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Encapsulation of bioactive compounds in nanoemulsion-based delivery systems

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Abstract

Encapsulation into nanoemulsion-based delivery systems of bioactive compounds characterized by low solubility in aqueous phase, represents an effective approach to improve the dispersion of the bioactives into food products, to protect them against degradation or interaction with other ingredients, to reduce the impact on organoleptic properties of the food and to improve their bioavailability. The aim of the present work is the fabrication of nanoemulsions, based on natural food ingredients, to be used as delivery systems of two different bioactive compounds, resveratrol and curcumin, with the ultimate goal of improving the antioxidant and/or antimicrobial activities of the encapsulated compounds. A preliminary screening study of the optimal emulsion ingredients was carried out through the construction of a pseudo-ternary phase diagram of kinetic stability. The formation of very fine emulsions in the nanometric range (< 200 nm) was achieved by high pressure homogenization treatment, choosing those formulations containing small amounts of emulsifier, in order to minimize the impact on the organoleptic properties. Resveratrol (0.01% wt) was encapsulated in peanuts oil-based nanoemulsions, using different emulsifiers, such as soy lecithin and sugar esters. Curcumin was encapsulated in stable solid fat nanoemulsions, using stearic acid as lipid phase, up to 0.1% wt concentration. The nanoemulsions were characterized in terms of physical stability of the mean droplet size (dynamic light scattering) and chemical stability of the encapsulated bioactive compounds (HPLC and UV-Vis spectra analysis) upon accelerated ageing conditions. The effectiveness of the delivery systems was evaluated in terms of antioxidant activity of the encapsulated compounds.

Keywords: Delivery systems; Nanoemulsions; Polyphenols; Antioxidant activity.

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1. Introduction

Many bioactive compounds are highly lipophilic and show a very low solubility in water, which makes the addition to the majority of foods difficult. Furthermore, poor solubility also means lower absorption in the gastrointestinal tract and, therefore, limited bioavailability. In the food industry, it has become apparent that there is a pressing need for edible delivery systems to encapsulate, protect and release bioactive compounds when developing functional foods.

The nanoencapsulation in o/w nanoemulsions-based delivery systems represents an effective approach for incorporating bioactive compounds, such as resveratrol and curcumin, in foods. The nanoemulsions, lipid droplets of nanometric size (50-200 nm) dispersed in an aqueous phase using a suitable emulsifier at the oil/water interface [1], are produced through an energy-intensive comminution process, such as high pressure homogenization [2]. Immobilization of active compounds in the lipid matrix of the nanoemulsions efficiently contributes (a) to improve the dispersability of the bioactive compounds in aqueous solutions, minimizing the tendency to separate the different phases (aqueous and lipid), (b) to protect the bioactive compounds from interaction with food ingredients, keeping their functional properties and preventing the deterioration of the food itself (i.e. oxidation of fat), (c) to minimize the impact on the organoleptic properties of food, as well as (d) to improve absorption and bioavailability, due to the subcellular size of the nanocapsules, which enhances passive transport mechanisms (i.e. related to the concentration gradient) across the cell membrane [3].

In the field of bioactive compounds, we are currently conducting extensive work on the nanoencapsulation of curcumin [4] and resveratrol, two phytochemicals with many beneficial effects on the human health (antioxidant, antimicrobial, anti-inflammatory, chemopreventive and anti-cancer activity), but poor bioavailability which limits their clinical use [5].

In particular, this study focused on the fabrication of stable nanoemulsions made of natural and food-acceptable ingredients, such as soy lecithin, sugar ester and modified starch, to encapsulate two polyphenolic compounds, curcumin and resveratrol, with the final goal of improving their dispersability in aqueous systems, of protecting them from degradation, as well as of enhancing their antioxidant activity.

2. Materials and Methods

Different ingredients were screened for the fabrication of the nanoemulsion-based delivery systems, such as emulsifiers of natural origin (soy lecithin, sugar ester, modified starch and vegetable proteins), and different lipid phases (peanut oil, palm oil and stearic acid). For any different formulation, we have experimentally identified the range of kinetic stability as a function of the ingredients used and their concentrations, through the definition of different pseudo-ternary phase diagrams of stability (as previously described [6]) of the primary emulsions of micrometric size, prepared by high speed homogenization, using an *Ultra Turrax T25* (IKA Labortechnik, *Jahnke und Kunkel, Germany*) at 24000 rpm and 10°C for 4 min. The most suitable formulations, in terms of emulsion stability and minimization of the amount of emulsifier employed, were processed by high pressure homogenization for the production of nanodelivery systems. For the fabrication of nanoemulsion-based delivery systems, for the encapsulation of both resveratrol and curcumin, a laboratory scale high pressure homogenizer, the Nano DeBEE Electric Bench-top Laboratory (BEE International, USA), was used. The primary emulsion was pumped by the intensifier pump through a small orifice, available in a variety of sizes (5 o 8 μm), at high pressure (100-300 MPa), producing an extremely high velocity jet. This constitutes the first step in the size-reduction process. The high velocity jet of product enters in a specially designed interaction cell, downstream to the orifice, where a combination of shear, cavitation and impact forces completely break apart the droplets, as well as prevent their recoalescence, reducing the mean emulsion droplet size to nanometric dimensions. The process conditions used were 10 high pressure homogenization (HPH) passes at 275 MPa. For the resveratrol encapsulation the temperature during processing was always kept

about 10°C. In the case of curcumin, encapsulated in solid lipid nanoparticles, the formation process was similar, but the process temperature was kept at 60°C, which is well above the lipid melting temperature, in order to produce a hot emulsion, whose rapid cooling generated the lipid nanoparticles.

The physical stability was evaluated by measuring the variation of the mean droplet diameter by Dynamic Light Scattering and turbidity over time under accelerated ageing conditions (high temperature and high-low pH). Moreover an extraction method, able to separate resveratrol from the oil and from the water phase, was developed for the determination, by HPLC analysis, of the encapsulation efficiency and of its chemical stability over time.

In the case of the curcumin-encapsulated emulsions, the chemical stability was measured by UV-Vis spectrophotometer, obtaining UV-Vis spectra in the range of 360-460 nm of the curcumin-loaded emulsions.

The antioxidant activity was determined by the ferric reducing/antioxidant power (FRAP) assay at 593 nm [7] using L-(+)-ascorbic acid as standard.

Each measurement was replicated three times.

3. Results and Discussion

During the screening phase, the formulations of kinetically stable O/W emulsions were identified in a pseudo-ternary phase diagram (Fig. 1), in terms of emulsifier(s), lipid, and water fractions, with the bioactives loaded in the lipid phase. Unstable systems included the formulations characterized by physical separation, such as the formation of two or three layers of excess water, emulsion and/or excess oil. On the other side the stable systems could be divided into three large categories: (a) the "Creams", including the emulsion systems with a macroscopically Non-Newtonian behavior, (b) the "Stable o/w emulsions", including all the formulations at low oil concentrations diluted to the point to exhibit a Newtonian flow behavior, and (c) the "Stable w/o emulsions", including the formulations at high oil concentrations with a Newtonian flow behavior.
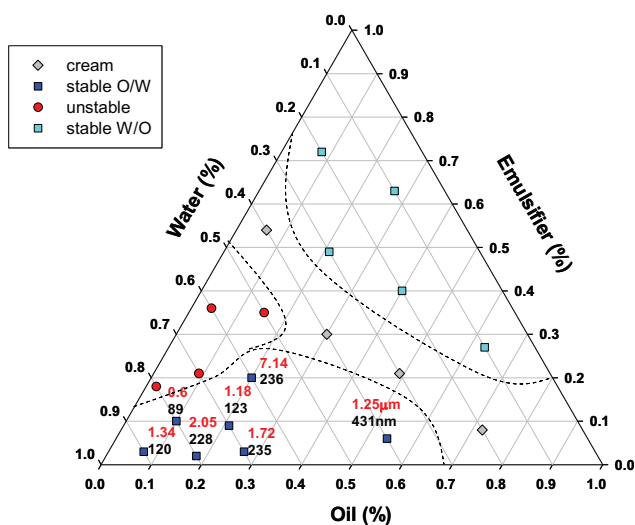

Fig. 1. Pseudo-ternary phase diagram of stability of tween 20 – glycerol monooleate – sunflower oil – water system homogenized by HSH. For stable emulsion formulations, the $x_{3,2}$ diameter of the droplets is reported in μm (red) and the z-diameter of the HPH treated emulsion is reported in nm (black).

Fig 1 shows a typical pseudo-ternary phase diagram, made of tween 20 – glycerol monooleate (in 1:1 proportion) – sunflower oil – water, with the indication of the different stability regions.

The "Stable o/w emulsions" were those of interest for the preparation of delivery systems, and they were considered as the primary emulsions for further processing by HPH and production of the secondary nanometric emulsions. It can be observed that, after HPH process, in the region corresponding to lower amounts of emulsifier and larger oil fractions, larger mean droplet sizes were obtained. It can be postulated that during the secondary homogenization, in this region the controlling step in determining particle size is represented by the fraction of emulsifier sufficient to cover the surface of droplet formed. In fact, when smaller droplets are formed, and hence with only partial surface coverage, they rapidly coalesce to form bigger particles until complete surface coverage is reached. Instead, in the region richer in emulsifier, final particle size is only determined by the efficiency with which energy is transferred to the fluid, see Fig 1.

Resveratrol (0.01% wt) was encapsulated in peanut oil-based nanoemulsions. As emulsifiers, different mixtures of a lipophilic compound, such as soy lecithin, and an hydrophilic compound such as sugar ester and defatted soy lecithin were used. The behaviour of the delivery systems based on natural emulsifiers was compared with that of a nanoemulsion-based delivery system consisting of artificial emulsifiers such as glycerol monooleate and tween 20. Table 1 shows the formulation and the mean droplet size of the nanoemulsions tested.

Table 1. Composition and mean droplet diameter of resveratrol nanoemulsions.

Delivery systems	Composition	Z-average (nm)
R/LSL-DSL	0.01% resveratrol 0.2% ethanol 1% lipophilic soy lecithin 0.5% defatted soy lecithin 6% peanut oil 92.29% water	211.8±0.3
R/LSL-SE	0.01% resveratrol 0.2% ethanol 1% lipophilic soy lecithin 0.3% sugar ester 9% peanut oil 89.49% water	137.5±0.2
R/T20-GMO	0.01% resveratrol 0.2% ethanol 1.5% tween 20 1.5% glycerol monooleate 7% peanut oil 89.79% water	128.2±0.1

All the nanoemulsions resulted physically stable over 4 weeks with neither visible creaming nor significant variation of the mean droplet size. Moreover, the nanoemulsions were also able to protect resveratrol from oxidation. For example, Fig 2 shows the effect of encapsulation on the light stability of resveratrol evaluated by the retention percentage, the ratio of the content of cis-resveratrol retained to the original trans-resveratrol in the sample. For the standard resveratrol, solubilised in ethanol, the formation of cis-resveratrol was observed after 10 min that the sample was exposed to UV light illumination and increased with the exposition time. On the contrary, for the formulations R/LSL-DSL and R/T20-GMO a low concentration of cis-resveratrol was formed only after 30 and 120 min of light exposition respectively. The formulation R/LSL-SE resulted as the most stable delivery system because even after 120 min of light exposure, there was no degradation of trans-resveratrol.
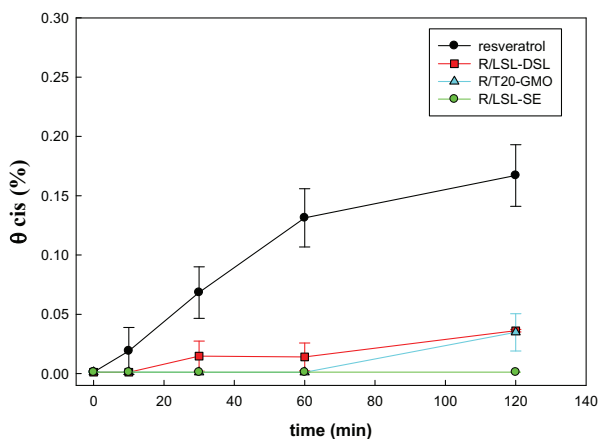
Fig. 2. The retention percentage of cis-resveratrol under UV light conditions for 2h.

Curcumin was encapsulated in solid lipid nanoparticles using stearic acid and/or palm oil as lipid phase. It was observed that the highest concentration of curcumin that could be encapsulated to avoid curcumin recrystallization and settling is about 0.1% wt. The results indicated that encapsulated curcumin was very stable when kept at refrigerated conditions (4°C) and at room temperature (30°C) for 20 days with constant mean droplet diameters (results not reported). Fig 3 shows the absorption curve of UV–Vis spectra among the curcumin-encapsulated emulsions stored at 4°C during 20 days study. It can be observed that the peaks of the absorbance at 420 nm remain quite constant over the storage time. This result highlights that the encapsulated curcumin was very stable and was not released from the delivery system.
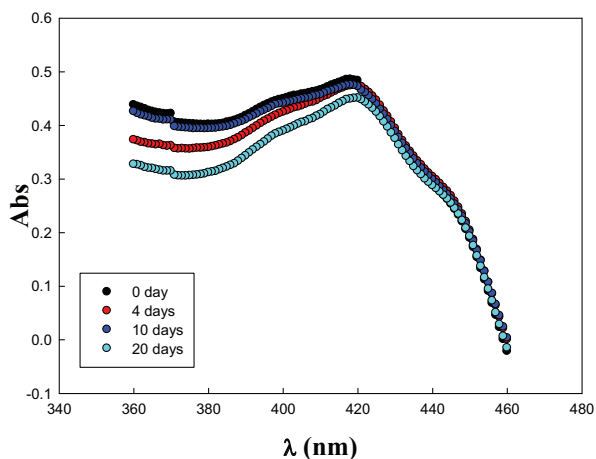

Fig. 3. The UV–Vis absorption of the curcumin-encapsulated emulsion after storage at 4°C for 20 days.

The effect of the delivery systems on the antioxidant activity of the encapsulated compounds was evaluated by comparing it with resveratrol and curcumin not encapsulated, see Table 2. Regarding

resveratrol, the nanoemulsion R/LSL-SE, which appeared to be the most chemically stable (no cis-resveratrol), showed an antioxidant activity comparable to that of the unencapsulated compound. For the other two formulations (R/LSL-DSL and R/T20-GMO), which showed a lower stability with the formation of low concentrations of cis-resveratrol, the antioxidant activity of the encapsulated resveratrol was decreased compared to the non-encapsulated compound.

On the other hand, for curcumin encapsulation the interpretation of results may be different. Although the antioxidant activity of the encapsulated compound is lower than non-encapsulated, this may be due to the fact that curcumin is encapsulated in a solid lipid matrix which not only protects better the compound but also prevents a good measure of the antioxidant activity.

Table 2. Antioxidant activity of resveratrol and curcumin-encapsulated emulsions using FRAP assay.

Sample	FRAP (*A 593 nm*)
Resveratrol not encapsulated	0.434±0.06
R/LSL-DSL	0.151±0.07
R/LSL-SE	0.443±0.04
R/T20-GMO	0.266±0.05
Curcumin not encapsulated	2.504±0.06
Curcumin encapsulated in solid lipid nanoemulsion	0.996±0.07

4. Conclusion

This study showed that nanoemulsion-based delivery systems can be efficiently used in the encapsulation of bioactive, improving their water dispersability, protecting them from degradation and preserving the antioxidant activity. The encapsulation of resveratrol (0.01% wt) in peanut oil-based nanoemulsions improved its stability, as shown by the significant reduction of the chemical degradation of trans-resveratrol to cis-. Curcumin (0.1% wt) was encapsulated in solid lipid nanoemulsions, that trapped the compound in a solid matrix, which contributed to improve its solubility in aqueous systems and to avoid the recrystallization and settling of the bioactive compound over time.

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