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Improvement of Oxidative Stability of Dry Emulsion Containing Antioxidants by Modifying Process Conditions

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The encapsulation of polyunsaturated fatty acids (PUFAs) in powder by spray drying oil-in-water (O/W) emulsion containing antioxidant is used both to modify their handling properties and to preserve their functional properties. In this study, the influence of the process conditions (e.g. during production of emulsion and drying) on the oxidative stability of encapsulated sunflower oil and on the antioxidant activity of α-tocopherol was investigated in relation with the microstructure and morphology of the dry particles produced. High drying rate conditions were found to be detrimental to the encapsulation efficiency since giving rise to hollow particles with the inner vacuole acting as an oxygen reservoir. Furthermore, the contamination of the initial emulsion by metal ions favoured a pro-oxidant behaviour of α-tocopherol. The control of the process conditions to limit both the iron content in the initial emulsion and the drying rate is therefore essential to take advantage of the antioxidant character of α-tocopherol and to ensure an efficient protection of PUFAs in dry emulsion.

1. Introduction

The encapsulation of active lipophilic compounds such as polyunsaturated fatty acids (PUFAs) in powders by spray drying oil-in-water (O/W) emulsions is used both to modify their handling properties when going to the powder form and to preserve their functional properties during storage since lipophilic compounds, rich in large unsaturated hydrocarbon chains, are very sensitive to lipid oxidation. Oxidation arises when PUFAs are in contact with initiators such as metals, light or heat and produces hydroperoxides which are primary oxidation products (Chaiyasit, 2007). Hydroperoxides have no smell and taste and are relatively stable at room temperature but are decomposed by transition metal cations or high temperature into alkoxyl radicals that contribute to the propagation of oxidation giving rise to secondary oxidation products responsible for undesirable odours and off-flavours associated with rancidity of lipids (Frankel, 1998). By providing a physical barrier, the encapsulation of PUFAs in a solid matrix is used to prevent or at least delay their degradation. In addition, the use of antioxidants is expected to enhance their oxidative stability. The effectiveness of antioxidants depends on their chemical structure and relative polarity corresponding to different partitioning between the oil and aqueous phase of the emulsion and to different mechanisms of action in limiting lipid oxidation such as radical scavenging, metal chelation or oxygen reduction.
The encapsulation efficiency depends therefore on many factors linked both to the formulation and to the conditions during the different steps of the process (Aliakbarian et al., 2015). Actually, during spray drying, both the atomization stage where the emulsion undergoes high shear stress and the drying kinetics are known to influence the dry particle structure and morphology and therefore the protection provided. Especially, the oil droplet breakup during atomization was found to lead to higher non-encapsulated surface oil content (Munoz-Ibanez et al., 2015, 2016). Furthermore, oil protection is lower when there are cracks or fissures at the particle surface due to an easier access of oxygen into the solid matrix or for hollow particles obtained under fast drying conditions as the air core can act as an oxygen reservoir (Partanen et al., 2008; Fernandes et al.,

2013). The control of the spray drying conditions is therefore important to get dry particles with a structure limiting the contact between oxygen and encapsulated oil.
The aim of this study was to investigate the influence of the spray drying conditions on the protection provided to encapsulated purified sunflower oil. For this purpose, the morphology and microstructure of the dry particles obtained under fast and slow drying conditions were analysed and the oil oxidative stability in the dry emulsions prepared with and without antioxidant (e.g. α-tocopherol) was evaluated under accelerated thermo-oxidation conditions.

2. Materials and methods

2.1 Materials

Commercial sunflower oil purchased from a local store (Cora, Fr) and stripped from its natural tocopherols (Hernandez Sanchez et al., 2016) was used as a model oil for PUFAs (11%w saturated-, 29%w mono-saturated- and 60%w poly-unsaturated- fatty acids). α-tocopherol (Sigma-Aldrich, Ge) was used as antioxidant and added to a concentration of about 500 ppm in stripped oil.
O/W emulsions containing 59.86 %w ultra-pure water (Millipore, Fr), 4 %w oil and 36%w maltodextrin DE12 (MD) (Glucidex®, Roquette, Fr) used as wall material were stabilized by adding 0.14 %w polyoxyethylene (20) sorbitan monolaurate (Tween®20) (Sigma-Aldrich, Fr).

2.2 Preparation of initial O/W emulsion

Initial O/W emulsions were prepared using a two-step protocol with first the production of a pre-emulsion without wall material and then the addition, under gentle stirring, of an aqueous solution of maltodextrin (41 %w). Pre-emulsions were prepared dispersing oil (with or without α-tocopherol) and Tween®20 in water first using a rotor-stator homogenizer (Ultra-Turrax T25 basic, IKA, FR) at 16,000 rpm for 3 min and second performing two passes in a high pressure homogenizer (1001 L PANDA, Soavi NIRO, FR) with two stages and a total pressure of 300 bar. Emulsions were prepared either in stainless steel container or in glass container to avoid contamination by metal ions during production and conservation along spray drying (e.g. about 2h).

2.3 Preparation of dry emulsion by spray drying

Initial O/W emulsions were spray dried just after production (delay inferior to 30 minutes) using a Niro Minor pilot scale spray dryer (NIRO, DK). Two series of spray drying conditions corresponding to slow and fast drying kinetics respectively were tested varying the liquid feed rate, the air inlet temperature and the rotation speed of the rotary atomizer (e.g. 30 $g.min^{-1}$, 170 °C, 22 700 rpm and 25 $g.min^{-1}$, 200 °C, 25 000 rpm respectively) with a drying air flow rate of 110 $kg.h^{-1}$ in both cases. The air outlet temperature was comprised between 95 and 100 °C or 110°C depending on the air inlet temperature (e.g. of 170 °C or 200 °C).

2.4 Characterization of liquid and dry emulsions

Size distribution was measured by laser light diffraction (Mastersizer 2000, Malvern, FR) in wet mode (Hydro 2000) for initial and reconstituted (4 g powder in 6 g hot water 50 °C) O/W emulsions after dispersion in purified water (refractive index 1.33 for dispersing water and 1.475 for oil), and in dry mode (Scirocco 2000, 4 bar) for dry emulsions (powders). From the volume size distribution obtained, the median diameter d_{50} and d_{10} and d_{90}, corresponding to the diameters for which respectively 50, 10 and 90% of the particles have a smaller size, were determined and the span was calculated as span = $(d_{90} - d_{10})/d_{50}$. The higher is the span value, the wider is the distribution.
Particle morphology was deduced from micrographs obtained by Scanning Electron Microscopy (SEM) (Quanta 200, FEI, US). The samples were mounted on aluminum stubs with double-sided sticky carbon tape and subjected to a gold sputter coating process. Coated samples were then observed under high vacuum (< 6.10^{-4} Pa) at accelerating voltage of 5 kV.

2.5 Oil oxidative stability and antioxidant content

Oil oxidative stability was evaluated from the evolution of conjugated dienes content (CD), as primary oxidation products, in samples stored under accelerated thermo-oxidation conditions. Just after production, 30 g of each powder were equally distributed in three open ceramic vessels (10 g powder/vessel) and randomly placed into a climatic test chamber (VCN 100, VÖTSCH Industrietechnik, FR) at 60 °C and 50 % relative humidity (RH).
The measurement of conjugated dienes content (CD) in powders required the extraction of the oil-organic phase. This was performed following the procedure described by Hernandez Sanchez (2016) as well as the measurement of the conjugated dienes content by UV spectrophotometry at 234 nm (Standard ISO

3656/2011). Results correspond to the mean value of specific absorbance variation ΔAS (calculated as ΔAS = AS(t) – AS(0)) for the three samples.
The α-tocopherol content in the oil phase of antioxidant enriched powders was measured with a HPLC system (Waters®, Milford, USA) on the extracted oil-organic phase after evaporation, addition of HPLC grade n-heptane and vortex and ultrasonic agitation (see Hernandez Sanchez et al., 2016).

3. Results and discussion

Four powders were produced with (a) and without (0) antioxidant, under low (L) and high (H) drying rate (Table 1). They were characterized for their morphology and microstructure and the oxidative stability of encapsulated oil was evaluated and compared to the one of crude oil.

Table 1: Conditions during production of dry emulsions.

Powder	Antioxidant (α-tocopherol)	Beaker for MD solution	Tinlet air (°C)	Emulsion feed rate ($g.min^{-1}$)	Rotation speed rotary atomizor (rpm)
P0H	-	Stainless Steel	200	25	25,000
P0L	-	Stainless Steel	170	30	27,500
PaH	500 ppm/oil	Stainless Steel	200	25	25,000
PaL	500 ppm/oil	Glass	170	30	27,500

3.1 Morphology and microstructure of dry emulsions

Although the four powders were obtained under different spray drying conditions, they had similar particle size distributions (Table 2). Median diameters were comprised between about 18 and 25 µm with 80 % of the particles with diameters between about 9 and 50 µm. Span values were low (e.g. about 1.5) corresponding to straight distributions.

Table 2: Characteristic volume diameters of particles in dry emulsions and of oil droplets in initial O/W emulsion and in reconstituted emulsions for powders produced under low (L) and high (H) drying rate, with (a) and without (0) antioxidant.

Powder	Oil droplet size								Powder particle size			
	Initial emulsion				Reconstituted emulsion							
	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)	Span	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)	span	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)	span
P0H	0.3	0.8	2.5	2.6	0.7	2.4	12.4	4.9	10.3	18.9	37.2	1.4
P0L	0.4	1.2	3.1	2.2	0.8	2.5	9.8	3.6	12.4	24.8	50.8	1.5
PaH	0.4	1.2	3.2	2.3	0.8	1.9	23.2	11.7	8.9	18.2	35	1.4
PaL	0.2	0.7	2.1	2.6	0.4	1.1	3.2	2.7	10.5	18.1	31.2	1.1

Anyway, from the micrographs giving a general view of the powders (Figure 1), it appears that, whilst mainly shrunken particles were obtained for powders P0L and PaL produced under low drying rate conditions, for powders P0H and PaH produced under high drying rate conditions both shrunken and larger spherical particles were obtained. These morphologies are typical for spray dried particles and consistent with the different spray drying conditions tested (Tonon et al., 2011). For the four powders, the particle surface was smooth, with some wrinkles for shrivelled particles, but without cracks or fissures. Some large bumps can be observed corresponding to the locations left by the non-encapsulated oil which was eliminated during the metallization of the samples under reduced pressure before observation. The inner microstructure observed on crushed particles showed that the holes corresponding to oil droplets were distributed throughout the thickness of the particle crust, but not in the close vicinity of the surface ensuring oil protection.
The influence of the atomization step on the emulsion microstructure can be estimated comparing the oil droplet size distribution in the initial emulsion and in the reconstituted emulsion that was assumed to be representative of the dry emulsion microstructure. The oil droplet size distributions of initial O/W emulsions that were spray dried were similar for the four powders showing the reproducibility of the protocol used for production of initial emulsions (Table 2). They all were monomodal with a median volume diameter d_{50} between 0.7 and 1.2 µm and 80 % of the droplets with diameters ranging from 0.2 to 3.2 µm. Their size was therefore compatible with a good dispersion in the solid matrix of dry particles (diameters between 9 and 50

µm). In any case, the reconstituted emulsion showed larger diameters and span value compared to the initial one. The shift to larger sizes and the widening of the size distribution were due on the one hand, to the disappearing of the smaller oil droplets (e.g. below about 0.2 µm) due to coalescence probably caused by the depletion phenomenon in presence of a large quantity of carbohydrate polymer in the emulsion (e.g. 36 %w maltodextrin) (Chenamai and McClements, 2001) and, on the other hand, to the appearing of some large oil droplets (e.g. above 10 µm) corresponding to the non-encapsulated oil at the surface of dry emulsion particles that was observed on SEM micrographs.

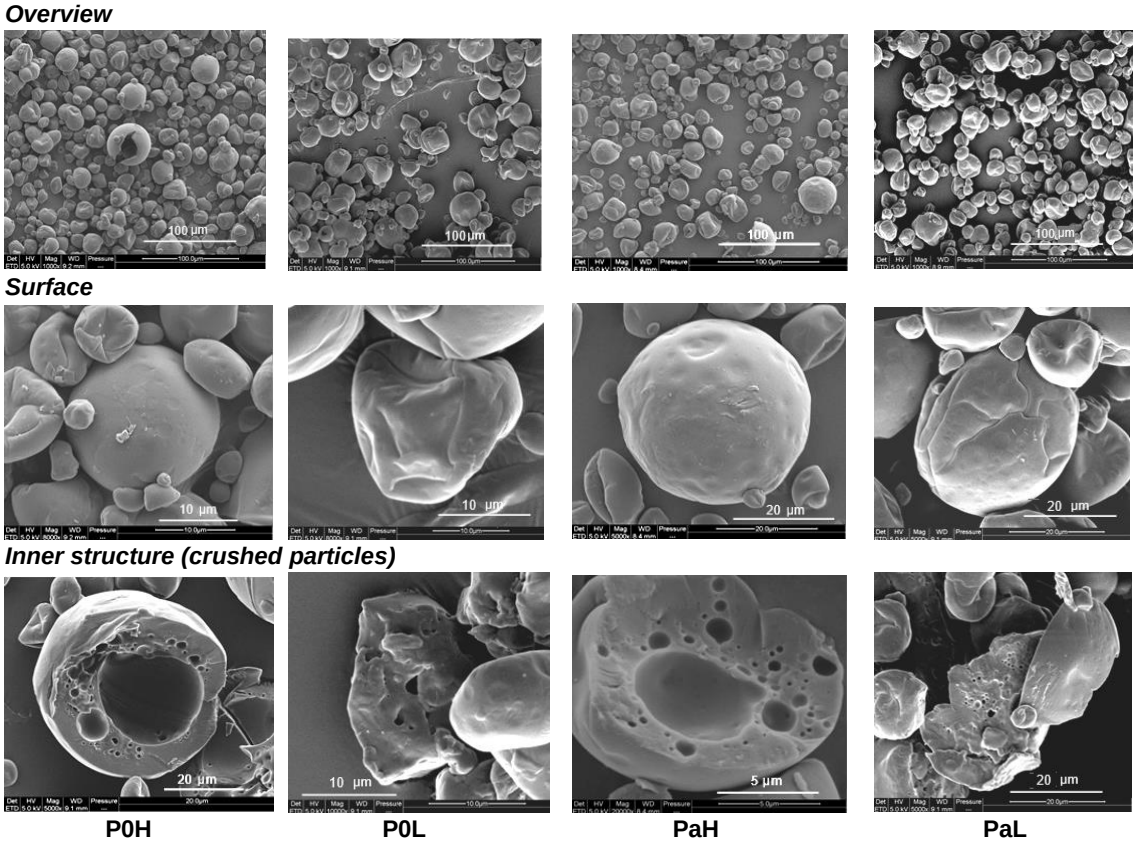

Figure 1: SEM micrographs of dry emulsion particles.

3.2 Oxidative stability of oil

The oxidative stability of encapsulated oil was estimated following the evolution of the conjugated dienes content (estimated from ΔAS) in powder samples stored under accelerated oxidation conditions (60 °C, 50 % RH) during about 20 days (Figure 2).

Whatever the powder, no lag phase was observed and oil oxidation started from the beginning of the test probably due to the initiation of oxidation during spray drying as it was demonstrated by Hernandez Sanchez et al. (2016). Nevertheless, the encapsulation in the powder solid matrix really provided protection to oil since the oxidation rate was significantly lower when compared to bulk oil (Figure 2a). Anyway, without antioxidant, the encapsulation efficiency was worst for powder P0H produced under high drying rate conditions. This may be attributed to the presence of hollow particles in powder P0H with the vacuole acting as an oxygen reservoir and favouring encapsulated oil oxidation.

Despite the fact that the addition of α-tocopherol in the formulation was expected to improve oil protection, the evolution observed for powder PaH rather highlighted a pro-oxidant effect (Figure 2b). This was attributed to the contamination of the emulsion by ferric ions during processing and conservation since α-tocopherol is known to become unstable regarding oxidation when in presence of metallic ions (Kamal-Eldin and Appelqvist, 1996). This hypothesis is supported by the greatly smaller oxidation rate observed for powder PaL prepared replacing stainless steel container with glass container to limit the iron contamination. In this case, the antioxidant effect of α-tocopherol combined to the absence of hollow particles led to an improved oil protection

with a very slow increase of the conjugated dienes content during the twelve first days of ageing whilst without antioxidant (P0L) the same maximum was reached within four days.

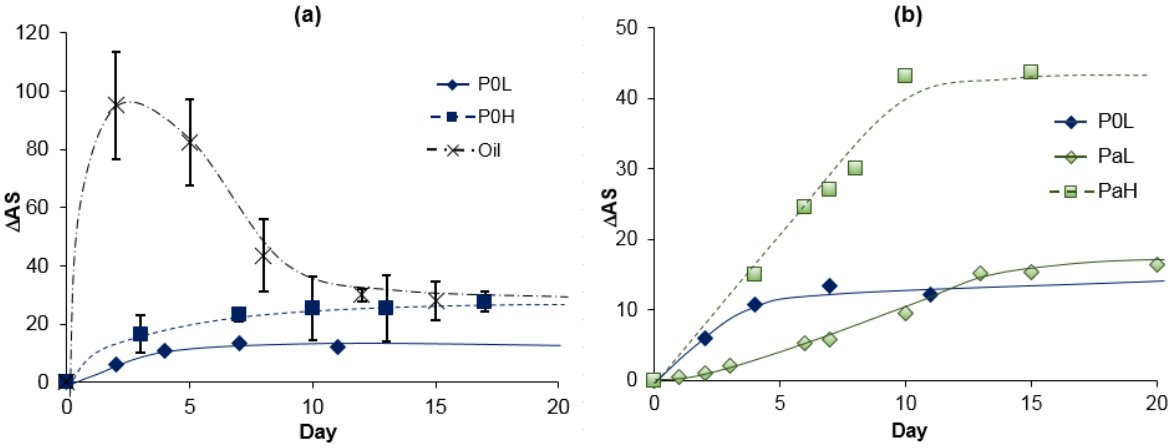

Figure 2: Evolution of conjugated dienes content during accelerated oxidation test in (a) stripped oil under gentle agitation and powders P0L and P0H (without antioxidant), and (b) powders PaL,PaH (with α-tocopherol) and P0L.

These findings are further evidenced by the evolution of the α-tocopherol content in the oil phase of powders PaL and PaH during ageing (Figure 3). For powder PaH, the antioxidant content at the beginning of the test was significantly lower than the theoretical value in the initial liquid emulsion (e.g. about 500 mg/kg oil) and it decreases rapidly within the six first days. This confirms that α-tocopherol started reacting with ferric ions during processing leading to a pro-oxidant behaviour during ageing. For powder PaL, most of the antioxidant added in the initial emulsion remained in the spray dried powder and it was consumed progressively during ageing ensuring an efficient oil protection within about ten days. In this case α-tocopherol totally disappeared after fifteen days.

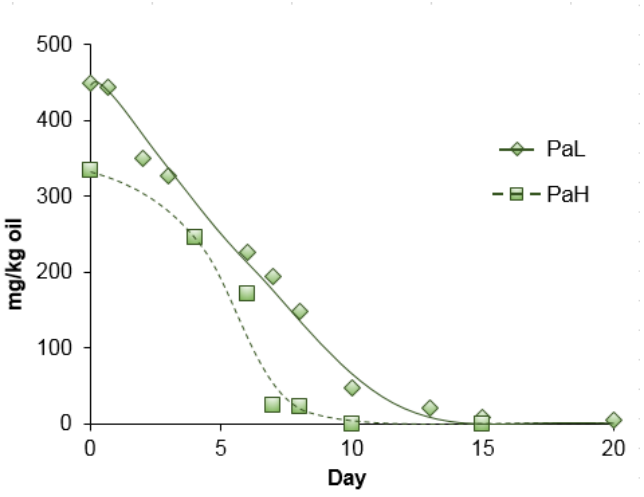

Figure 3: Evolution of the α-tocopherol content in the oil phase of powders PaL and PaH during ageing.

4. Conclusions

For an efficient encapsulation of PUFAs by spray drying, it is essential to consider interactions between formulation, process and structure. Especially, the formulation of the initial emulsion and the drying rate must be adapted to get dry particles with a microstructure ensuring oil protection e.g. limiting the contact with oxygen. When α-tocopherol is added, the expression of its antioxidant activity requires avoiding metal ions contamination or adding a metal chelator.

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