPSEC analysis and the loss of tocopherols, described in a previous report (Holgado *et al.*, 2019).

Statistical analysis

Analyses were carried out in triplicate samples. One-factor ANOVA was applied using 24.0 SPSS Statistics program (SPSS Inc., Chicago, IL, USA). Tukey's test was used for multiple comparisons. Significance was defined at $p < 0.05$.

Results and discussion

Physicochemical characteristics of the DMO samples

The homogenisation conditions applied rendered samples with varying oil droplet size and different oil fraction distribution in the encapsulation matrix (Table 1). The ME was not high, but showed normal values for samples obtained by freeze-drying (Bakry *et al.*, 2016). The reconstituted emulsions from CAS samples presented a unimodal size distribution, smaller values for the mean oil droplet size and droplets in a much narrower size range than the MD samples, whose reconstituted emulsions showed a bimodal distribution (not shown). However, the ME was higher for the MD samples even when the average surface area (A_N) for the lipid fraction was far greater for the CAS samples, as previously reported (Holgado *et al.*, 2013). The homogenisation pressure exerted greater influence on the oil droplet size in the CAS sample, showing that its encapsulating matrix imparted a greater stabilizing effect on the lipid phase than the MD sample. As a result, the ME increased proportionally more in the CAS sample when the homogenisation pressure was increased.

The water activity of the samples was very low and similar for the two types of DMO, showing values that were around 0.05. The Tg values were higher for the CAS samples, indicating a greater structural stability (Table 1). In fact, significant differences in the ME were not observed in the CAS samples along the oxidation assay, which indicates that the matrix did not undergo changes in its structure that gave rise to changes in the oil distribution. In contrast, a significant rise in the ME up to 95% occurred in the MD samples from 100 d of storage, as also found in a recent study (Holgado et al., 2019).

Initial DMO samples

Results for the initial oil and the oil extracts from the DMO samples are listed in Table 2. Very slight increases in the content of oxTGM and the PV were found in the DMO samples, indicating slight oxidation during sample preparation. Significant losses of tocopherols were

also observed and these were significantly greater in the MD samples. The free oil in the MD samples was slightly more oxidised than the encapsulated oil, whereas this fact was not clear in the CAS samples. Regardless of the type of matrix, significant changes in the oxidative state of the encapsulated fraction were not observed when the homogenisation pressure was increased. In fact, the content of oxTGM and also the PV remained unchanged. However, the incipient oxidation of the free oil of the CAS sample was lower when the homogenisation pressure was increased. This fact was not clear in the MD sample as the oxTGM content did not show significant differences, but slightly higher PV was found in the MD70 sample.

Oxidised DMO samples

In the four samples studied, the free oil fraction presented an oxidative pattern characteristic of bulk oils, i.e. of a continuous phase, whereas oxidation in the encapsulated fraction occurred discontinuously, as reported in a previous study (Holgado *et al*., 2019). In this respect, the free oil showed a well-defined induction period after which complete depletion of tocopherols and onset of significant polymerization took place (Figure 1). On the other hand, significant levels of polymers were found in the encapsulated oil when the tocopherol content was still elevated, which was indicative of droplets with different oxidation states (Velasco *et al.*, 2006; 2009a; Morales *et al.*, 2015).

In the CAS samples, the PV increased similarly in the two oil fractions during the early stages of oxidation (Figure 1a). In addition, tocopherols were completely depleted at similar oxidation periods in both fractions (Figure 1c). Therefore, the encapsulation matrix did not seem to provide greater protection to the encapsulated fraction. From the results obtained by the three analytical determinations applied, it was evident that the CAS70 sample was more stable against oxidation than the CAS15 sample (Figure 1a-c).

In the MD samples, the PV increased more slowly in the encapsulated oil than in the free oil during the early stages of oxidation. This fact was more evident in the MD70 sample,

although it was also observed in the MD15 sample when the PV was up to 200 meq/kg (Figure 1a'). Therefore, the MD matrix seemed to protect the encapsulated oil against oxidation. While the PV showed greater oxidative stability for the MD70 sample at the beginning of the assay, the increase of polymers and the losses of tocopherols indicated the opposite at the end (Figure 1b'-c'). In fact, the total loss of tocopherols and the onset of substantial polymerization in the free oil occurred before in the MD70 sample than in MD15. However, when the end of the induction period of any given oil is reached, the oxidation level is extremely high from the point of view of rancidity perception (Velasco *et al.*, 2010). For practical purposes, it would be more appropriate to examine the oxidative development during the first stages of the process and not at the end of the induction period. According to a recent report (Holgado et al., 2019), oxidation extent up to levels of PV of 100 meq/kg has shown to be a good maximum limit to evaluate oxidation in DMOs.

Table 3 lists the periods at which the PV reached 100 meq/kg and also, for comparative purposes, those when tocopherols were exhausted. The PV exhibited similar or greater oxidative stability for the encapsulated oil fraction than for the free oil, showing the protective role of the two encapsulation matrices against the diffusion of oxygen. In addition, the PV during early oxidation also showed greater oxidative stability for the samples prepared with 70 MPa, i.e. for those with smaller oil droplet size, than those made with 15 MPa.

The greater oxidative stability of the samples containing smaller oil droplet sizes is in agreement with reported studies in which only the total oil fraction was analysed (Ishido *et al.*, 2002; Minemoto *et al.*, 2002; Nakazawa *et al.*, 2008; Ramakrishnan *et al.*, 2014; Abd Ghani *et al.*, 2016; Shiga *et al.*, 2017). The results of the present study showed that a significant reduction in the oil droplet size enhanced the oxidative stability of the encapsulated oil and also that of the free oil fraction (Table 3). Therefore, the improved oxidative stability of the DMOs can not only be attributed to a decrease of the surface oil

content as it was concluded in previous studies (Ishido *et al.*, 2002; Minemoto *et al.*, 2002; Nakazawa *et al.*, 2008; Ramakrishnan *et al.*, 2014; Abd Ghani *et al.*, 2016; Shiga *et al.*, 2017). According to Drusch and Berg (2008), the free oil fraction is mainly constituted by the surface oil, formed by discrete oil deposits that are not stabilised by an interfacial layer and are localized on the particle surface, porous and capillaries. In addition, inner droplets close to the particle surface that are accessible to the organic solvent also contribute to this fraction. It is highly likely that these droplets were completely covered and protected by the wall and that they were accessible to the organic solvent when the surface oil was first removed during the oil extraction. This inner oil sub-fraction would have to constitute an important part of the free oil to explain the increase in stability observed when the droplet size was reduced. Conversely, studies on fat localisation have shown by applying different approaches that the surface oil is by far the main part of the extractable oil (Gejl-Hansen & Flink, 1977; Drusch & Berg, 2008; Foerster *et al.*, 2016).

Foerster *et al.* (2016) observed that the free oil constituted a continuous film that covered the powder particles in a dried emulsion model containing similar composition to the CAS samples of the present work. This would be consistent with the fact that the free oil oxidised like a continuous lipid phase. However, if the free fraction had also been formed by individual droplets, its oxidation would have proceeded discontinuously in a similar way to the encapsulated fraction. Although the onset of substantial polymerization when tocopherol is completely lost is characteristic of the oxidation of oils in continuous phase, an uneven loss of the antioxidant could be due to oil droplets or oil sub-fractions oxidising at different rates. That was the case of the free oil in the MD samples (Figure 1c'), but it was not observed in the CAS samples. In addition, the levels of polymers accumulated during the induction period were different between samples prepared with different homogenisation pressure regardless of the type of matrix (Figure 1b-b'). This fact could also be indicative of oil droplets with

different oxidation states, i.e. droplets with low oxidation and droplets within advanced oxidation containing substantial polymerisation compounds. Therefore, the results seem to indicate that part of the free oil fraction also oxidised discontinuously.

Not less interesting was the fact that the oxidative stability of the microencapsulated oil also increased when the oil droplet size was reduced. An increase in oxidative stability as a result of a reduction in the surface oil content would only apply to the free oil, but obviously not to the encapsulated fraction. The interfacial layer of the oil droplets, often neglected, may play a very significant role in creating an additional oxygen barrier. The oil-matrix interface has a composition different from the bulk encapsulation matrix and thus may be a more or less effective barrier to oxygen diffusion than the bulk wall material (Reineccius & Yan, 2016). If the interfacial film were effective by providing additional protection, a significant increase in the oxidative stability of the encapsulated oil could be explained by a decrease in the oil droplet size. Similarly, the droplets that contribute to the free oil fraction and preserve their interfacial layer would also be more stable against lipid oxidation with a decrease in their particle size and would also contribute to the increased oxidative stability observed in the free oil fraction.

Conclusions

A reduction of the oil droplet size by applying greater homogenisation pressure in the emulsification step gave rise to increased oxidative stability of the free and encapsulated oil fractions in two matrices of different chemical nature. Therefore, the increase in oxidative stability was not only due to a reduction of the surface oil content as concluded in most studies in which only the total oil fraction was evaluated. This would only apply to the free oil fraction, but not to the encapsulated oil. Results suggested that the free fraction of freeze-dried microencapsulated oil was also formed by droplets that maintained their interfacial layer and were protected by the solid matrix. The interfacial layer may have provided additional

protection to the droplets of the encapsulated oil. In conclusion, if the encapsulation matrix and the interfacial film are effective as oxygen barriers, a reduction of the oil droplet size of the parent emulsion by an increase in the homogenisation pressure will result in capsules more stable against lipid oxidation.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the research, authorship or publication of this article.

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Figure legends

Figure 1 Oxidation of the free (FRE) and encapsulated (ENC) oil fractions in the CAS (a-c) and MD (a'-c') samples obtained from emulsions prepared with 15 or 70 MPa. The samples were oxidised at 30 ºC in the dark and 0.0% RH. Data correspond to mean values and the error bars show the standard deviation (n=3).

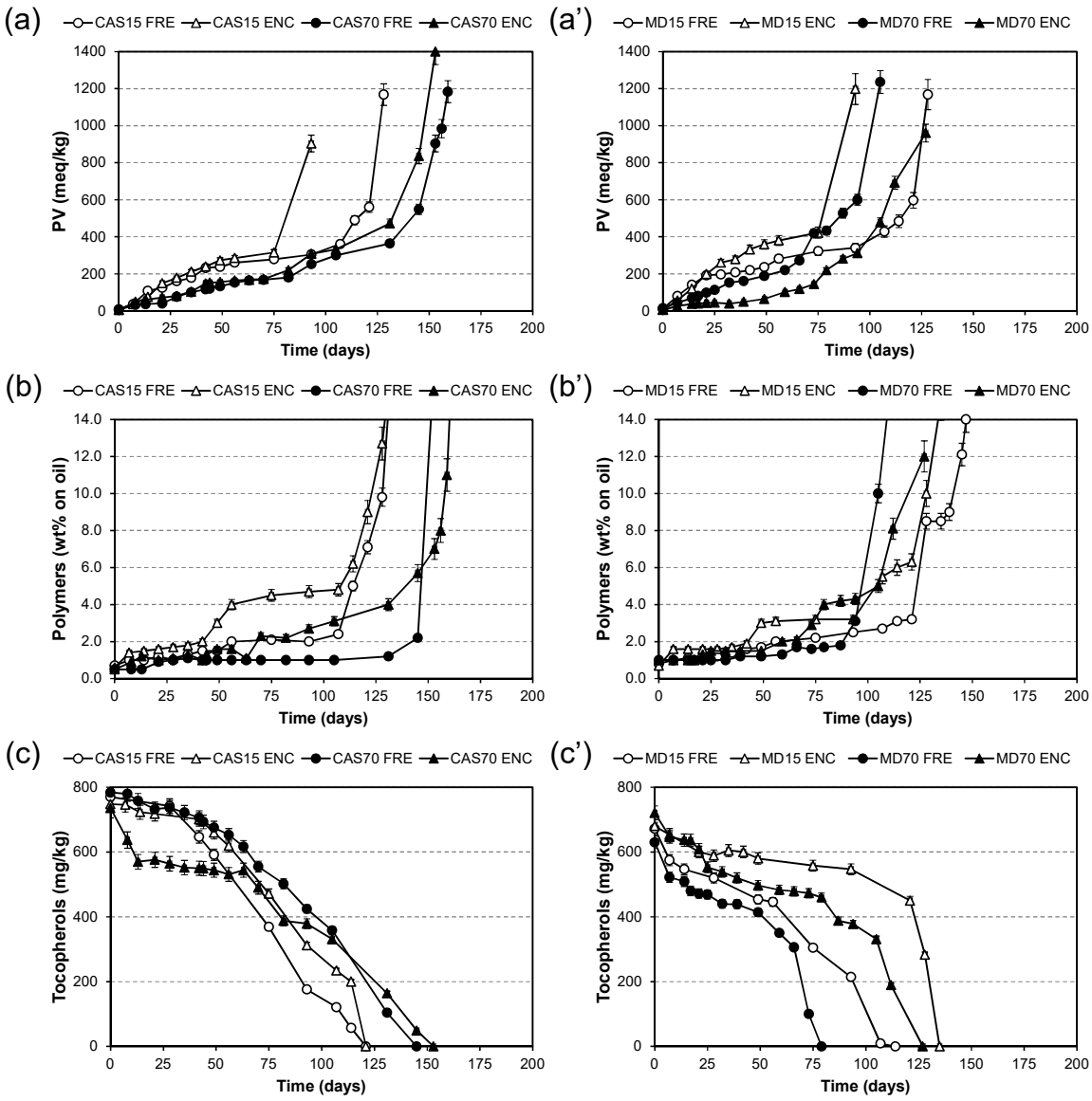
(a)
CAS15 FRE
CAS15 ENC
CAS70 FRE
CAS70 ENC
PV (meq/kg)
Time (days)
(a')
MD15 FRE
MD15 ENC
MD70 FRE
MD70 ENC
PV (meq/kg)
Time (days)
(b)
CAS15 FRE
CAS15 ENC
CAS70 FRE
CAS70 ENC
Polymers (wt% on oil)
Time (days)
(b')
MD15 FRE
MD15 ENC
MD70 FRE
MD70 ENC
Polymers (wt% on oil)
Time (days)
(c)
CAS15 FRE
CAS15 ENC
CAS70 FRE
CAS70 ENC
Tocopherols (mg/kg)
Time (days)
(c')
MD15 FRE
MD15 ENC
MD70 FRE
MD70 ENC
Tocopherols (mg/kg)
Time (days)

Figure 1

Table 1 Physicochemical characteristics of the dried microencapsulated oil samples.

	CAS15	**CAS70**	**MD15**	**MD70**
ME (%)	48.1 ± 2.9 a	61.2 ± 1.1 b	65.7 ± 3.8 b	77.1 ± 2.3 c
Oil droplet size:				
$d_{(v, 0.5)}$ (μm)	1.29 ± 0.15 b	0.85 ± 0.02 a	4.23 ± 0.01 c	3.77 ± 0.04 d
$d_{(v, 0.9)}$ - $d_{(v, 0.1)}$ (μm)	5.54 ± 0.04 b	3.47 ± 0.06 a	19.41 ± 0.26 c	20.50 ± 0.82 c
d_{32} (μm)	0.90 ± 0.03 b	0.55 ± 0.04 a	1.85 ± 0.01 d	1.48 ± 0.02 c
A_N (μm^{-1})	6.67 ± 0.22 c	10.95 ± 0.80 d	3.24 ± 0.02 b	4.05 ± 0.05 a
Water activity	0.04 ± 0.00 a	0.06 ± 0.00 b	0.08 ± 0.01 c	0.04 ± 0.00 a
Glass Transition Temperature (ºC)	55.8 ± 0.6 b	61.5 ± 0.3 c	40.6 ± 0.5 a	40.7 ± 0.4 a

CAS, samples prepared with sodium caseinate and lactose; MD, samples containing maltodextrin, sucrose and gelatine; the number after the sample name means the homogenisation pressure applied in the emulsification step; ME, microencapsulation efficiency; $d_{(v, 0.5)}$, mean droplet size; $d_{(v, 0.9)}$ - $d_{(v, 0.1)}$, range of droplet sizes; d_{32}, Sauter mean diameter; A_N, average surface area. Results are expressed as mean ± standard deviation (n=3). Different letters for each determination denote significance differences ($P < 0.05$).

Table 2 Characterisation of the initial oil and the oils extracted from the initial dried microencapsulated oil samples.

Sample	TPC (wt% on oil)	PC distribution (wt% on oil)				PV (meq O_2/kg)	Toc (mg/kg)
		TGO+TGD	oxTGM	DAG	FFA*		
Oil	4.9 ± 0.1 a	0.7 ± 0.1 ab	2.4 ± 0.1 a	1.4 ± 0.1 ab	0.4 ± 0.0 a	3.0 ± 0.1 a	891 ± 12 f
CAS15 FRE	5.6 ± 0.1 b	0.7 ± 0.1 ab	2.9 ± 0.2 bc	1.5 ± 0.1 ab	0.5 ± 0.0 ab	8.1 ± 0.4 d	780 ± 22 e
CAS15 ENC	5.4 ± 0.1 b	0.6 ± 0.1 ab	2.5 ± 0.1 ab	1.5 ± 0.1 ab	0.8 ± 0.0 d	5.5 ± 0.3 bc	745 ± 20 de
CAS70 FRE	4.9 ± 0.1 a	0.5 ± 0.1 a	2.3 ± 0.1 a	1.5 ± 0.1 ab	0.6 ± 0.0 bc	4.3 ± 0.3 ab	780 ± 18 e
CAS70 ENC	4.9 ± 0.1 a	0.5 ± 0.1 a	2.4 ± 0.1 a	1.4 ± 0.1 ab	0.7 ± 0.0 cd	5.1 ± 0.2 bc	737 ± 22 cde
MD15 FRE	5.9 ± 0.1 c	0.8 ± 0.1 b	3.2 ± 0.2 c	1.4 ± 0.1 ab	0.5 ± 0.1 ab	10.6 ± 0.6 e	668 ± 15 ab
MD15 ENC	5.5 ± 0.1 b	0.7 ± 0.1 ab	2.6 ± 0.1 ab	1.6 ± 0.1 b	0.6 ± 0.0 bc	5.8 ± 0.7 c	686 ± 20 bc
MD70 FRE	5.4 ± 0.1 b	0.7 ± 0.0 ab	2.9 ± 0.1 bc	1.4 ± 0.1 ab	0.4 ± 0.1 a	13.6 ± 0.7 f	631 ± 18 a
MD70 ENC	4.9 ± 0.1 a	0.8 ± 0.1 b	2.3 ± 0.2 a	1.3 ± 0.1 a	0.5 ± 0.0 ab	5.2 ± 0.6 bc	725 ± 22 cd

FRE, free oil fraction; ENC, encapsulated oil fraction; TPC, total polar compounds; TGO, triacylglycerol oligomers; TGD, triacylglycerol dimers; oxTGM, oxidised triacylglycerol monomers; DAG, diacylglycerols, FFA*, free fatty acids and other polar minor oil components; PV, peroxide value; Toc, tocopherols. Results are expressed as mean ± standard deviation (n=3). Different letters for each determination denote significance differences ($P < 0.05$).

Table 3 Oxidation time periods at which tocopherols were completely depleted and when the PV reached 100 meq kg^{-1} oil.

Sample	Time $_{Toc=0}$ (d)		Time $_{PV=100}$ (d)	
	Free	**Encapsulated**	**Free**	**Encapsulated**
CAS15	114-121	114-121	7-14	14-21
CAS70	131-145	145-153	35	35
MD15	107-114	128-135	7-14	7-14
MD70	73-79	112-127	21	59